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Annual Report

Institute of Medical Science
University of Tokyo

2022

Preface

It is our pleasure to present Annual Report 2022 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 the present threat, 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 and other types of 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 last year to continue the center program for the next term of six years. By utilizing this platform, we are supporting 32 international joint research projects in fiscal year 2022. 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 2022. 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 2023

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

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〈as of December, 2022〉

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International Vaccine Design Center　国際ワクチンデザインセンター

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Research Center for Asian Infectious Diseases ····· 214

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Assistant Professor	Miho Asahara, M.D., Ph.D.	助　教	博士(医学)	浅　原　美　保
Project Assistant Professor	Miki shinokawa, M.D.	特任助教		篠　川　美　希

Department of Joint Surgery ····· 255

関節外科

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Assistant Professor	Kumiko Ono, M.D., D.M.Sc.	助　教	博士(医学)	大　野　久美子

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脳腫瘍外科

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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

Department of Urology ····· 260

泌尿器科

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Project Senior Assistant Professor	Sayuri Takahashi, M.D., Ph.D.	特任講師	博士(医学)	高　橋　さゆり
Assistant Professor	Yuta Takeshima, M.D., Ph.D.	助　教	博士(医学)	竹　島　雄　太
Project Assistant Professor	Mariko Tabata, M.D.	特任助教		田　畑　真梨子
Project Assistant Professor	Daiji Watanabe, M.D.	特任助教		渡　邉　大　仁

Department of Medical Informatics ····· 262

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	Haruomi Yamaguchi, M.D., D.M.Sc.	助　教	博士(医学)	山　口　晴　臣

Department of Cell Processing and Transfusion ····· 263

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
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Assistant Professor	Toyotaka Kawamata, M.D., Ph.D.	助　教	博士(医学)	川　俣　豊　隆

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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
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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-derived products from microorganisms and viruses. Recent reports have demonstrated that imbalances in TLR responses can result in the development of autoimmune diseases. Nucleic acid(NA) -sensing TLRs detect not only bacterial and viral NAs, but also host-derived NAs. To prevent excessive immune responses to host-derived NA, there may exist regulatory mechanisms that control TLR expression, localization, and function. Based on this hypothesis, it is believed that TLRs are involved not only in autoimmune diseases, but also in the pathogenesis of a variety of other diseases. Our research endeavors to uncover the regulatory mechanisms that control TLR-mediated recognition of pathogenic ligands, as well as the identification of endogenous ligands. Our research goal is to clarify the pathogenic mechanisms of histiocytosis and autoimmune diseases that are thought to be mediated by TLRs.

1. Endosomal abnormalities in dendritic cells cause autoimmune liver diseases

Shin-Ichiroh Saitoh[1], Kenichi Harada[5], Yoshiko Mori Saitoh[1], Ge-Hong Sun-Wada[6], Tamami Denda[3], Yasunori Ota[3], Hiroshi Sagara[4], Yuji Watanabe[4], Yoh Wada[7], and Kensuke Miyake[1, 2]

[1]Division of Infectious Genetics, Department of Microbiology and Immunology. [2]Laboratory of Innate Immunity, [3]Department of Pathology, Research Hospital, [4]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan. [5]Department of Human Pathology, Kanazawa University School of Medicine, Kanazawa, 920-8640, Japan. [6]Department of Biochemistry, Faculty of Pharmaceutical Sciences, Doshisha Women's College, Kohdo, Kyotanabe, Kyoto 610-0395, Japan. [7]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 autoimmune liver diseases with unknown etiologies. Although T cells are thought to drive these liver diseases, little is known about the underlying mechanism of T cell activation in these liver diseases. Since antigen presentation is regulated by endosome maturation which Rab7a controls, we investigated the changes in the immune response that occur upon blocking endosome maturation by Rab7a deficiency in dendritic cells (DCs). As a result, DC-specific Rab7a-deficient mice developed AIH and PBC. Failure to suppress Vps34-dependent endosome fusion due to Rab7a deficiency markedly enhanced cross-presentation by forming giant endosomes and altering MHC class Ⅰ transport. MHC class Ⅰ was accumulated in the giant endosomes. Hyperactivated CD8+ T cells caused fibrosis around portal veins and central veins, a hallmark of AIH. Female mice had a

worse condition of PBC. αβ T cell ablation protected the mice against AIH, but not PBC, where cytotoxic γδ T cells were localized around the bile duct. αβ- and γδ-T cell deficiency in the mice ameliorated both AIH and PBC. This study revealed that endosomal abnormalities in DCs strongly enhanced cross-presentation and γδ T cell activation resulting in autoimmune liver diseases. CD8+ T cells and γδ T cells are potential therapeutic targets for AIH and PBC.

2. Nucleosides drive histiocytosis in SLC29A3 disorders by activating TLR7

Takuma Shibata[1], Ryota Sato[1], Masato Taoka[2], Shin-Ichiroh Saitoh[1], Mayumi Komine[3], Kiyoshi Yamaguchi[4], Susumu Goyama[5], Yuji Motoi[1], Jiro Kitaura[6], Kumi Izawa[6], Yoshio Yamauchi[2], Yumiko Tsukamoto[7], Takeshi Ichinohe[8], Etsuko Fujita[3], Ryosuke Hiranuma[1], Ryutaro Fukui[1], Yoichi Furukawa[4], Toshio Kitamura[9], Toshiyuki Takai[10], Arinobu Tojo[11], Mamitaro Ohtsuki[3], Umeharu Ohto[12], Toshiyuki Shimizu[12], Manabu Ozawa[13], Nobuaki Yoshida[13], Toshiaki Isobe[2], Eicke Latz[14], Kojiro Mukai[15], Tomohiko Taguchi[15], Kensuke Miyake[1]*

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Loss-of-function mutations in *SLC29A3* cause lysosomal nucleoside storage and histiocytosis: phagocyte accumulation in multiple organs. However, little is known about the mechanism by which lysosomal nucleoside storage drives histiocytosis. Herein, histiocytosis in *Slc29a3*$^{-/-}$ mice was shown to depend on Toll-like receptor 7 (TLR7), which senses a combination of nucleosides and oligoribonucleotides (ORNs). TLR7 increased phagocyte numbers by driving the proliferation of Ly6C^{hi} immature monocytes and their maturation into Ly6C^{low} phagocytes in *Slc29a3*$^{-/-}$ mice. Downstream of TLR7, FcRγ and DAP10 were required for monocyte proliferation. Histiocytosis is accompanied by inflammation in SLC29A3 disorders. However, TLR7 in nucleoside-laden splenic macrophages failed to activate inflammatory responses. Enhanced production of pro-inflammatory cytokines was observed only after stimulation with ssRNAs, which would increase lysosomal ORNs. Patient-derived monocytes harboring the G208R *SLC29A3* mutation showed enhanced survival and proliferation in a TLR8 antagonist-sensitive manner. These results demonstrated that non-inflammatory TLR7/8 responses to lysosomal nucleoside stress drive SLC29A3 disorders.

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

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production and multiple organ damage. We found that inhibition of Toll-like receptor 7 (TLR7) rescues NZBWF1 mice from lethal nephritis by reducing the activation of B cells and monocytes. Immunohistochemistry analysis of the kidneys revealed that Ly6C-negative/

FcγRIV-positive patrolling monocytes (PatMCs) infiltrated into glomeruli. To clarify the role for PatMCs in nephritis, we focused on the molecules expressing in PatMCs. We performed RNA-sequencing and antibody array screening by comparing PatMC and Ly6C-positive classical monocytes, with or without TLR7 inhibition. In results, expression of several lupus-related molecules, for example, IL-10, PD-L2, and PECAM-1 were induced by TLR7 signaling. We are analyzing the mechanisms of these molecules as further study.

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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. Redundant and specific roles of A-type lamins and lamin B receptor in herpes simplex virus 1 infection

Kosuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasushi Kawaguchi

We investigated whether A-type lamins (lamin A/C) and lamin B receptor (LBR) are redundant during herpes simplex virus 1 (HSV-1) infection in HeLa cells expressing lamin A/C and LBR. Lamin A/C and LBR double knockout (KO) in HSV-1-infected HeLa cells significantly impaired expressions of HSV-1 early and late genes, maturation of replication compartments, marginalization of host chromatin to the nuclear periphery, enlargement of host cell nuclei, and viral DNA replication. Phenotypes of HSV-1-infected HeLa cells were restored by the ectopic expression of lamin A/C or LBR in lamin A/C and LBR double KO cells. Of note, lamin A/C single KO, but not LBR single KO, promoted the aberrant accumulation of virus particles outside the inner nuclear membrane (INM) and viral replication, as well as decreasing the frequency of virus particles inside the INM without affecting viral gene expression and DNA replication, time-spatial organization of replication compartments and host chromatin, and nuclear enlargement. These results indicated that lamin A/C and LBR had redundant and specific roles during HSV-1 infection. Thus, lamin A/C and LBR redundantly regulated the dynamics of the nuclear architecture, including the time-spatial organization of replication compartments and host chromatin, as well as promoting nuclear enlargement for efficient HSV-1 gene expression and DNA replication. In contrast, lamin A/C inhibited HSV-1 nuclear export through the INM during viral nuclear egress, which is a unique property of lamin A/C. IMPORTANCE This study demonstrated that lamin A/C and LBR had redundant functions associated with HSV-1 gene expression and DNA replication by regulating the dynamics of the nuclear architecture during HSV-1 infection. This is the first report to demonstrate the redundant roles of lamin A/C and LBR as well as the involvement of LBR in the regulation of these viral and cellular features in HSV-1-infected cells. These findings provide evidence for the specific property of lamin A/C to inhibit HSV-1 nuclear egress, which has long been considered but without direct proof.

2. Role of the orphan transporter SLC35E1 in the nuclear egress of herpes simplex virus 1

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This study developed a system consisting of two rounds of screening cellular proteins involved in the nuclear egress of herpes simplex virus 1 (HSV-1). Using this system, we first screened cellular proteins that interacted with the HSV-1 nuclear egress complex (NEC) consisting of UL34 and UL31 in HSV-1-infected cells, which are critical for the nuclear egress of HSV-1, by tandem affinity purification coupled with mass spectrometry-based proteomics technology. Next, we performed CRISPR/Cas9-based screening of live HSV-1-infected reporter cells under fluorescence microscopy using single guide RNAs targeting the cellular proteins identified in the first proteomic screening to detect the mislocalization of the lamin-associated protein emerin, which is a phenotype for defects in HSV-1 nuclear egress. This study focused on a cellular orphan transporter SLC35E1, one of the cellular proteins identified by the screening system. Knockout of SLC35E1 reduced HSV-1 replication and induced membranous invaginations containing perinuclear enveloped virions (PEVs) adjacent to the nuclear membrane (NM), aberrant accumulation of PEVs in the perinuclear space between the inner and outer NMs and the invagination structures, and mislocalization of the NEC. These effects were similar to those of previously reported mutation(s) in HSV-1 proteins and depletion of cellular proteins that are important for HSV-1 de-envelopment, one of the steps required for HSV-1 nuclear egress. Our newly established screening system enabled us to identify a novel cellular protein required for efficient HSV-1 de-envelopment. IMPORTANCE The identification of cellular protein(s) that interact with viral effector proteins and function in important viral procedures is necessary for enhancing our understanding of the mechanics of various viral processes. In this study, we established a new system consisting of interactome screening for the herpes simplex virus 1 (HSV-1) nuclear egress complex (NEC), followed by loss-of-function screening to target the identified putative NEC-interacting cellular proteins to detect a defect in HSV-1 nuclear egress. This newly established system identified SLC35E1, an orphan transporter, as a novel cellular protein required for efficient HSV-1 de-envelopment, providing an insight into the mechanisms involved in this viral procedure.

3. Role of the arginine cluster in the disordered domain of Herpes Simplex Virus 1 UL34 for the recruitment of ESCRT-III for viral primary envelopment

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During the nuclear export of nascent nucleocapsids of herpesviruses, the nucleocapsids bud through the inner nuclear membrane (INM) by acquiring the INM as a primary envelope (primary envelopment). We recently reported that herpes simplex virus 1 (HSV-1) nuclear egress complex (NEC), which consists of UL34 and UL31, interacts with an endosomal sorting complex required for transport III (ESCRT-III) adaptor ALIX and recruits ESCRT-III machinery to the INM for efficient primary envelopment. In this study, we identified a cluster of six arginine residues in the disordered domain of UL34 as a minimal region required for the interaction with ALIX, as well as the recruitment of ALIX and an ESCRT-III protein CHMP4B to the INM in HSV-1-infected cells. Mutations in the arginine cluster exhibited phenotypes similar to those with ESCRT-III inhibition reported previously, including the mislocalization of NEC, induction of membranous invagination structures containing enveloped virions, aberrant accumulation of enveloped virions in the invaginations and perinuclear space, and reduction of viral replication. We also showed that the effect of the arginine cluster in UL34 on HSV-1 replication was dependent primarily on ALIX. These results indicated that the arginine cluster in the disordered domain of UL34 was required for the interaction with ALIX and the recruitment of ESCRT-III machinery to the INM to promote primary envelopment. IMPORTANCE Herpesvirus UL34 homologs contain conserved amino-terminal domains that mediate vesicle formation through interactions with UL31 homologs during primary envelopment. UL34 homologs also comprise other domains adjacent to their membrane-anchoring regions, which differ in length, are variable in herpesviruses, and do not

form distinguished secondary structures. However, the role of these disordered domains in infected cells remains to be elucidated. In this study, we present data suggesting that the arginine cluster in the disordered domain of HSV-1 UL34 mediates the interaction with ALIX, thereby leading to the recruitment of ESCRT-III machinery to the INM for efficient primary envelopment. This is the first study to report the role of the disordered domain of a UL34 homolog in herpesvirus infections.

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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 immuno-preventive and/or therapeutic agents against infectious diseases, cancer and allergy as well as other non-communicable diseases.

1. *Making innate sense of mRNA vaccine adjuvanticity.*

Successful vaccines contain two essential immunological components: a protective antigen and an adjuvant. Adjuvants are essential for optimal antigen-specific immune responses, the so-called 'immunogenicity', but are often a cause of reactogenicity (even toxicity) that results in local and systemic inflammation. Therefore, to ensure vaccine efficacy and safety, it is critical to understand the molecular and cellular mechanism(s) by which adjuvants provoke the immune system. By introducing papers, we describe that there seems to be more room to improve the immunogenicity and reduce the reactogenicity of LNP-mRNA vaccine formulations by further study of immunization methods (including delivery systems and devices) and their built-in adjuvanticity.

2. *Anti-tumor immunity by transcriptional synergy between TLR9 and STING activation*

Agonists for TLR9 and stimulator of IFN genes (STING) offer therapeutic applications as both anti-tumor agents and vaccine adjuvants, though their clinical applications are limited; the clinically available TLR9 agonist is a weak IFN inducer and STING agonists induce undesired type 2 immunity. Yet, combining TLR9 and STING agonists overcame these limitations by synergistically inducing innate and adaptive IFNγ to become an advantageous type 1 adjuvant, suppressing type 2 immunity, in addition to exerting robust anti-tumor activities when used as a monotherapeutic agent for cancer immunotherapy. Here, we sought to decipher the immunological mechanisms behind the synergism mediated by TLR9 and STING agonists and found that their potent anti-tumor immunity in a Pan02 peritoneal dissemination model of pancreatic cancer was achieved only when agonists for TLR9 and STING were administered locally, and was via mechanisms involving CD4 and CD8 T cells as well as the co-operative action of IL-12 and type I IFNs. Rechallenge studies of long-term cancer survivors suggested that the elicitation of Pan02-specific memory responses provides protection against the secondary tumor challenge. Mechanistically, we found that TLR9 and STING agonists synergistically induce IL-12 and type I IFN production in murine APCs. The synergistic effect of the TLR9 and STING agonists on IL-12p40 was at protein, mRNA and promoter activation levels, and transcriptional regulation was mediated by a 200 bp region sit-

uated 983 bp upstream of the IL-12p40 transcription initiation site. Such intracellular transcriptional synergy may hold a key in successful cancer immunotherapy and provide further insights into dual agonism of innate immune sensors during host homeostasis and diseases.

3. *Machine Learning-Assisted Screening of Herbal Medicine Extracts as Vaccine Adjuvants*

Adjuvants are important vaccine components, composed of a variety of chemical and biological materials that enhance the vaccine antigen-specific immune responses by stimulating the innate immune cells in both direct and indirect manners to produce a variety cytokines, chemokines, and growth factors. It has been developed by empirical methods for decades and considered difficult to choose a single screening method for an ideal vaccine adjuvant, due to their diverse biochemical characteristics, complex mechanisms of, and species specificity for their adjuvanticity. We therefore established a robust adjuvant screening strategy by combining multiparametric analysis of adjuvanticity in vivo and immunological profiles in vitro (such as cytokines, chemokines, and growth factor secretion) of various library compounds derived from hot-water extracts of herbal medicines, together with their diverse distribution of nano-sized physical particle properties with a machine learning algorithm. By combining multiparametric analysis with a machine learning algorithm such as rCCA, sparse-PLS, and DIABLO, we identified that human G-CSF and mouse RANTES, produced upon adjuvant stimulation in vitro, are the most robust biological parameters that can predict the adjuvanticity of various library compounds. Notably, we revealed a certain nano-sized particle population that functioned as an independent negative parameter to adjuvanticity. Finally, we proved that the two-step strategy pairing the negative and positive parameters significantly improved the efficacy of screening and a screening strategy applying principal component analysis using the identified parameters. These novel parameters we identified for adjuvant screening by machine learning with multiple biological and physical parameters may provide new insights into the future development of effective and safe adjuvants for human use.

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Department of Microbiology and Immunology

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

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Project Assistant Professor	Michelle S.J. Lee, Ph.D.	特任助教　博士(医学)	リー　ミシェル

Our lab focuses on the elucidation of host-pathogen interactions. We mainly work on malaria disease but cover various infectious organisms such as Leishmania parasites, and respiratory viruses, to be able to understand their way of causing pathology and eventually create successful vaccines against them. Our recent topics include how to bolster B cell memory responses against pathogens.

1. Novel adjuvant discovery and development

Adjuvants are known as must-have vaccine components for the potentiation of vaccine responses. As a member of IMSUT International Vaccine Design Center (https://vdesc.ims.u-tokyo.ac.jp/en/), we have been involved in the screening of herbal medicine extracts as safe and ready-to-use adjuvants for current human vaccines. We have been systematically screening innate and adaptive immune signaling molecules taking part in the mode of action (MOA) of adjuvants and vaccines. One of the recent findings involves understanding how the combination of TLR9 and STING agonists synergistically induce innate and adaptive responses to become an advantageous type 1 adjuvant while suppressing type 2 immunity which leads to generation of robust anti-tumor responses.

Our recent projects focus on the investigation of B cell development and pathways involved in the germinal center (GC) formation for the generation of potent antibody responses against infections and during vaccinations. We have found that TBK1, the famous innate immune signaling kinase for controlling anti-viral immune responses and nucleic-acid mediated type-I interferon responses, is very important for the generation of GC which confers sterile immunity to reinfections.

2. Elucidation of malaria-mediated pathologies

Malaria killed 50% more children last year due to Covid-19-mediated lockdowns and restrictions which prevented remedies to reach those who needed them. Our lab has been investigating cerebral malaria immunopathology by using imaging techniques such as CUBIC clearance of the brain. The research has been ongoing for the investigation of olfactory bulb-mediated pathology in experimental cerebral malaria models in mice. We have made significant progress in the understanding of new cell types in the olfactory bulb and signaling molecules taking part.

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Department of Microbiology and Immunology

Division of Systems Virology
システムウイルス学分野

Professor	Kei Sato, Ph.D.	教　授　博士(医学)	佐　藤　　佳
Assistant Professor	Jumpei Ito, Ph.D., D.V.M.	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. Evolution of SARS-CoV-2

Daichi Yamasoba, Izumi Kimura, Hirofumi Aso, Keiya Uriu, Yusuke Kosugi, Shigeru Fujita, Yuuka Masuda, Lin Pan, Naoko Misawa, Mai Suganami, Adam Strange, Naomi Ohsumi, Mika Chiba, Ryo Yoshimura, Kyoko Yasuda, Keiko Iida, Jumpei Ito, 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 660 million people are infected with this virus and more than 6.7 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.

2. Evolution of gene regulatory network driven by hominoid-specific endogenous retroviruses

Jumpei Ito, Kei Sato

Mammalian germ cells stem from primordial germ cells (PGCs). Although the gene regulatory network controlling the development of germ cells such as PGCs is critical for ensuring gamete integrity, substantial differences exist in this network among mammalian species, suggesting that this network has been modified during mammalian evolution. Here, we show that a hominoid-specific group of endogenous retroviruses, LTR5_Hs, works as enhancers in human in vitro-induced PGCs, PGC-like cells (PGCLCs). LTR5_Hs are preferentially bound by transcription factors that are highly expressed in PGCLCs (KLF4, TFAP2C, NANOG, and CBFA2T2), suggesting that these transcription factors contribute to the epigenetic activation of LTR5_Hs in PGCLCs. Comparative transcriptome analysis between humans and macaques suggests that the expression of many genes in PGCLCs is upregulated by LTR5_Hs insertions in the hominoid lineage. Together, this study suggests that LTR5_Hs insertions may have finetuned the gene regulatory network in PGCs and coordinately altered the gene expression in PGCs during hominoid evolution.

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Akatsuki Saito, Takashi Irie, Rigel Suzuki, Tadashi Maemura, Hesham Nasser, Keiya Uriu, Yusuke Kosugi, Kotaro Shirakawa, Kenji Sadamasu, Izumi Kimura, Jumpei Ito, Jiaqi Wu, Kiyoko Iwatsuki-Horimoto, Mutsumi Ito, Seiya Yamayoshi, Samantha Loeber, Masumi Tsuda, Lei Wang, Seiya Ozono, Erika P. Butlertanaka, Yuri L. Tanaka, Ryo Shimizu, Kenta Shimizu, Kumiko Yoshimatsu, Ryoko Kawabata, Takemasa Sakaguchi, Kenzo Tokunaga, Isao Yoshida, Hiroyuki Asakura, Mami Nagashima, Yasuhiro Kazuma, Ryosuke Nomura, Yoshihito Horisawa, Kazuhisa Yoshimura, Akifumi Takaori-Kondo, Masaki Imai, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Shinya Tanaka, So Nakagawa, Terumasa Ikeda, Takasuke Fukuhara, Yoshihiro Kawaoka, Kei Sato. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature 602(7896):300-306 (2022).

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Department of Cancer Biology

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

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Project Senior Assistant Professor	Atsushi Takano, M.D., D.M.Sc.	特任講師	博士(医学)	高　野　　淳
Assistant Professor	Takeshi Ito, Ph.D.	助　教	博士(医学)	伊　東　　剛
Assistant Professor	Masaru Kasai, Ph.D.	助　教	博士(医科学)	笠　井　　優

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 genetic susceptibility to various human diseases are being investigated.

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

Takeshi Ito, Yutaka Kasai, Yumi Tsuboi, Yoshiaki Kanamoto, Yue Guo, Mai Mizusawa, Mizuki Tominaga, Miko Komiya, Kaito Akiyama , Jialiang Nie, Tomoko Masuda, Hiromi Ichihara, Motoi Oba, Daisuke Matsubara[1] and Yoshinori Murakami; [1]Department of Diagnostic Pathology, University of Tsukuba, Tsukuba.

Disruption of cell adhesion is a critical step to invasion and metastasis of human cancer and their acquired resistance to anti-cancer drugs 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 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 (1).

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 and their significance in

cancer diagnosis and treatment is being investigated in collaboration partly with a pharmaceutical company.

2. Studies for establishing novel diagnostic and therapeutic approaches to small cell lung cancer and neuroendocrine cancers

Takeshi Ito, Motoi Oba, Tomoko Masuda, Daisuke Matsubara[1], Goh Tanaka[2], Akihisa Mitani[2], Takahide Nagase[2] and Yoshinori Murakami; [2]Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo.

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 were 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, in collaboration partly with a pharmaceutical company. Together with generation of novel diagnostic and therapeutic approaches, molecular features of SCLC and its four subgroups expressing Ascl1, NeuroD1, POU2F3 and YAP1, were investigated with others (2,3).

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

Ken Akashi, Yoshiaki Kanamoto, Atsushi Takano, Ayaka Sato, Takeshi Ito 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 the clinical significance of circulating tumor DNA from Human Papilloma Virus (HPV)-derived sequences in human papillomavirus-related p16-positive oropharyngeal cancer 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). Possible in involvement of Vitamin D in colon cancer and additional potential biomarkers were also investigated in collaboration with others (5, 16, 17).

4. Genomic-epidemiological studies of human cancers and various diseases and phenotypes using the materials and information of Biobank Japan and a population-based cohort in Japan.

Takayuki Morisaki, Yoshinori Murakami, Masaru Koido[3], Yoichiro Kamatani[3] and Koichi Matsuda[4]; [3]Laboratory of Complex Trait Genomics and [4]Laboratory of Clinical Genome Sequencing, Graduate School of Frontier Sciences, The University of Tokyo

To unveil genomic and environmental factors and their interaction involved in human cancer, large numbers of patients suffered from gastric (6), biliary tract (7), urinary tract urothelial cancer (8), breast and ovarian cancer (9) or lymphoma (10), as well as cancer-free controls within the same cohorts, were analyzed for DNA sequencing at the specific loci of hereditary cancer genes and prevalence and characterization of germline mutations in cancer patients and control populations were determined in collaboration with Dr. Yukihide Momozawa's group in Riken, Center for Integrative Medical Sciences. In gastric cancer, this collaborative study identified that Helicobacter pylori infection modifies gastric cancer risk associated with germline pathogenic variants in homologous recombination pathway genes, providing a typical example of genomic and environmental interaction in specific cancer (6).

Furthermore, to understand the mechanisms of complex diseases and various specific human phenotypes, we carried out genome-wide association studies (GWAS) and their downstream analysis for survival time of individuals (11), retinal and renal complications of type 2 diabetes (12) and atrial fibrillation (13). To promote genetic studies for preventing and treating cancer and complex diseases in academia and company worldwide, we released genotyping data and serum metabolome data of patients from BioBank Japan.

For single gene disorders, patients and family members with genetic vascular diseases, including

Loeys-Dietz syndrome (14) and Ehlers-Danlos syndrome (15) were diagnosed 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

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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., Zhong, Z., Yoda, M., Hwang, J., 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 (CMS)

and myasthenia gravis (MG), which are characterized by fatigable 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 mechanism-based therapies for NMJ disorders.

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

Eguchi, T., Tezuka, T., Mao, Y., 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 pathogenic 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, including those related to congenital myasthenic syndromes (see below).

3. Pathophysiological mechanisms underlying *DOK7* myasthenia.

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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 formation, maintenance and 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 increase NMJ number and enhance its function together with lifespan 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 and regenerates 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 enhanced MuSK activation and 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 rein-

nervation 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) or constitutively active form (T287D) of CaMKIIβ, an isoform of CaMKII, by AAV (adeno-associated virus) enhanced or reduced muscle mass and strength, respectively, 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 found that Dok-1/-2 and Dok-3 play distinctive roles in cell fusion and proliferation during osteoclastogenesis and cooperatively protect mice from osteopenia. Additionally, we recently demonstrated that Dok-1/-2 and Dok-3 play distinctive roles in intestinal tumor growth and malignant progression. Interestingly, Dok-3 deficiency in non-tumor cells induces malignant conversion of benign tumors without intensifying mutagenesis in tumors, providing a new insight into the regulation of tumor malignant progression. We are currently investigating molecular mechanisms underlying the Dok-3-mediated suppression of malignant progression of intestinal tumors, which may lead to developing new therapeutic approaches against non-tumor cell-driven malignant progression. 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., Wu, W., Jozawa, H., 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 previously 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 cell type-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, neurodegeneration, 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 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.

Inoue-Yamauchi, A., Hwang, J., Eguchi, T., Tsoumpra, M., 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 NMJ formation. We are investigating in vivo and in vitro effects, including therapeutic ones in mouse models of human diseases, of hit compounds or down- or up-regulation of candidate genes, and continue the ongoing screenings to further collect appropriate hit compounds and candidate genes that may be involved in the regulation of NMJ formation. We are also investigating target proteins for the hit compounds to understand their modes of actions.

Publication

Arimura S, Inoue-Yamauchi A, Katayama K, Kanno T, Jozawa H, Imoto S, Yamanashi Y. Loss of Dok-3 in non-tumor cells induces malignant transformation of benign epithelial tumor cells of the intestine. *Cancer Research Communications*, 2:1590-1600, 2022

Department of Cancer Biology

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

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There is some evidence supporting the important role of senescent cells in aging and healthy life span. However, little is known about the molecular basis underlying aging-related pathologies. Our research is focused on understanding the common pathologies underlying a variety of aging-related diseases. We are currently interested in the role of p16-positive senescent cells in the age-dependent decline of various organ functions and the mechanism of senescent cell accumulation with aging. Furthermore, we are focusing on the mechanism underlying accumulation of abnormal proteins as a cause of aging. By understanding the degradation mechanisms of misfolded proteins, we are promoting research on abnormal cellular functions caused by the accumulation of protein aggregates, especially in the pathogenesis of neurodegenerative diseases. We also study the molecular linkage between DNA methylation and maintenance of genome stability.

1. Blocking PD-L1/PD-1 improves senescence surveillance and aging phenotypes

Teh-Wei Wang, Sae Aratani, Yoshimi Imawari, Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Dan Li, Satoru Meguro, Leung Wingchi, Miho Ishiura, Harris Alexander Stephen, Satotaka Omori, Liu Xiangyang, Koji Nishikawa, Satoshi Kawakami, Yuki Okamura, Yue Zhang, Jiayi Yao, Xinyi Zeng, Toshiro Migita, Yoichi Furukawa[1], Seiya Imoto[2], Akihiko Yoshimura[3], Yoshikazu Johmura[4], and Makoto Nakanishi:
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Several lines of evidence suggest that with aging, senescent cells accumulate in various tissues, leading to excessive inflammation and, consequently, an imbalance in tissue homeostasis. A simple explanation for this accumulation is the age-dependent increase in senescence-inducing stimuli such as telomere shortening and DNA damage. Immune system disorders may also be involved, as it has recently been reported that senescent hepatocytes induced by oncogenes and senescent stellate cells induced by injury are eliminated by activated T cells and natural killer cells, respectively. However, little is known about exactly how the immune system monitors cellular senescence during natural aging. We have shown that senescent cells heterogeneously express the immune checkpoint PD-L1 and that PD-L1$^+$ senescent cells accumulate with age *in vivo*. PD-L1$^-$ cells are sensitive to T cell surveillance, whereas PD-L1$^+$ cells are resistant even in the presence of senescence-associated secretory phenotypes (SASP). Interestingly, single-cell analysis of p16$^+$ cells *in vivo* revealed that PD-L1 expression correlated with higher levels of SASP. Consistent with this, administration of PD-1 antibody to naturally aging mice or NASH-induced mice reduces not only the total number of p16$^+$ cells *in vivo* but also the PD-L1$^+$ population in an activated CD8$^+$ T cell-dependent manner, ameliorating various aging-related diseases.

These results suggest that the heterogeneous expression of PD-L1 plays an important role in the accumulation of senescent cells and inflammation associated with aging, and the elimination of PD-L1^{+} senescent cells by immune checkpoint blockade may be a promising strategy for anti-aging therapy.

2. Loss of LONRF2, a bona fide protein quality control ubiquitin-ligase, causes late-onset neurological deficits

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Many age-related disorders are causally associated with protein misfolding and certain environmental stresses may trigger misfolding of mature proteins. To avoid this, all cells have evolved Protein Quality Control (PQC) systems such as translational control, molecular chaperone activity, and proteolytic destruction either by the proteasome or via autophagy. For example, cleaved protein products generated by stalled ribosomes on defective mRNAs have been found to be targeted for degradation by molecular pathways initiated on the ribosome. If the ribosome cannot reach the correct termination codon, the protein will inevitably be cleaved and likely defective. Thus, it may be advantageous for the cell to degrade such incomplete nascent chains. Protein misfolding is a major cause of neurodegenerative diseases. Post-mitotic neurons are highly susceptible to protein aggregates that are not diluted by mitosis. Therefore, post-mitotic cells may have a specific protein quality control (PQC) system. We analyzed cellular senescence as an excellent post-mitotic model and found that LONRF2 is a bona fide protein quality control ubiquitin ligase induced in post-mitotic senescent cells. Under unperturbed conditions, LONRF2 is predominantly expressed in neurons. LONRF2 binds and ubiquitylates abnormally structured TDP-43 and hnRNP M1 and artificially misfolded proteins. LONRF2$^{-/-}$ mice exhibit age-dependent TDP-43-mediated motor neuron degeneration and cerebellar ataxia. Mouse iPS cell-derived motor neurons lacking LONRF2 showed reduced survival, shortening of neurites, and accumulation of pTDP-43 and G3BP1 after long-term culture. These phenotypes as well as the shortening of neurites in human ALS patients-derived motor neurons are rescued by ectopic expression of LONRF2. Our findings reveal that LONRF2 is a PQC ligase whose loss may contribute to motor neuron degeneration and motor deficits.

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

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DNA methylation at CpG dinucleotide is an epigenetic modification that regulates various biological processes, including gene silencing, genome stability, cellular development, and differentiation. DNA methylation is stably maintained during cell proliferation. DNA methyltransferase 1 (DNMT1) plays a key role in the maintenance of DNA methylation by catalyzing the conversion of hemi-methylated DNA to a fully methylated state. In addition, recent studies have also suggested the potential de novo function of DNMT1. 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

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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. DNA damage that causes hair graying in mice

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All living things experience an increase in entropy, manifested as a loss of genetic and epigenetic information. In yeast, epigenetic information is lost over time due to the relocalization of chromatin-modifying proteins to DNA breaks, causing cells to lose their identity, a hallmark of yeast aging. Using a DNA damage inducing model named "ICE", we found that the induction of DNA double strand breaks (DSBs) with faithful DNA repair advances the expression of aging phenotypes with epigenetic changes. To study the fate and dynamics of DNA-damaged stem cells in tissues and the resultant impact in the expression of aging phenotypes, we studied the fate of melanocyte stem cells which acquired DNA DSBs and demonstrated that those cells disappear from the niche, causing the loss of mature melanocytes for hair pigmentation. This is consistent with our previous report in which we demonstrated that genotoxic stress abrogates renewal of melanocyte stem cells by triggering their ectopic differentiation (Inomata K et al. Cell, 2009). The aberrant differentiation and the resultant loss of the cell lineage in tissues may partially explain the age-associated loss of lineage-specific epigenetic information in ICE mice. We are currently testing whether the selective induction of DNA double strand breaks in melanocyte stem cells similarly causes hair graying.

2. Fate tracing of DNA-damaged hair follicle stem cells and their seno-differentiation clearance out of the niche

Miranda-Salmeron M, Matsumura H, Muroyama Y, Kato T, Higa M, Tan L, Kawamura Y, Nanba D, Mohri Y, and Nishimura EK.

Hair follicles, mammalian mini-organs that grow hair, miniaturize during aging, leading to hair thin-

ning and loss. In the event of severe genotoxicity such as DNA double-strand breaks (DSBs), stem cells are largely believed to choose between cell death (apoptosis) or irreversible cell cycle arrest (senescence) to prevent further damage to neighboring healthy cells and tissues. Accumulation of these senescent cells across organs has been implicated in disease and aging-related morbidities such as cancer. However, the exact fate and dynamics of sublethally damaged cells in tissues during aging/chemotherapy - and the development of alopecia - and where exactly senescent cells exist in tissues are still largely unknown because of the lack of any single perfect marker of senescent cells. Previous work from our group demonstrated that various stem cells in the skin will aberrantly commit to differentiation in response to DNA damage by abrogating their self-renewal capabilities to discard unfit/stressed/aged stem cells. We are testing the unique hypothesis that the tissue youth is achieved through rapid, dynamic clearance of DNA-damaged cells out of the epithelia as a robust genomic quality control mechanism. We are evaluating a combination of recently devised mouse lines that can induce DSBs in a small number of stem cells to visualize and trace the exact fate, senescent state, and dynamics of those individual cells in epithelial tissue such as the hair follicle. Upon hair follicle stem cell (HFSC) activation, DNA-damaged cells were observed at the epidermal level, hinting to their transdermal exit out of the niche. Remarkably, while DNA damaged HFSCs exhibited gH2AX foci, SAβ-galactosidase activity was not significantly increased in such cells. We are in the process of characterizing the identity of those DNA-damaged HFSCs and their fate switching in the HFSC niche that leads to hair follicle miniaturization and hair loss. Taken together, our findings demonstrate a tissue-autonomous mechanism within the hair follicle niche that can effectively discard DNA-damaged cells.

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. Gene expression profiling of EpiSCs with DSBs reveals that DNA damage response (DDR)-p53-Notch/p21 axis is specifically induced in EpiSCs with DSBs. Stem cell fate analysis showed that 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. Early differential diagnosis of acral melanomas from nevi.

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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. We are currently increasing the number of cases to further evaluate the accuracy and efficiency of method.

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Department of Basic Medical Sciences

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

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

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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 stronger 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.

2. SIPA1L1/SPAR1 interacts with the neurabin family of proteins and is involved in GPCR signaling

Kobayashi, S., Arima-Yoshida, F., Matsuura, K.[4], Akiyama, T.[4] and Manabe, T.: [4]Laboratory of Molecular and Genetic Information, Institute for Quantitative Biosciences, University of Tokyo, Tokyo, Japan

SIPA1L1 (also known as SPAR1) has been proposed to regulate synaptic functions that are important in maintaining normal neuronal activities, such as regulating spine growth and synaptic scaling, as a component of the PSD-95/NMDA-receptor complex. However, its physiological role remains poorly understood. Here, we performed expression analyses using super-resolution microscopy in the mouse brain and demonstrated that SIPA1L1 is mainly localized to general submembranous regions in neurons, but surprisingly, not to the postsynaptic density (PSD). Our screening for physiological interactors of SIPA1L1 in the mouse brain identified spinophilin and neurabin-1, regulators of GPCR signaling, but rejected PSD-95/NMDA-receptor-complex components. Furthermore, sipa1l1 (-/-) mice showed normal spine-size distribution and NMDA receptor-dependent synaptic plasticity. Nevertheless, sipa1l1 (-/-) mice showed aberrant responses to α2-adrenergic receptor (a spinophilin target)- or adenosine A1 receptor (a neurabin-1 target)-agonist stimulation, and striking behavioral anomalies, such as hyperactivity, enhanced anxiety, learning impairments, social interaction deficits, and enhanced epileptic-seizure susceptibility. Our findings revealed unexpected properties of SIPA1L1, suggesting a possible association of SIPA1L1 deficiency with neuropsychiatric disorders related to dysregulated GPCR signaling, such as epilepsy, attention-deficit hyperactivity disorder (ADHD), autism, or fragile X syndrome.

3. Loss of calsyntenin paralogs disrupts interneuron stability and mouse behavior

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Calsyntenins (CLSTNs) are important synaptic molecules whose molecular functions are not fully understood. Although mutations in calsyntenin genes have been associated with psychiatric disorders in humans, their function is still unclear. One of the reasons why the function of CLSTNs in the nervous system has not been clarified is the functional redundancy among the three paralogs. Therefore, to investigate the functions of mammalian CLSTNs, we generated triple knockout (TKO) mice lacking all CLSTN paralogs and examined their behavior. The mutant mice tended to freeze in novel environments and exhibited hypersensitivity to stress. Consistent with this, glucose levels under stress were significantly higher in the mutant mice than in the wild-type controls. In particular, phenotypes such as decreased motivation, which had not been reported in single CLSTN KO mice, were newly discovered. The TKO mice generated in this study represent an important mouse model for clarifying the function of CLSTN in the future.

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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

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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 Ohe, Yuji Kubota, Kiyoshi Yamaguchi[1], Yusuke Takagi, Junichiro Nashimoto, Hiroko Kozuka-Hata[2], Masaaki Oyama[2], Yoichi Furukawa[1] and Mutsuhiro Takekawa: [1]Division of Clinical Genome Research, IMSUT, [2]Medical Proteomics Laboratory, IMSUT

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.

Growth factor-induced, ERK-mediated induction of immediate-early genes (IEGs) is crucial for cell growth and tumorigenesis. Although IEG expression is mainly regulated at the level of transcription elongation by RNA polymerase-II (Pol-II) promoter-proximal pausing and its release, the role of ERK in this process remains unknown. This year, by developing a unique screening strategy using yeast cells, we identi-

fied negative elongation factor (NELF)-A, which is a core component of the NELF complex and is critical for the stabilization of Pol-II pausing, as an ERK substrate. Upon growth factor stimulation, ERK phosphorylates NELF-A, which dissociates NELF from paused Pol-II at the promoter-proximal regions of IEGs, allowing Pol-II to resume elongation and produce full-length transcripts. Furthermore, we found that in cancer cells, protein phosphatase 2A (PP2A) efficiently dephosphorylates NELF-A, thereby preventing aberrant IEG expression induced by ERK-activating oncogenes (e.g., EGFR, Ras, and BRAF). However, when PP2A inhibitor proteins (e.g., SET and CIP2A) are overexpressed, as is frequently observed in human cancers, decreased PP2A activity combined with oncogene-mediated ERK activation conspire to induce NELF-A phosphorylation and IEG upregulation, resulting in tumor progression. Our data delineate previously unexplored roles of ERK and PP2A inhibitor proteins in carcinogenesis.

2. Qualitative differences in disease-associated MEK mutants reveal molecular signatures and aberrant signaling-crosstalk in cancer

Yuji Kubota, Yuko Fujioka[1], Ashwini Patil[2], Yusuke Takagi, Daisuke Matsubara[3], Masatomi Iijima[4], Isao Momose[4], Ryosuke Naka, Kenta Nakai[2], Nobuo N. Noda[1], and Mutsuhiro Takekawa: [1]Division of Biological Molecular Mechanisms, Institute for Genetic Medicine, Hokkaido University, [2]Laboratory of Functional Analysis In Silico, Human Genome Center, IMSUT, [3]Molecular Pathology Laboratory, IMSUT, [4]Institute of Microbial Chemistry, Microbial Chemistry Research Foundation.

Dysregulation of the ERK pathway is frequently observed in human cancers. Indeed, many key ERK pathway molecules are known oncogenes. Gain-of-function mutations or gene amplification of RTKs (e.g., EGFR), Ras, and BRaf are common in a variety of cancers. Furthermore, MEK1 mutations are also observed in many tumors. These oncogenes ultimately hyperactivate ERK signaling and generate aberrant gene expression patterns that provoke cell overgrowth and/or a differentiation abnormality, thereby inducing cancer development and progression. Targeting of these oncogenes for cancer therapy is therefore of particular interest, and their specific inhibitors are currently in clinical use. Although some of these ERK pathway-targeted drugs are clinically effective, their efficacy is limited by intrinsic and/or acquired resistance of cancer cells to these drugs. Furthermore, MEK mutations promote resistance to allosteric MEK inhibitors. The precise molecular mechanisms underlying such drug resistance, however, remain ill-defined.

Besides their somatic mutations in sporadic cancers, recent clinical sequencing studies identified germline mutations of key components of ERK signaling (e.g., Ras, Raf, and MEK) in a group of congenital developmental disorders termed RASopathies. RASopathies, including Noonan, Costello, Leopard, and cardio-facio-cutaneous (CFC) syndromes, share many overlapping clinical manifestations such as cranio-facial dysmorphisms, cardiomyopathies, and neurocognitive impairment. Interestingly, congenital mutations of the *MEK1* gene in patients with CFC syndrome do not necessarily increase the risk of cancer development. Therefore, cancer- and RASopathy-associated MEK mutants may differentially alter ERK signaling processes and consequent biological outcomes. Although increased catalytic activity of the disease-associated MEK mutants has been reported, the detailed biological properties of individual MEK mutants are, however, still obscure. Moreover, despite the importance of MEK1 in the etiology of cancer and RASopathies, no protein structure of any disease-associated MEK mutant is known.

This year, we resolved the crystal structure of a cancer-derived MEK1 mutant, and revealed the molecular mechanisms underlying abnormal kinase activities of the cancer- and RASopathy-associated MEK1 mutants. Furthermore, we found that differences in biochemical properties between cancer- and RASopathy-associated MEK1 mutants produce qualitative changes in ERK signaling dynamics and downstream transcriptional programs. Transcriptome analyses revealed that the two MEK1 mutant classes elicit distinct gene expression patterns that are specifically related to the pathophysiology of cancer or RASopathies. We identified cancer- and RASopathy-signature genes that may serve as diagnostic markers or therapeutic targets for these diseases. In particular, two AKT-inhibitor molecules, PHLDA1 and 2, are simultaneously upregulated by oncogenic ERK signaling, and mediate cancer-specific ERK-AKT crosstalk. The combined expression of PHLDA1/2 is critical to confer resistance to ERK pathway-targeted therapeutics on cancer cells. Coadministration of bortezomib overcomes this drug resistance by disrupting PHLDA1/2-mediated, cancer-specific ERK-AKT crosstalk. Our data delineate qualitative differences in biological properties between cancer- and RASopathy-associated MEK1 mutants, and provide vital insights into the etiology, diagnosis, and therapeutic strategy of cancers and RASopathies.

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

Daisuke Yoshioka, Hisashi Miriizumi, Noriko Nishizumi-Tokai, Takanaori Nakamura, Yuji Kubota, 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. Identification of novel genes whose expression is controlled by MAPK signaling pathways.

Junichiro Nashimoto, Jue Wang, Mariko Saito, Yusuke Takagi, Noriko Nishizumi-Tokai, Yuji Kubota, and Mutsuhiro Takekawa

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.

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

Shunske Fukuta, Natsumi Mikami, Saeko Kawataki, Hisashi Moriizumi, Noriko Nishizumi-Tokai, 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 various cell lines deficient for one of

SAPK signaling molecules (e.g., GADD45, MTK1, and others) , we investigated the regulation and function of these molecules MTK, and uncovered their roles 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.

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Department of Basic Medical Sciences

Division of RNA and Gene Regulation
RNA 制御学分野

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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. A distinct mammalian disome collision interface harbors K63-linked polyubiquitination of uS10 to trigger hRQT-mediated subunit dissociation

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Translational stalling events that result in ribosome collisions induce Ribosome-associated Quality Control (RQC) in order to degrade potentially toxic truncated nascent proteins. For RQC induction, the collided ribosomes are first marked by the Hel2/ZNF598 E3 ubiquitin ligase to recruit the RQT complex for subunit dissociation. In yeast, uS10 is polyubiquitinated by Hel2, whereas eS10 is preferentially monoubiquitinated by ZNF598 in human cells for an unknown reason. Here, we characterize the ubiquitination activity of ZNF598 and its importance for human RQT-mediated subunit dissociation using the endogenous XBP1u and poly(A) translation stallers. Cryo-EM analysis of a human collided disome reveals a distinct composite interface, with substantial differences to yeast collided disomes. Biochemical analysis of collided ribosomes shows that ZNF598 forms K63-linked polyubiquitin chains on uS10, which are decisive for mammalian RQC initiation. The human RQT (hRQT) complex composed only of ASCC3, ASCC2 and TRIP4 dissociates collided ribosomes dependent on the ATPase activity of ASCC3 and the ubiquitin-binding capacity of ASCC2. The hRQT-mediated subunit dissociation requires the K63-linked polyubiquitination of uS10, while monoubiquitination of eS10 or uS10 is not sufficient. Therefore, we conclude that ZNF598 functionally marks collided mammalian ribosomes by K63-linked polyubiquitination of uS10 for the trimeric hRQT complex-mediated subunit dissociation.

1.2. Two modes of Cue2-mediated mRNA cleavage with distinct substrate recognition initiate No-go decay

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Ribosome collisions are recognized by E3 ubiquitin ligase Hel2/ZNF598, leading to RQC (Ribosome-associated Quality Control) and to endonucleolytic cleavage and degradation of the mRNA termed NGD (No-Go Decay). NGD in yeast requires the Cue2 endonuclease and occurs in two modes, either coupled to RQC (NGD^{RQC+}) or RQC-uncoupled (NGD^{RQC-}). This is mediated by an unknown mechanism of substrate recognition by Cue2. Here, we show that the ubiquitin-binding activity of Cue2 is required for NGD^{RQC-} but not for NGD^{RQC+}, and that it involves the first two N-terminal Cue domains. In contrast, Trp122 of Cue2 is crucial for NGD^{RQC+}. Moreover, the colliding ribosome association factor Mbf1 and its interaction with uS3 is crucial for NGD^{RQC+} by the *SDD1*-staller. We propose that in Cue2-dependent cleavage upstream of the collided ribosomes (NGD^{RQC-}), polyubiquitination of eS7a is recognized by two N-terminal Cue domains of Cue2. In contrast, for the cleavage within collided ribosomes (NGD^{RQC+}), the UBA domain, Trp122 and the interaction between Mbf1 and uS3 are critical.

1.3. The mechanism of Ufmlyation and its relation with RQC

Ishimura, R., El-Gowily, A. H., Noshiro, D., Komatsu-Hirota, S., Ono, Y., Shindo, M., Hatta, T., Abe, M., Uemura, T., Lee-Okada, H. C., Mohamed, T. M.,

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Protein modification by ubiquitin-like proteins (UBLs) amplifies limited genome information and regulates diverse cellular processes, including translation, autophagy and antiviral pathways. Ubiquitin-fold modifier 1 (UFM1) is a UBL covalently conjugated with intracellular proteins through ufmylation, a reaction analogous to ubiquitylation. Ufmylation is involved in processes such as endoplasmic reticulum (ER)-associated protein degradation, ribosome-associated protein quality control at the ER and ER-phagy. However, it remains unclear how ufmylation regulates such distinct ER-related functions. Here we identify a UFM1 substrate, NADH-cytochrome b5 reductase 3 (CYB5R3), that localizes on the ER membrane. Ufmylation of CYB5R3 depends on the E3 components UFL1 and UFBP1 on the ER and converts CYB5R3 into its inactive form. Ufmylated CYB5R3 is recognized by UFBP1 through the UFM1-interacting motif, which plays an important role in the further uyfmylation of CYB5R3. Ufmylated CYB5R3 is degraded in lysosomes, which depends on the autophagy-related protein Atg7- and the autophagy-adaptor protein CDK5RAP3. Mutations of CYB5R3 and genes involved in the UFM1 system cause hereditary developmental disorders, and ufmylation-defective Cyb5r3 knock-in mice exhibit microcephaly. Our results indicate that CYB5R3 ufmylation induces ER-phagy, which is indispensable for brain development.

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.

2.1. Sensing of individual stalled 80S ribosomes by Fap1 for non-functional rRNA turnover

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Cells can respond to stalled ribosomes by sensing ribosome collisions and employing quality control pathways. How ribosome stalling is resolved without collisions, however, has remained elusive. Here, focusing on non-colliding stalling exhibited by decoding-defective ribosomes, we identified Fap1 as a stalling sensor triggering 18S non-functional rRNA decay

via poly-ubiquitination of uS3. Ribosome profiling revealed enrichment of Fap1 at the translation initiation site but also the association with elongating individual ribosomes. Cryo-EM structures of Fap1-bound ribosomes elucidated Fap1 probing the mRNA simultaneously at both the entry and exit channels suggesting an mRNA stasis sensing activity, and Fap1 sterically hinders the formation of canonical collided di-ribosomes. Our findings indicate that individual stalled ribosomes are the potential signal for ribosome dysfunction, leading to accelerated turnover of the ribosome itself.

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 the 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.

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1. Narita M.# Denk T.# Matsuo Y. Sugiyama T. Kikuguchi C. Ito S. Sato N. Suzuki T. Hashimoto S. Machová I. Tesina P. Beckmann R. Inada T.* (#Equal contribution) A distinct mammalian disome collision interface harbors K63-linked polyubiquitination of uS10 to trigger hRQT-mediated subunit dissociation. *Nat Commun.* doi:10.1038/s41467-022-34097-9 (2022)
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7. 実験医学月刊特集「RNAワクチンの先の基礎研究」mRNA医薬開発の基盤となる衝突リボソーム品質管理の分子機構と生理機能　2022年02月18日
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Human Genome Center

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

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

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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. Computational inference of cooperative transcriptional regulators from 3D genomic contacts in germinal center B cells

Sung-Joon Park and Kenta Nakai

During the process of cell differentiation, the higher-order chromosomal structure is intricately remodeled to ensure lineage-specific transcription. In the case of peripheral B-cell differentiation, upon activation by antigen, mature naive B cells (NBs) enter the germinal center reaction along with complex and multifaceted genomic/epigenomic modifications including drastic changes in the 3D chromatin architecture. To understand the impact of chromatin remodeling in germinal center B cells (GCBs) differentiation, we analyzed muti-omics NGS data including Hi-C, Histone ChIP-seq, and RNA-seq with human NB, GCB, and malignant lymphoma cells. We first developed computational models to infer potential transcription factors that bind the promoter regions and long-range contact (LRC) regions. Next, we employed a graph-embedding approach to detect the gene regulatory modules by incorporating cofactors that link promoter-binding TFs and LRC-binding TFs for a certain gene. The in-silico models revealed the distinct transcriptional regulatory roles of promoter-binding and LRC-binding TFs in the context of differentially expressed genes (DEGs) during GCB differentiation. Furthermore, the DEG-TF-Cofactor modules we detected exhibited highly correlated expression patterns in the healthy and malignant B cells. These results provide further explanation for the importance of long-range-contact-mediated transcriptional regulation.

2. Comprehensive Comparison of Gene Expression Diversity among a Variety of Human Stem Cells

Yukiyo Yamatani and Kenta Nakai

Several factors, including tissue origins and culture conditions, affect the gene expression of undifferentiated stem cells. However, understanding the basic identity across different stem cells has not been pursued well despite its importance in stem cell biology. Thus, we aimed to rank the relative importance of multiple factors to gene expression profile among undifferentiated human stem cells by analyzing publicly available RNA-seq datasets. We first conducted batch effect correction to avoid undefined variance in the dataset as possible. Next, we clustered stem cell samples, followed by a dimensionality reduction of their gene expression profile. Then, we highlighted the relative impact of biological and technical factors among undifferentiated stem cell types: a more influence on tissue origins in induced pluripotent stem cells (iPSCs) than in other stem cell types; a stronger impact of culture condition in embryonic stem cells (ESCs) and somatic stem cell types, including mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). In addition, we found that a characteristic gene module, which was enriched in histones, exhibits higher expression across different stem cell types that were annotated by the specific stem cell and growth conditions. This tendency was also observed in mouse undifferentiated stem cell RNA-seq data. We further detected 16 zinc finger family genes that exhibit a co-expression pattern with the histone genes. Altogether, we characterized the major factor, the relative strength of its impacts in each stem cell type, and the impact of culture conditions on histones and zinc finger family genes, which might be highly involved in stem cell identity. Our findings could allow experimental researchers to obtain general insights into stem cell quality, such as the balance of differentiation potentials that undifferentiated stem cells possess.

3. Tc1-Mariner Superfamily of DNA Transposons is Enriched at the Enhancer Boundaries in Ciona

Satoshi Otaki and Kenta Nakai

A long-time stratification of experimental studies on tissue specific enhancers enlisted in the early developmental stage of Ciona allows us to have clues to know different stretch of DNA sequences are involved to cause spaciotemporal gene expression patterns. Less likely found than on mammalian genomes, there are transposable elements interspersed on Ciona genome. There are a growing number of evidence accumulated to suggest some of transposable elements or its remnants reside in host genome playing a role of promoters or enhancers for transcriptional regulation in several model organisms. However, there are now few studies about cis-regulatory elements comprised of transposable elements on Ciona genome. Therefore, we have manually surveyed cis-regulatory regions from literature published from 1997 to 2020. These experimentally validated cis-regulatory regions were mapped on the reference genomes to be modelized as database resources. In our study, though transposable elements are located rarely in exons, one of transposable elements of DNA/TcMar-Tc1 abundantly overlaps with cis-regulatory regions, and we observed an enrichment peak of DNA/TcMar-Tc1 which is a few kb away from neurogenic tissue specific enhancers obtained from construct truncation studies. Furthermore, this transposable element of DNA/TcMar-Tc1 is found to be located just upstream of functional center unit of enhancers. Some of truncation study reveal that this transposable element does not repress aimed gene expression but seems more secure to activate it like a co-working enhancer. Human genome is known to harbor DNA sequence embedded by horizontal transfers of retrovirus genome in ancient time, but many of its sequence are stabilized in the host genome. These retro-elements like LTR elements or its remnants derived from some ERVs work like enhancers in human diseases or neurological disorders, such as multiple sclerosis, amyotrophic lateral sclerosis, and schizophrenia. DNA/TcMar-Tc1 closely located to transcription unit in Ciona has some important role such as demarcation for its boundaries or cis-regulatory elements, but further investigation is required.

4. A variety of nucleotide/amino acid sequence analyses based on deep learning and related methods

Leyi Wei[1] and Kenta Nakai
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We have collaborated in a variety of nucleotide/protein sequence analyses. First, we benchmarked 12 available methods for the imputation of scRNA-seq data, based on six simulated and two real datasets. We found that deep learning-based approaches generally exhibit better overall performance than model-based ones. We built an online platform that integrates all available state-of-the-art imputation methods for comparison and visualization analysis. Second, we predicted protein-peptide binding residues via interpretable deep learning; more specifically, we used a BERT-based contrastive learning framework. Since we used a well pre-trained protein language model, we did not need to design effective features for prediction basically but if we integrate the traditional features with our learned features, we could outperform existing methods. Third, we devel-

oped a multi-scale deep biological language learning model that enables the interpretable prediction of DNA methylations bed on genomic sequences only. Our model not only outperforms existing methods but also can capture both sequential and functional semantic information from background genomes. Since the model can explain what it learnt, its results will be useful for in-depth analysis of their biological functions. Fourth, by combining all the above works and expanding them, we developed an automated and interpretable platform for biological sequence prediction, functional annotation, and visualization analysis. The platform is accessible as a one-stop-shop web server where researchers are expected to develop a new deep-learning architecture to answer any biological question.

5. Housekeeping enhancers in the human genome

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Enhancers are cis-regulatory elements that regulate cell type-specific gene expression patterns. Recent evidence suggests that a large number of enhancers are active in multiple organs in diverse developmental contexts. However, due to the specificity and complexity of the experiments, the validation of enhancers is not easy to determine in many cell types. To fill this gap, bioinformatics methods have been developed to predict enhancer-gene interactions using various classes of sequencing data, e.g., histone modification and chromatin conformation. In this study, we used the interactions predicted by the ABC method in 50 cell types to characterize the human enhancers. We show that even though most of the predictions are active in only one cell, there are a significant number of conserved elements active among all the 50 cell types. Most of these conserved elements overlap with promoter regions; however, we found around 700 housekeeping enhancers with distinctive characteristics that differentiate them from cell type-specific enhancers. Housekeeping enhancers are rich in G/C nucleotides, and they regulate genes in a longer distance than cell type-specific ones. Besides their low number, as compared with cell type-specific enhancers, housekeeping enhancers regulate around 50% of the protein-coding genes and their distribution correlates across chromosomes. Overall, our work unveils a new type of enhancers conserved in multiple cell types, which will broaden our understanding of the epigenetics behind gene regulation.

6. Integrative Single-cell Analyses of Human Haematopoietic Progenitors Reveals a Putative Dendritic Cell Progenitor in Granulocyte-Monocyte-Dendritic Cell Progenitor

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The human dendritic cells (DC) population comprises a heterogeneous family of immune cells, including plasmacytoid DC (pDC) and two subsets of conventional DC (cDC1 and cDC2). Despite the well characterization of mature DC, the origins and differentiation pathways of human DC are still not clearly elucidated. In this study, eight haematopoietic datasets were integrated with Mutual Nearest Neighbors (Haghverdi *et al*., 2018). A group of bone marrow-derived cells among granulocyte-monocyte-dendritic cell progenitors (GMDP) were found to have the ability to differentiate into cDC via in silico trajectory inference. The group of cells have hallmarks of the DC lineage, including IRF8, CD74, FLT3, and MHC class II expression. A suggested marker panel $CD34^{+}$ $CD123^{int}CD2^{int}CD127^{lo}CD11c^{+}$ was identified using Hypergate (Becht et al., 2019). In the future, we intend to further analyze the group of cells using combinations of flow cytometry, CyTOF and single cell RNA-sequencing, in order to find out its differentiation ability and functional features.

7. Gene Regulatory Network Comparison of Photosensitive Related Cells Between Five Species by Single Cell RNA-seq data

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The visual system plays an important role in supporting the vertebrates adapted to various environment on the earth. Although the single cell expression profiles of photosensitive related cells from ascidian and vertebrates have been determined, a systematic comparison of developmental mechanisms of homologous cell types between species is needed. Here, we reconstruct the gene regulatory network (GRN) in the photosensitive related cells from five representative species based on gene-gene co-expression correlation by using single-cell RNA-seq data. We compared the GRN for each homologous cell type from these species to identify conserved genetic network. By combining RNA velocity and GRN analysis, we identified the key regulators for retinal cell differentiation in zebrafish and mouse. Altogether, our analysis provides a new understanding of evolutionary molecular mechanisms in photosensitive related cells.

8. Identification of macrophage polarization hysteresis genes using multi-omic datasets

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Macrophages are plastic innate immune cells that can be polarized into classical pro-inflammatory phenotype (M1) and alternative anti-inflammatory phenotype (M2). Accumulating evidence shows that current macrophages state also relies only on its polarization memory (named as macrophage hysteresis). However, the macrophage polarization memory is still not fully investigated. To contribute to this unfulfilled field, we utilized public time-course RNAseq and single cell RNAseq datasets and selected potential memory genes of M1 and M2 phenotypes using traditional machine learning and graph embedding methods. By checking the expression profile of the gene list from a separate single cell RNAseq dataset, we confirmed the functional importance of these genes for macrophage. To further explain the macrophage memory and hysteresis phenomenon, we explored the histone modification among M0, M1, and repolarized M0 macrophages using public ATACseq datasets. We found several TFBS which highly related to inflammatory state are significantly enriched in hysteresis regions. We also applied enhancer prediction using ABC model. These results provided more explanation from epigenetic perspective. Finally, we explore memory genes on patient cancer datasets to further explore the biological meaning of macrophage hysteresis. Overall, our results might improve the progress toward the understanding of macrophage immunity.

9. Spatial Transcriptome Analysis of the Adult Brain of *Ciona intestinalis*

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As one of the closest relatives of vertebrates, the ascidians is a simply but comparable model system to provide insights into the evolutionary process of the chordate nervous system. However, the entire transcriptional profiles of nervous system of adult ascidians have yet to be determined and functionally investigated. Here, we performed 10X Visium spatial transcriptomics system on around 2,000 spots from the adult brain of Ciona. Using unsupervised clustering and Gene Ontology (GO) analysis, we identified four main tissues: body wall muscle, dorsal strand, neural gland, and ciliated groove. Furthermore, we characterized micrometer-scale spatial variable genes and the spatial correlation between genes by a deep generative model and a multivariate normal likelihood ratio test. Together, our analysis sheds the light on spatial gene expression patterns that define the organization of the Ciona adult brain.

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

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In the medical treatment for patients with respiratory failure including the 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, we are developing effective next-generation sequencing (NGS) protocols and a data analysis platform. By collecting bronchoalveolar lavage fluid samples of more than 100 patients with COVID-19 and integrating the information from medical records, we tuned our pipeline for meta-genome analysis and quantified the time-course viral DNA and RNA sequencing data. We found severe bacterial and viral infections known to the involvement of such as myocarditis, pericarditis, and pneumonia. Furthermore, we developed a graph-based machine learning method to predict severity using microbial co-occurrence networks that have been profiled from the patient data, which needs further verification. We believe that the information on bacterial and viral microorganisms significantly present in patients with COVID-19 is a useful resource to establish a therapeutic strategy.

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

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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, mecha-

nisms 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), and 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.

12. Single-cell transcriptome analysis of ocular-like cell lineages derived from human pluripotent stem cells

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The recent cell culturing protocol of the so-called SEAM (self-formed ectodermal autonomous multizone), generating 2D eye-like circular colonies from induced pluripotent stem cells (iPSCs), demonstrates that the cells in each zone have different morphologies, e.g., neuroectoderm, ocular-surface ectoderm, and neural crest. To understand the underlying molecular mechanism for the differentiation potency, we are profiling cell populations and their characteristics using time-course scRNA-seq data of SEAM. We confirmed that the undifferentiated iPSCs undergo specific ocular-like cell lineages, including lens cells, retinal cells, and ocular-surface ectoderm progenitors, along with significant marker gene expression. We believe that this single-cell atlas advances studying gene regulation for ocular lineage differentiation.

13. Intercellular crosstalk in adult dental pulp is mediated by heparin-binding growth factors Pleiotrophin and Midkine

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The cell heterogeneity and intercommunication in dental pulp (DP) are not well understood. To address this lack of knowledge, we performed an in-silico analysis of publicly available single-cell RNA-seq data from DP. We compared the cell composition in DP against five other reference tissues: blood, bone marrow, adipose tissue, lung, and skin. We identified a DP-specific population of fibroblasts expressing higher levels of heparin-binding growth factors, pleiotrophin (PTN) and midkine (MDK), as compared with fibroblasts from other tissues. We performed cell-cell crosstalk analysis of these DP-specific fibroblasts finding extensive communication with other cell types such as Schwann cells and odontoblasts. Moreover, the analysis revealed that the communication was mostly carried through PTN and MDK receptor binding, which suggests the importance of these molecules in cell proliferation and differentiation in DP. Together, our analysis extends our understanding of cell heterogeneity in DP and their crosstalk communication, which we expect has a potential role in designing and developing therapeutic targeting molecules in dental treatment.

Publication list

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Human Genome Center

Laboratory of Molecular Medicine
ゲノム医科学分野

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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. Single-Cell Analysis Reveals a CD4+ T-cell Cluster That Correlates with PD-1 Blockade Efficacy

CD4+ T-cell immunity helps clonal proliferation, migration, and cancer cell killing activity of CD8+ T cells and is essential in antitumor immune responses. To identify CD4+ T-cell clusters responsible for antitumor immunity, we simultaneously analyzed the naïve-effector state, Th polarization, and T-cell receptor clonotype based on single-cell RNA-sequencing data. Unsupervised clustering analysis uncovered the presence of a new CD4+ T-cell metacluster in the CD62Llow CD4+ T-cell subpopulation, which contained multicellular clonotypes associated with efficacy of programmed death-ligand 1 (PD-1) blockade therapy. The frequency of these cells in the peripheral blood significantly correlated with progression-free survival and overall survival of patients with lung cancer after PD-1 blockade therapy. These findings suggest that CD62Llow CCR4-CCR6+ CD4+ T cells form a novel metacluster with predictive potential of the immune status and sensitivity to PD-1 blockade, which may pave the way for personalized antitumor immunotherapy strategies for patients.

2. ICGC-ARGO precision medicine: targeted therapy according to longitudinal assessment of tumour heterogeneity in colorectal cancer.

Colorectal cancer is characterised by high molecular heterogeneity and genomic alterations in common cancer drivers, including RAS, BRAF, and mismatch repair genes, which are routinely assessed to inform precision treatments. However, constant clonal evolution is common and leads to therapeutic resistance. Longitudinal molecular analysis of integrated tissue and liquid biopsies is essential to monitor the molecular evolution of colorectal cancer during the continuum of care and inform sequential adaptive therapies based on real-time genomic changes. We present two patients with metastatic colorectal cancer, who after referral to the Precision Medicine Tumour Board underwent assessment using tumour tissue and circulating-tumour DNA (ctDNA), and responded to targeted therapy based on these molecular profiles.

3. Evolutionary Analysis of Cancer

The process of cancer development by genome mutation can be regarded as the "evolution" of cancer. We have conducted several analyses to elucidate the evolution of cancer. Population genetics has been used to elucidate the evolution of various species. Using the theory of population genetics, we are develop-

ing a method to detect selection related to intratumor heterogeneity (ITH) in cancer. In addition, various genomic mutations occur in cancer, and theoretical analysis can clarify the timing of their mutations in the development of cancer. Using these methods, we have analyzed the evolution of various cancers.

4. Verification of the Effect of Mindfulness Meditation on Heart Rate Variability Using Mobile Health Technology

Mindfulness meditation (hereafter referred to as "meditation") is a modern form of mental training that has been re-edited from traditional Buddhist religious practice to remove its religious overtones. We will examine whether continuous meditation can change heart rate patterns in daily life, based on heart rate data obtained from a smartwatch. For this purpose, we will also collect information on sleep and step counts, collect information on stress and activity status in real time using a smartphone application, and collect information on stress, exercise, sleep, etc. through a post-questionnaire survey, and conduct an integrated analysis of these data. The results obtained from this study will make it possible to objectively measure the effects of meditation on stress reduction using mobile health technology.

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Human Genome Center

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

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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. Expansion of Cancer Risk Profile for BRCA1 and BRCA2 Pathogenic Variants.

Importance: The clinical importance of genetic testing of BRCA1 and BRCA2 in breast, ovarian, prostate, and pancreatic cancers is widely recognized. However, there is insufficient evidence to include other cancer types that are potentially associated with BRCA1 and BRCA2 in clinical management guidelines.

Objective: To evaluate the association of BRCA1 and BRCA2 pathogenic variants with additional cancer types and their clinical characteristics in 100 914 individuals across 14 cancer types.

Design, setting, and participants: This case-control analysis to identify cancer types and clinical characteristics associated with pathogenic variants in BRCA1 and BRCA2 included DNA samples and clinical information from 63 828 patients with 14 common cancer types and 37 086 controls that were sourced from a multi-institutional hospital-based registry, BioBank Japan, between April 2003 and March 2018. The data were analyzed between August 2019 and October 2021.

Main outcomes and measures: Germline pathogenic variants in coding regions and 2 bp flanking intronic sequences in BRCA1 and BRCA2 were identified by a multiplex polymerase chain reaction-based target sequence method. Associations of (likely) pathogenic variants with each cancer type were assessed by comparing pathogenic variant carrier frequency between patients in each cancer type and controls.

Results: A total of 65 108 patients (mean [SD] age at diagnosis, 64.1 [11.6] years; 27 531 [42.3%] female) and 38 153 controls (mean [SD] age at registration, 61.8 [14.6] years; 17 911 [46.9%] female) were included in this study. A total of 315 unique pathogenic variants were identified. Pathogenic variants were associated with $P < 1 \times 10\text{-}4$ with an odds ratio (OR) of greater than 4.0 in biliary tract cancer (OR, 17.4; 95% CI, 5.8-51.9) in BRCA1, esophageal cancer (OR, 5.6; 95% CI, 2.9-11.0) in BRCA2, and gastric cancer (OR, 5.2; 95% CI, 2.6-10.5) in BRCA1, and (OR, 4.7; 95% CI, 3.1-7.1) in BRCA2 in addition to the 4 established cancer types. We also observed an association with 2 and 4 other cancer types in BRCA1 and BRCA2, respectively. Biliary tract, female breast, ovarian, and prostate cancers showed enrichment of carrier patients according to the increased number of reported cancer types in relatives.

Conclusions and relevance: The results of this large-scale registry-based case-control study suggest

that pathogenic variants in BRCA1 and BRCA2 were associated with the risk of 7 cancer types. These results indicate broader clinical relevance of BRCA1 and BRCA2 genetic testing.

Hereditary cancer variants and homologous recombination deficiency in biliary tract cancer

Background & aims: The heritability and actionability of variants in homologous recombination-related genes in biliary tract cancers (BTCs) are uncertain. Although associations between BTC and BRCA germline variants have been reported, homologous recombination deficiency has not been investigated in BTCs.

Methods: We sequenced germline variants in 27 cancer-predisposing genes in 1,292 BTC cases and 37,583 controls without a personal nor family history of cancer. We compared pathogenic germline variant frequencies between cases and controls and documented the demographic and clinical characteristics of carriers. In addition, whole-genome sequencing of 45 BTC tissues was performed to evaluate homologous recombination deficiency status.

Results: Targeted sequencing identified 5,018 germline variants, which were classified into 317 pathogenic, 3,611 variants of uncertain significance, and 1,090 benign variants. Seventy-one BTC cases (5.5%) had at least one pathogenic variant among 27 cancer-predisposing genes. Pathogenic germline variants enriched in BTCs were present in BRCA1, BRCA2, APC, and MSH6 ($p < 0.00185$). PALB2 variants were marginally associated with BTC ($p = 0.01$). APC variants were predominantly found in ampulla of Vater carcinomas. Whole-genome sequencing demonstrated that three BTCs with pathogenic germline variants in BRCA2 and PALB2, accompanied by loss of heterozygosity, displayed homologous recombination deficiency. Conversely, pathogenic germline variants in without a second hit or other homologous recombination-realted genes such as ATM and BRIP1 showed homologous recombination-proficient phenotypes.

Conclusions: In this study, we describe the heritability and actionability of variants in homologous recombination-related genes, which could be used to guide screening and therapeutic strategies for BTCs.

Impact and implications: We found that 5.5% of biliary tract cancers (BTCs) in a Japanese population possessed hereditary cancer-predisposing gene alterations, including in BRCA and genes associated with colorectal cancer. Two hits in homologous recombination-related genes were required to confer a homologous recombination-deficient phenotype. PARP inhibitors and DNA-damaging regimens may be effective strategies against BTCs exhibiting homologous recombination deficiency. Hence, in this study, genome-wide sequencing has revealed a potential new therapeutic strategy that could be applied to a subset of BTCs.

Deciphering colorectal cancer genetics through multi-omic analysis of 100,204 cases and 154,587 controls of European and east Asian ancestries

Colorectal cancer (CRC) is a leading cause of mortality worldwide. We conducted a genome-wide association study meta-analysis of 100,204 CRC cases and 154,587 controls of European and east Asian ancestry, identifying 205 independent risk associations, of which 50 were unreported. We performed integrative genomic, transcriptomic and methylomic analyses across large bowel mucosa and other tissues. Transcriptome- and methylome-wide association studies revealed an additional 53 risk associations. We identified 155 high-confidence effector genes functionally linked to CRC risk, many of which had no previously established role in CRC. These have multiple different functions and specifically indicate that variation in normal colorectal homeostasis, proliferation, cell adhesion, migration, immunity and microbial interactions determines CRC risk. Crosstissue analyses indicated that over a third of effector genes most probably act outside the colonic mucosa. Our findings provide insights into colorectal oncogenesis and highlight potential targets across tissues for new CRC treatment and chemoprevention strategies.

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Human Genome Center

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

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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, Kawabata K, 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 Action Plan for Whole Genome Analysis of Ministry of Health, Labour and Welfare

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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

Identifying the host genetic factors underlying se-

vere COVID-19 is an emerging challenge. Here we conducted a genome-wide association study (GWAS) involving 2,393 cases of COVID-19 in a cohort of Japanese individuals collected during the initial waves of the pandemic, with 3,289 unaffected controls. We identified a variant on chromosome 5 at 5q35 (rs60200309-A), close to the dedicator of cytokinesis 2 gene (DOCK2), which was associated with severe COVID-19 in patients less than 65 years of age. This risk allele was prevalent in East Asian individuals but rare in Europeans, highlighting the value of genome-wide association studies in non-European populations. RNA-sequencing analysis of 473 bulk peripheral blood samples identified decreased expression of DOCK2 associated with the risk allele in these younger patients. DOCK2 expression was suppressed in patients with severe cases of COVID-19. Single-cell RNA-sequencing analysis (n = 61 individuals) identified cell-type-specific downregulation of DOCK2 and a COVID-19-specific decreasing effect of the risk allele on DOCK2 expression in non-classical monocytes. Immunohistochemistry of lung specimens from patients with severe COVID-19 pneumonia showed suppressed DOCK2 expression. Moreover, inhibition of DOCK2 function with CPYPP increased the severity of pneumonia in a Syrian hamster model of SARS-CoV-2 infection, characterized by weight loss, lung oedema, enhanced viral loads, impaired macrophage recruitment and dysregulated type I interferon responses. We conclude that DOCK2 has an important role in the host immune response to SARS-CoV-2 infection and the development of severe COVID-19, and could be further explored as a potential biomarker and/or therapeutic target.

b. COVID-19 risk assessment at 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

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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. Comparison of the 2021 International Society for Stem Cell Research (ISSCR) guidelines for "laboratory-based human stem cell research, embryo research, and related research activities" and the corresponding Japanese regulations

This paper presents a comparison of the 2021 guidelines for stem cell research and clinical translation outlined by the International Society for Stem Cell Research (ISSCR) with the current regulations in Japan regarding the performance of such research. This paper provides a convenient English-language summary of the Japanese regulations, and illustrates the difference between the ISSCR guidelines and Japanese regulations regarding the conditions of implementation of study activities using human embryos or stem cells, for researchers outside Japan. The regulations governing the performance of research activities using human embryos or stem cells in Japan are relatively complex and comprise a range of laws and guidelines; the specific rules applied depend on the characteristics of each study. Therefore, even similar research activities may differ in terms of not only the guidelines or laws implemented, but also the procedures required. Such situations may confuse researchers.

2. Learning to listen: A complementary approach to informed consent for patients with visual impairments

This forum describes an exploratory approach for assisting individuals with visual impairment during the informed consent (IC) process to participate in a cutting-edge trial. Our approach has been developed to focus on potential participants' preparedness to give IC, along with the creation of supporting audio material.

3. Patient and public involvement in mobile health-based research for hay fever: a qualitative study of patient and public involvement implementation process

Patient and public involvement (PPI) plays an important role in promoting effective execution of health science research, as well as in the establishment of a social agreement and infrastructure for the care of various diseases, including cancer, chronic diseases,

and allergic illnesses. Hay fever is one of the most common allergic diseases, affecting more than 30 million people in Japan. It is known for its myriad factors and diverse presentations. Previously, we developed a mobile health (mHealth) smartphone application (app) for hay fever—AllerSearch—released in February 2018. This app is capable of collecting relevant digital phenotypes and user-provided information, which are used in providing tailored, evidence-based suggestions. To our knowledge, no other studies have been conducted on the implementation of PPI in mHealth. Since hay fever presents with a wide variety of symptoms and risk factors, PPI principles appear well-suited for eliciting insights from the patient/public population and for incorporating new, expert perspectives into the research process. In this study, we included PPI contributors in the research plan, app development, and evaluation. Most notably, the survey questionnaire and user interface of the app was tailored based on PPI feedback. The updated AllerSearch app was released during this study period. Since hay fever is a widespread and variable illness, the multifaceted input from patients and public experts enabled by PPI implementation holds promise for improving society-wide healthcare and in empowering a culture toward medical involvement.

4. A comparative analysis of attitudes toward stem cell research and regenerative medicine between six countries - A pilot study

Breakthroughs in stem cell research (SCR) and regenerative medicine (RM) have attracted significant public attention worldwide. Simultaneously, scientific communities and science policies have tried to establish appropriate governance of SCR and RM. In this context, effective communication between scientific communities and the public is regarded as a key factor. However, the diversity of public attitudes and interests has not been sufficiently examined, especially the differences across countries. We conducted an international comparison of public attitudes toward SCR and RM. We circulated an internet questionnaire among people in six countries: Japan, South Korea, the United States, the UK, Germany, and France. We collected 100 valid responses from each country, and a total of 600 responses were obtained. Our key findings are the diversity of interests in RM, which can be expressed as user pragmatism, governance and handling of RM, risk, and benefit, and scientific interests. The priority of interests varied across the six countries, and the variations may be influenced by the political, social, cultural, and media contexts of SCR and RM in each country. The implications can contribute to a deeper understanding of the diversity of public attitudes, and bring about an appropriate examination of a wide range of ethical and social concerns of SCR and RM in global contexts.

5. Public attitudes in the clinical application of genome editing on human embryos in Japan: a cross-sectional survey across multiple stakeholders

Recent advances in genome editing technology are accompanied by increasing public expectations on its potential clinical application, but there are still scientific, ethical, and social considerations that require resolution. In Japan, discussions pertaining to the clinical use of genome editing in human embryos are underway. However, understanding of the public's sentiment and attitude towards this technology is limited which is important to help guide the debate for prioritizing policies and regulatory necessities. Thus, we conducted a cross-sectional study and administered an online questionnaire across three stakeholder groups: the general public, patients and their families, and health care providers. We received responses from a total of 3,511 individuals, and the attitudes were summarized and compared among the stakeholders. Based on the distribution of responses, health care providers tended to be cautious and reluctant about the clinical use of genome editing, while patients and families appeared supportive and positive. The majority of the participants were against the use of genome editing for enhancement purposes. Participants expressed the view that clinical use may be acceptable when genome editing is the fundamental treatment, the risks are negligible, and the safety of the technology is demonstrated in human embryos. Our findings suggest differences in attitudes toward the clinical use of genome editing across stakeholder groups. Taking into account the diversity of the public's awareness and incorporating the opinion of the population is important. Further information dissemination and educational efforts are needed to support the formation of the public's opinion.

6. Relationship Between High Organ Donation Rates and COVID-19 Vaccination Coverage

Besides attaining the goal of self-protection, the rollout of vaccination programs also encourages altruistic practices. Therefore, the progress in vaccination against coronavirus disease (COVID-19) in each country may be related to the prevalence of cooperative and altruistic practices in health care. I hypothesized that in countries where organ donation is popular, individuals would exhibit a greater tendency to become vaccinated. I examined the correlation between the level of progress of COVID-19 vaccination and the status of organ donation just before the pandemic in Organization for Economic Co-operation and Development (OECD) countries. Publicly available statistical information on the progress of immunization and organ donation was used. Univariate and multivariate analyses were conducted to examine common drivers of immunization and organ dona-

tion. In OECD countries, progress in vaccination was found to be significantly correlated with the status of organ donation in each country. This relationship was stable after the summer (September 1: Pearson's r = 0.442, October 1: 0.457, November 1: 0.366). The results of the univariate and multivariate analyses showed that high trust in medical professionals was significantly correlated with both the "progress of vaccinations" and "organ donations." Progress in COVID-19 vaccination and organ donation status for transplantation have similar trends, and both may involve people's trust in medical personnel and public health systems. Similar to the efforts to obtain organ donors, governments around the world need to take further steps to ensure that vaccination programs are supported by people's trust and sense of solidarity.

7. Factors that Lead to Stagnation in Direct Patient Reporting of Adverse Drug Reactions: An Opinion Survey of the General Public and Physicians in Japan

Data collection from patients regarding adverse drug reactions (ADRs) in Japan have greatly stagnated. To examine the factors underlying this stagnation, we investigated the awareness of and opinions about the direct ADR reporting system among the general public and physicians. We conducted questionnaire surveys of general citizens and physicians throughout Japan and included the following topics: (1) awareness of the direct patient ADR reporting system, (2) attitude toward this system, (3) reasons for negative opinions of this system, (4) awareness of the physician ADR reporting system, and (5) respondent demographics. Responses were received from 845 citizens and 300 physicians. Most citizens (83.7%) were unaware of the direct patient ADR reporting system. While many citizens supported the idea of the system, 26.7% expressed negative/hesitant opinions. Prominent reasons for negative/hesitant opinions included the patient burden for reporting their own ADRs and expectations that physicians would make reports. Among the general public, the physician reporting system was better known (43.6%). In contrast, many physicians were aware of the direct patient ADR reporting system (65.0%). However, only 46.7% of physicians had supported this system; prominent reasons for disapproval included skepticism toward patients' judgment and the regulatory authorities' assessment. Our survey suggests that stagnation in the reporting system is affected by the attitudes of the general public and physicians. In addition to government measures to improve awareness and eliminate reporting hurdles, the involvement of medical staff in patient reporting needs to be improved.

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Human Genome Center

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

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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 Privacy Preserving Technologies for Medical Data

a. Differentially Private SNPs Ranking Publication of GWAS

i) Discrete Fourier Transform-based Method

Akihito Yamamoto[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informtatics, Institute of Medical Science, the University of Tokyo

As the amount of data containing human genome information increases, these data will be further utilized in medicine. If the statistics obtained from large-scale analyses are released unchanged, there is a risk of identifying individuals. Although there are several privacy-preserving techniques to release and utilize genomic statistics, most have the problem of poor accuracy at high privacy levels and do not provide correct results especially with an increased number of outputs. In addition, existing methods with relatively high accuracy are computationally intensive and hardly applicable to a large cohort such as those containing 10^6 SNPs. We propose innovative differentially private methods with both efficiency and high accuracy to release the top K significant SNPs based on genomic statistics data [4]. First, we enhance the Fourier perturbation algorithm (FPA), which was proposed in the context of histogram publication, for use with genomic statistics. Then, we propose a new extended FPA with more accurate privacy guarantees and provide a proof that this method achieves ε-differential privacy. Furthermore, we present novel methods combining DFT with the Laplace and exponential mechanisms. These methods take only $O(m \log m)$ time for a dataset containing m SNPs. We also theoretically guarantee that the value of sensitivity for these methods is smaller than that for existing methods and therefore can provide more accurate outputs. Our proposed algorithms can be conducted in less than 20 seconds even for a large cohort, and our experiments using real data show that our methods can achieve 1.5 to 8 times higher accuracy than state-of-the-art methods especially when K is large. Because retrieving multiple significant SNPs from large cohorts in genomic analysis is preferred, our proposed methods are remarkably advisable rather than existing methods.

ii) Compressive Mechanism with Haar Wavelet Transform

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To promote the use of personal genome information in medicine, it is important to analyze the relationship between diseases and the human genomes. Statistical analysis using genomic data is often conducted, but there is a privacy concern with respect to releasing the statistics as they are. Existing methods to address this problem using the concept of differential privacy cannot provide accurate outputs under strong privacy guarantees, making them less practical. We investigate the application of a compressive mechanism to genomic statistical data and propose two approaches [2]. The first is to apply the normal compressive mechanism to the statistics vector along with an algorithm to determine the number of nonzero entries in a sparse representation. The second is to alter the mechanism based on the data, aiming to release significant single nucleotide polymorphisms with a high probability. In this algorithm, we apply the compressive mechanism with the input as a sparse vector for significant data and the Laplace mechanism for nonsignificant data. By using the Haar wavelet transform for the compressive mechanism, we can determine the number of nonzero elements and the amount of noise. In addition, we give theoretical guarantees that our proposed methods achieve ε-differential privacy. We evaluated our methods in terms of accuracy and rank error compared with the Laplace and exponential mechanisms. The results show that our second method in particular can guarantee high privacy assurance as well as utility.

b. Differentially Private Publication of Transmission Disequilibrium Test

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To achieve the provision of personalized medicine, it is very important to investigate the relationship between diseases and human genomes. 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. We propose new efficient differentially private methods for a transmission disequilibrium test, which is a family-based association test [9]. 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 demonstrate 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 is the first in the world that can be applied to a large cohort, such as those with 10^6 SNPs. In addition, we examine a suitable dataset to apply our approximation algorithm.

c. Differentially Private Data Sharing with k-Anonymity

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As the amount of biomedical and healthcare data increases, data mining for medicine becomes more and more important for health improvement. Privacy concerns in data utilization have also been growing. The key concepts for privacy protection are *k*-anonymity and differential privacy, but *k*-anonymity alone cannot protect personal presence information, and differential privacy alone would leak the identity. To promote data sharing throughout the world, universal methods to release the entire data while satisfying both concepts are required, but such a method does not yet exist. Therefore, we propose a novel privacy-preserving method, (ε, *k*)-Randomized Anonymization [1]. We first present two methods that compose the Randomized Anonymization method. They perform *k*-anonymization and randomized response in sequence and have adequate randomness and high privacy guarantees, respectively. Then, we show the algorithm for (ε, *k*)-Randomized Anonymization, which can provide highly accurate outputs with both *k*-anonymity and differential privacy. In addition, we describe the analysis procedures for each method using an inverse matrix and expectation-maximization (EM) algorithm. In the experiments, we used real data to evaluate our methods' anonymity, privacy level, and accuracy. Furthermore, we show several examples of analysis results to demonstrate high utility of the proposed methods.

2. Development of Biomedical Database Technologies

a. Optimally Confining Lattice Polymers

Robert Daniel Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informtatics, Institute of Medical Science, the University of Tokyo

We introduce the Lattice Polymer Confinement Problem (LPCP), where provided a graph *G* corresponding to a solid or hole-containing finite lattice, and provided a finite set of lattice polymers modeled as Self-Avoiding Walks (SAWs), the objective is to delete the fewest possible number of vertices in *G* to satisfy a bound on a sum over the configuration entro-

pies of each polymer [7]. We also propose a novel Self-Avoiding Walk (SAW) centrality measure for a vertex in a lattice or graph as a variation on the standard notion of betweenness centrality. We show that LPCP is NP-hard as well as APX-hard. On the other hand, we prove the existence of a polynomial time parameterized approximation algorithm for LPCP where the treewidth of the graph is assumed as a parameter. We moreover establish a deterministic algorithm for SAW centrality with multiplicative error $1\pm\varepsilon$. Finally, we analyze variations on LPCP, including a variant where we delete edges in lieu of vertices, and variant with rigid lattice polymers (e.g., lattice proteins) where every embedding must satisfy a set of consecutive dihedral angles for adjacent bonds.

b. String Editing under Pattern Constraints

Robert Daniel Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informtatics, Institute of Medical Science, the University of Tokyo

We introduce the novel Nearest Pattern Constrained String (NPCS) problem of finding a minimum set of character mutation, insertion, and deletion edit operations sufficient to modify a string to contain all contiguous words in a pattern set and no contiguous words in a forbidden pattern set. We show that NPCS is fixed-parameter tractable [3].

c. Graph Database Technologies

i) Hardness of Bounding Influence via Graph Modification

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We consider the problem of minimally modifying graphs and digraphs by way of exclusively deleting vertices, exclusively deleting edges, or exclusively adding new edges, with or without connectivity constraints for the resulting graph or digraph, to ensure that centrality-based influence scores of all vertices satisfy either a specified lowerbound or upperbound [5]. Here, we classify the hardness of exactly or approximately solving this problem for: (1) all vertex- and edge-deletion cases for betweenness, harmonic, degree, and in-degree centralities; (2) all vertex-deletion cases for eigenvector, Katz, and PageRank centralities; (3) all edge-deletion cases for eigenvector, Katz, and PageRank centralities under a connectivity or weak-connectivity constraint; and (4) a set of edge-addition cases for harmonic, degree, and in-degree centralities. We show that some of our results, in particular multiple results concerning betweenness, eigenvector, Katz, and PageRank centralities, hold for planar graphs and digraphs. Finally, under a variety of constraints, we establish that no polynomial time constant factor approximation algorithm can exist for computing the cardinality of a minimum set of vertices or minimum set of edges whose deletion ensures a lowerbound betweenness centrality score, or a lower- or upperbound eigenvector, Katz, or PageRank centrality score (unless $P = NP$).

ii) Proper Colorability of Segment Intersection Graphs

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We consider the vertex proper coloring problem for highly restricted instances of geometric intersection graphs of line segments embedded in the plane [6]. We show that, provided a graph in the class PURE-k-DIR corresponding to intersection graphs of segments lying in at most k directions with all parallel segments disjoint, and provided a k-coloring for this graph, it is NP-complete to decide if the graph admits a $(k-1)$-coloring $\forall k \geq 4$. Furthermore, we show that this result holds under the constraint that all segments are of unit length in the case where $k = 4$, and under the constraint that segments have at most three distinct lengths $\forall k \geq 5$. More generally, we establish that the problem of properly 3-coloring an arbitrary graph can be reduced in linear time to the problem of properly 3-coloring a PURE-4-DIR graph where all segments are of unit length, yielding a method for explicit construction of hard 3-colorability instances for this graph class.

3. Development of Artificial Intelligence Technologies for Text Data

a. A Comprehensive Analysis of Subword Contextual Embeddings for Languages with Rich Morphology

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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 [10]. We experimented with crosslingual, multilingual, and monolingual BERT models, and three non-BERT based 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 are shown to 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, 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 proposed BERT based model achieved new state-of-the-art results on both DEP and NER tasks for the Turkish language.

b. Developing Language Resources and NLP Tools for the North Korean Language

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Since the division of Korea, the two Korean languages have diverged significantly over the last 70 years. However, due to the lack of linguistic source of the North Korean language, there is no DPRK-based language model. Consequently, scholars rely on the Korean language model by utilizing South Korean linguistic data. We first present a large-scale dataset for the North Korean language. We use the dataset to train a BERT-based language model, DPRK-BERT. Second, we annotate a subset of this dataset for the sentiment analysis task. Finally, we compare the performance of different language models for masked language modeling and sentiment analysis tasks [8].

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Human Genome Center

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

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Project Associate Professor	Kosuke Fujimoto, M.D., Ph.D.	特任准教授　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 diseases.

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In recent years, intestinal dysbiosis has been detected in a variety of diseases. It has become clear that dysbiosis is involved in the pathogenesis of these diseases. We collected fecal samples from 10 Crohn's disease patients in remission and performed metagenomic analysis. Disease-specific defective bacteria and gene pathways have been identified. Functional analysis of the defective bacteria is currently underway. Graft-versus-host disease is a serious side effect after bone marrow transplantation in leukemia patients. We are collecting fecal samples over time and performing metagenomic analysis in patients with bone marrow transplantation. We have identified bacteria that increase in the gut after bone marrow transplantation and have obtained data that they increase the risk of GVHD. Functional analysis of those bacteria is currently underway.

2. Development of a microbiome digital twin to predict disease states based on metagenome analysis of intestinal microflora

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We are developing a digital twin that predicts disease states using metagenomic data of the intestinal microbiota and gene pathway analysis data as teaching data. For this purpose, we collected fecal samples from 18 Parkinson's disease patients and performed metagenomic analysis. Currently, under collaboration with Fujitsu, we are comparing these data with metagenomic data of 100 healthy subjects and performing machine learning and deep learning.

3. Development of phage therapy

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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 compiling an comprehensive catalog of enzymes derived from phage infecting *Acinetobacter baumannii* for the purpose of developing next-generation phage therapies against Gram-negative bacteria. We are also developing a platform for the creation of artificial phage capable of killing *Escherichia coli*.

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

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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*. It has shown strong antibacterial activity against the bacteria wuth IgA-degrading enzyme.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.

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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

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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. The oncogene-dependent resistance to reprogramming unveils cancer therapeutic targets

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The resistance to transcription factor-mediated reprogramming into pluripotent stem cells is one of the distinctive features of cancer cells. Here we dissect the profiles of reprogramming factor binding and the subsequent transcriptional response in cancer cells to reveal its underlying mechanisms. Using clear cell sarcomas (CCSs), we show that the driver oncogene EWS/ATF1 misdirects the reprogramming factors to cancer-specific enhancers and thereby impairs the transcriptional response toward pluripotency that is otherwise provoked. Sensitization to the reprogramming cue is observed in other cancer types when the corresponding oncogenic signals are pharmacologically inhibited. Exploiting this oncogene dependence of the transcriptional "stiffness," we identify mTOR signaling pathways downstream of EWS/ATF1 and discover that inhibiting mTOR activity substantially attenuates the propagation of CCS cells in vitro and in vivo. Our results demonstrate that the early transcriptional response to cell fate perturbations can be a faithful readout to identify effective therapeutics targets in cancer cells.

2. MYCL-mediated reprogramming expands pancreatic insulin-producing cells

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β cells have a limited capacity for regeneration, which predisposes towards diabetes. Here, we show that, of the MYC family members, *Mycl* plays a key role in proliferation of pancreatic endocrine cells. Genetic ablation of *Mycl* causes a reduction in the proliferation of pancreatic endocrine cells in neonatal mice. By contrast, the expression of *Mycl* in adult mice stimulates the proliferation of β and α cells, and the cells persist after withdrawal of *Mycl* expression. A subset of the expanded α cells give rise to insulin-producing cells after this withdrawal. Transient *Mycl* expression in vivo is sufficient to normalize the hyperglycaemia of diabetic mice. In vitro expression of *Mycl* similarly provokes active replication in islet cells, even in those from aged mice. Finally, we show that *MYCL* stimulates the division of human adult cadaveric islet cells. Our results demonstrate that the induction of *Mycl* alone expands the functional β-cell population, which may provide a regenerative strategy for β cells.

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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

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Toll-like receptor 7 (TLR7) is an innate immune RNA sensor that is expressed in B cells, dendritic cells, and monocytes/macrophages. This receptor responds not only to pathogen-derived single-stranded RNA (ssRNA), but also to self-derived ssRNA, and drives autoimmune diseases such as SLE and psoriasis. A lupus-prone mouse strain, Y-linked autoimmune accelerator (Yaa), has a duplicate copy of the TLR7 gene that results in TLR7 hyperactivation, leading to lupus-like states. The TLR7 agonist imiquimod drives lupus nephritis in mice, whereas lupus nephritis spontaneously developed in the lupus-prone strain, New Zealand Black/New Zealand White F1 mice (NZBWF1 mice) is ameliorated by a small chemical TLR7 inhibitor. TLR7 might play unique pathogenic roles in patrolling monocytes because they express abundant TLR7. We previously reported that the anti-TLR7 mAb inhibits TLR7 responses in B cells, dendritic cells, and monocyte/macrophages. The anti-TLR7 mAb binds to cell surface TLR7, which is internalized into the endosomal compartment. Because TLR7 shuttles between cell surface and the endosomal compartment, endosomal TLR7 comes out of the cell surface and becomes accessible to the anti-TLR7 mAb. Therefore, the TLR7-mAb immune complex gradually increases with the mAb treatment. When endosomal TLR7 is mostly complexed with the anti-TLR7 mAb, endosomal TLR7 responses are inhibited. The inhibitory effect of the anti-TLR7 was also observed in vivo, rescuing Unc93b1D34A/D34A mice from TLR7-dependent autoimmune hepatitis. Here, we investigated the pathogenic role of TLR7 in NZBWF1 mice using an anti-TLR7 inhibitory mAb This mAb ameliorated lupus nephritis in NZBWF1 mice by acting on B cells and monocytes/macrophages, thereby reducing IgG deposition in glomeruli and diminishing autoantibody production. These findings suggested that the activation and differentiation of autoreactive B cells in NZBWF1 mice is TLR7-de-

pendent. Furthermore, the numbers of Ly6Clow patrolling monocytes, which are thought to be tissue macrophages in the circulation, TLR7-dependently increased in the spleen, circulation, and kidneys. Transcriptome and FACS analyses revealed increased expression of lupus-associated molecules such as IL-10, which promotes nephritis, in monocytes that accumulated in the spleen. These results suggested that TLR7 is a therapeutic target for SLE and that anti-TLR7 mAb is a promising therapeutic tool targeting both B cells and monocytes in SLE.

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

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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. TLR7/8 stress response drives histiocytosis in SLC29A3 disorders

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SLC29A3, also known as ENT3, is a lysosomal transmembrane protein that transports nucleosides from the lysosomes to the cytoplasm. Loss-of-function mutations in *SLC29A3* cause lysosomal nucleoside storage and histiocytosis: phagocyte accumulation in multiple organs. However, little is known about the mechanism through which lysosomal nucleoside storage drives histiocytosis. Herein, histiocytosis in *Slc29a3*$^{-/-}$ mice was demonstrated to depend on TLR7, which senses a combination of nucleosides and oligoribonucleotides. TLR7 responded to lysosomal nucleoside storage and enhanced proliferation of Ly6C^{hi} CX3CR1^{low} immature monocytes and their maturation into Ly6C^{low} phagocytes in *Slc29a3*$^{-/-}$ mice. Because accumulated nucleosides primarily originated from cell corpse phagocytosis, TLR7 in immature monocytes recognized nucleoside storage as lysosomal stress and increased phagocyte numbers. This non-inflammatory compensatory response is referred to as the TLR7 stress response where Syk, GSK3β, β-catenin, and mTORC1 serve as downstream signaling molecules. In SLC29A3 disorders, histiocytosis accompanies inflammation. Nucleoside storage failed to induce pro-inflammatory cytokine production in *Slc29a3*$^{-/-}$ mice, but enhanced ssRNA-dependent pro-inflammatory cytokine production in Ly6C^{hi} classical monocytes and peripheral macrophages, not proliferating immature monocytes. Patient-derived monocytes harbouring G208R *SLC29A3* mutation showed higher survival and proliferation in the presence of M-CSF and produced larger amounts of IL-6 upon ssRNA stimulation than did those derived from healthy subjects. A TLR8 antagonist inhibited the survival/proliferation of patient-derived macrophages. These results demonstrated that TLR7/8 responses to lysosomal nucleoside stress drive SLC29A3 disorders.

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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

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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. CRISPR/Cas9-Mediated Highly Efficient Gene Targeting in Embryonic Stem Cells for Developing Gene-Manipulated Mouse Models

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The CRISPR/Cas9 system has made it possible to develop genetically modified mice by direct genome editing using fertilized zygotes. However, although the efficiency in developing gene-knockout mice by inducing small indel mutation would be sufficient enough, the efficiency of embryo genome editing for making large-size DNA knock-in (KI) is still low. Therefore, in contrast to the direct KI method in embryos, gene targeting using embryonic stem cells (ESCs) followed by embryo injection to develop chimera mice still has several advantages (e.g., high throughput targeting in vitro, multi-allele manipulation, and Cre and flox gene manipulation can be carried out in a short period). In addition, strains with difficult-to-handle embryos in vitro, such as BALB/c, can also be used for ESC targeting. This protocol describes the optimized method for large-size DNA (several kb) KI in ESCs by applying CRISPR/Cas9-mediated genome editing followed by chimera mice production to develop gene-manipulated mouse models.

2. Splice factor polypyrimidine tract-binding protein 1 (Ptbp1) primes endothelial inflammation in atherogenic disturbed flow conditions

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NF-κB-mediated endothelial activation drives leu-

kocyte recruitment and atherosclerosis, in part through adhesion molecules Icam1 and Vcam1. The endothelium is primed for cytokine activation of NF-κB by exposure to low and disturbed blood flow (LDF)but the molecular underpinnings are not fully understood. In an experimental in vivo model of LDF, platelets were required for the increased expression of several RNA-binding splice factors, including polypyrimidine tract binding protein (Ptbp1). This was coordinated with changes in RNA splicing in the NF-κB pathway in primed cells, leading us to examine splice factors as mediators of priming. Using Icam1 and Vcam1 induction by tumor necrosis factor (TNF)-α stimulation as a readout, we performed a CRISPR Cas9 knockout screen and identified a requirement for Ptbp1 in priming. Deletion of Ptbp1 had no effect on cell growth or response to apoptotic stimuli, but reversed LDF splicing patterns and inhibited NF-κB nuclear translocation and transcriptional activation of downstream targets, including Icam1 and Vcam1. In human coronary arteries, elevated PTBP1 correlates with expression of TNF pathway genes and plaque. In vivo, endothelial-specific deletion of Ptbp1 reduced Icam1 expression and myeloid cell infiltration at regions of LDF in atherosclerotic mice, limiting atherosclerosis. This may be mediated, in part, by allowing inclusion of a conserved alternative exon in Ripk1 leading to a reduction in Ripk1 protein. Our data show that Ptbp1, which is induced in a subset of the endothelium by platelet recruitment at regions of LDF, is required for priming of the endothelium for subsequent NF-κB activation, myeloid cell recruitment and atherosclerosis.

3. Genetic loss of function of Ptbp1 does not induce glia-to-neuron conversion in retina

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Direct reprogramming of glia into neurons is a potentially promising approach for the replacement of neurons lost to injury or neurodegenerative disorders. Knockdown of the polypyrimidine tract-binding protein Ptbp1 has been recently reported to induce efficient conversion of retinal Müller glia into functional neurons. Here, we use a combination of genetic lineage tracing, single-cell RNA sequencing (scRNA-seq), and electroretinogram analysis to show that selective induction of either heterozygous or homozygous loss-of-function mutants of Ptbp1 in adult retinal Müller glia does not lead to any detectable level of neuronal conversion. Only a few changes in gene expression are observed in Müller glia following Ptbp1 deletion, and glial identity is maintained. These findings highlight the importance of using genetic manipulation and lineage-tracing methods in studying cell-type conversion.

4. MYCL-mediated reprogramming expands pancreatic insulin-producing cells

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β cells have a limited capacity for regeneration, which predisposes towards diabetes. Here, we show that, of the MYC family members, Mycl plays a key role in proliferation of pancreatic endocrine cells. Genetic ablation of Mycl causes a reduction in the proliferation of pancreatic endocrine cells in neonatal mice. By contrast, the expression of Mycl in adult mice stimulates the proliferation of β and α cells, and the cells persist after withdrawal of Mycl expression. A subset of the expanded α cells give rise to insulin-producing cells after this withdrawal. Transient Mycl expression in vivo is sufficient to normalize the hyperglycaemia of diabetic mice. In vitro expression of Mycl similarly provokes active replication in islet cells,

even in those from aged mice. Finally, we show that MYCL stimulates the division of human adult cadaveric islet cells. Our results demonstrate that the induction of Mycl alone expands the functional β-cell population, which may provide a regenerative strategy for β cells.

5. Efficient simultaneous double DNA knock-in in murine embryonic stem cells by CRISPR/Cas9 ribonucleoprotein-mediated circular plasmid targeting for generating gene-manipulated mice

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Gene targeting of embryonic stem (ES) cells followed by chimera production has been conventionally used for developing gene-manipulated mice. Although direct knock-in (KI) using murine zygote via CRISPR/Cas9-mediated genome editing has been reported, ES cell targeting still has merits, e.g., high throughput work can be performed in vitro. In this study, we first compared the KI efficiency of mouse ES cells with CRISPR/Cas9 expression vector and ribonucleoprotein (RNP), and confirmed that KI efficiency was significantly increased by using RNP. Using CRISPR/Cas9 RNP and circular plasmid with homologous arms as a targeting vector, knock-in within ES cell clones could be obtained efficiently without drug selection, thus potentially shortening the vector construction or cell culture period. Moreover, by incorporating a drug-resistant cassette into the targeting vectors, double DNA KI can be simultaneously achieved at high efficiency by a single electroporation. This technique will help to facilitate the production of genetically modified mouse models that are fundamental for exploring topics related to human and mammalian biology.

6. Trim41 is required to regulate chromosome axis protein dynamics and meiosis in male mice

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Meiosis is a hallmark event in germ cell development that accompanies sequential events executed by numerous molecules. Therefore, characterization of these factors is one of the best strategies to clarify the mechanism of meiosis. Here, we report tripartite motif-containing 41 (TRIM41), a ubiquitin ligase E3, as an essential factor for proper meiotic progression and fertility in male mice. Trim41 knockout (KO) spermatocytes exhibited synaptonemal complex protein 3 (SYCP3) overloading, especially on the X chromosome. Furthermore, mutant mice lacking the RING domain of TRIM41, required for the ubiquitin ligase E3 activity, phenocopied Trim41 KO mice. We then examined the behavior of mutant TRIM41 (ΔRING-TRIM41) and found that ΔRING-TRIM41 accumulated on the chromosome axes with overloaded SYCP3. This result suggested that TRIM41 exerts its function on the chromosome axes. Our study revealed that Trim41 is essential for preventing SYCP3 overloading, suggesting a TRIM41-mediated mechanism for regulating chromosome axis protein dynamics during male meiotic progression.

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Center for Experimental Medicine and Systems Biology

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

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Genome engineering technologies such as clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) nucleases (CRISPR-Cas) have widely used in life science 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 improving this tool for translational research such as gene therapy and virus diagnostics. We are 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 process by CRISPR-Cas3

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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 single-stranded DNA (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 double-stranded DNA (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.

Application for the genetic engineering in mice and rats with CRISPR-Cas3

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Genome editing technologies are highly effective tools for genetic engineering in various organisms including experimental animals. Type I-E CRISPR-Cas3 uses an RNA-guided multi Cas-protein complex, Cas-

cade, which detects and degrades foreign nucleic acids via the helicase-nuclease Cas3 protein. However, it is unclear whether the system can be used for genome editing in fertilized eggs.

We applied the CRISPR-Cas3 system with several modification to generate genetically modified animals, and could generated knockout mice and rats in several genetic loci with optimizing method for the introduction into embryos. These results provides a molecular mechanism for collateral ssDNA cleavage and target dsDNA degradation by CRISPR-Cas3, and informing future genome editing tools in experimental animals.

CRISPR-Cas3-based diagnostics for virus detection and genetic screening

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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. In addition, we are also optimizing protocols for the cancer detection through liquid biopsy and genetic screening for hereditary diseases.

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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

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The 'Core Laboratory for Developing Advanced Animal Models' supports 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 make 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 of the Core

'Core Laboratory for Developing Advanced Animal models' was launched in 2020 to provide gene-manipulated mice or rats models to domestic or international academic institutions. Two divisions, the Division of Stem Cell Pathology and the Division of Genome engineering, and one laboratory, the Laboratory of Reproductive Systems Biology, all of which belong to the Center for Experimental Medicine and Systems Biology, comprise 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 zygotes 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 rats, e.g., reporter KI or humanized rat models, the direct zygote genome editing technique, termed Combi-CRISPR, is applicable.

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

We provide cutting-edge animal production techniques through our core lab and 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. Therefore, researchers earning KAKENHI, Grant-in-Aid for Scientific Research, can apply to this

platform.

Number of mice or rat strains we developed in 2022

In 2022, our core provided 9 or 11 strains of gene-manipulated mice through the core lab or AdAMS, respectively. In the rat case, 1 or 6 strains of gene-manipulated rats have also been provided through the core lab or AdAMS, respectively.

Advanced Clinical Research Center

Division of Infectious Disease
感染症分野

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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

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

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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 have been in total more than 700 COVID-19 patients admitted to our hospital so far. 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, Amato Ohtani[1], Kazuhiko Ikeuchi[1], Eisuke Adachi[1], Shinya Yamamoto, Takeya Tsutsumi, Aya Ishizaka, Taketoshi Mizutani[2], Ai Tachikawa-Kawana[3], Ken Ishii[4], Yoshihiro Kawaoka[5], Hiroshi Yotsuyanagi

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Several laboratories at IMSUT and external institutes have continued COVID-19-related research during 2022, 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 protec-

tive and pathogenic immune responses of the disease. Specifically, we are performing gut microbiome analysis as well as single-cell RNA sequencing (scRNA-seq) to obtain a bias-free and comprehensive imaging of immune responses in peripheral blood mononuclear cells (PBMCs) from patients with COVID-19. We also running analysis of change in the nasal microbiome after COVID-19 vaccination.

3. Analysis of genetic sequence of hepatitis viruses.

Ayako Sedohara, Kazuaki Takahashi, Kazuhiko Ikeuchi[1], Eisuke Adachi[1], Michiko Koga, Takeya Tsutsumi, 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, Aya Ishizaka, Taketoshi Mizutani[2],Kazuhiko Ikeuchi[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 as desired. We are also conducting research from the perspective of the microbiome.

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

Michiko Koga, Kazuhiko Ikeuchi[1], 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 (RS), 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 8.4% in 2021.

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

Michiko Koga, Takahiro Tanaka[1], Aya Ishizaka, Akari Fukuda, 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[1], 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 (H3K27 me3) 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, Kazuhiko Ikeuchi, Eisuke Adachi[1], Tetsuro Matano[3,6], Hiroshi Yotsuyanagi
[6] Department of AIDS Vaccine Development, IMSUT Hspital, 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. We reported the relevance between gut dysbiosis and chronic inflammation in people living with HIV infection (PLWH). Currently, we are running analysis of longitudinal change in the gut microbiome and its relationship with clinical condition.

9. 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 to support the revision of the malaria guidelines by the World Health Organization. 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.

10. Analysis of rifaximin effect on the small intestinal microbiome

Kazuhiko Ikeuchi[1], Takeya Tsutsumi, Aya Ishizaka, Taketoshi Mizutani[2], Ayako Sedohara, Michiko Koga, Satoru Tamaoki[7], Hiroshi Yotsuyanagi
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Rifaximin is a poorly absorbed broad-spectrum antibiotic used for hepatic encephalopathy, but rifaximin does not alter the stool microbiota. Because the antimicrobial effect of rifaximin increases by micellization with bile acids, we hypothesized that rifaximin alters the microbiota in the duodenum and jejunum, where bile acids are abundant. Using CCl_4-induced liver fibrosis mice, we showed that rifaximin decrease Lactobacillaceae in the duodenum and jejunum, which is known to increase in patients with hepatic encephalopathy. Rifaximin may exert its effect by altering the duodenal and jejunal microbiota. The changes in the duodenal and small intestinal microbiota were not associated with that of stool, suggesting that the analysis of stool microbiota is insufficient to evaluate upper intestinal microbiota.

11. Analysis of transmission route of HCV among HIV patients

Kazuhiko Ikeuchi[1], Kazuya Okushin[8], Makoto Saito, Eisuke Adachi[1], Michiko Koga, Takeya Tsutsumi Tomoyuki Takura[9], Hiroshi Yotsuyanagi
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Hepatitis C virus (HCV) has been mainly transmitted through injection drug use, but recently, sexual transmission among men who have sex with men (MSM), which is also a major route of HIV transmission, is increasing. However, the prevalence of HIV and the incidence of other sexually transmitted infections (STIs) among HCV patients have been rarely reported. Using a healthcare insurance claim data of employees and their dependents covering seven-million people in Japan, we evaluated HIV prevalence among HCV patients. HIV prevalence among young

male HCV patients was very high in Tokyo. HCV/HIV co-infected patients were more likely to acquire HIV before HCV, which is a known feature of MSM. They also had a higher incidence of STIs. These findings suggest that HCV might be prevalent as an STI among MSM particularly in Tokyo.

We also analyzed the long-term incidence of HCV among people living with HIV (PLWH) in the IMSUT hospital. In the multivariable analysis, MSM, IDU, and syphilis infection during study period were associated with HCV incidence, suggesting that HCV could be transmitted by both injection drug and sexual contact.

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Advanced Clinical Research Center

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

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Research Projects
The aim of our research is the application of findings in 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 human carcinogenesis, 2) discovery of Wnt inhibitors 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 human carcinogenesis

Kiyoshi Yamaguchi, Yoichi Furukawa

Aberrant Wnt/β-catenin signaling has been found in the various types of cancer, particularly colon and liver 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 gene expression and ChIP-seq analyses using colorectal cancer and hepatoma cells identified *MOSPD1* (motile sperm domain containing 1) and *ODAM* (odontogenic, ameloblast associated) that are downstream in the Wnt/β-catenin signaling pathway. We additionally searched for regulatory regions by the pathway and found potential Wnt-response elements (WREs) located far from each TSS. To ensure the involvement of these WREs in the transcriptional regulation of *MOSPD1* and *ODAM*, we conducted chromatin immunoprecipitation (ChIP) and chromatin conformation capture (3C) assays. As a result, ChIP assay showed an association of TCF7L2 with the WREs and 3C assay corroborated the interaction of the WREs with the promoter regions of each gene. Furthermore, CRISPR/Cas9-mediated disruption of these WREs significantly decreased the expression of *MOSPD1* and *ODAM* in cancer cells with augmented β-catenin/TCF transcriptional activity (or carrying mutant *β-catenin*). These data suggested that β-catenin/TCF up-regulates *MOSPD1* and *ODAM* through the binding to the WREs, and that these distal regions function as enhancers. Understanding of the biological role of MOSPD1 and ODAM will uncover a new role of the pathway involved in human carcinogenesis.

2. Discovery of Wnt inhibitors 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, several hit compounds that inhibit Wnt/β-catenin signaling activity have been identified, and their target(s) are currently under investigation. To identify protein(s) that interact with the compounds, we have pulled down proteins from cell lysates by the compound-immobilized beads and analyzed them using mass spectrometry.

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 previously established a novel mouse model of intrahepatic cholangiocarcinoma (ICC) using liver-specific expression of oncogenic *Kras* and homozygous *Pten* deletion. We also established another ICC mouse model carrying a cancer-associated mutant allele of *Fbxw7* in combination with an oncogenic *Kras* allele.

In addition, we developed liver-specific knockin mice carrying cancer associated hotspot mutations of *IDH1* or *IDH2*. By crossing these mice with *Kras* mutant mice, we have investigated roles of the *IDH1* and *IDH2* mutations in hepatocarcinogenesis. Although *IDH1* and *IDH2* mutations cooperate with oncogenic *Kras* mutation to promote ICC and hepatocellular carcinoma (HCC), the incidence of ICC and HCC varies with *IDH1* and *IDH2* mutations. We are currently investigating the cellular origin of ICC caused by *IDH1* and *IDH2* mutations by cell lineage tracing. 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 appendiceal PMPs 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.

We further performed genome-wide DNA methylome analysis of 15 appendiceal PMP samples using Infinium 850K BeadChip. As a result, we clarified that the 15 PMPs are classified into at least two epigenotypes, unique methylation epigenotype and normal-like methylation epigenotype. We also identified a set of hypermethylation marker genes in the 15 PMPs. These findings may help the understanding of the molecular mechanism(s) of PMP and contribute to the development of therapeutic strategies for this life-threatening disease.

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 two projects: 1) genetic diagnosis of patients with suspected hereditary cancer predisposition and 2) implementation of precision medicine for patients with rare or intractable cancer.

In the first project, we applied NGS technology for molecular diagnostics of hereditary colon cancer syndromes such as familial adenomatous polyposis (FAP), Lynch syndrome (LS), and polymerase proofreading-associated polyposis (PPAP). In addition to a short-read sequencing, we took advantage of MinION, a long-read sequencer of Oxford nanopore platform, for the detection of pathogenic structural variants (SVs) because not only single nucleotide variations (SNVs) and short insertions and deletions (indels), but also structural variations (SVs) are responsible for the predisposition of hereditary cancer. Using MinION, we have successfully identified the breakpoints of a pathogenic SVs that are difficult to identify by short-read sequencing technology.

In the second project, we have been 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, gastric, cervical cancer, and pheochromocytoma 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 IMSUT Tumor Board (ITB). ITB is composed of members of various specialties, with physicians, medical oncologists, genetic counsellors, geneticists, bioinformaticians, and experts of ethics, and is held online every two weeks.

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Advanced Clinical Research Center

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

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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 approved as the world's first oncolytic virus product for brain tumors in June 2021 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 not only destroys tumor cells by its lytic activity but also shows robust antitumor effect by eliciting systemic and specific antitumor immunity, and is expected as a promising 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 highly selective replication in tumor cells while maintaining safety in normal tissues, (2) a high stability of the viral genome, (3) a potent oncolytic activity in a wide range of cancer cells, (4) cell-to-cell spread of the virus minimally affected by antiviral antibodies, (5) presence of antiviral drugs that serve as fail safe, (6) a high capacity for incorporating large or multiple transgenes owing to its large genome size (<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, enables 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).

Since 2003, translational research of G47Δ was initiated totally by this laboratory, including invention, preclinical tests, clinical lot manufacturing and clinical trials. G47Δ was 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 a standard treatment option for cancer in the near future. The clinical trials of G47Δ for malig-

nant 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.

Publications

1. Jing Y, Kobayashi M, Vu H, Kasahara A, Chen X, Pham L, Kurayoshi K, Tadokoro Y, Ueno M, Todo T, Nakada M, Hirao A: Therapeutic advantage of targeting lysosomal membrane integrity supported by lysophagy in malignant glioma. Cancer Sci 113 (8):2716-2726, 2022
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Advanced Clinical Research Center

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

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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 the 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

Masanori Nojima, Fumitaka Nagamura

At IMSUT Hospital, we work together with the 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 the results of the collaboration between Division of Advanced Medicine Promotion and Center for Translational Research. In 2022, we supported 4 sponsor-investigator clinical trials and 3 non-IND clinical studies.

2. 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.

3. Statistical consulting for basic research

Masanori Nojima

For basic researchers, we suggest exploratory statistical approach and molecular epidemiological approach.

4. 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.

5. A review of criteria strictness in "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials"

Masanori Nojima, Motoki Amai, Fumitaka Nagamura

To assess safety in vaccine development, stricter grading scales, such as the "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" issued by the U.S. Food and Drug Administration (FDA grading scale), are required. However, concern exists that their strictness may lead to an overestimation of some adverse events (AEs). We analyzed the details of AEs in a phase I clinical trial of a preventive vaccine for infectious diseases. In this trial, we observed the high occurrence of Grade 1 or greater AEs in hemoglobin changes from baseline value, and hypernatremia, and hypokalemia by FDA grading scale. The range considered as non-AE according to the FDA grading scale shifted or became narrower when compared to reference intervals, especially for a Japanese cohort. Regarding a decrease in hemoglobin from baseline, the criterion of "any decrease" used for a Grade 1 AE was too strict and we suggest this be omitted. Upper and lower limits of AE criteria for sodium and potassium should be equal to, or 10–20% above, the reference interval consistent with other toxicities determined by laboratory tests. Consideration should be given to the issues surrounding the criteria that determine AEs before conducting clinical trials.

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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,] Yoku Hayakawa[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. This specific effect of IL33 on gastric tissue is also confirmed by 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,] Yoku Hayakawa[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. To elucidate the role of Sox9 in gastric diseases, we established stomach specific Sox9 knockout mice (TFF1-cre; $Sox9^{f/f}$ mice). We found these mice developed gastritis and gastric tumor in the antrum suggesting critical role of Sox9 in the homeostasis of stomach. We are further characterizing molecular mechanisms using SOX9 deficient organoid and RNAseq.

3. Role of acetylcholine signaling in the inflammatory bowel diseases

Aya Yamashita[1], Yoshihiro Hirata, Sozaburo Ihara[1], Yoku Hayakawa[1], Hayato Nakagawa[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. 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.

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

Hisayoshi Natomi, Hayato Nakagawa[3], Yoshihiro Hirata. [3]Department of Gastroenterology, Mie University

Primary sclerosing cholangitis is a rare form of biliary inflammation which can progress to cirrhosis and cancer. We are currently investigating 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.

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

Ryosuke Muroyama[4], Naoya Kato[4], Yoshihiro Hirata. [4]Department of Gastroenterology, Chiba University

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.

6. Analysis of primary biliary cholangitis mouse model

Jiaqi Zhang[4], Ryo Nakagawa[4], Naoya Kato[4], Hayato Nakagawa[3], Yoshihiro Hirata

Primary biliary cholangitis is a rare autoimmune cholestatic liver disease and its cause is not well understood. UDCA administration is the only established therapy, but many advanced cases does not respond to treatment, and eventually requires liver transplantation. We have generated transgenic mice which develop immune cell infiltration, bile duct destruction in the liver with elevated serum autoantibody, all of which are characteristics of human PBC. Using this mouse model, we now try to unveil molecular pathogenesis and establish novel treatment strategy.

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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. Public survey on industrial use of research data generated from provided biological samples

Ayako Kamisato, Kazuyo Arisawa

Registration of clinical research data in a database and utilization of the data, including industrial use, are currently promoted as a national policy. However, the research participants from whom the data is derived usually provide biological samples and information free of charge. Furthermore, research participants do not obtain any benefits even if the companies gain economic benefits from this data. Therefore, research participants may have a different awareness of the use of data from conventional non-commercial academic use. Research participants who provide samples and information are essential for promoting the industrial use of research data. Therefore, it is crucial to understand the general public's perceptions of who may become research participants in the future.

We conducted an Internet survey of the general public in Japan, which included 2,202 respondents with 18.2% response rate. The results showed the following: more than 50% of the respondents were reluctant to provide biological samples for industrial use; a certain percentage (12.4%-34.0%) of the respondents believed that samples and data were attributed to donors; more than 50% of the respondents thought companies should divide gained profits to the donors; 50.0% of the respondents believed the companies should return earnings to the donors by money, and 22.9%-37.0 thought it should return profits to society. From these results, we found that researchers must provide clear explanations to the research participants in the informed consent process. In addition, the government also needs to develop legislation, such as clarifying the legal status of samples and data.

2. Public survey on genomic medicine

Ayako Kamisato, Kazuyo Arisawa

Since genomic mutations are involved in many diseases' onset, aggravation, and side effects, utilizing genomic information will enable the provision of medical care optimized for each individual. In addition, we can create effective medicines if we can identify genetic mutations involved in the onset and aggravation of diseases for which there are currently no therapeutic drugs. Furthermore, with the development of genome/omics analysis research and diagnostic technology, it is expected that it will become possible not only to diagnose diseases that have already developed but also to predict and diagnose diseases at a high probability before the onset. In this way, utilizing genomic information contributes to the

prevention, early detection of diseases and improvement of patients' QOL. Therefore, in December 2019, the "Whole Genome Analysis Action Plan (1st edition)" was formulated by the Ministry of Health, Labor and Welfare (MHLW) to promote whole genome analysis in the fields of cancer and intractable diseases. Based on this plan, whole-genome analysis of patients has been carried out extensively.

However, on the other hand, germline genetic information is characterized as follows; it does not change throughout life, is passed on to offspring, and is shared with blood relatives. Therefore, if genetic information is handled inappropriately, patients and their relatives may suffer unfair discrimination and social disadvantages in various non-medical situations, such as insurance, employment, marriage, and education.

For these reasons, we conducted a public survey with the National Museum of Emerging Science and Innovation (Miraikan) on the utilization of genome information. From this survey, we found that 60% of the respondents were worried about "discrimination based on genomic information," 56% about "information leakage and misuse," and 40% about "impact on relatives." We reported this survey's results at the "Expert Committee on Promotion of Whole Genome Analysis" meeting established by the MHLW (7th meeting).

However, in this questionnaire survey, many respondents were visitors to the National Museum of Emerging Science and Innovation and patients who belong to a patient group and their families. Therefore, the results may be different for the general public. Consequently, we conducted a questionnaire survey targeting the general public to ascertain the level of awareness of genomes, concerns and interests in the use and application of genome information, and awareness of genome information database registration and secondary use. We are currently analyzing the survey results.

3. Ethical support for "The Center of Well-being Regional Society Innovation project."

Ayako Kamisato, Kazuyo Arisawa

"The Center of Well-being Regional Society Innovation project," promoted by Hirosaki University (Hirosaki COI-NEXT), is one of the projects of the JST Program on Open Innovation Platform for Industry-Academia Co-Creation. The goal of this project is to build and utilize a big data platform on medical and health. To achieve this goal, one of the wide-ranging efforts of Hirosaki COI-NEXT is the implementation of a large-scale health survey called the "Iwaki Health Checkup Project" (hereinafter referred to as "Iwaki Health Checkup"), targeting residents of the Iwaki district of Hirosaki City. In this checkup, Hirosaki University collaborates with companies and research institutes to collect 2,000 to 3,000 items of health and medical data per person. The aim is to quickly find preventive and therapeutic methods for dementia and lifestyle-related diseases by analyzing such big data from multiple angles. Our laboratory is in charge of ethical support for the Iwaki health checkup. In 2022, we received 47 consultations from researchers at universities and companies.

4. The REC Education program (RECs) for Research Ethics Committees (REC) members and researchers

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo

We have constructed the REC Education program for Research Ethics Committees (REC) members with support from the Japan Agency for Medical Research and Development (AMED) since FY 2016. So far, we have produced fifteen video programs and released them on our website. Two thousand two hundred fifty-two members and 778 institutions have registered with our program as of 31 May 2022. In 2022, we revised the video programs in accordance with revisions of the "Ethical Guidelines for Medical and Biological Research Involving Human Subjects" and the "Act on the Protection of Personal Information."

Also, we have constructed the REC Education program for researchers and produced five video programs with support from the AMED since FY 2019. Currently, we have 2,939 members registered with this program as of 31 May 2022.

5. Production of educational tool for researchers who use human Embryonic Stem Cells

Ayako Kamisato

Kamisato produced an educational video for researchers on "Guidelines on the Utilization of Human Embryonic Stem Cells." This video was adopted by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and is now posted on the ministry's website.

出版物

・神里彩子, 有澤和代. 「提供試料から生成された研究データの産業利用に対する一般市民の意識―アンケート調査結果からの考察―」臨床薬理　53(6) 235-242 (2022年11月)

・神里彩子「「「人を対象とする生命科学・医学研究に関する指針」におけるゲノム研究の取扱い―

ゲノム指針特有規定のその後」医事法研究第6号（甲斐克則責任編集、信山社、2022年12月）
・神里彩子「再生医療の倫理」日本医師会雑誌第151巻第4号（日本医師会、2022年7月）
・神里彩子「医学研究・先端医療技術に関する政府指針」甲斐克則・手嶋豊編『医事法判例百選　第3版』別冊ジュリストNo.258（有斐閣、2022年7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
Assistant Professor	Shigehiro Kojima, M.D.	助　教	博士(生命医科学)	小　島　成　浩

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 joined this new division. This year, on October 1th, Dr. Kojima also joined this 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

Prognostic differences between oligometastatic and polymetastatic disease after resection in patients with colorectal cancer and hepatic or lung metastases: Retrospective analysis of a large cohort at a single institution.

Horie T, Kanemitsu Y, Takamizawa Y, Moritani K, Tsukamoto S, Shida D.

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Ishibashi F, Shida D, Suzuki S, Nagai M, Mochida K, Morishita T.

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Optimal criteria for predicting lymph node metastasis in esophageal squamous cell carcinoma by anatomical location using preoperative computed tomography: a retrospective cohort study.

Shiomi S, Yajima S, Yoshimura S, Urabe M, Ri M, Okumura Y, Yagi K, Aikou S, Nomura S, Seto Y.

Surg Today. 2022 Aug;52(8):1185-1193.

Survival Prediction Capabilities of Preoperative Inflammatory and Nutritional Status in Esophageal Squamous Cell Carcinoma Patients.

Sugawara K, Yagi K, Okumura Y, Aikou S, Yamashita H, Seto Y.

World J Surg. 2022 Mar;46(3):639-647.

Lymphatic invasion is a prognostic factor of pathological N0 esophageal squamous cell carcinoma.

Toriumi T, Yagi K, Ri M, Yajima S, Okumura Y, Aikou S, Yamashita H, Nomura S, Seto Y.

Dis Esophagus. 2022 Jul 12;35(7):doab087. doi: 10.1093/dote/doab087.PMID: 34937084

A design of forceps-type coincidence radiation detector for intraoperative LN diagnosis: clinical impact estimated from LNs data of 20 esophageal cancer patients.

Takahashi M, Yoshimura S, Takyu S, Aikou S, Okumura Y, Yagi K, Fukayama M, Momose T, Seto Y, Yamaya T.

Ann Nucl Med. 2022 Mar;36(3):285-292.

The type of gastrectomy affects skeletal muscle loss and the long-term outcomes of elderly patients with gastric cancer: a retrospective study using computed tomography images.

Okamoto A, Aikou S, Iwata R, Oya S, Kawasaki K, Okumura Y, Yagi K, Yamashita H, Nomura S, Seto Y.

Surg Today. 2022 May;52(5):812-821.

Identifying multiple swollen lymph nodes on preoperative computed tomography is associated with poor prognosis along with pathological extensive nodal metastasis in locally advanced gastric cancer.

Ri M, Yamashita H, Gonoi W, Okumura Y, Yagi K, Aikou S, Seto Y.

Eur J Surg Oncol. 2022 Feb;48(2):377-382.

Advanced Clinical Research Center

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

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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 maintains myeloid leukemia stem cells through inhibition of beta-catenin degradation

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The identification of characteristic differences between cancer stem cells and their normal counterparts remains a key challenge for cancer treatment. Here, we investigated the role of immunoglobulin superfamily member 8 (Igsf8, also known as EWI-2, PGRL, and CD316) on normal and malignant hematopoietic stem cells, mainly using the conditional knockout model. Deletion of Igsf8 did not affect steady state hematopoiesis, but it led to a significant improvement of survival in mouse myeloid leukemia models. Deletion of Igsf8 significantly depletes leukemia stem cells (LSCs) through enhanced apoptosis and beta-catenin

degradation. At a molecular level, we found that activation of beta-catenin in LSCs depends on Igsf8, which promotes the association of FZD4 with its co-receptor LRP6 in the presence of Igsf8. Similarly, IGSF8 inhibition blocks the colony-forming ability of LSCs and improves the survival of recipients in xenograft models of myeloid leukemia. 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. An Immune Checkpoint Molecule DNAM-1/CD112 Axis Is a Novel Target of NK-Cell Therapy in Acute Myeloid Leukemia.

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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 T[1, 2], Kohara C[1], Watanabe E[3], Takahashi S[4], Ozawa G[4], Suzuki K[5], Mizukami M[5], Nagai E[5], Jimbo K[1], Kaito Y[1], Isobe M[1,2], Kato S[1,2], Takahashi S[1,2],Chiba A[6], Miyake S[6], Tojo A[1,7], Yasuhito Nannya[1,2]

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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 re-

constitution 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. Genetic deletion and pharmacologic inhibition of E3 ubiquitin ligase HOIP impairs the propagation of myeloid leukemia

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We investigated the role of Hoip, a catalytic subunit of linear ubiquitin chain assembly complex (LUBAC), in adult hematopoiesis and myeloid leukemia by using both conditional deletion of Hoip and small-molecule chemical inhibitors of Hoip. Conditional deletion of Hoip led to significantly longer survival and marked depletion of leukemia burden in murine myeloid leukemia models. Nevertheless, a competitive transplantation assay showed the reduction of donor-derived cells in the bone marrow of recipient mice was relatively mild after conditional deletion of Hoip. Although both Hoip-deficient hematopoietic stem cells (HSCs) and leukemia stem cells (LSCs) impaired the maintenance of quiescence, conditional deletion of Hoipinduced apoptosis in LSCs but not HSCs in vivo. Structure-function analysis revealed that LUBAC ligase activity and the interaction of LUBAC subunits were critical for the propagation of leukemia. Hoip regulated oxidative phosphorylation pathway independently of nuclear factor kappa B pathway in leukemia, but not in normal hematopoietic cells. Finally, the administration of thiolutin, which inhibits the catalytic activity of Hoip, improved the survival of recipients in murine myeloid leukemia and suppressed propagation in the patient-derived xenograft model of myeloid leukemia. Collectively, these data indicate that inhibition of LUBAC activity may be a valid therapeutic target for myeloid leukemia.

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Currently, organ transplantation is the only effective treatment for patients with end-stage organ failure. Unfortunately, the limited number of transplantable organs hinders the extensive application of this treatment. On the other hand, recent development of regenerative medicine that aims to generate transplantable organs on a dish has attracted much attention. Regenerative medicine is a challenging scientific field that attempts to convert knowledge from developmental biology and stem cell biology into clinical application. Our established novel organoid culture technologies reconstruct functional human organs derived from stem cells, including human induced pluripotent stem cells (hiPSCs), and finally aim to develop a substitute for organ transplantation therapy. Currently, we are trying to conduct the transplantation of human liver primordia (liver buds [LBs]) generated from hiPSCs to treat liver diseases, such as metabolic disorders and liver fibrosis. Moreover, we expand the application of our technologies to reconstruct artificial cancer tissue (cancer organoid) with a refractory 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

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Hepatocytes play crucial roles in maintaining homeostasis in living organisms by carrying out various metabolic functions, including ammonia detoxification. Urea cycle disorders commonly accompanied by hyperammonemia, are genetically inherited diseases after a single gene defect of the urea cycle enzymes or transporters. Ornithine transcarbamylase (OTC) is a rate-limiting enzyme in the urea cycle, and OTC deficiency (OTCD) is the most common urea cycle disorder in humans. Unlike other liver diseases, most urea cycle disorder patients show normal liver histology. Therefore, the transplantation of human induced pluripotent stem cell derived liver bud (hiPSC-LB) that possess OTC enzyme activity might serve as a promising regenerative medicine approach for OTCD treatment. To this end, we are currently accelerating the efficacy and safety verification of our hiPSC-LB

transplantation for OTCD treatment. As we need to establish a robust hiPSC-LB production method, we first improved the ECM coating of culture dishes to expand human iPSCs (*Biology Methods&Protocols*, 2022 bpac034). Next, the efficacy of hiPSC-LB transplantation was evaluated in a severe OTCD animal model with an immunocompromised background. After the renal capsule transplantation of hiPSC-LBs in immunodeficient OTCD mice, the hyperammonemia condition was significantly improved, suggesting the efficacy of iPSC-LBs transplantation for OTCD treatment. Simultaneously, we also optimize hiPSC-LBs transplantation end-to-end protocol for clinical trials on OTCD patients.

Since we consider the broad adaptation of hiPSCs-LB transplantation for various liver disease therapies, we need to upscale our current hiPSC-LB production. To guarantee mass production safety, we established a highly sensitive method for detecting undifferentiated cells after differentiation induction (*Stem Cell Rev Rep*. 2022 18(8):2995-3007). Concurrently with this, we also developed a liver surface transplantation method that could reduce the immune response after allogeneic transplantation (*Int J Mol Sci*, 22(21):11589, 2021, *Cells*, 10(2):476.). Overall, with our exceptionally unique technologies, our efforts to establish a new treatment for liver cirrhosis, one of the most common liver diseases worldwide, has advanced to the next level.

2. Liver repopulation with hiPSC derived proliferative progenitors

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Liver transplantation is a proven therapy for genetic liver disorders. Yet, its clinical application faces the persistent shortage of transplantable livers. Fortunately, hiPSCs derived hepatic lineages are considered as promising cellular sources substitute for transplantation therapy. Although various progresses have been made in the differentiation of hiPSCs into hepatic lineages, previously reported methods failed to achieve long-term survival and engraftment of functional liver tissue after in vivo transplantation. We previously developed a two-step protocol for hepatocyte differentiation and defined a culture condition for inducing proliferative hepatic progenitor cells (*Hepatology*, 2022). Based on these results, we further generated proliferative hepatoblast, bipotential liver progenitor cells, derived from hiPSCs. We confirmed that this proliferative hepatoblast exhibits differentiation capacity into hepatocytes and cholangiocytes under in vitro and in vivo condition. Moreover, under in vivo environment, engrafted hepatoblasts would differentiate to mature hepatocytes, form functional liver tissue, and survive for more than 40 weeks. Currently, we are focusing on developing novel therapeutic strategies for liver diseases based on hepatoblast transplantation.

3. Modeling liver diseases with hiPSC-derived organoid

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Liver diseases account for major global health care and financial burden. Therefore, there is an urgent need to develop preventive therapy for liver disease. Lacking in complex cellular properties, the current human cell-based liver ex vivo models still could not accurately recapitulate liver physiology and disease progression. Based on our thorough fetal liver development study, we successfully generated several lineages of liver progenitor cells from hiPSCs, including hepatocytes, kupffer cells, hepatic stellate cells, and sinusoidal endothelial cells. We then utilized three-dimensional (3D) culture technologies to fuse those aforementioned liver progenitor cell lineages and were able to generate novel functional liver organoids with complex cellular properties. Importantly, this new liver organoid with complex cellular properties could simulate critical pathological processes during liver disease progression upon stimulation, such as the inflammation-mediated liver injury and activation of hepatic stellate cells. In the future, this recapitulation model would allow a more precise understanding of the cell-to-cell interactions during liver disease progression. It would also give us a novel platform to explore potential targets for alleviating liver disease progression.

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

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To overcome the critical shortage of organ donors, the generation of hiPSC-derived liver organs with structures and functions is highly anticipated. However, the current generation method of hiPSC-organoid is still limited in size and therefore remains insufficient as an alternative for organ transplantation. Blood perfusion is a critical event for organ growth by supplying nutrients and oxygen. However, tissue blood perfusion is still lacking in the present organoid culture system. We are developing perfusion culture systems using two approaches; hiPSC-liver buds (LBs) associated with reconstructed blood vessel, and the decellularized liver tissue infused with hiPSC-LBs.

From our first approach, we generated the hiPSC-derived macrovessel using collagen gels, hiPSC-derived vascular smooth muscle cells (SMC), and vascular endothelial cells (EC). Although we clarified that hiPSC-derived macrovessel is histologically similar to the vascular structure of *in vivo* blood vessels, EC-seeded macrovessels did not show angiogenesis after coculturing with hiPSC-LBs. Therefore, we established a new induction method to differentiate hiPSC into specific EC lineages that exist around the fetal liver. We demonstrated that hiPSC-derived macrovessel containing those specific ECs had higher angiogenic potentials. Recently, under an optimized culture condition, we successfully connected the hiPSC-derived macrovessel with the microvessels within hiPSC-LBs. In order to establish an organoid perfusion system, we are trying to confirm the perfusion circulation into hiPSC-LBs and ultimately will conduct the perfusion culture of LBs.

As the second approach, we utilize decellularized liver tissue which retains *in vivo* vascular structures. The decellularization technique has been established to prepare the scaffold for organ reconstitution. Decellularized organs potentially retain the architecture of the original tissue, including the extracellular matrix. A recent report shows how the recellularized liver using hepatocytes could exert liver-specific functions after transplantation. However, the vascular structures within this recellularized liver remain unreconstructed, which might explain its reported limited hepatocyte functions. Our current study attempts to generate a more functional recellularized liver by adding oxygen supply into our perfusion culture system of the recellularized liver containing hiPSC-LBs.

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

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Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis, with a 5-year survival rate of about 10% due to delayed diagnosis, drug resistance, and recurrence. Organoid technologies have been applied to investigate the properties of PDACs; unfortunately, current organoid technology is still unable to recapitulate tumor microenvironment (TME), which is thought to be crucial for the poor prognosis of PDAC. Based on the organ bud technology developed in our regenerative medicine studies, we generated multicellular spheroids by coculturing primary PDAC cells isolated from Japanese pancreatic cancer patients with hiPSC-mesenchymal cells (MCs) and -endothelial cells (ECs). This technology then enabled us to generate a human primary pancreatic cancer organoid equipped with its appropriate TME. Our PDAC organoid recapitulates the tissue structure of clinical tissue compared to conventional organoids and, in particular, possesses various types of cancer-associated fibroblasts (CAFs). Moreover, the PDAC organoid showed strong resistance to anti-cancer drugs and re-proliferative capacity after drug treatment indicating its close resemblance to frequent relapse in PDAC patients. Thus, using this novel cancer organoid, we could investigate the mechanisms of recurrence more meticulously by understanding the characteristics of contained CAFs within PDAC organoids. We believe that applying this new cancer organoid in drug screening and biological analysis would contribute to the development of more effective therapies against PDAC.

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

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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 hiPSCs-derived liver tissue in collaboration with Japan Aerospace Exploration Agency (JAXA). In particular, we attempt to establish a new technique for generating three-dimensional organs containing large blood vessels. After we prepared hiPSC-LBs and artificial vessels on the earth, we placed those organoids into the culture container and launched to the International Space Station "KIBO". We confirmed that hiPSC-LBs were successfully assembled to the artificial vessel under microgravity, as how *in silico* simulation suggested. After culturing hiPSC-LBs for a predetermined period in the incubator installed in "KIBO", the sample were then transported back to the earth. Adherence and fusion of hiPSC-LBs to the artificial vessels were observed in the post-flight samples cultured in orbit. Moreover, endothelial cells started to extend their filopodia-like structure. Using qRT-PCR analysis of ground controls and post-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 the post-flight samples, indicating how the space environment could provide an optimal condition for tissue reconstruction. These findings will uncover the effects of gravity on cell growth and differentiation. We hope these space experiment results will contribute to the subsequent development and understanding of (1) The development of a new technique in human three-dimensional tissue preservation and transportation, which is crucial to the practical use of regenerative medicine products. (2) The establishment of a novel technique for generating human organs joined with large blood vessels. (3) The development of a new three-dimensional culture device simulating the microgravity environment on earth.

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

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The biliary system consisting of intrahepatic bile duct tubule (IHBD), extrahepatic bile ducts (EHBDs), and gallbladder, is a 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, the generation of tubular-shaped IHBD within hiPSC-liver organoids and the induction method of EHBD organoids from hiPSC cells have not been reported so far.

First, to generate liver organoid containing BD-like structures, we focus on an environmental cue for the formation of IHBDs in the fetus, specifically, the interaction between liver progenitors with the portal vein (PV). To recapitulate the fetal PV-BD tissue interaction, we developed a new co-culture system in which the hiPSC-liver progenitors are located in next to the hiPSC-blood vessel (BV). In this condition, hiPSC-liver progenitors differentiated into cholangiocytes and formed duct structures. Currently, we are examining their secretory functions *in vitro* and *in vivo* tissue reconstitution after organoid transplantation to immunodeficient mice.

Second, to generate EHBDs, we induced EHBD progenitor cells from the endoderm cells derived from hiPSCs and performed 3D culture to induce the formation of epithelial tissue structures. By refining the optimal culture condition, we try to establish tubular organoids that show secretory functions similar to IHBDs and EHBDs. Our final goal is to eventually connect these two tubular structures with hiPSC-derived hepatocytes on a dish to generate Hepatobiliary Tubular Organoids (HBTO) that possess a long-term hepatic function *in vitro* as well as *in vivo*.

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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. Epigenetic traits inscribed in chromatin accessibility in aged hematopoietic stem cells

Naoki Itokawa[1,2,9], Motohiko Oshima[2,9], Shuhei Koide[2], Naoya Takayama[3], Wakako Kuribayashi[1,2], Yaeko Nakajima-Takagi[2], Kazumasa Aoyama[1,2], Satoshi Yamazaki[4,5], Kiyoshi Yamaguchi[6], Yoichi Furukawa[6], Koji Eto[3,7] and Atsushi Iwama[1,2,8]

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Hematopoietic stem cells (HSCs) exhibit considerable cell-intrinsic changes with age. Here, we present an integrated analysis of transcriptome and chromatin accessibility of aged HSCs and downstream progenitors. Alterations in chromatin accessibility preferentially take place in HSCs with aging, which gradually resolve with differentiation. Differentially open accessible regions (open DARs) in aged HSCs are enriched for enhancers and show enrichment of binding motifs of the STAT, ATF, and CNC family transcription factors that are activated in response to

external stresses. Genes linked to open DARs show significantly higher levels of basal expression and their expression reaches significantly higher peaks after cytokine stimulation in aged HSCs than in young HSCs, suggesting that open DARs contribute to augmented transcriptional responses under stress conditions. However, a short-term stress challenge that mimics infection is not sufficient to induce persistent chromatin accessibility changes in young HSCs. These results indicate that the ongoing and/or history of exposure to external stresses may be epigenetically inscribed in HSCs to augment their responses to external stimuli.

2. Unraveling unique features of plasma cell clones in POEMS syndrome with single cell analysis

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POEMS syndrome is a rare monoclonal plasma cell disorder, with unique symptoms distinct from those of other plasma cell neoplasms, including high serum VEGF levels. Because the prospective isolation of POEMS clones has not yet been successful, their real nature remains unclear. Herein, we performed single-cell RNA-Seq of BM plasma cells from patients with POEMS syndrome and identified POEMS clones that had Ig λ light chain (IGL) sequences (IGLV1-36, -40, -44, and -47) with amino acid changes specific to POEMS syndrome. The proportions of POEMS clones in plasma cells were markedly smaller than in patients with multiple myeloma (MM) and patients with monoclonal gammopathy of undetermined significance (MGUS). Single-cell transcriptomes revealed that POEMS clones were $CD19^{+}$, $CD138^{+}$, and MHC class II^{lo}, which allowed for their prospective isolation. POEMS clones expressed significantly lower levels of c-MYC and CCND1 than MM clones, accounting for their small size. VEGF mRNA was not upregulated in POEMS clones, directly indicating that VEGF is not produced by POEMS clones. These results reveal unique features of POEMS clones and enhance our understanding of the pathogenesis of POEMS syndrome.

3. 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], Bahityar 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]

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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 dys-

plastic 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-deficient 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.

4. YAP1/TAZ activity maintains vascular integrity and organismal survival

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Radiation therapy is one of the major treatment modalities for patients with cancers. However, ionizing radiation (IR) damages not only cancer cells but also the surrounding vascular endothelial cells (ECs). Hippo pathway effector genes Yap1 and Taz are the two transcriptional coactivators that have crucial roles in tissue homeostasis and vascular integrity in various organs. However, their function in adult ECs at the steady state and after IR is poorly understood. Here, we report sex- and context-dependent roles of endothelial YAP1/TAZ in maintaining vascular integrity and organismal survival. EC-specific Yap1/Taz deletion compromised systemic vascular integrity, resulting in lethal circulation failure preferentially in male mice. Furthermore, EC-specific Yap1/Taz deletion induced acute lethality upon sublethal IR that was closely associated with exacerbated systemic vascular dysfunction and circulation failure. Consistent with these findings, RNA-seq analysis revealed downregulation of tight junction genes in Yap1/Taz deleted ECs. Collectively, our findings highlight the importance of endothelial YAP1/TAZ for maintaining adult vascular function, which may provide clinical implications for preventing organ injury after radiation therapy.

5. A high prevalence of myeloid malignancies in progeria with Werner syndrome is associated with p53 insufficiency

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Werner syndrome (WS) is a progeroid syndrome caused by mutations in the WRN gene, which encodes the RecQ type DNA helicase for the unwinding of unusual DNA structures and is implicated in DNA replication, DNA repair, and telomere maintenance. patients with WS are prone to develop malignant neoplasms, including hematological malignancies. However, the pathogenesis of WS-associated hematological malignancies remains uncharacterized. Here we investigated the somatic gene mutations in WS-associated myelodysplastic syndrome/acute myeloid leukemia (MDS/AML). Whole-exome sequencing (WES) of 4 patients with WS with MDS/AML revealed that all patients had somatic mutations in TP53 but no other recurrent mutations in MDS/AML. TP53 mutations were identified at low allele frequencies at more than one year before the MDS/AML stage. All 4 patients had complex chromosomal abnormalities including those that involved TP53. Targeted sequencing of nine patients with WS without apparent blood abnormalities did not detect recurrent mutations in MDS/AML except for a PPM1D mutation. These results suggest that patients with WS are apt to acquire TP53 mutations and/or chromosomal abnormalities involving TP53, rather than other MDS/AML-related mutations. TP53 mutations are frequently associated with prior exposure to chemotherapy; however, all four patients with WS with TP53 mutations/deletions had not received any prior chemotherapy, suggesting a pathogenic link between WRN mutations and p53 insufficiency. These results indicate that WS hematopoietic stem cells with WRN insufficiency acquire competitive fitness by inactivating p53, which may cause complex chromosomal abnormalities and the subsequent development of myeloid malignancies. These findings promote our understanding of the pathogenesis of myeloid malignancies associated with progeria.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Transplantation
幹細胞移植分野

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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. Higher Cryopreserved CD34(+) Cell Dose Is Associated with Decreased Hepatic Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome after Single-Unit Cord Blood Transplantation in Adults Given Prophylactic Ursodeoxycholic Acid and Intravenous Heparin

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Hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), is one of the most serious complications to occur early after allogeneic hematopoietic cell transplantation (HCT). However, detailed data on VOD/SOS after cord blood transplantation (CBT) are not available. The objective of this retrospective study was to evaluate the incidence, risk factors, and clinical impact of VOD/SOS after single-unit unrelated CBT for adult patients at our institution. We retrospectively evaluated the incidence, risk factors, and outcomes of VOD/SOS after 390 single-unit unrelated CBTs performed in 332 adults under a prophylactic strategy of ursodeoxycholic acid (UDCA) and i.v. heparin at our institution between 1998 and 2021. VOD/SOS was observed in 24 of the 390 CBTs. The cumulative incidence of VOD/SOS was 5.9% at 30 days and 6.2% at 100 days after CBT. Multivariate analysis showed that cryopreserved CD34(+) cell dose >/=1.0 x 10(5)/kg was significantly associated with a decreased risk of VOD/SOS after CBT (hazard ratio [HR], 0.33; 95% confidence interval [CI], 0.12 to 0.91; P = .032). In multivariate analysis, the development of VOD/SOS was significantly associated with higher overall mortality (HR, 6.19; 95% CI, 3.61 to 10.65; P < .001), treatment failure (HR, 4.79; 95% CI, 2.95 to 7.76; P < .001), and nonrelapse mortality (HR, 12.60; 95% CI, 6.90 to 23.00; P < .001). Our study shows that the incidence of hepatic VOD/SOS was relatively low after unrelated single-unit CBT under a prophylactic strategy of UDCA and i.v. heparin. A higher cryopreserved cord blood CD34(+) cell dose was associated with a reduction in VOD/SOS, suggesting that selection of a higher cord blood unit CD34(+) cell dose could be efficient in preventing hepatic VOD/SOS in adults undergoing single-unit CBT.

2. Optimal time and threshold of absolute lymphocyte count recovery as a prognostic factor after single-unit cord blood transplantation in adults.

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We retrospectively evaluated the optimal time and threshold of absolute lymphocyte count (ALC) recovery as a prognostic factor in 174 adult patients who received single-unit cord blood transplantation (CBT) at our institute. We analyzed the impact of ALC >/=300, >/=600, and >/=900/mul by 30 and 60 days on transplant outcomes. Multivariate analysis showed that only ALC >/=300/mul at 60 days was significantly associated with overall mortality (hazard ratio, 0.24; p = 0.001) following CBT. The optimal time point to use ALC recovery as a prognostic tool following CBT could be later than those following adult donor transplantation.

3. Total body irradiation-based versus busulfan-based myeloablative conditioning for single-unit cord blood transplantation in adults.

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Comparative studies between total body irradiation (TBI)-based and busulfan-based myeloablative conditioning (MAC) regimens for cord blood transplantation (CBT) have been limited. We retrospectively analyzed the results of single-unit CBT in 333 adult patients who received either TBI-based (n = 258) or busulfan-based (n = 75) MAC regimens at our institute. After adjusting for significant variables in the univariate analysis, there were no significant differences in neutrophil recovery (hazard ratio (HR), 0.88; p = .460), grade III-IV acute graft-versus-host disease (GVHD) (HR: 1.40, p = .410), extensive chronic GVHD (HR: 0.73, p = .380), relapse (HR: 0.61, p = .270), non-relapse mortality (HR: 1.38, p = .420), overall survival (HR: 1.18, p = .637), or event-free survival (HR: 1.08, p = .773), although platelet recovery was lower with marginal significance for the busulfan-based regimen (HR: 0.67, p = .068). In subgroup analysis, TBI-based regimens were superior to busulfan-based regimens in terms of survival for acute lymphoblastic leukemia, but not for myeloid malignancies. Further investigation is warranted even for CBT.

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Division of Stem Cell Processing
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Stem cells represent a valuable cell source in the field of regenerative medicine. Hematopoietic stem cells represent a valuable cell source for transplantation medicine, whereas pluripotent stem cells are newly emerging types of stem cells that have been utilized either for basic research or to develop a curative treatment for various diseases. We have been focusing especially on the utilization of induced pluripotent stem cells as a research platform to elucidate the pathophysiology of intractable diseases based on their proper modeling. Our goal is to establish safe and efficacious treatment for patients suffering from various types of incurable diseases.

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

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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, but how the oncogenic RAS mutations impact non-transformed hematopoietic progenitor cells (HPCs) remains uncertain. We previously generated KRAS mutant ($KRAS^{G13C/WT}$) and wild-type isogenic ($KRAS^{WT/WT}$) human induced pluripotent stem cells (hiPSCs) from the same RALD patients. Compared with $KRAS^{WT/WT}$ hiPSC-derived hematopoietic progenitor cells (hiPSC-HPC), we found that $KRAS^{G13C/WT}$ hiPSC-HPC exhibited obvious aberrant cell-cycle and apoptosis responses, compatible with "dysregulated expansion," demonstrated by molecular and biological assessment. With screening platforms established for therapeutic intervention, selective activity against $KRAS^{G13C/WT}$ hiPSC-HPC expansion in several candidate compounds, most notably in a MEK- and a BCL-2/BCL-xL inhibitor. The combination of these two compounds could selectively inhibit the growth of primary $KRAS^{G13C/WT}$ HPC. Moreover, we used genome-editing technologies to build a screening platform for other KRAS or NRAS mutation types in RALD. Meanwhile, we developed a feeder-free protocol to differentiate hiPSC-HPC. The purity of generated hiPSC-HPC was as high as 90%, and the cell number was ten times that of the previous protocol. Now, we are trying to generate hiPSC-HPC with KRAS or NRAS mutation and establish a high-throughput screening platform for developing ideal treatment strategies for RALD.

Center for Stem Cell Biology and Regenerative Medicine

Division of Experimental Pathology
幹細胞病理学分野

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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. The oncogene-dependent resistance to reprogramming unveils cancer therapeutic targets

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The resistance to transcription factor-mediated reprogramming into pluripotent stem cells is one of the distinctive features of cancer cells. Here we dissect the profiles of reprogramming factor binding and the subsequent transcriptional response in cancer cells to reveal its underlying mechanisms. Using clear cell sarcomas (CCSs), we show that the driver oncogene EWS/ATF1 misdirects the reprogramming factors to cancer-specific enhancers and thereby impairs the transcriptional response toward pluripotency that is otherwise provoked. Sensitization to the reprogramming cue is observed in other cancer types when the corresponding oncogenic signals are pharmacologically inhibited. Exploiting this oncogene dependence of the transcriptional "stiffness," we identify mTOR signaling pathways downstream of EWS/ATF1 and discover that inhibiting mTOR activity substantially attenuates the propagation of CCS cells in vitro and in vivo. Our results demonstrate that the early transcriptional response to cell fate perturbations can be a faithful readout to identify effective therapeutics targets in cancer cells.

2. MYCL-mediated reprogramming expands pancreatic insulin-producing cells

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β cells have a limited capacity for regeneration, which predisposes towards diabetes. Here, we show that, of the MYC family members, *Mycl* plays a key role in proliferation of pancreatic endocrine cells. Genetic ablation of *Mycl* causes a reduction in the proliferation of pancreatic endocrine cells in neonatal mice. By contrast, the expression of *Mycl* in adult mice stimulates the proliferation of β and α cells, and the cells persist after withdrawal of *Mycl* expression. A subset of the expanded α cells give rise to insulin-producing cells after this withdrawal. Transient *Mycl* expression in vivo is sufficient to normalize the hyperglycaemia of diabetic mice. In vitro expression of *Mycl* similarly provokes active replication in islet cells, even in those from aged mice. Finally, we show that *MYCL* stimulates the division of human adult cadaveric islet cells. Our results demonstrate that the induction of *Mycl* alone expands the functional β-cell population, which may provide a regenerative strategy for β cells.

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Center for Stem Cell Biology and Regenerative Medicine

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幹細胞生物学分野

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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. Blocking PD-L1-PD-1 improves senescence surveillance and ageing phenotypes.

Teh-Wei Wang [1], Yoshikazu Johmura [2 3], Narumi Suzuki [1], Satotaka Omori [1], Toshiro Migita [1], Kiyoshi Yamaguchi [4], Seira Hatakeyama [4], Satoshi Yamazaki [5 6], Eigo Shimizu [7], Seiya Imoto [7], Yoichi Furukawa [4], Akihiko Yoshimura [8], Makoto Nakanishi [9]

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The accumulation of senescent cells is a major cause of age-related inflammation and predisposes to a variety of age-related diseases[1]. However, little is known about the molecular basis underlying this accumulation and its potential as a target to ameliorate the ageing process. Here we show that senescent cells heterogeneously express the immune checkpoint protein programmed death-ligand 1 (PD-L1) and that PD-L1$^+$ senescent cells accumulate with age in vivo. PD-L1$^-$ cells are sensitive to T cell surveillance, whereas PD-L1$^+$ cells are resistant, even in the presence of senescence-associated secretory phenotypes (SASP). Single-cell analysis of p16$^+$ cells in vivo revealed that PD-L1 expression correlated with higher levels of SASP. Consistent with this, administration of programmed cell death protein 1 (PD-1) antibody to naturally ageing mice or a mouse model with normal livers or induced nonalcoholic steatohepatitis reduces the total number of p16$^+$ cells in vivo as well as the PD-L1$^+$ population in an activated CD8$^+$ T cell-dependent manner, ameliorating various ageing-related phenotypes. These results suggest that the heterogeneous expression of PD-L1 has an important role in the accumulation of senescent cells and inflammation associated with ageing, and the elimination of PD-L1$^+$ senescent cells by immune checkpoint blockade may be a promising strategy for anti-ageing therapy.

2. Hematopoietic progenitor cells specifically induce a unique immune response in dental pulp under conditions of systemic inflammation Heliyon.

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Teeth are exposed to various stimuli, including bacterial, thermal, and physical stimuli. Therefore, immune cells present in the normal dental pulp and the immune response to these stimuli have been studied. However, the relationship between systemic inflammation, such as that induced by viral infection, and changes occurring in dental pulp is not well known. This study aimed to investigate the immunological and hematological responses to systemic inflammation in dental pulp. Poly(I:C), a toll-like receptor 3 agonist, was injected into mice every two days to simulate a systemic inflammatory state in which type I interferon (IFN-I) was produced. The untreated normal state was defined as a steady state, and the states of acute and chronic inflammation were defined according to the period of administration. Changes in the abundance and dynamics of hematopoietic and immune cells in dental pulp, bone marrow and peripheral blood were quantitatively investigated in the steady state and under conditions of inflammation induced by IFN-l. We found that dental pulp in the steady state contained only a few hematopoietic cells, but a greater variety of immune cells than previously reported. B cells were also found in the steady state. An increase in multipotent progenitor cell levels was observed in the dental pulp during both acute and chronic inflammation. The increased multipotent progenitor cells in the dental pulp during acute inflammation tended to differentiate into the myeloid lineage. On the other hand, there was an influx of B cells into the dental pulp during chronic inflammation. These results revealed that a unique immune response is induced in the dental pulp by systemic inflammation, which would lead to a significant change in the perspective of dentists on the utility of dental pulp in the management of systemic diseases.

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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. Generation of *Tfap2c-T2A-tdTomato* knock-in reporter rats via adeno-associated virus-mediated efficient gene targeting

Mami Oikawa[1,2], Mayuko Nagae[2,3], Naoaki Mizuno[4], Kenyu Iwatsuki[1,2,5], Fumika Yoshida[2], Naoko Inoue[3], Yoshihisa Uenoyama[3], Hiroko Tsukamura[3], Hiromitsu Nakauchi[4,6], Masumi Hirabayashi[2,7], Toshihiro Kobayashi[1,2]

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Gene editing in mammalian zygotes enables us to generate genetically modified animals rapidly and efficiently. In this project, we compare multiple gene targeting strategies in rat zygotes by generating a novel knock-in reporter rat line to visualize the expression pattern of transcription factor AP-2 gamma (*Tfap2c*). The targeting vector is designed to replace the stop codon of *Tfap2c* with *T2A-tdTomato* sequence. We show that the combination of electroporation-mediated transduction of CRISPR/Cas9 components with adeno-associated virus-mediated transduction of the targeting vector is the most efficient in generating the targeted rat line. The Tfap2c-T2A-tdTomato fluorescence reflects the endogenous expression pattern of Tfap2c in preimplantation embryo, germline, placenta, and forebrain during rat embryo development. The reporter line generated here will be a reliable resource for identifying and purifying *Tfap2c* expressing cells in rats, and the gene targeting strategy we used can be widely applied for generating desired animals.

2. Induction of functional primordial germ cell-like cells from rat pluripotent stem cells

Mami Oikawa, Hisato Kobayashi[7], Makoto Sanbo,

Naoaki Mizuno, Kenyu Iwatsuki, Tomoya Takashima[7,8], Keiko Yamauchi[2], Fumika Yoshida[2], Takuya Yamamoto[9,10,11], Takashi Shinohara[12], Hiromitsu Nakauchi, Kazuki Kurimoto[7], Masumi Hirabayashi, Toshihiro Kobayashi

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The *in vitro* generation of germ cells from pluripotent stem cells (PSCs) can have a substantial effect on future reproductive medicine and animal breeding. A decade ago, in vitro gametogenesis was established in the mouse. However, induction of primordial germ cell-like cells (PGCLCs) to produce gametes has not been achieved in any other species. In this project, we demonstrate the induction of functional PGCLCs from rat PSCs. We show that epiblast-like cells in floating aggregates form rat PGCLCs. The gonadal somatic cells support maturation and epigenetic reprogramming of the PGCLCs. When rat PGCLCs are transplanted into the seminiferous tubules of germline-less rats, functional spermatids-that is, those capable of siring viable offspring-are generated. Insights from our rat model will elucidate conserved and divergent mechanisms essential for the broad applicability of *in vitro* gametogenesis.

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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. DNA damage that causes hair graying in mice

Mohri Y, Nishimura EK, Hayano M[1,2], Yang JH[1], Oberdoerffer P[1], Sinclair DA[1].
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All living things experience an increase in entropy, manifested as a loss of genetic and epigenetic information. In yeast, epigenetic information is lost over time due to the relocalization of chromatin-modifying proteins to DNA breaks, causing cells to lose their identity, a hallmark of yeast aging. Using a DNA damage inducing model named "ICE", we found that the induction of DNA double strand breaks (DSBs) with faithful DNA repair advances the expression of aging phenotypes with epigenetic changes. To study the fate and dynamics of DNA-damaged stem cells in tissues and the resultant impact in the expression of aging phenotypes, we studied the fate of melanocyte stem cells which acquired DNA double-strand breaks (DSBs) and demonstrated that those cells disappear from the niche, causing the loss of mature melanocytes for hair pigmentation. This is consistent with our previous report in which we demonstrated that genotoxic stress abrogates renewal of melanocyte stem cells by triggering their ectopic differentiation (Inomata K et al. Cell, 2009). The aberrant differentiation and the resultant loss of the cell lineage in tissues may partially explain the age-associated loss of lineage-specific epigenetic information in ICE mice. We are currently testing whether the selective induction of DNA double strand breaks in melanocyte stem cells similarly causes hair graying.

2. Fate tracing of DNA-damaged hair follicle stem cells and their seno-differentiation clearance out of the niche

Miranda-Salmeron M, Matsumura H, Muroyama Y, Kato T, Higa M, Tan L, Kawamura Y, Nanba D, Mohri Y, and Nishimura EK.

Hair follicles, mammalian mini-organs that grow hair, miniaturize during aging, leading to hair thinning and loss. In the event of severe genotoxicity such as DNA double-strand breaks (DSBs), stem cells are largely believed to choose between cell death (apoptosis) or irreversible cell cycle arrest (senescence) to prevent further damage to neighboring healthy cells and tissues. Accumulation of these senescent cells across organs has been implicated in disease and aging-related morbidities such as cancer. However, the exact fate and dynamics of sublethally damaged cells in tissues during aging/chemotherapy - and the de-

velopment of alopecia - and where exactly senescent cells exist in tissues are still largely unknown because of the lack of any single perfect marker of senescent cells. Previous work from our group demonstrated that various stem cells in the skin will aberrantly commit to differentiation in response to DNA damage by abrogating their self-renewal capabilities to discard unfit/stressed/aged stem cells. We are testing the unique hypothesis that the tissue youth is achieved through rapid, dynamic clearance of DNA-damaged cells out of the epithelia as a robust genomic quality control mechanism. We are evaluating a combination of recently devised mouse lines that can induce DSBs in a small number of stem cells to visualize and trace the exact fate, senescent state, and dynamics of those individual cells in epithelial tissue such as the hair follicle. Upon hair follicle stem cell (HFSC) activation, DNA-damaged cells were observed at the epidermal level, hinting to their transdermal exit out of the niche. Remarkably, while DNA damaged HFSCs exhibited gH2AX foci, SAβ-gal was not detected in such cells. We are in the process of characterizing the identity of those DNA-damaged HFSCs and their fate switching in the HFSC niche that leads to hair follicle miniaturization and hair loss. Taken together, our findings demonstrate a tissue-autonomous mechanism within the hair follicle niche that can effectively discard DNA-damaged cells.

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. Gene expression profiling of EpiSCs with DSBs reveals that DNA damage response (DDR)-p53-Notch/p21 axis is specifically induced in EpiSCs with DSBs. Stem cell fate analysis showed that 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. Early differential diagnosis of 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. We are currently increasing the number of cases to further evaluate the accuracy and efficiency of method.

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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) and several companies, 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.

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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 (SORPAria, Aria, AriaIII) from BD Biosciences. For cell analysis, the FACS Core Laboratory is equipped with four benchtop analyzers (Verse, Calibur, CantoII from BD Biosciences and CytoFLEX LX from Beckman Coulter).

FCM usage performance in 2022

FCM analysis and sorting is performed either by the FACS Core staff or by trained users. There were about 3,000 cases of FCM use in 2022.

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

Professor	Kei Sato, Ph.D.	教　授	博士(医学)	佐　藤　　佳
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Visiting Professor	Seiya Yamayoshi, D.V.M. Ph.D.	客員教授	博士(医学)	山　吉　誠　也
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The aim of this laboratory is to launch an interdisciplinary research platform to comprehensively understand the behavior of viruses from macroscale to microscale. COVID pandemic alarmed the importance of understanding viral transmissibility and spreading pathway. These knowledges are brought from epidemiology and public health (science at macroscale). Viral surveillance, molecular phylogenetic and bioinformatics provide information of the variant currently spreading (science at macroscale). "Science at mesoscale", the use of animal models and cell cultures, performing experiments, and assessing clinical data, provide the knowledge of viral pathogenicity, features and drug efficacy. When certain variants that are resistant to antivirals or vaccines emerged, the molecular mechanisms of actions should be understood. For that, the understanding based on structural biology is essential (science at micro scale). Our study will launch the platform to perform multiscale investigation of viruses.

A broadly protective human monoclonal antibody targeting the sialidase activity of influenza A and B virus neuraminidases

Atsuhiro Yasuhara, Seiya Yamayoshi, Maki Kiso, Yuko Sakai-Tagawa, Moe Okuda, Yoshihiro Kawaoka

Improved vaccines and antiviral agents that provide better, broader protection against seasonal and emerging influenza viruses are needed. The viral surface glycoprotein hemagglutinin (HA) is a primary target for the development of universal influenza vaccines and therapeutic antibodies. The other major surface antigen, neuraminidase (NA), has been less well studied as a potential target and fewer broadly reactive anti-NA antibodies have been identified. In this study, we isolate three human monoclonal antibodies that recognize NA from A/H1N1 subtypes, and find that one of them, clone DA03E17, binds to the NA of A/H3N2, A/H5N1, A/H7N9, B/Ancestral-lineage, B/Yamagata-lineage, and B/Victoria-lineage viruses. DA03E17 inhibits the neuraminidase activity by direct binding to the enzyme active site, and provides in vitro and in vivo protection against infection with several types of influenza virus. This clone could, therefore, be useful as a broadly protective therapeutic agent. Moreover, the neutralizing epitope of DA03E17 could be useful in the development of an NA-based universal influenza vaccine.

Age-Stratified Seroprevalence of SARS-CoV-2 Antibodies before and during the Vaccination Era, Japan, February 2020–March 2022

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Kato, Hideaki Nakajima, Norio Sugaya, Yuki Seki, Asef Iqbal, Isamu Kamimaki, Masahiko Yamazaki, Yoshihiro Kawaoka, Yuki Furuse

Japan has reported a relatively small number of COVID-19 cases. Because not all infected persons receive diagnostic tests for COVID-19, the reported number must be lower than the actual number of infections. We assessed SARS-CoV-2 seroprevalence by analyzing >60,000 samples collected in Japan (Tokyo Metropolitan Area and Hokkaido Prefecture) during February 2020–March 2022. The results showed that ≈3.8% of the population had become seropositive by January 2021. The seroprevalence increased with the administration of vaccinations; however, among the elderly, seroprevalence was not as high as the vaccination rate. Among children, who were not eligible for vaccination, infection was spread during the epidemic waves caused by the SARS-CoV-2 Delta and Omicron variants. Nevertheless, seroprevalence for unvaccinated children <5 years of age was as low as 10% as of March 2022. Our study underscores the low incidence of SARS-CoV-2 infection in Japan and the effects of vaccination on immunity at the population level.

Attenuated fusogenicity and pathogenicity of SARS-CoV-2 Omicron variant

Rigel Suzuki, Daichi Yamasoba, Izumi Kimura, Lei Wang, Mai Kishimoto, Jumpei Ito, Yuhei Morioka, Naganori Nao, Hesham Nasser, Keiya Uriu, Yusuke Kosugi, Masumi Tsuda, Yasuko Orba, Michihito Sasaki, Ryo Shimizu, Ryoko Kawabata, Kumiko Yoshimatsu, Hiroyuki Asakura, Mami Nagashima, Kenji Sadamasu, Kazuhisa Yoshimura, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Hirofumi Sawa, Terumasa Ikeda, Takashi Irie, Keita Matsuno, Shinya Tanaka, Takasuke Fukuhara, Kei Sato.

The emergence of the Omicron variant of SARS-CoV-2 is an urgent global health concern1. In this study, our statistical modelling suggests that Omicron has spread more rapidly than the Delta variant in several countries including South Africa. Cell culture experiments showed Omicron to be less fusogenic than Delta and than an ancestral strain of SARS-CoV-2. Although the spike (S) protein of Delta is efficiently cleaved into two subunits, which facilitates cell-cell fusion2,3, the Omicron S protein was less efficiently cleaved compared to the S proteins of Delta and ancestral SARS-CoV-2. Furthermore, in a hamster model, Omicron showed decreased lung infectivity and was less pathogenic compared to Delta and ancestral SARS-CoV-2. Our multiscale investigations reveal the virological characteristics of Omicron, including rapid growth in the human population, lower fusogenicity and attenuated pathogenicity.

High body temperature increases gut microbiota-dependent host resistance to influenza A virus and SARS-CoV-2 infection.

Nagai M, Moriyama M, Ishii C, Mori H, Watanabe H, Nitta Y, Arimitsu N, Nishimoto M, Nakahara T, Yamada T, Ishikawa D, Ishikawa T, Hirayama A, Kimura I, Nagahara A, Naito T, Fukuda S, and Ichinohe T.

While a common symptom of influenza and coronavirus disease 2019 (COVID-19) is fever, its physiological role on host resistance to viral infection remains less clear. Here, we demonstrate that exposure of mice to the high ambient temperature of 36 °C increase host resistance to viral pathogens including influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). High heat-exposed mice increase basal body temperature over 38 °C to enable more bile acids production in a gut microbiota-dependent manner. The gut microbiota-derived deoxycholic acid (DCA) and its plasma membrane-bound receptor Takeda G-protein-coupled receptor 5 (TGR5) signaling increase host resistance to influenza virus infection by suppressing virus replication and neutrophil-dependent tissue damage. Furthermore, the DCA and its nuclear farnesoid X receptor (FXR) agonist protect Syrian hamster from lethal SARS-CoV-2 infection. Moreover, we demonstrate that certain bile acids are reduced in the plasma of COVID-19 patients who developed moderate I/II disease compared with minor illness group. These findings uncover an unexpected mechanism by which virus-induced high fever increases host resistance to influenza virus and SARS-CoV-2 in a gut microbiota-dependent manner.

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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. Role of the arginine cluster in the disordered domain of Herpes Simplex Virus 1 UL34 for the recruitment of ESCRT-III for viral primary envelopment

Jun Arii[1], Kosuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Yoshitaka Nakayama, Akihisa Kato, Yasuko Mori[1], Yasushi Kawaguchi: [1]Division of Clinical Virology, Center for Infectious Diseases, Kobe University Graduate School of Medicine

During the nuclear export of nascent nucleocapsids of herpesviruses, the nucleocapsids bud through the inner nuclear membrane (INM) by acquiring the INM as a primary envelope (primary envelopment). We recently reported that herpes simplex virus 1 (HSV-1) nuclear egress complex (NEC), which consists of UL34 and UL31, interacts with an endosomal sorting complex required for transport III (ESCRT-III) adaptor ALIX and recruits ESCRT-III machinery to the INM for efficient primary envelopment. In this study, we identified a cluster of six arginine residues in the disordered domain of UL34 as a minimal region required for the interaction with ALIX, as well as the recruitment of ALIX and an ESCRT-III protein CHMP4B to the INM in HSV-1-infected cells. Mutations in the arginine cluster exhibited phenotypes similar to those with ESCRT-III inhibition reported previously, including the mislocalization of NEC, induction of membranous invagination structures containing enveloped virions, aberrant accumulation of enveloped virions in the invaginations and perinuclear space, and reduction of viral replication. We also showed that the effect of the arginine cluster in UL34 on HSV-1 replication was dependent primarily on ALIX. These results indicated that the arginine cluster in the disordered domain of UL34 was required for the interaction with ALIX and the recruitment of ESCRT-III machinery to the INM to promote primary envelopment. IMPORTANCE Herpesvirus UL34 homologs contain conserved amino-terminal domains that mediate vesicle formation through interactions with UL31 homologs during primary envelopment. UL34 homologs also comprise other domains adjacent to their membrane-anchoring regions, which differ in length, are variable in herpesviruses, and do not form distinguished secondary structures. However, the role of these disordered domains in infected cells remains to be elucidated. In this study, we present data suggesting that the arginine cluster in the disordered domain of HSV-1 UL34 mediates the interaction with ALIX, thereby leading to the recruitment of

ESCRT-III machinery to the INM for efficient primary envelopment. This is the first study to report the role of the disordered domain of a UL34 homolog in herpesvirus infections.

2. Role of the orphan transporter SLC35E1 in the nuclear egress of herpes simplex virus 1

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This study developed a system consisting of two rounds of screening cellular proteins involved in the nuclear egress of herpes simplex virus 1 (HSV-1). Using this system, we first screened cellular proteins that interacted with the HSV-1 nuclear egress complex (NEC) consisting of UL34 and UL31 in HSV-1-infected cells, which are critical for the nuclear egress of HSV-1, by tandem affinity purification coupled with mass spectrometry-based proteomics technology. Next, we performed CRISPR/Cas9-based screening of live HSV-1-infected reporter cells under fluorescence microscopy using single guide RNAs targeting the cellular proteins identified in the first proteomic screening to detect the mislocalization of the lamin-associated protein emerin, which is a phenotype for defects in HSV-1 nuclear egress. This study focused on a cellular orphan transporter SLC35E1, one of the cellular proteins identified by the screening system. Knockout of SLC35E1 reduced HSV-1 replication and induced membranous invaginations containing perinuclear enveloped virions (PEVs) adjacent to the nuclear membrane (NM), aberrant accumulation of PEVs in the perinuclear space between the inner and outer NMs and the invagination structures, and mislocalization of the NEC. These effects were similar to those of previously reported mutation(s) in HSV-1 proteins and depletion of cellular proteins that are important for HSV-1 de-envelopment, one of the steps required for HSV-1 nuclear egress. Our newly established screening system enabled us to identify a novel cellular protein required for efficient HSV-1 de-envelopment. IMPORTANCE The identification of cellular protein(s) that interact with viral effector proteins and function in important viral procedures is necessary for enhancing our understanding of the mechanics of various viral processes. In this study, we established a new system consisting of interactome screening for the herpes simplex virus 1 (HSV-1) nuclear egress complex (NEC), followed by loss-of-function screening to target the identified putative NEC-interacting cellular proteins to detect a defect in HSV-1 nuclear egress. This newly established system identified SLC35E1, an orphan transporter, as a novel cellular protein required for efficient HSV-1 de-envelopment, providing an insight into the mechanisms involved in this viral procedure.

3. Redundant and specific roles of A-type lamins and lamin B receptor in herpes simplex virus 1 infection

Kosuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasushi Kawaguchi

We investigated whether A-type lamins (lamin A/C) and lamin B receptor (LBR) are redundant during herpes simplex virus 1 (HSV-1) infection in HeLa cells expressing lamin A/C and LBR. Lamin A/C and LBR double knockout (KO) in HSV-1-infected HeLa cells significantly impaired expressions of HSV-1 early and late genes, maturation of replication compartments, marginalization of host chromatin to the nuclear periphery, enlargement of host cell nuclei, and viral DNA replication. Phenotypes of HSV-1-infected HeLa cells were restored by the ectopic expression of lamin A/C or LBR in lamin A/C and LBR double KO cells. Of note, lamin A/C single KO, but not LBR single KO, promoted the aberrant accumulation of virus particles outside the inner nuclear membrane (INM) and viral replication, as well as decreasing the frequency of virus particles inside the INM without affecting viral gene expression and DNA replication, time-spatial organization of replication compartments and host chromatin, and nuclear enlargement. These results indicated that lamin A/C and LBR had redundant and specific roles during HSV-1 infection. Thus, lamin A/C and LBR redundantly regulated the dynamics of the nuclear architecture, including the time-spatial organization of replication compartments and host chromatin, as well as promoting nuclear enlargement for efficient HSV-1 gene expression and DNA replication. In contrast, lamin A/C inhibited HSV-1 nuclear export through the INM during viral nuclear egress, which is a unique property of lamin A/C. IMPORTANCE This study demonstrated that lamin A/C and LBR had redundant functions associated with HSV-1 gene expression and DNA replication by regulating the dynamics of the nuclear architecture during HSV-1 infection. This is the first report to demonstrate the redundant roles of lamin A/C and LBR as well as the involvement of LBR in the regulation of these viral and cellular features in HSV-1-infected cells. These findings provide evidence for the

specific property of lamin A/C to inhibit HSV-1 nuclear egress, which has long been considered but without direct proof.

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Associate Professor　Takeshi Ichinohe, Ph.D.

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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. High body temperature increases gut microbiota-dependent host resistance to influenza A virus and SARS-CoV-2 infection.

Nagai M, Moriyama M, Ishii C, Mori H, Watanabe H, Nitta Y, Arimitsu N, Nishimoto M, Nakahara T, Yamada T, Ishikawa D, Ishikawa T, Hirayama A, Kimura I, Nagahara A, Naito T, Fukuda S, and Ichinohe T.

While a common symptom of influenza and coronavirus disease 2019 (COVID-19) is fever, its physiological role on host resistance to viral infection remains less clear. Here, we demonstrate that exposure of mice to the high ambient temperature of 36 °C increase host resistance to viral pathogens including influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). High heat-exposed mice increase basal body temperature over 38 °C to enable more bile acids production in a gut microbiota-dependent manner. The gut microbiota-derived deoxycholic acid (DCA) and its plasma membrane-bound receptor Takeda G-protein-coupled receptor 5 (TGR5) signaling increase host resistance to influenza virus infection by suppressing virus replication and neutrophil-dependent tissue damage. Furthermore, the DCA and its nuclear farnesoid X receptor (FXR) agonist protect Syrian hamster from lethal SARS-CoV-2 infection. Moreover, we demonstrate that certain bile acids are reduced in the plasma of COVID-19 patients who developed moderate I/II disease compared with minor illness group. These findings uncover an unexpected mechanism by which virus-induced high fever increases host resistance to influenza virus and SARS-CoV-2 in a gut microbiota-dependent manner.

2. Inactivation of novel coronavirus and alpha variant by photo-renewable CuxO/TiO2 nanocomposites.

Tatsuma T, Nakakido M, Ichinohe T, Kuroiwa Y, Tomioka K, Liu C, Miyamae N, Onuki T, Tsumoto K, Hashimoto K, and Wakihara T.

In order to reduce infection risk of novel coronavirus (SARS-CoV-2), we developed photocatalysts with nanoscale rutile TiO2 (4–8 nm) and CuxO (1–2 nm or less). Their extraordinarily small size leads to high dispersity and good optical transparency, besides large active surface area. Those photocatalysts can be applied to white and translucent latex paints and a

transparent varnish. Although Cu2O clusters involved in the paint coating undergo gradual aerobic oxidation in the dark, the oxidized clusters are re-reduced under >380 nm light. The paint coating inactivated novel coronavirus and its alpha (B.1.1.7) variant under irradiation with fluorescent light for 3 h. The coating also exhibited antivirus effects on influenza A virus, feline calicivirus and bacteriophage Qβ. The photocatalysts would be applied to practical coatings and lower the risk of coronavirus infection via solid surfaces.

International Vaccine Design Center

Division of Systems Immunology (Human Immune-Profiling Team)

ヒト免疫プロファイリング系・数理免疫学分野

Professor　Kei Sato, Ph.D.

教　授　博士(医学)　　佐　藤　　佳

The aim of this laboratory is to launch an interdisciplinary research network to "quantitatively" understand the behaviors of pathogens and the immune reaction against pathogen infection. Our study will connect microbiology and immunology, which will lead to the development of novel vaccines in the future.

International Vaccine Design Center

Division of Human Immunology (Human Immune-Profiling Team)

ヒト免疫プロファイリング系・ヒト免疫学分野

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Visiting Professor	Noriko Toyama-Sorimachi, Ph.D.	客員教授	博士(医学)	反　町　典　子
Project Senior Assistant Professor	Toshihiko Kobayashi, Ph.D.	特任講師	博士(医学)	小　林　俊　彦

The laboratory is consisted of two groups working on vaccine and immunometabolism lead by Ken Ishii and Noriko Sorimachi, respectively to conduct novel research on vaccine immunology and immunometabolism towards human immune-profiling to understand why and how our immune system respond to infection and other immunological disorders. In FY 2022, we reported two independent phase-I clinical trials for seasonal influenza vaccine with a novel adjuvant HP-bCD, and phase-Ib study for mono-therapeutic application of humanized CpG ODN K3, for cancer patients.

1. *Safety and immunogenicity of a quadrivalent seasonal influenza vaccine adjuvanted with hydroxypropyl-β-cyclodextrin: A phase 1 clinical trial*

Objectives: Hydroxypropyl-β-cyclodextrin (HP-β-CyD), an oligosaccharide used as an excipient in pharmaceutical preparation, was recently reported to function as a vaccine adjuvant to co-administered antigens. In this study, we investigated the safety and immunogenicity of a seasonal influenza vaccine adjuvanted with HP-β-CyD (FluCyD-vac) in healthy adults compared with those of a standard seasonal influenza vaccine (Flu-vac).
Methods: We conducted a single-blinded randomized phase 1 clinical trial study, and used two quadrivalent split seasonal influenza vaccines: FluCyD-vac containing 9 μg of HA/strain and 20% w/v of HP-β-CyD, and Flu-vac containing 15 μg of hemagglutinin (HA)/strain only. All participants were randomly assigned to receive a single dose of Flu/CyD-vac or Flu-vac at a ratio of 2:1. We assessed solicited and unsolicited adverse events (AEs) and immune responses using hemagglutination inhibition (HI) titers. In addition, we assessed T-cell function in peripheral blood mononuclear cells (PBMCs), after stimulation with HA vaccine strains, using flow cytometry.
Results: Among 36 healthy volunteers enrolled in the study (FluCyD-vac, n = 24; Flu-vac, n = 12), FluCyD-vac was well tolerated. Most of the solicited AEs were mild local skin reactions at the injection site. No serious AEs were reported in either group. HI titers 21 days after vaccination with FluCyD-vac were comparable with those of Flu-vac and sufficient to meet international criteria, despite reduced HA antigen doses. When PBMCs were stimulated with the four HA antigens in the vaccine, tumor necrosis factor (TNF)-α-producing CD4+ T cells were enhanced in the FluCyD-vac group.
Conclusion: FluCyD-vac was well-tolerated and immunogenic, despite containing 40% less HA antigens than Flu-vac. This study showed that HP-β-CyD is a potentially safe, novel adjuvant for human influenza vaccine.
Clinical trial registry: UMIN000028530.

2. *CpG ODN (K3)-toll-like receptor 9 agonist-induces Th1-type immune response and enhances cytotoxic activity in advanced lung cancer patients: a phase I study*

Background: Cytosine-phosphate-guanine oligodeoxynucleotide (CpG ODN) (K3)-a novel synthetic single-stranded DNA immune adjuvant for cancer immunotherapy-induces a potential Th1-type immune response against cancer cells. We conducted a phase I study of CpG ODN (K3) in patients with lung cancer to assess its safety and patients' immune responses.
Methods: The primary endpoint was the proportion of dose-limiting toxicities (DLTs) at each dose level. Secondary endpoints included safety profile, an immune response, including dynamic changes in immune cell and cytokine production, and progression-free survival (PFS). In a 3 + 3 dose-escalation design, the dosage levels for CpG ODN (K3) were 5 or 10 mg/body via subcutaneous injection and 0.2 mg/kg via intravenous administration on days 1, 8, 15, and 29.
Results: Nine patients (eight non-small-cell lung cancer; one small-cell lung cancer) were enrolled. We found no DLTs at any dose level and observed no serious treatment-related adverse events. The median observation period after registration was 55 days (range: 46-181 days). Serum IFN-α2 levels, but not inflammatory cytokines, increased in six patients after the third administration of CpG ODN (K3) (mean value: from 2.67 pg/mL to 3.61 pg/mL after 24 hours). Serum IFN-γ (mean value, from 9.07 pg/mL to 12.7 pg/m after 24 hours) and CXCL10 levels (mean value, from 351 pg/mL to 676 pg/mL after 24 hours) also increased in eight patients after the third administration. During the treatment course, the percentage of T-bet-expressing CD8+ T cells gradually increased (mean, 49.8% at baseline and 59.1% at day 29, $p = 0.0273$). Interestingly, both T-bet-expressing effector memory (mean, 52.7% at baseline and 63.7% at day 29, $p = 0.0195$) and terminally differentiated effector memory (mean, 82.3% at baseline and 90.0% at day 29, $p = 0.0039$) CD8+ T cells significantly increased. The median PFS was 398 days.
Conclusions: This is the first clinical study showing that CpG ODN (K3) activated innate immunity and elicited Th1-type adaptive immune response and cytotoxic activity in cancer patients. CpG ODN (K3) was well tolerated at the dose settings tested, although the maximum tolerated dose was not determined.
Trial registration: UMIN-CTR number 000023276.

3. *Metabolic regulation of macrophage's inflammatory functions by a lysosome-resident amino acid transporter, SLC15A4; a therapeutic target for controlling macrophage-mediated inflammatory diseases*.

Background: Controlling inflammation is critical for alleviation of immune-mediated diseases including infection-related subsequent complications. The endolysosome system plays critical roles in inflammatory responses since endolysosomes function as signal transduction hubs to convert various environmental danger signals into gene expression. Such endolysosome-dependent signals cause metabolic adaptation of immune cells which required for efficient orchestration of inflammatory responses including the termination of inflammation and the progression of tissue repair. However, the precise mechanisms of endolysoosme-dependent metabolic regulation are still largely unclear.

SLC15A4 is an endolysosome-resident amino acid transporter that regulates innate immune responses, and is genetically associated with inflammatory diseases such as systemic lupus erythematosus (SLE) and colitis. SLC15A4-deficient mice showed the amelioration of symptoms of these model diseases, and thus SLC15A4 is a promising therapeutic target of SLE and colitis. In this study, we investigated SLC15A4's functions in endolysosome-dependent metabolic regulation of macrophages and their impact on inflammatory responses.
Methods: We used BioID to identify molecules that are in close-proximity to SLC15A4. Based on the results of BioID, we compared metabolic properties between *Sls15a4*$^{+/+}$ and *SLC15A4*$^{-/-}$ mouse-derived macrophages using Seahorse flux analyses and mass-spectrometry-based metabolomics analyses.
Results: We identified 9 proteins involved in the mTOR signaling pathway, including RagA, RagB, RagC, and Lamtor1/2 by BioID, and further investigated the functional linkage between SLC15A4 and mTORC1. We revealed that SLC15A4 loss disturbed the coupling of glycolysis and the TCA cycle, and SLC15A4-deficient macrophages preferred to use glutamine rather than glucose as a carbon source for the TCA cycle. SLC15A4-deficient macrophages produced low levels of itaconate and pro-inflammatory IL-12 cytokine members.
Conclusions: Our findings reveal a novel mechanism of metabolic regulation in which an amino acid transporter acts as a gatekeeper that protects immune cells' ability to acquire an M1-prone metabolic phenotype in inflammatory tissues by mitigating metabolic stress. SLC15A4 pivotally and widely affects inflammatory and metabolic signaling at the endolysosome, and loss of SLC15A4 inhibits multiple inflammatory signals in macrophages.

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International Vaccine Design Center

Division of Infection Immunology (Human Immune-Profiling Team)

ヒト免疫プロファイリング系・感染免疫学分野

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As a member of the International Vaccine Design Center of IMSUT (VDeSC), we focus on the elucidation of host-pathogen interactions in the context of malaria and Leishmania parasites, and the respiratory viruses, to be able to understand their way of causing pathology to create successful vaccines against them. Our recent topics include how to bolster B cell memory responses against pathogens.

1. Novel adjuvant discovery and development

Adjuvants are known as must-have vaccine components for the potentiation of vaccine responses. As a member of IMSUT International Vaccine Design Center (https://vdesc.ims.u-tokyo.ac.jp/en/), we have been involved in the screening of herbal medicine extracts as safe and ready-to-use adjuvants for current human vaccines. We have been systematically screening innate and adaptive immune signaling molecules taking part in the mode of action (MOA) of adjuvants and vaccines. One of the recent findings involves understanding how the combination of TLR9 and STING agonists synergistically induce innate and adaptive responses to become an advantageous type 1 adjuvant while suppressing type 2 immunity which leads to generation of robust anti-tumor responses.

Our recent projects focus on the investigation of B cell development and pathways involved in the germinal center (GC) formation for the generation of potent antibody responses against infections and during vaccinations. We have found that TBK1, the famous innate immune signaling kinase for controlling anti-viral immune responses and nucleic-acid mediated type-I interferon responses, is very important for the generation of GC which confers sterile immunity to reinfections.

2. Elucidation of malaria-mediated pathologies

Malaria killed 50% more children last year due to Covid-19-mediated lockdowns and restrictions which prevented remedies to reach those who needed them. Our lab has been investigating cerebral malaria immunopathology by using imaging techniques such as CUBIC clearance of the brain. The research has been ongoing for the investigation of olfactory bulb-mediated pathology in experimental cerebral malaria models in mice. We have made significant progress in the understanding of new cell types in the olfactory bulb and signaling molecules taking part.

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International Vaccine Design Center

Division of Vaccine Engineering (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・ワクチン工学分野

Project Professor　Kouhei Tsumoto, Ph.D.

特任教授　博士（工学）　津　本　浩　平

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. Development of a high-throughput method to screen novel antiviral materials

Nakakido M, Tanaka N, Shimojo A, Miyamae N, Tsumoto K.

Respiratory infectious diseases pose a serious threat worldwide, and novel antiviral materials are highly demanded. Photocatalytic nanoparticles have been developed to inhibit indirect transmission of pathogens by acting as surface coating materials. During development of such antiviral materials, researchers use bacteriophages as model viruses due to their safety and experimental efficiency. Screening methods are used to identify potential antiviral materials, and better screening technologies will accelerate the discovery of antiviral treatments. In this study, we constructed a novel platform to evaluate antiviral activity of surface coating materials using the M13 bacteriophage and phagemid system derived from phage display technology. The evaluation results generated by this system for the two tested antiviral materials were comparable to those for the materials tested on the Qβ bacteriophage and influenza virus using traditional screening methods. The experimental system developed in this study provides rapid and effective screening and can be applied to the development of novel antiviral materials.

2. Oligo (N-methylalanine) as a Peptide-Based Molecular Scaffold with a Minimal Structure and High Density of Functionalizable Sites

Yokomine M, Morimoto J, Fukuda Y, Shiratori Y, Kuroda D, Ueda T, Takeuchi K, Tsumoto K, Sando S.

Functionalizable synthetic molecules with nanometer sizes and defined shapes in water are useful as molecular scaffolds to mimic the functions of biomacromolecules and develop chemical tools for manipulating biomacromolecules. Herein, we propose oligo (N-methylalanine) (oligo-NMA) as a peptide-based molecular scaffold with a minimal structure and a

high density of functionalizable sites. Oligo-NMA forms a defined shape in water without hydrogen-bonding networks or ring constraints, which enables the molecule to act as a scaffold with minimal atomic composition. Furthermore, functional groups can be readily introduced on the nitrogens and α-carbons of oligo-NMA. Computational and NMR spectroscopic analysis suggested that the backbone structure of oligo-NMA is not largely affected by functionalization. Moreover, the usefulness of oligo-NMA was demonstrated by the design of protein ligands. The ease of synthesis, minimal structure, and high functionalization flexibility makes oligo-NMA a useful scaffold for chemical and biological applications.

3. Biophysical Characterization of the Contribution of the Fab Region to the IgG-FcγRIIIa Interaction

Kosuge H, Nagatoishi S, Kiyoshi M, Ishii-Watabe A, Terao Y, Ide T, Tsumoto K.

The cell-surface receptor FcγRIIIa is crucial to the efficacy of therapeutic antibodies as well as the immune response. The interaction of the Fc region of IgG molecules with FcγRIIIa has been characterized, but until recently, it was thought that the Fab regions were not involved in the interaction. To evaluate the influence of the Fab regions in a biophysical context, we carried out surface plasmon resonance analyses using recombinant FcγRIIIa ligands. A van't Hoff analysis revealed that compared to the interaction of the papain-digested Fc fragment with FcγRIIIa, the interaction of commercially available, full-length rituximab with FcγRIIIa had a more favorable binding enthalpy, a less favorable binding entropy, and a slower off rate. Similar results were obtained from analyses of IgG1 molecules and an IgG1-Fc fragment produced by Expi293 cells. For further validation, we also prepared a maltose-binding protein-linked IgG1-Fc fragment (MBP-Fc). The binding enthalpy of MBP-Fc was nearly equal to that of the IgG1-Fc fragment for the interaction with FcγRIIIa, indicating that such alternatives to the Fab domains as MBP do not positively contribute to the IgG-FcγRIIIa interactions. Our investigation strongly suggests that the Fab region directly interacts with FcγRIIIa, resulting in an increase in the binding enthalpy and a decrease in the dissociation rate, at the expense of favorable binding entropy.

4. Mirror-image streptavidin with specific binding to L-biotin, the unnatural enantiomer

Suganuma M, Kubo T, Ishiki K, Tanaka K, Suto K, Ejima D, Toyota M, Tsumoto K, Sato T, Nishikawa Y.

The streptavidin-biotin system is known to have a very high affinity and specificity and is widely used in biochemical immunoassays and diagnostics. However, this method is affected by endogenous D-biotin in serum sample measurements (biotin interference). While several efforts using alternative high-affinity binding systems (e.g., genetically modified streptavidin and biotin derivatives) have been attempted, these efforts have all led to reduction in affinity. To solve this interference issue, the enantiomer of streptavidin was synthesized, which enabled specific binding to L-biotin. We successfully obtained a functional streptavidin molecule by peptide synthesis using D-amino acids and an in vitro folding technique. Several characterizations, including size exclusion chromatography (SEC), circular dichroism spectra (CD), and heat denaturation experiments collectively confirmed the higher-order enantiomer of natural streptavidin had been formed with comparable stability to the natural protein. L-biotin specific binding of this novel molecule enabled us to avoid biotin interference in affinity measurements using the Biacore system and enzyme-linked immunosorbent assay (ELISA). We propose the enantiomer of streptavidin as a potential candidate to replace the natural streptavidin-biotin system, even for in vivo use.

5. Experimental Comparison of Bond Lifetime and Viscoelastic Relaxation in Transient Networks with Well-Controlled Structures

Katashima T, Kudo R, Naito M, Nagatoishi S, Miyata K, Chung UI, Tsumoto K, Sakai T.

We demonstrate an experimental comparison of the bond lifetime, estimated using surface plasmon resonance (SPR), and the viscoelastic relaxation time of transient networks with well-controlled structures (dynamically cross-linked Tetra-PEG gel). SPR and viscoelastic measurements revealed that the temperature dependences of the two characteristic times are in agreement, while the viscoelastic response is delayed with respect to the lifetime by a factor of 2-3, dependent on the network strand length. Polymers cross-linked by temporary interactions form transient networks, which show fascinating viscoelasticity with a single relaxation mode. However, the molecular understanding of such simple viscoelasticity has remained incomplete because of the difficulty of experimentally evaluating bond lifetimes and heterogeneous structures in conventional transient networks. Our results suggest that bond dissociation and recombination both contribute to the macromechanical response. This report on direct bond-lifetime-viscoelastic-relaxation time comparison provides important information for the molecular design of transient network materials.

6. Antibody recognition of complement factor H reveals a flexible loop involved in atypical hemolytic uremic syndrome pathogenesis

Yokoo T, Tanabe A, Yoshida Y, Caaveiro JMM, Nakakido M, Ikeda Y, Fujimura Y, Matsumoto M, Entzminger K, Maruyama T, Okumura CJ, Nangaku M, Tsumoto K.

Atypical hemolytic uremic syndrome (aHUS) is a disease associated with dysregulation of the immune complement system, especially of the alternative pathway (AP). Complement factor H (CFH), consisting of 20 domains called complement control protein (CCP1-20), downregulates the AP as a cofactor for mediating C3 inactivation by complement factor I. However, anomalies related to CFH are known to cause excessive complement activation and cytotoxicity. In aHUS, mutations and the presence of anti-CFH autoantibodies (AAbs) have been reported as plausible causes of CFH dysfunction, and it is known that CFH-related aHUS carries a high probability of end-stage renal disease. Elucidating the detailed functions of CFH at the molecular level will help to understand aHUS pathogenesis. Herein, we used biophysical data to reveal that a heavy-chain antibody fragment, termed VHH4, recognized CFH with high affinity. Hemolytic assays also indicated that VHH4 disrupted the protective function of CFH on sheep erythrocytes. Furthermore, X-ray crystallography revealed that VHH4 recognized the Leu1181-Leu1189CCP20 loop, a known anti-CFH AAbs epitope. We next analyzed the dynamics of the C-terminal region of CFH and showed that the epitopes recognized by anti-CFH AAbs and VHH4 were the most flexible regions in CCP18-20. Finally, we conducted mutation analyses to elucidate the mechanism of VHH4 recognition of CFH and revealed that VHH4 inserts the Trp1183C-CP20 residue of CFH into the pocket formed by the complementary determining region 3 loop. These results suggested that anti-CFH AAbs may adopt a similar molecular mechanism to recognize the flexible loop of Leu1181-Leu1189CCP20, leading to aHUS pathogenesis.

7. Structure and role of the linker domain of the iron surface-determinant protein IsdH in heme transportation in Staphylococcus aureus

Valenciano-Bellido S, Caaveiro JMM, Morante K, Sushko T, Nakakido M, Nagatoishi S, Tsumoto K.

Staphylococcus aureus is a major cause of deadly nosocomial infections, a severe problem fueled by the steady increase of resistant bacteria. The iron surface determinant (Isd) system is a family of proteins that acquire nutritional iron from the host organism, helping the bacterium to proliferate during infection, and therefore represents a promising antibacterial target. In particular, the surface protein IsdH captures hemoglobin (Hb) and acquires the heme moiety containing the iron atom. Structurally, IsdH comprises three distinctive NEAr-iron Transporter (NEAT) domains connected by linker domains. The objective of this study was to characterize the linker region between NEAT2 and NEAT3 from various biophysical viewpoints and thereby advance our understanding of its role in the molecular mechanism of heme extraction. We demonstrate the linker region contributes to the stability of the bound protein, likely influencing the flexibility and orientation of the NEAT3 domain in its interaction with Hb, but only exerts a modest contribution to the affinity of IsdH for heme. Based on these data, we suggest that the flexible nature of the linker facilitates the precise positioning of NEAT3 to acquire heme. In addition, we also found that residues His45 and His89 of Hb located in the heme transfer route toward IsdH do not play a critical role in the transfer rate-determining step. In conclusion, this study clarifies key elements of the mechanism of heme extraction of human Hb by IsdH, providing key insights into the Isd system and other protein systems containing NEAT domains.

8. Addition of arginine hydrochloride and proline to the culture medium enhances recombinant protein expression in Brevibacillus choshinensis: The case of RBD of SARS-CoV-2 spike protein and its antibody

Matsunaga R, Tsumoto K.

Brevibacillus choshinensis is a gram-positive bacterium that is known to efficiently secrete recombinant proteins. However, the expression of these proteins is often difficult depending upon the expressed protein. In this study, we demonstrated that the addition of arginine hydrochloride and proline to the culture medium dramatically increased protein expression. By culturing bacterial cells in 96-well plates, we were able to rapidly examine the expression conditions and easily scale up to 96 mL of culture for production. Although functional expression of the receptor binding domain (RBD) of the SARS-CoV-2 spike protein without any solubility-enhancing tag in bacterial strains (including Escherichia coli) has not been reported to date, we succeeded in efficiently producing RBD which showed a similar CD spectrum to that of RBD produced by eukaryotic cell expression systems. Furthermore, RBD from the omicron variant (B.1.1.529) was also produced. Physicochemical analyses indicated that omicron RBD exhibited markedly increased instability compared to the wild-type. We also revealed that the Fab format of the anti-SARS-CoV-2 antibody C121 can be produced in large quantities using the same expression system. The obtained C121 Fab bound to wild-type RBD but not to omicron RBD. These results strongly suggest that the Breviba-

cillus expression system is useful for facilitating the efficient expression of proteins that are difficult to fold and will thus contribute to the rapid physicochemical evaluation of functional proteins.

9. Ladder observation of bovine serum albumin by high resolution agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Nagatoishi S, Tsumoto K, Arakawa T, Akuta T.

A commercially available bovine serum albumin (BSA) was examined by agarose native gel electrophoresis using two different agarose sources, UltraPure and MetaPhor agarose. While UltraPure agarose up to 5 % showed no clear separation of BSA oligomers, MetaPhor agarose clearly demonstrated oligomer bands above 4 %, indicating that the latter agarose has greater molecular sieving effects and is hence characterized to have high resolution for size differences, as probed by a greater slope of Ferguson plot. Physical properties are different between two agaroses. In general, UltraPure agarose has physical strength, while MetaPhor agarose is considerably fragile, but MetaPhor agarose solution is less viscous so that even 10 % gel can be made. Cause of oligomers was shown to be not associated with inter-chain disulfide bonds, but is due to association of native or native-like molecules.

10. Residue-based program of a β-peptoid twisted strand shape via a cyclopentane constraint

Kim J, Kobayashi H, Yokomine M, Shiratori Y, Ueda T, Takeuchi K, Umezawa K, Kuroda D, Tsumoto K, Morimoto J, Sando S.

N-Substituted peptides, such as peptoids and β-peptoids, have been reported to have unique structures with diverse functions, like catalysis and manipulation of biomolecular functions. Recently, the preorganization of monomer shape by restricting bond rotations about all backbone dihedral angles has been demonstrated to be useful for de novo design of peptoid structures. Such design strategies are hitherto unexplored for β-peptoids; to date, no preorganized β-peptoid monomers have been reported. Here, we report the first design strategy for β-peptoids, in which all four backbone dihedral angles (ω, ϕ, θ, ψ) are rotationally restricted on a per-residue basis. The introduction of a cyclopentane constraint realized the preorganized monomer structure and led to a β-peptoid with a stable twisted strand shape.

11. PRELP Regulates Cell-Cell Adhesion and EMT and Inhibits Retinoblastoma Progression

Hopkins J, Asada K, Leung A, Papadaki V, Davaapil H, Morrison M, Orita T, Sekido R, Kosuge H, Reddy MA, Kimura K, Mitani A, Tsumoto K, Hamamoto R, Sagoo MS, Ohnuma SI.

Retinoblastoma (RB) is the most common intraocular pediatric cancer. Nearly all cases of RB are associated with mutations compromising the function of the RB1 tumor suppressor gene. We previously demonstrated that PRELP is widely downregulated in various cancers and our in vivo and in vitro analysis revealed PRELP as a novel tumor suppressor and regulator of EMT. In addition, PRELP is located at chromosome 1q31.1, around a region hypothesized to be associated with the initiation of malignancy in RB. Therefore, in this study, we investigated the role of PRELP in RB through in vitro analysis and next-generation sequencing. Immunostaining revealed that PRELP is expressed in Müller glial cells in the retina. mRNA expression profiling of PRELP-/- mouse retina and PRELP-treated RB cells found that PRELP contributes to RB progression via regulation of the cancer microenvironment, in which loss of PRELP reduces cell-cell adhesion and facilitates EMT. Our observations suggest that PRELP may have potential as a new strategy for RB treatment.

12. Analysis of bovine serum albumin unfolding in the absence and presence of ATP by SYPRO Orange staining of agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Kurosawa Y, Nagatoishi S, Tsumoto K, Arakawa T, Akuta T.

An attempt was made to specifically stain unfolded proteins on agarose native gels. SYPRO Orange is routinely used to detect unfolded protein in differential scanning fluorimetry, which is based on the enhanced fluorescence intensity upon binding to the unfolded protein. We demonstrated that this dye barely bound to the native proteins, resulting in no or faint staining of the native bands, but bound to and stained the unfolded proteins, on agarose native gels. Using bovine serum albumin (BSA), it was shown that staining did not depend on whether BSA was thermally unfolded in the presence of SYPRO Orange or stained after electrophoresis. On the contrary, SYPRO Orange dye stained protein bands in the presence of sodium dodecylsulfate (SDS) due to incorporation of the dye into SDS micelles that bound to the unfolded proteins. This staining resulted in detection of new, intermediately unfolded structure of BSA during thermal unfolding. Such intermediate structure occurred at higher temperature in the presence of ATP.

13. Human antibody recognition and neutralization mode on the NTD and RBD domains of SARS-CoV-2 spike protein

Otsubo R, Minamitani T, Kobiyama K, Fujita J, Ito T, Ueno S, Anzai I, Tanino H, Aoyama H, Matsuura Y, Namba K, Imadome KI, Ishii KJ, Tsumoto K, Kamitani W, Yasui T.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). Variants of concern (VOCs) such as Delta and Omicron have developed, which continue to spread the pandemic. It has been reported that these VOCs reduce vaccine efficacy and evade many neutralizing monoclonal antibodies (mAbs) that target the receptor binding domain (RBD) of the glycosylated spike (S) protein, which consists of the S1 and S2 subunits. Therefore, identification of optimal target regions is required to obtain neutralizing antibodies that can counter VOCs. Such regions have not been identified to date. We obtained 2 mAbs, NIBIC-71 and 7G7, using peripheral blood mononuclear cells derived from volunteers who recovered from COVID-19. Both mAbs had neutralizing activity against wild-type SARS-CoV-2 and Delta, but not Omicron. NIBIC-71 binds to the RBD, whereas 7G7 recognizes the N-terminal domain of the S1. In particular, 7G7 inhibited S1/S2 cleavage but not the interaction between the S protein and angiotensin-converting enzyme 2; it suppressed viral entry. Thus, the efficacy of a neutralizing mAb targeting inhibition of S1/2 cleavage was demonstrated. These results suggest that neutralizing mAbs targeting blockade of S1/S2 cleavage are likely to be cross-reactive against various VOCs.

14. Molecular basis for thermal stability and affinity in a VHH: Contribution of the framework region and its influence in the conformation of the CDR3

Kinoshita S, Nakakido M, Mori C, Kuroda D, Caaveiro JMM, Tsumoto K.

The camelid single domain antibody, referred to VHH or Nanobody, is considered a versatile tool for various biotechnological and clinical applications because of its favorable biophysical properties. To take advantage of these characteristics and for its application in biotechnology and therapy, research on VHH engineering is currently vigorously conducted. To humanize a camelid VHH, we performed complementarity determining region (CDR) grafting using a humanized VHH currently in clinical trials, and investigated the effects of these changes on the biophysical properties of the resulting VHH. The chimeric VHH exhibited a significant decrease in affinity and thermal stability and a large conformational change in the CDR3. To elucidate the molecular basis for these changes, we performed mutational analyses on the framework regions revealing the contribution of individual residues within the framework region. It is demonstrated that the mutations resulted in the loss of affinity and lower thermal stability, revealing the significance of bulky residues in the vicinity of the CDR3, and the importance of intramolecular interactions between the CDR3 and the framework-2 region. Subsequently, we performed back-mutational analyses on the chimeric VHH. Back-mutations resulted in an increase of the thermal stability and affinity. These data suggested that back-mutations restored the intramolecular interactions, and proper positioning and/or dynamics of the CDR3, resulting in the gain of thermal stability and affinity. These observations revealed the molecular contribution of the framework region on VHHs and further designability of the framework region of VHHs without modifying the CDRs.

15. Repression of the PRELP gene is relieved by histone deacetylase inhibitors through acetylation of histone H2B lysine 5 in bladder cancer

Shozu K, Kaneko S, Shinkai N, Dozen A, Kosuge H, Nakakido M, Machino H, Takasawa K, Asada K, Komatsu M, Tsumoto K, Ohnuma SI, Hamamoto R.

Proline/arginine-rich end leucine-rich repeat protein (PRELP) is a member of the small leucine-rich proteoglycan family of extracellular matrix proteins, which is markedly suppressed in the majority of early-stage epithelial cancers and plays a role in regulating the epithelial-mesenchymal transition by altering cell-cell adhesion. Although PRELP is an important factor in the development and progression of bladder cancer, the mechanism of PRELP gene repression remains unclear. Here, we show that repression of PRELP mRNA expression in bladder cancer cells is alleviated by HDAC inhibitors (HDACi) through histone acetylation. Using ChIP-qPCR analysis, we found that acetylation of lysine residue 5 of histone H2B in the PRELP gene promoter region is a marker for the de-repression of PRELP expression. These results suggest a mechanism through which HDACi may partially regulate the function of PRELP to suppress the development and progression of bladder cancer. Some HDACi are already in clinical use, and the findings of this study provide a mechanistic basis for further investigation of HDACi-based therapeutic strategies.

16. Performance Comparison of Spectral Distance Calculation Methods

Oyama T, Suzuki S, Horiguchi Y, Yamane A, Akao K, Nagamori K, Tsumoto K.

Circular dichroism (CD) spectroscopy is a widely

used technique for assessing the higher-order structure (HOS) of biopharmaceuticals, including antibody drugs. Since the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use established quality control guidelines, objective evaluation of spectral similarity has been required in order to assess structural comparability. Several spectral distance quantification methods and weighting functions to increase sensitivity have been proposed, but not many reports have compared their performance for CD spectra. We constructed comparison sets that combine actual spectra and simulated noise and performed a comprehensive performance evaluation of each spectral distance calculation method and weighting function under conditions that consider spectral noise and fluctuations from pipetting errors. The results showed that using the Euclidean distance or Manhattan distance with Savitzky-Golay noise reduction is effective for spectral distance assessment. For the weighting function, it is preferable to combine the spectral intensity weighting function and the noise weighting function. In addition, the introduction of the external stimulus weighting function should be considered to improve the sensitivity. It is crucial to select the weighting function based on the balance between spectral changes and noise distributions for robust, sensitive antibody HOS similarity assessment.

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International Vaccine Design Center

Division of Adjuvant Innovation (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・アジュバント開発分野

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Visiting Professor	Jun Kunisawa, Ph.D.		客員教授	博士(薬学)	國　澤　　純

The laboratory is consisted of two groups working on vaccine and adjuvant lead by Ken Ishii and Jun Kunisawa, respectively to conduct novel research on vaccine immunology towards rational vaccine design. In FY 2022, we reported various papers related to immunology on vaccine and adjuvant R&D.

1. *Making innate sense of mRNA vaccine adjuvanticity.*

Successful vaccines contain two essential immunological components: a protective antigen and an adjuvant. Adjuvants are essential for optimal antigen-specific immune responses, the so-called 'immunogenicity', but are often a cause of reactogenicity (even toxicity) that results in local and systemic inflammation. Therefore, to ensure vaccine efficacy and safety, it is critical to understand the molecular and cellular mechanism(s) by which adjuvants provoke the immune system. By introducing papers, we describe that there seems to be more room to improve the immunogenicity and reduce the reactogenicity of LNP-mRNA vaccine formulations by further study of immunization methods (including delivery systems and devices) and their built-in adjuvanticity.

2. *Anti-tumor immunity by transcriptional synergy between TLR9 and STING activation*

Agonists for TLR9 and stimulator of IFN genes (STING) offer therapeutic applications as both anti-tumor agents and vaccine adjuvants, though their clinical applications are limited; the clinically available TLR9 agonist is a weak IFN inducer and STING agonists induce undesired type 2 immunity. Yet, combining TLR9 and STING agonists overcame these limitations by synergistically inducing innate and adaptive IFNγ to become an advantageous type 1 adjuvant, suppressing type 2 immunity, in addition to exerting robust anti-tumor activities when used as a monotherapeutic agent for cancer immunotherapy. Here, we sought to decipher the immunological mechanisms behind the synergism mediated by TLR9 and STING agonists and found that their potent anti-tumor immunity in a Pan02 peritoneal dissemination model of pancreatic cancer was achieved only when agonists for TLR9 and STING were administered locally, and was via mechanisms involving CD4 and CD8 T cells as well as the co-operative action of IL-12 and type I IFNs. Rechallenge studies of long-term cancer survivors suggested that the elicitation of Pan02-specific memory responses provides protection against the secondary tumor challenge. Mechanistically, we found that TLR9 and STING agonists synergistically induce IL-12 and type I IFN produc-

tion in murine APCs. The synergistic effect of the TLR9 and STING agonists on IL-12p40 was at protein, mRNA and promoter activation levels, and transcriptional regulation was mediated by a 200 bp region situated 983 bp upstream of the IL-12p40 transcription initiation site. Such intracellular transcriptional synergy may hold a key in successful cancer immunotherapy and provide further insights into dual agonism of innate immune sensors during host homeostasis and diseases.

3. *Machine Learning-Assisted Screening of Herbal Medicine Extracts as Vaccine Adjuvants*

Adjuvants are important vaccine components, composed of a variety of chemical and biological materials that enhance the vaccine antigen-specific immune responses by stimulating the innate immune cells in both direct and indirect manners to produce a variety cytokines, chemokines, and growth factors. It has been developed by empirical methods for decades and considered difficult to choose a single screening method for an ideal vaccine adjuvant, due to their diverse biochemical characteristics, complex mechanisms of, and species specificity for their adjuvanticity. We therefore established a robust adjuvant screening strategy by combining multiparametric analysis of adjuvanticity in vivo and immunological profiles in vitro (such as cytokines, chemokines, and growth factor secretion) of various library compounds derived from hot-water extracts of herbal medicines, together with their diverse distribution of nano-sized physical particle properties with a machine learning algorithm. By combining multiparametric analysis with a machine learning algorithm such as rCCA, sparse-PLS, and DIABLO, we identified that human G-CSF and mouse RANTES, produced upon adjuvant stimulation in vitro, are the most robust biological parameters that can predict the adjuvanticity of various library compounds. Notably, we revealed a certain nano-sized particle population that functioned as an independent negative parameter to adjuvanticity. Finally, we proved that the two-step strategy pairing the negative and positive parameters significantly improved the efficacy of screening and a screening strategy applying principal component analysis using the identified parameters. These novel parameters we identified for adjuvant screening by machine learning with multiple biological and physical parameters may provide new insights into the future development of effective and safe adjuvants for human use.

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International Vaccine Design Center

Division of Mucosal Vaccines (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・粘膜ワクチン分野

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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.

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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. We confirmed that mice deficient with this molecule showed reduced levels of antigen-specific IgA antibody responses in the vaginal washes despite intact levels of serum IgG titers. We are currently testing chemokine receptor expression which involved for the regulation of antigen specific IgA responses.

2. Human salivary protein-derived peptides specific-salivary SIgA antibodies enhanced by nasal double DNA adjuvant in mice play an essential role in preventing *Porphyromonas gingivalis* colonization *in vitro*

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We have previously shown that fimbriae-bore from *Poryphyromonas gingivalis* (Pg), one of the putative periodontopathogenic bacteria specifically bound to a peptide domain (stat23, prp21) shared on statherin or acidic proline-rich protein 1 (PRP1) molecule of human salivary proteins (HSPs). In this study, we first investigated whether nasal administration of double DNA adjuvant (dDA) consisting of DNA plasmid expressing Flt3 ligand and CpG oligodeoxynucleotide plus sta23 or prp21 peptide as an antigen (Ag) in mice could enhance stat23- or prp21-specific secretory IgA (SIgA) antibody (Ab) responses in the saliva of mice. Significant elevated levels of salivary SIgA Ab to stat23 or prp21 in mice given nasal stat23 or prp21 with dDA were seen compared to those in mice given Ag alone. Of interest, mice given the mixture of stat23 and prp21 (double Ags) plus dDA, nasally, resulted in stat23- and prp21-specific salivary SIgA Ab induction, which is mediated through significantly increased numbers of $CD11c^+$ dendritic cells populations and marked Th1 and Th2 cytokines production by $CD4^+$ T cells in the mucosal inductive and effector tissues. Furthermore, when mice were nasally immunized with double Ags plus dDA, stat23- and prp21-specific salivary SIgA Ab responses were enhanced, and the SIgA Ab-enriched saliva showed significantly reduced numbers of live Pg cells binding to human whole saliva-coated hydroxyapatite beads (wsHAPs) as compared with those in mice given double Ags alone or naïve mice. Additionally, saliva from IgA knock-out mice given nasally double Ags plus dDA indicated no decrease of live Pg binding to wsHAPs. These findings show that HSP-derived peptides-specific salivary SIgA Abs induced by nasal administration of stat23 and prp21 peptides plus dDA, play an essential role in the preventing Pg attachment and colonization on the surface of teeth, suggesting that the SIgA may interrupt and mask fimbriae-binding domains in HSPs on the teeth.

3. Utilizing mast cells in a positive manner to overcome inflammatory and allergic diseases

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Mast cells (MCs) are immune cells widely distributed in the body, accompanied by diverse phenotypes and functions. Committed mast cell precursors

(MCPs) leave the bone marrow and enter the blood circulation, homing to peripheral sites under the control of various molecules from different microenvironments, where they eventually differentiate and mature. Partly attributable to the unique maturation mechanism, MCs display high functional heterogeneity and potentially plastic phenotypes. High plasticity also means that MCs can exhibit different subtypes to cope with different microenvironments, which we call "the peripheral immune education system". Under the peripheral immune education system, MCs showed a new character from previous cognition in some cases, namely regulation of allergy and inflammation. In this review, we focus on the mucosal tissues, such as the gastrointestinal tract, to gain insights into the mechanism underlying the migration of MCs to the gut or other organs and their heterogeneity, which is driven by different microenvironments. In particular, the immunosuppressive properties of MCs let us consider that positively utilizing MCs may be a new way to overcome inflammatory and allergic disorders.

Intestinal homeostasis and inflammation: gut microbiota at the crossroads of pancreas–intestinal barrier axis

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The pancreas contains exocrine glands, which release enzymes (e.g., amylase, trypsin, and lipase) that are important for digestion, and islets, which produce hormones. Digestive enzymes and hormones are secreted from the pancreas into the duodenum and bloodstream, respectively. Growing evidence suggests that the roles of pancreas extend to not only the secretion of digestive enzymes and hormones but also to the regulation of intestinal homeostasis and inflammation (e.g., mucosal defense to pathogens and pathobionts). Organ crosstalk between the pancreas and intestine is linked to a range of physiological, immunological, and pathological activities, such as the regulation of the gut microbiota by the pancreatic proteins and lipids, the retroaction of the gut microbiota on the pancreas, the relationship between inflammatory bowel disease, and pancreatic diseases. Thus, the pancreas–intestinal barrier axis and the control of commensal bacteria in intestinal inflammation need to be further elucidated.

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Center for Gene and Cell Therapy

Division of Molecular and Medical Genetics
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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, and promote the advantage of molecular pathological analysis, genetic diagnosis, and gene/cell therapy, based on the development of basic technologies for gene and cell therapy, aiming to conduct comprehensive translational research on personalized genomic medicine.

Advanced methods for AAV vector preparation and purification for *in vivo* gene therapy

Toward full-scale commercialization of AAV vectors, which are expected to be highly safe and capable of long-term expression, as products for gene therapy, we are promoting the establishing of basic technologies for the development, production and purification of novel mutants. We have developed functionally enhanced cells as host cells necessary for vector production and are investigating expression culture methods using bioreactors and analyzing virus particles secreted in the cell culture supernatant. Regarding purification methods, in addition to the development of new ultracentrifugation technology, we are promoting process development that combines ultrafiltration, ammonium sulfate precipitation, ion exchange, gel filtration, and hydrophobic chromatography according to the purpose. In order to stably produce high-quality vectors with little contamination of intermediates and empty particles, we utilize analytical ultracentrifuges and cryo-electron microscopes, and promote the development of next-generation analytical technologies that reflect trends in FDA and domestic regulatory guidelines. In addition, as a technology related to AAV vectors, we are developing non-viral drug delivery system (DDS) by applying empty particles that do not contain viral genomes. While proceeding with functional analysis of novel capsid mutants, we have developed a technology for encapsulating or binding plasmids, DNA fragments, and artificial nucleic acids into empty particles, and are proposing its application to nucleic acid medicine.

Molecular pathophysiological analysis and treatment development for rare intractable diseases

We are developing treatments for intractable neuromuscular diseases by applying vector-related technologies to gene and cell therapy. To develop a treatment for Duchenne muscular dystrophy, for which there is no fundamental treatment, we are verifying the safety and efficacy of small dystrophin and the method of sustained gene expression by immune tolerance induction treatment using mouse and dog disease model animals and primates.

Development of treatment methods using somatic stem cells

Since stem cell therapy or a combination of gene therapy and stem cell therapy may be appropriate depending on the disease state and genetic predisposition, we are promoting the development of cell therapy technology in parallel with gene therapy. We have demonstrated the safety and efficacy of stem cell therapy for Duchenne muscular dystrophy and acute cerebral infarction in disease model animals and are promoting efforts toward clinical trials.

Search for biomarkers

In order to search for biomarkers required for pathological evaluation in clinical trials, we are conducing comprehensive analysis using model animals of Duchenne muscular dystrophy and identifying many candidate substances. We aim for practical application by combining clinical research with clinical trials of gene and cell therapy.

Development of next-generation AAV vaccine

Taking this opportunity of SARS-CoV-2, in order to deal with infectious diseases that may become a threat in the future, the Japan Agency for Medical Research and Development (AMED) has launched the Center for Advanced Research and Development Strategy (SCARDA). The vaccine/new modality R&D project and the world top level R&D base (Flagship Base) formation project have started, and we are also conducting research within that framework. By applying our unique AAV vector-related technology as a "next-generation AAV vaccine modality (drug discovery method)", we aim to commercialize vaccines against various infectious diseases and neurodegenerative diseases that are both safe and effective. AAV vectors are expected to be excellent vaccine modalities due to their stability and high immunity acquisition effect. Furthermore, we will develop an efficient gene expression method that avoids neutralizing antibodies and promote the development of new nucleic acid vaccines. In the operation of a small-scale GMP manufacturing facility capable of manufacturing investigational viral vector vaccines, during normal times, it operates as a facility for researching and manufacturing vectors for gene therapy, accumulating the latest technology and evidence, and in the event of an infectious disease, we will promote operation as a rapid and flexible early vaccine production facility.

Gene and cell therapy for cancer

Utilizing stem cells that have the property of accumulating in cancer, we have developed vector-producing tumor target cells. Systemically administered cells accumulate in cancer. Furthermore, since the therapeutic gene is then amplified, it is expected to be effective in treating infiltrating lesions and metastatic foci, for which conventional anticancer drug therapy and radiotherapy are not effective.

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We are currently working on the development of gene and cell therapies for intractable cancers and chronic diseases. Specifically, we are conducting development research into T-lymphocyte therapy for post-transplant viral infections, mesenchymal cell and hematopoietic stem cells gene therapy, as well as oncolytic virus therapy, genetically engineered T-lymphocyte therapy, and adeno-associated virus vectors for gene therapy of neurodegenerative diseases and haemophilia.

Application of adeno-associated virus (AAV) vector for muscular dystrophy

Among muscular dystrophies, there are some that develop due to abnormalities in glycan metabolism, such as Fukuyama muscular dystrophy, which is the second most common childhood muscular dystrophy in Japan. Abnormalities in isoprenoid synthase domain-containing protein (ISPD), a synthase of CDP-ribitol required for biosynthesis of glycans and glycosylation, were found to be the cause of the disease by the use of an ISPD-deficient mice model. It was also discovered that ISPD gene therapy using an AAV vector in this mouse model could suppress the progression of the disease. As there are very few examples of drug development research targeting the glycan biosynthetic pathway, this discovery is a breakthrough in the development of treatments for diseases caused by glycosylation abnormalities. This is therefore expected to make a major contribution to the development of treatments for rare intractable diseases such as muscular dystrophy and congenital glycan deficiency.

Development of an oncolytic virus therapy for malignant glioma

Clinical development of G47Δ, a herpes simplex virus type 1 containing three engineered viral genes for 3rd-generation cancer treatment has progressed. In a phase II physician-initiated clinical trial conducted at the University of Tokyo Institute of Medical Science Hospital in patients with glioblastoma, G47Δ demonstrated high therapeutic efficacy and safety as a treatment of malignant glioma. Based on the results of this phase II trial G47Δ received approval for manufacturing and marketing as a therapeutic option against malignant glioma. Cancer viral therapy is a method that directly destroys cancer cells by the use of viruses and is expected to be an innovative method of cancer treatment. G47Δ is the first virotherapy product to be put into practical use in Japan and the first virotherapy product for brain tumors in developed countries in the world.

CAR-T cell therapy for solid cancer

PRIME CAR-T cells, next-generation immune

function modulating chimeric antigen receptor-expressing T cells with extremely high therapeutic efficacy against solid tumors, have been developed. CAR-T cell therapy, in which Chimeric Antigen Receptor (CAR) gene transfection is used to produce T cells that have enhanced reactivity to cancer, is attracting attention as an extremely promising new technology. While this CAR-T cell therapy has shown remarkable therapeutic effects against hematological cancers, including leukemia, malignant lymphoma, etc., it has so far been considered ineffective against solid cancers, including gastric cancer, lung cancer, colon cancer, breast cancer, etc., which account for most cancers. Therefore, in order to add the ability to control immune function to the CART-T cells, newly developed cells were engineered with the ability to simultaneously produce both a cytokine, IL-7 and a chemokine, CCL-19. These new CART-T cells were shown induce marked infiltration of T cells and dendritic cells into the tumor and synergistically exert an extremely potent anti-cancer effect in cooperation with the host T cells. In addition, it was shown that mice treated with these CAR-T cells also developed long-term immune memory against cancer, making it possible to prevent cancer recurrence. While conventional CAR-T cells have attracted only attention for their ability to kill cancer cells directly, PRIME CAR-T cells also serve as a "delivery system" for molecules that control the body's own immune function. The PRIME CAR-T cell technology will lead to a ground-breaking treatment for solid tumors that expands the scope of CAR-T cell therapy, which has only been effective against blood cancers.

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Laboratory Animal Research Center

Division of Animal Genetics
先進動物ゲノム研究分野

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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 science 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.

Characterization of several severe combined immunodeficiency rats for humanized models

Ryuya Iida, Yoshiki Miyasaka[1], Kousuke Hattori, Alejandro Soto-Gutierrez[2], Kazuki Takeishi[3], Kazuto Yoshimi, Saeko Ishida, and Tomoji Mashimo

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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.

Developing the next generation of CAR-T cells with CRISPR-Cas3 technology

Tomoaki Fujii, Koji Asano, Kazuto Yoshimi, and Tomoji Mashimo

Chimeric Antigen Receptor (CAR)-T cell therapy is promising cancer immunotherapy. Conventionally,

CAR-T cells are produced from autologous T cells, but this can be high costs, long manufacturing periods, and difficulties in ensuring uniform quality of cell sources. We reported that genome editing using CRISPR-Cas3 system is possible in human cells. The novel genomic editing system can produce fewer off-target and mosaic mutations compared to CRISPR-Cas9, which is the most widely used genome editing tools. Our research aims are to use CRISPR-Cas3 to generate a safe and effective CAR-T therapy. To overcome the limitations of producing 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 and we found that there were no off-target mutations observed in its cells. In addition, this system can also generate targeted deletions of the target genes in human primary T cells. These results suggest that the CRISPR-Cas3 system could be a powerful genetic tool for generating allogenic CAR-T cells.

Generation of several genetically engineered mice and rat models via genome editing technologies

Kosuke Hattori, Yuko Yamauchi, Natsuki Matsushita[1], Kazuto Kobayashi[2], Kazuto Yoshimi, Tomoji Mashimo

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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 developed the two KI strategies with CRISPR/Cas9 for the large genomic regions in rodents. One is the long single strand DNA (lssDNA)-mediated KI method. Microinjection and electroporation of originally synthesized lssDNAs with gRNA and Cas9 mRNA could produce several types of KI mice and rats with a good efficiency such as GFP-tagging, floxed and repeat sequence replacement. In addition, we have also 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 mice and rats via these genome editing strategies with several collaborators.

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Laboratory Animal Research Center

Animal Center
動物センター

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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 20,095 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 geneticall-engineered mice, biohazardous experiments area and equipment room which has X-ray Irradiator, MRI, CT and IVIS imaging system. In 2022, 498 researchers from 43 laboratories are engaged in this facility with about 20,095 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 2022, 44strains of embryos and 43 strains of sperm were stored, and 113 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
Visiting Associate Professor	Takeshi Annoura, Ph.D.		客員准教授	医学博士	案　浦　　健
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

Squirrel monkeys (*Saimiri boliviensis*) and 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 well known as the best candidates for malaria model in primates. In our 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 sexes. Their gestation period is about 130 days. Ten newborns were given in reproductive groups of squirrel monkeys in 2022, 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. The construction work to install a large cage unit is under its way in our squirrel monkey room, and is scheduled to be completed by the end of March 2023.

Research using non-human primates

Notable aspect of our laboratory is the unique International Joint Usage and Research Center capability of conducting infection experiment using squirrel monkeys, owl monkeys, and cynomolgus monkeys. Our laboratory is currently in the process of renovating the 3rd building equipped with BSL3 animal experimental rooms, which allows for experiments on mosquito-borne infectious diseases in primates (scheduled to be completed next spring or next summer). Our BSL3 animal experimental rooms still await completion, however, experimental rooms up to BSL2 for infection experiments in primates have completed renovation 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.

Research on the control of snakebite envenoming

Snakebite envenoming is still a serious health problem in many tropical and subtropical countries. It was recognized by the World Health Organization (WHO) as a neglected tropical disease in 2009, and was elevated into Category A of the Neglected Tropical Diseases list in 2017. Amami laboratory used to be an important facility for research and development of antivenom serum for Habu (*Protobothrops flavoviridis*), which is a species endemic to Japan. Even now, we are continuing research aimed to elucidate the detail

components of Habu venom through genome analysis, etc. and that will contribute to control of snakebite envenoming in the world.

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
Project Professor	Kouhei Tsumoto, Ph.D.	特任教授	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	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. Integrative analysis of cancer cell signaling networks by high-resolution proteomics and systems biology

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.

1-1. 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.

1-2. 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.

1-3. 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, Kouhei Tsumoto, Jun-ichiro Inoue, Tetsu Akiyama and Masaaki Oyama.

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.

1-4. System-level analysis of CagA-dependent signaling network dynamics by Helicobacter pylori infection

Hiroko Kozuka-Hata, Masato Suzuki, Kotaro Kiga, Shinya Tasaki, Jun-ichiro Inoue, Tadashi Yamamoto, Chihiro Sasakawa and Masaaki Oyama.

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 analyzing the regulatory mechanisms at the system-level.

2. Mass spectrometry-based annotation of the human short ORFeome

Masaaki Oyama, Hiroko Kozuka-Hata, Sumio Sugano, Tadashi Yamamoto and Jun-ichiro Inoue.

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).

3. Shotgun proteomics deciphered age/division of labor-related functional specification of three honeybee (*Apis mellifera* L.) exocrine glands

Toshiyuki Fujita, Hiroko Kozuka-Hata, Yutaro Hori, Jun Takeuchi, Takeo Kubo and Masaaki Oyama.

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.

4. 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 Ultimate3000 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.

5. Stress-dependent cell stiffening by tardigrade tolerance proteins that reversibly form a filamentous network and gel

Akihiro Tanaka, Tomomi Nakano, Kento Watanabe, Kazutoshi Masuda, Gen Honda, Shuichi Kamata, Reitaro Yasui, Hiroko Kozuka-Hata, Chiho Watanabe, Takumi Chinen, Daiju Kitagawa, Satoshi Sawai, Masaaki Oyama, Miho Yanagisawa and Takekazu Kunieda.

Tardigrades are able to tolerate almost complete dehydration by entering a reversible ametabolic state called anhydrobiosis and resume their animation upon rehydration. Dehydrated tardigrades are exceptionally stable and withstand various physical extremes. Although trehalose and late embryogenesis abundant (LEA) proteins have been extensively studied as potent protectants against dehydration in other anhydrobiotic organisms, tardigrades produce high amounts of tardigrade-unique protective proteins. Cytoplasmic-abundant heat-soluble (CAHS) proteins are uniquely invented in the lineage of eutardigrades, a major class of the phylum Tardigrada and are essential for their anhydrobiotic survival. However, the precise mechanisms of their action in this protective role are not fully understood. In the present study, we first postulated the presence of tolerance proteins that form protective condensates via phase separation in a stress-dependent manner and searched for tardigrade proteins that reversibly form condensates upon dehydration-like stress. Through a comprehensive search using a desolvating agent, trifluoroethanol (TFE), we identified 336 proteins, collectively dubbed "TFE-Dependent ReversiblY condensing Proteins (T-DRYPs)." Unexpectedly, we rediscovered CAHS proteins as highly enriched in T-DRYPs, 3 of which were major components of T-DRYPs. We revealed that these CAHS proteins reversibly polymerize into many cytoskeleton-like filaments depending on hyperosmotic stress in cultured cells and undergo reversible gel-transition in vitro. Furthermore, CAHS proteins increased cell stiffness in a hyperosmotic stress-dependent manner and counteract the cell shrinkage caused by osmotic pressure, and even improved the survival against hyperosmotic stress. The conserved putative helical C-terminal region is necessary and sufficient for filament formation by CAHS proteins, and mutations disrupting the secondary structure of this region impaired both the filament formation and the gel transition. On the basis of these results, we propose that CAHS proteins are novel cytoskeleton-like proteins that form filamentous networks and undergo gel-transition in a stress-dependent manner to provide on-demand physical stabilization of cell integrity against deformative forces during dehydration and could contribute to the exceptional physical stability in a dehydrated state.

<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.

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1. Current advances in biopharmaceutical informatics: guidelines, impact and challenges in the computational developability assessment of antibody therapeutics

Khetan R, Curtis R, Deane CM, Hadsund JT, Kar U, Krawczyk K, Kuroda D, Robinson SA, Sormanni P, Tsumoto K, Warwicker J and Martin ACR.

Therapeutic monoclonal antibodies and their derivatives are key components of clinical pipelines in the global biopharmaceutical industry. The availabil-

ity of large datasets of antibody sequences, structures, and biophysical properties is increasingly enabling the development of predictive models and computational tools for the "developability assessment" of antibody drug candidates. Here, we provide an overview of the antibody informatics tools applicable to the prediction of developability issues such as stability, aggregation, immunogenicity, and chemical degradation. We further evaluate the opportunities and challenges of using biopharmaceutical informatics for drug discovery and optimization. Finally, we discuss the potential of developability guidelines based on *in silico* metrics that can be used for the assessment of antibody stability and manufacturability.

2. 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.

3. Development of an Outward Proton Pumping Rhodopsin with a New Record in Thermostability by Means of Amino Acid Mutations

Yasuda S, Akiyama T, Kojima K, Ueta T, Hayashi T, Ogasawara S, Nagatoishi S, Tsumoto K, Kunishima N, Sudo Y, Kinoshita M and Murata T.

We have developed a methodology for identifying further thermostabilizing mutations for an intrinsically thermostable membrane protein. The methodology comprises the following steps: (1) identifying thermostabilizing single mutations (TSSMs) for residues in the transmembrane region using our physics-based method; (2) identifying TSSMs for residues in the extracellular and intracellular regions, which are in aqueous environment, using an empirical force field FoldX; and (3) combining the TSSMs identified in steps (1) and (2) to construct multiple mutations. The methodology is illustrated for thermophilic rhodopsin whose apparent midpoint temperature of thermal denaturation Tm is ~91.8 °C. The TSSMs previously identified in step (1) were F90K, F90R, and Y91I with ΔTm ~5.6, ~5.5, and ~2.9 °C, respectively, and those in step (2) were V79K, T114D, A115P, and A116E with ΔTm ~2.7, ~4.2, ~2.6, and ~2.3 °C, respectively (ΔTm denotes the increase in Tm). In this study, we construct triple and quadruple mutants, F90K + Y91I + T114D and F90K + Y91I + V79K + T114D. The values of ΔTm for these multiple mutants are ~11.4 and ~13.5 °C, respectively. Tm of the quadruple mutant (~105.3 °C) establishes a new record in a class of outward proton pumping rhodopsins. It is higher than Tm of Rubrobacter xylanophilus rhodopsin (~100.8 °C) that was the most thermostable in the class before this study.

4. Development of a high-throughput method to screen novel antiviral materials

Nakakido M, Tanaka N, Shimojo A, Miyamae N and Tsumoto K.

Respiratory infectious diseases pose a serious threat worldwide, and novel antiviral materials are highly demanded. Photocatalytic nanoparticles have been developed to inhibit indirect transmission of pathogens by acting as surface coating materials. During development of such antiviral materials, researchers use bacteriophages as model viruses due to their safety and experimental efficiency. Screening methods are used to identify potential antiviral materials, and better screening technologies will accelerate the discovery of antiviral treatments. In this study, we constructed a novel platform to evaluate antiviral activity of surface coating materials using the M13 bacteriophage and phagemid system derived from phage display technology. The evaluation results generated by this system for the two tested antiviral materials were comparable to those for the materials tested on the Qβ bacteriophage and influenza virus using traditional screening methods. The experimental system developed in this study provides rapid and effective screening and can be applied to the development of novel antiviral materials.

5. Oligo(N-methylalanine) as a Peptide-Based Molecular Scaffold with a Minimal Structure and High Density of Functionalizable Sites

Yokomine M, Morimoto J, Fukuda Y, Shiratori Y, Kuroda D, Ueda T, Takeuchi K, Tsumoto K and Sando S.

Functionalizable synthetic molecules with nanometer sizes and defined shapes in water are useful as molecular scaffolds to mimic the functions of biomacromolecules and develop chemical tools for manipulating biomacromolecules. Herein, we propose oligo(N-methylalanine) (oligo-NMA) as a peptide-based molecular scaffold with a minimal structure and a high density of functionalizable sites. Oligo-NMA forms a defined shape in water without hydrogen-bonding networks or ring constraints, which enables the molecule to act as a scaffold with minimal atomic composition. Furthermore, functional groups can be readily introduced on the nitrogens and α-carbons of oligo-NMA. Computational and NMR spectroscopic analysis suggested that the backbone structure of oligo-NMA is not largely affected by functionalization. Moreover, the usefulness of oligo-NMA was demonstrated by the design of protein ligands. The ease of synthesis, minimal structure, and high functionalization flexibility makes oligo-NMA a useful scaffold for chemical and biological applications.

6. Biophysical Characterization of the Contribution of the Fab Region to the IgG-FcγRIIIa Interaction

Kosuge H, Nagatoishi S, Kiyoshi M, Ishii-Watabe A, Terao Y, Ide T and Tsumoto K.

The cell-surface receptor FcγRIIIa is crucial to the efficacy of therapeutic antibodies as well as the immune response. The interaction of the Fc region of IgG molecules with FcγRIIIa has been characterized, but until recently, it was thought that the Fab regions were not involved in the interaction. To evaluate the influence of the Fab regions in a biophysical context, we carried out surface plasmon resonance analyses using recombinant FcγRIIIa ligands. A van't Hoff analysis revealed that compared to the interaction of the papain-digested Fc fragment with FcγRIIIa, the interaction of commercially available, full-length rituximab with FcγRIIIa had a more favorable binding enthalpy, a less favorable binding entropy, and a slower off rate. Similar results were obtained from analyses of IgG1 molecules and an IgG1-Fc fragment produced by Expi293 cells. For further validation, we also prepared a maltose-binding protein-linked IgG1-Fc fragment (MBP-Fc). The binding enthalpy of MBP-Fc was nearly equal to that of the IgG1-Fc fragment for the interaction with FcγRIIIa, indicating that such alternatives to the Fab domains as MBP do not positively contribute to the IgG-FcγRIIIa interactions. Our investigation strongly suggests that the Fab region directly interacts with FcγRIIIa, resulting in an increase in the binding enthalpy and a decrease in the dissociation rate, at the expense of favorable binding entropy.

7. Mirror-image streptavidin with specific binding to L-biotin, the unnatural enantiomer

Suganuma M, Kubo T, Ishiki K, Tanaka K, Suto K, Ejima D, Toyota M, Tsumoto K, Sato T and Nishikawa Y.

The streptavidin-biotin system is known to have a very high affinity and specificity and is widely used in biochemical immunoassays and diagnostics. However, this method is affected by endogenous D-biotin in serum sample measurements (biotin interference). While several efforts using alternative high-affinity binding systems (e.g., genetically modified streptavidin and biotin derivatives) have been attempted, these efforts have all led to reduction in affinity. To solve this interference issue, the enantiomer of streptavidin was synthesized, which enabled specific binding to L-biotin. We successfully obtained a functional streptavidin molecule by peptide synthesis using D-amino acids and an in vitro folding technique. Several characterizations, including size exclusion chromatography (SEC), circular dichroism spectra (CD), and heat denaturation experiments collectively confirmed the higher-order enantiomer of natural streptavidin had been formed with comparable stability to the natural protein. L-biotin specific binding of this novel molecule enabled us to avoid biotin interference in affinity measurements using the Biacore system and enzyme-linked immunosorbent assay (ELISA). We propose the enantiomer of streptavidin as a potential candidate to replace the natural streptavidin-biotin system, even for in vivo use.

8. Experimental Comparison of Bond Lifetime and Viscoelastic Relaxation in Transient Networks with Well-Controlled Structures

Katashima T, Kudo R, Naito M, Nagatoishi S, Miyata K, Chung UI, Tsumoto K and Sakai T.

We demonstrate an experimental comparison of the bond lifetime, estimated using surface plasmon resonance (SPR), and the viscoelastic relaxation time of transient networks with well-controlled structures (dynamically cross-linked Tetra-PEG gel). SPR and viscoelastic measurements revealed that the temperature dependences of the two characteristic times are in agreement, while the viscoelastic response is delayed with respect to the lifetime by a factor of 2-3, dependent on the network strand length. Polymers cross-linked by temporary interactions form transient

networks, which show fascinating viscoelasticity with a single relaxation mode. However, the molecular understanding of such simple viscoelasticity has remained incomplete because of the difficulty of experimentally evaluating bond lifetimes and heterogeneous structures in conventional transient networks. Our results suggest that bond dissociation and recombination both contribute to the macromechanical response. This report on direct bond-lifetime-viscoelastic-relaxation time comparison provides important information for the molecular design of transient network materials.

9. Antibody recognition of complement factor H reveals a flexible loop involved in atypical hemolytic uremic syndrome pathogenesis

Yokoo T, Tanabe A, Yoshida Y, Caaveiro JMM, Nakakido M, Ikeda Y, Fujimura Y, Matsumoto M, Entzminger K, Maruyama T, Okumura CJ, Nangaku M and Tsumoto K.

Atypical hemolytic uremic syndrome (aHUS) is a disease associated with dysregulation of the immune complement system, especially of the alternative pathway (AP). Complement factor H (CFH), consisting of 20 domains called complement control protein (CCP1-20), downregulates the AP as a cofactor for mediating C3 inactivation by complement factor I. However, anomalies related to CFH are known to cause excessive complement activation and cytotoxicity. In aHUS, mutations and the presence of anti-CFH autoantibodies (AAbs) have been reported as plausible causes of CFH dysfunction, and it is known that CFH-related aHUS carries a high probability of end-stage renal disease. Elucidating the detailed functions of CFH at the molecular level will help to understand aHUS pathogenesis. Herein, we used biophysical data to reveal that a heavy-chain antibody fragment, termed VHH4, recognized CFH with high affinity. Hemolytic assays also indicated that VHH4 disrupted the protective function of CFH on sheep erythrocytes. Furthermore, X-ray crystallography revealed that VHH4 recognized the Leu1181-Leu1189CCP20 loop, a known anti-CFH AAbs epitope. We next analyzed the dynamics of the C-terminal region of CFH and showed that the epitopes recognized by anti-CFH AAbs and VHH4 were the most flexible regions in CCP18-20. Finally, we conducted mutation analyses to elucidate the mechanism of VHH4 recognition of CFH and revealed that VHH4 inserts the Trp1183C-CP20 residue of CFH into the pocket formed by the complementary determining region 3 loop. These results suggested that anti-CFH AAbs may adopt a similar molecular mechanism to recognize the flexible loop of Leu1181-Leu1189CCP20, leading to aHUS pathogenesis.

10. Structure and role of the linker domain of the iron surface-determinant protein IsdH in heme transportation in Staphylococcus aureus

Valenciano-Bellido S, Caaveiro JMM, Morante K, Sushko T, Nakakido M, Nagatoishi S and Tsumoto K.

Staphylococcus aureus is a major cause of deadly nosocomial infections, a severe problem fueled by the steady increase of resistant bacteria. The iron surface determinant (Isd) system is a family of proteins that acquire nutritional iron from the host organism, helping the bacterium to proliferate during infection, and therefore represents a promising antibacterial target. In particular, the surface protein IsdH captures hemoglobin (Hb) and acquires the heme moiety containing the iron atom. Structurally, IsdH comprises three distinctive NEAr-iron Transporter (NEAT) domains connected by linker domains. The objective of this study was to characterize the linker region between NEAT2 and NEAT3 from various biophysical viewpoints and thereby advance our understanding of its role in the molecular mechanism of heme extraction. We demonstrate the linker region contributes to the stability of the bound protein, likely influencing the flexibility and orientation of the NEAT3 domain in its interaction with Hb, but only exerts a modest contribution to the affinity of IsdH for heme. Based on these data, we suggest that the flexible nature of the linker facilitates the precise positioning of NEAT3 to acquire heme. In addition, we also found that residues His45 and His89 of Hb located in the heme transfer route toward IsdH do not play a critical role in the transfer rate-determining step. In conclusion, this study clarifies key elements of the mechanism of heme extraction of human Hb by IsdH, providing key insights into the Isd system and other protein systems containing NEAT domains.

11. Addition of arginine hydrochloride and proline to the culture medium enhances recombinant protein expression in Brevibacillus choshinensis: The case of RBD of SARS-CoV-2 spike protein and its antibody

Matsunaga R and Tsumoto K.

Brevibacillus choshinensis is a gram-positive bacterium that is known to efficiently secrete recombinant proteins. However, the expression of these proteins is often difficult depending upon the expressed protein. In this study, we demonstrated that the addition of arginine hydrochloride and proline to the culture medium dramatically increased protein expression. By culturing bacterial cells in 96-well plates, we were able to rapidly examine the expression conditions and easily scale up to 96 mL of culture for production. Although functional expression of the recep-

tor binding domain (RBD) of the SARS-CoV-2 spike protein without any solubility-enhancing tag in bacterial strains (including Escherichia coli) has not been reported to date, we succeeded in efficiently producing RBD which showed a similar CD spectrum to that of RBD produced by eukaryotic cell expression systems. Furthermore, RBD from the omicron variant (B.1.1.529) was also produced. Physicochemical analyses indicated that omicron RBD exhibited markedly increased instability compared to the wild-type. We also revealed that the Fab format of the anti-SARS-CoV-2 antibody C121 can be produced in large quantities using the same expression system. The obtained C121 Fab bound to wild-type RBD but not to omicron RBD. These results strongly suggest that the Brevibacillus expression system is useful for facilitating the efficient expression of proteins that are difficult to fold and will thus contribute to the rapid physicochemical evaluation of functional proteins.

12. Ladder observation of bovine serum albumin by high resolution agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Nagatoishi S, Tsumoto K, Arakawa T and Akuta T.

A commercially available bovine serum albumin (BSA) was examined by agarose native gel electrophoresis using two different agarose sources, UltraPure and MetaPhor agarose. While UltraPure agarose up to 5 % showed no clear separation of BSA oligomers, MetaPhor agarose clearly demonstrated oligomer bands above 4 %, indicating that the latter agarose has greater molecular sieving effects and is hence characterized to have high resolution for size differences, as probed by a greater slope of Ferguson plot. Physical properties are different between two agaroses. In general, UltraPure agarose has physical strength, while MetaPhor agarose is considerably fragile, but MetaPhor agarose solution is less viscous so that even 10 % gel can be made. Cause of oligomers was shown to be not associated with inter-chain disulfide bonds, but is due to association of native or native-like molecules.

13. Correction: Antibody recognition of complement factor H reveals a flexible loop involved in atypical hemolytic uremic syndrome pathogenesis

Yokoo T, Tanabe A, Yoshida Y, Caaveiro JMM, Nakakido M, Ikeda Y, Fujimura Y, Matsumoto M, Entzminger K, Maruyama T, Okumura CJ, Nangaku M and Tsumoto K.

No abstract available

14. Residue-based program of a β-peptoid twisted strand shape via a cyclopentane constraint

Kim J, Kobayashi H, Yokomine M, Shiratori Y, Ueda T, Takeuchi K, Umezawa K, Kuroda D, Tsumoto K, Morimoto J and Sando S.

N-Substituted peptides, such as peptoids and β-peptoids, have been reported to have unique structures with diverse functions, like catalysis and manipulation of biomolecular functions. Recently, the preorganization of monomer shape by restricting bond rotations about all backbone dihedral angles has been demonstrated to be useful for de novo design of peptoid structures. Such design strategies are hitherto unexplored for β-peptoids; to date, no preorganized β-peptoid monomers have been reported. Here, we report the first design strategy for β-peptoids, in which all four backbone dihedral angles (ω, ϕ, θ, ψ) are rotationally restricted on a per-residue basis. The introduction of a cyclopentane constraint realized the preorganized monomer structure and led to a β-peptoid with a stable twisted strand shape.

15. Nucleic acid-triggered tumoral immunity propagates pH-selective therapeutic antibodies through tumor-driven epitope spreading

Furuya G, Katoh H, Atsumi S, Hashimoto I, Komura D, Hatanaka R, Senga S, Hayashi S, Akita S, Matsumura H, Miura A, Mita H, Nakakido M, Nagatoishi S, Sugiyama A, Suzuki R, Konishi H, Yamamoto A, Abe H, Hiraoka N, Aoki K, Kato Y, Seto Y, Yoshimura C, Miyadera K, Tsumoto K, Ushiku T and Ishikawa S.

Important roles of humoral tumor immunity are often pointed out; however, precise profiles of dominant antigens and developmental mechanisms remain elusive. We systematically investigated the humoral antigens of dominant intratumor immunoglobulin clones found in human cancers. We found that approximately half of the corresponding antigens were restricted to strongly and densely negatively charged polymers, resulting in simultaneous reactivities of the antibodies to both densely sulfated glycosaminoglycans (dsGAGs) and nucleic acids (NAs). These anti-dsGAG/NA antibodies matured and expanded via intratumoral immunological driving force of innate immunity via NAs. These human cancer-derived antibodies exhibited acidic pH-selective affinity across both antigens and showed specific reactivity to diverse spectrums of human tumor cells. The antibody-drug conjugate exerted therapeutic effects against multiple cancers in vivo by targeting cell surface dsGAG antigens. This study reveals that intratumoral immunological reactions propagate tumor-oriented immunoglobulin clones and demonstrates a new therapeutic modality for the universal treatment of human malig-

nancies.

16. PRELP Regulates Cell-Cell Adhesion and EMT and Inhibits Retinoblastoma Progression

Hopkins J, Asada K, Leung A, Papadaki V, Davaapil H, Morrison M, Orita T, Sekido R, Kosuge H, Reddy MA, Kimura K, Mitani A, Tsumoto K, Hamamoto R, Sagoo MS and Ohnuma SI.

Retinoblastoma (RB) is the most common intraocular pediatric cancer. Nearly all cases of RB are associated with mutations compromising the function of the RB1 tumor suppressor gene. We previously demonstrated that PRELP is widely downregulated in various cancers and our in vivo and in vitro analysis revealed PRELP as a novel tumor suppressor and regulator of EMT. In addition, PRELP is located at chromosome 1q31.1, around a region hypothesized to be associated with the initiation of malignancy in RB. Therefore, in this study, we investigated the role of PRELP in RB through in vitro analysis and next-generation sequencing. Immunostaining revealed that PRELP is expressed in Müller glial cells in the retina. mRNA expression profiling of PRELP-/- mouse retina and PRELP-treated RB cells found that PRELP contributes to RB progression via regulation of the cancer microenvironment, in which loss of PRELP reduces cell-cell adhesion and facilitates EMT. Our observations suggest that PRELP may have potential as a new strategy for RB treatment.

17. Analysis of bovine serum albumin unfolding in the absence and presence of ATP by SYPRO Orange staining of agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Kurosawa Y, Nagatoishi S, Tsumoto K, Arakawa T and Akuta T.

An attempt was made to specifically stain unfolded proteins on agarose native gels. SYPRO Orange is routinely used to detect unfolded protein in differential scanning fluorimetry, which is based on the enhanced fluorescence intensity upon binding to the unfolded protein. We demonstrated that this dye barely bound to the native proteins, resulting in no or faint staining of the native bands, but bound to and stained the unfolded proteins, on agarose native gels. Using bovine serum albumin (BSA), it was shown that staining did not depend on whether BSA was thermally unfolded in the presence of SYPRO Orange or stained after electrophoresis. On the contrary, SYPRO Orange dye stained protein bands in the presence of sodium dodecylsulfate (SDS) due to incorporation of the dye into SDS micelles that bound to the unfolded proteins. This staining resulted in detection of new, intermediately unfolded structure of BSA during thermal unfolding. Such intermediate structure occurred at higher temperature in the presence of ATP.

18. Human antibody recognition and neutralization mode on the NTD and RBD domains of SARS-CoV-2 spike protein

Otsubo R, Minamitani T, Kobiyama K, Fujita J, Ito T, Ueno S, Anzai I, Tanino H, Aoyama H, Matsuura Y, Namba K, Imadome KI, Ishii KJ, Tsumoto K, Kamitani W and Yasui T.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). Variants of concern (VOCs) such as Delta and Omicron have developed, which continue to spread the pandemic. It has been reported that these VOCs reduce vaccine efficacy and evade many neutralizing monoclonal antibodies (mAbs) that target the receptor binding domain (RBD) of the glycosylated spike (S) protein, which consists of the S1 and S2 subunits. Therefore, identification of optimal target regions is required to obtain neutralizing antibodies that can counter VOCs. Such regions have not been identified to date. We obtained 2 mAbs, NIBIC-71 and 7G7, using peripheral blood mononuclear cells derived from volunteers who recovered from COVID-19. Both mAbs had neutralizing activity against wild-type SARS-CoV-2 and Delta, but not Omicron. NIBIC-71 binds to the RBD, whereas 7G7 recognizes the N-terminal domain of the S1. In particular, 7G7 inhibited S1/S2 cleavage but not the interaction between the S protein and angiotensin-converting enzyme 2; it suppressed viral entry. Thus, the efficacy of a neutralizing mAb targeting inhibition of S1/2 cleavage was demonstrated. These results suggest that neutralizing mAbs targeting blockade of S1/S2 cleavage are likely to be cross-reactive against various VOCs.

19. Molecular basis for thermal stability and affinity in a VHH: Contribution of the framework region and its influence in the conformation of the CDR3

Kinoshita S, Nakakido M, Mori C, Kuroda D, Caaveiro JMM and Tsumoto K.

The camelid single domain antibody, referred to VHH or Nanobody, is considered a versatile tool for various biotechnological and clinical applications because of its favorable biophysical properties. To take advantage of these characteristics and for its application in biotechnology and therapy, research on VHH engineering is currently vigorously conducted. To humanize a camelid VHH, we performed complementarity determining region (CDR) grafting using a humanized VHH currently in clinical trials, and investigated the effects of these changes on the biophysical properties of the resulting VHH. The chimeric

VHH exhibited a significant decrease in affinity and thermal stability and a large conformational change in the CDR3. To elucidate the molecular basis for these changes, we performed mutational analyses on the framework regions revealing the contribution of individual residues within the framework region. It is demonstrated that the mutations resulted in the loss of affinity and lower thermal stability, revealing the significance of bulky residues in the vicinity of the CDR3, and the importance of intramolecular interactions between the CDR3 and the framework-2 region. Subsequently, we performed back-mutational analyses on the chimeric VHH. Back-mutations resulted in an increase of the thermal stability and affinity. These data suggested that back-mutations restored the intramolecular interactions, and proper positioning and/or dynamics of the CDR3, resulting in the gain of thermal stability and affinity. These observations revealed the molecular contribution of the framework region on VHHs and further designability of the framework region of VHHs without modifying the CDRs.

20. Repression of the PRELP gene is relieved by histone deacetylase inhibitors through acetylation of histone H2B lysine 5 in bladder cancer

Shozu K, Kaneko S, Shinkai N, Dozen A, Kosuge H, Nakakido M, Machino H, Takasawa K, Asada K, Komatsu M, Tsumoto K, Ohnuma SI and Hamamoto R.

Background: Proline/arginine-rich end leucine-rich repeat protein (PRELP) is a member of the small leucine-rich proteoglycan family of extracellular matrix proteins, which is markedly suppressed in the majority of early-stage epithelial cancers and plays a role in regulating the epithelial-mesenchymal transition by altering cell-cell adhesion. Although PRELP is an important factor in the development and progression of bladder cancer, the mechanism of PRELP gene repression remains unclear.

Results: Here, we show that repression of PRELP mRNA expression in bladder cancer cells is alleviated by HDAC inhibitors (HDACi) through histone acetylation. Using ChIP-qPCR analysis, we found that acetylation of lysine residue 5 of histone H2B in the PRELP gene promoter region is a marker for the de-repression of PRELP expression.

Conclusions: These results suggest a mechanism through which HDACi may partially regulate the function of PRELP to suppress the development and progression of bladder cancer. Some HDACi are already in clinical use, and the findings of this study provide a mechanistic basis for further investigation of HDACi-based therapeutic strategies.

Keywords: Bladder cancer; Extracellular matrix proteins; Gene expression; H2BK5ac; HDACi; PRELP.

21. Performance Comparison of Spectral Distance Calculation Methods

Oyama T, Suzuki S, Horiguchi Y, Yamane A, Akao K, Nagamori K and Tsumoto K.

Circular dichroism (CD) spectroscopy is a widely used technique for assessing the higher-order structure (HOS) of biopharmaceuticals, including antibody drugs. Since the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use established quality control guidelines, objective evaluation of spectral similarity has been required in order to assess structural comparability. Several spectral distance quantification methods and weighting functions to increase sensitivity have been proposed, but not many reports have compared their performance for CD spectra. We constructed comparison sets that combine actual spectra and simulated noise and performed a comprehensive performance evaluation of each spectral distance calculation method and weighting function under conditions that consider spectral noise and fluctuations from pipetting errors. The results showed that using the Euclidean distance or Manhattan distance with Savitzky-Golay noise reduction is effective for spectral distance assessment. For the weighting function, it is preferable to combine the spectral intensity weighting function and the noise weighting function. In addition, the introduction of the external stimulus weighting function should be considered to improve the sensitivity. It is crucial to select the weighting function based on the balance between spectral changes and noise distributions for robust, sensitive antibody HOS similarity assessment.

<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 collect 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 microscopy. 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 12 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]Department of Retinal Biology and Pathology, Graduate School of Medicine, The University of Tokyo. 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 work regarding the composition of phospholipids in the photoreceptor cells and photoreceptor degeneration are also performed and revealed that the perturbation of mitochondrial functions in regard with PC synthesis defect are the main cause of photoreceptor death. (ref. Nagata *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. Maeda[7], in [7]Research Organization for Nano and Life In-

novation, Waseda University.

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. Ishikawa[8], [8]Laboratory of Reproductive Systems Biology, about the structure of young and aged mouse oocyte zona pellucida. Scanning electron microscopy are also used in the observation of the surface structure of electrochemically active microorganisms with Dr. Kobayashi[9], [9]Frontier Research Center for Energy and Resource (FRCER), Graduate School of Engineering, The University of Tokyo.

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Research Center for Asian Infectious Diseases
アジア感染症研究拠点

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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 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 conducting a

joint research program on avian influenza virus between Yoshihiro Kawaoka at IMSUT and Hualan Chen at 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 our 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. 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. Furthermore, we found that several compounds inhibit viral entry of SARS-CoV-2. This year, we demonstrated that two existing drugs significantly prevent infection with SARS-CoV-2 mutants, including Omicron, by inhibiting TMPRSS2-dependent cell surface entry and TMPRSS2-independent endosomal entry. These compounds might lead to the development of an antiviral drug against SARS-CoV-2 entry both through the cell surface pathway and the endosomal pathway. On the other hand, SARS-CoV-2 Omicron variant has been recently reported to exhibit decreased usage of the TMPRSS2-mediated cell surface entry pathway and increased usage of the endosomal entry pathway, which might cause altered cell tropism and less tissue damage. In addition, J. Gohda's group previously found that metalloproteinases are specifically involved in SARS-CoV-2 viral cell surface entry in some cell-types. This year, we performed comprehensive analyses of the usage of viral entry pathways, including the third entry pathway mediated by the host metalloproteinases, in Omicron infection. The study using various cell types showed that Omicron displayed the enhanced endosome entry and the reduced metalloproteinase-dependent as well as TMPRSS2-dependent cell surface entry. Furthermore, we showed that Omicron's fusogenic activity mediated by metalloproteinases was markedly reduced. We also demonstrated that the H655Y mutation in the Omicron spike determined its relative usage of the three entry pathways. These finding may not only clarify the biological and pathological phenotypes of Omicron, but increase the understanding of disease progression in infections with other SARS-CoV-2 variants.

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 has identified several existing drugs as a new LRA candidate. This year, we tried to clarify the molecular mechanism of re-activation of latent HIV-1 provirus by these drugs. As a result, one of the candidate drugs may re-activate HIV-1 proviral transcription through the different mechanism by which the existing LRAs induce the proviral re-activation. Furthermore, we are currently trying to identify compounds that block the release of HIV-1 viral particles from infected cells to "kill" the reactivated reservoirs by a cytopathic effect through accumulation of cytotoxic viral proteins in the cells without releasing infectious viral particles.

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 major findings this year are: (1) Our routine surveillance in China indicated that the Eurasian avian-like H1N1 (EA H1N1) swine influenza viruses circulated widely in pigs, and obtained different internal genes from different swine influenza viruses, forming various new genotypes. We found that a naturally isolated swine influenza reassortant, A/swine/Liaoning/265/2017, a representative strain of one of the predominant genotypes in recent years, is lethal in mice and transmissible in ferrets due to the acquisition of key mutations in PA. Our study provides important insights for monitoring field strains with pandemic potential. (2) We evaluated the replicative ability and pathogenicity of sublineages BA.1, BA.2, BA.4, and BA.5 of SARS-CoV-2 Omicron variants in wild-type Syrian hamsters, human ACE2 (hACE2) transgenic hamsters, and hACE2 transgenic mice. We observed no obvious differences among BA.1, BA.2, BA.4, and BA.5 isolates in terms of growth ability or pathogenicity in rodent models, but these isolates were less pathogenic than a previously circulating Delta (B.1.617.2 lineage) isolate. In addition, we analyzed the efficacy of antiviral drugs and antibodies against Omicron variants. The susceptibilities of BA.1, BA.2, BA.2.75, BA.4.6, BA.5, BQ.1.1, and XBB to remdesivir, molnupiravir, and nirmatrelvir were similar to those of the ancestral strain and other variants of concern. The effectiveness of monoclonal antibodies (REGN10987–REGN10933, COV2-2196–COV2-2130, and S309) varied with the type of omicron strain. None of the monoclonal antibodies tested was effective against all omicron strains.

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)
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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. Two activating phosphorylation sites of Pbs2 MAP2K in the yeast HOG pathway are differentially dephosphorylated by four PP2C phosphatases Ptc1-Ptc4

Kazuo Tatebayashi

The budding yeast *Saccharomyces cerevisiae* survive greatly fluctuating osmotic conditions in natural environment. To cope with an increased external osmolarity, yeast cells elicit a coordinated adaptive response. These adaptive responses are governed by the Hog1 MAP kinase (MAPK), which is activated via the High Osmolarity Glycerol (HOG) signaling pathway. The HOG pathway consists of a core module of three tiers of protein kinases termed a MAP kinase (MAPK), a MAPK kinase (MAPKK, MAP2K), and a MAPKK kinase (MAPKKK, MAP3K). In addition, the upstream part of the HOG pathway comprises the functionally redundant, but mechanistically distinct, SHO1 and SLN1 branches. When yeast cells are exposed to extracellular high osmolarity, the osmosensors in the SHO1 and SLN1 branches independently detect osmostress to activate cognate MAP3Ks. 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 system activates the functionally redundant MAP3Ks Ssk2 and Ssk22 (Ssk2/22). Activated Ste11 and Ssk2/22 phosphorylate and activate the MAP2K Pbs2. Activated Pbs2, in turn, phosphorylates the MAPK Hog1 at T174 and Y176 in its activation loop for its activation.

Unregulated continuous activation of the HOG pathway is deleterious to cell growth, probably by preventing cell cycle progression. Therefore, a mechanism is needed that appropriately inactivates the HOG pathway. Two groups of the protein phosphatases are involved in the HOG pathway inactivation. The first group contains the members of the protein tyrosine phosphatases (PTP), namely, Ptp2 and Ptp3, which dephosphorylate Hog1 at Y176. The second group contains the members of the serine/threonine protein phosphatase type 2 (PP2C), namely, Ptc1, Ptc2, Ptc3, and Ptc4. Of these, Ptc1, Ptc2, and Ptc3 had been proposed as negative regulators of the HOG pathway, because their overexpression rescued the lethality of the *sln1Δ* cell by inhibiting the constitutive activation of the HOG pathway. Furthermore, overexpression of either Ptc1 or Ptc2 inactivated Hog1 *in vivo*, and purified Ptc1 and Ptc2 dephosphorylated

T174 *in vitro*. In contrast, it was concluded that these phosphatases did not inhibit Pbs2 in vivo, because overexpression of either Ptc1 or Ptc2 did not reduce Hog1 phosphorylation at Y176, which served as an indicator of the Pbs2 activity. Thus, it had been proposed that these type 2C phosphatases inactivate Hog1, but not Pbs2. The phosphatases that dephosphorylate Pbs2 had not been identified yet.

The results of overexpression experiments must be regarded cautiously, because at high level of phosphatase expression, non-physiological substrates might be sufficiently dephosphorylated. For that reason, we consider the gene inactivation experiments more reliable. Furthermore, estimating Pbs2 activity indirectly from the extent of Hog1 phosphorylation might be difficult, as the efficiencies of Pbs2 phosphorylating at Hog1 T174 and Y176 may be significantly different.

This year, we examined the phosphorylation status of Pbs2 at the activating phosphorylation sites Ser-514 and Thr-518 (S514 and T518) in various mutants, both in the unstimulated and osmostressed conditions, using the assay method we developed recently. Thus, we found that Ptc1-Ptc4 collectively regulate Pbs2 negatively, but each Ptc acts differently to the two phosphorylation sites in Pbs2. T518 is predominantly dephosphorylated by Ptc1, whereas the effect of Ptc2-Ptc4 could be seen only when Ptc1 is absent. On the other hand, S514 can be dephosphorylated by any of Ptc1-4 to an appreciable extent. We also show that Pbs2 dephosphorylation by Ptc1 requires the adaptor protein Nbp2 that recruits Ptc1 to Pbs2.

2. A novel multifunctional role for Hsp70 in binding post-translational modifications on client proteins.

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The maintenance of a correctly folded proteome (proteostasis) is critical for cell survival. Cells maintain proteostasis under both basal and stress conditions through the expression of molecular chaperones such as Hsp70 and its associated co-chaperone regulators. Importantly, Hsp70 stabilizes and activates of a wide range of signaling molecules including those involved in processes such as DNA damage response, cell cycle control, autophagy, and nutrient sensing. The Hsp70 client proteins involved in these cellular processes tend to be either highly posttranslationally modified (PTMs) or regulate PTMs on other proteins. In turn, these PTMs tightly regulate a multitude of protein properties including subcellular localization, enzymatic activity, and protein interactions.

This year, we have utilized XL-MS to comprehensively understand the Hsp70 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.

Publication

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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. Clonal progression mechanisms of Myeloproliferative neoplasms using whole genome sequencing across disease stages

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Myeloproliferative tumors have a chronic course in true polycythemia vera and essential thrombocythemia, some of which may progress to myelofibrosis and acute myelogenous leukemia. The genomic aberrant changes that occur in such cases remain to be elucidated. We performed whole-genome sequencing of bone marrow fluid samples from 23 patients with myeloproliferative tumors that had converted from chronic to acute myelogenous leukemia before and after conversion. Almost all of the patients had received JAK inhibitors. The results showed that in most cases, driver mutations found in myeloid tumors appeared as subclones of mutations characteristic of MPNs. The mutation signature showed the same pattern between the chronic phase and acute transformation, suggesting that the mutagenicity was identical for both phases. Interestingly, there were two cas-

es of JAK2 mutation-positive MPNs that progressed to JAK2-negative secondary AML, both with a common parental clone, a result contrary to the conventional hypothesis of secondary AML emerging from completely different clones.

2. Single cell derived colony sequencing of paroxysmal nocturnal hemoglobinuria hematopoiesis reveals trajectory of clone development and clonal selection.

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To explore the mode of clonal expansion in paroxysmal nocturnal hemoglobinuria, we performed whole-genome sequencing of a large number of single-cell-derived hematopoietic cell clones from the bone marrow of two patients with PNH. Using the mutation data of each clone obtained by whole-genome sequencing for each patient, we generated a tree diagram and estimated the timing of mutation acquisition using a Bayesian statistical approach, and found that the early PIGA mutant clones were born decades before clinical PNH occurred. Furthermore, we showed that the acquisition of driver mutations increases the rate of subsequent mutation acquisition. Furthermore, we succeeded in estimating the number of PNH clones from the birth of the patient to the present. The results showed that the number of PNH clones increased slowly, in contrast to the sudden increase in clones in neoplastic diseases such as MDS and MPN. Furthermore, the results refute the conventional clonal selection hypothesis that the mutations found concomitantly in PIGA were present in PNH clones from the early stages of the disease and were acquired late in the disease to contribute to the increase in the number of clones.

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

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Azacitidine is a mainstay of therapy for MDS-related diseases. 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 a total of 449 patients with MDS or related myeloid neoplasms. They were analyzed for gene mutations in pre- ($n = 449$) and post- ($n = 289$) treatment bone marrow samples using targeted-capture sequencing to assess the impact of gene mutations and their post-treatment clone size on treatment outcomes. In Cox proportional hazard modeling, multi-hit *TP53* mutation (HR, 2.03; 95% CI, 1.42-2.91; $P<.001$), *EZH2* mutation (HR, 1.71; 95% CI, 1.14-2.54; $P = .009$), and *DDX41* mutations (HR, 0.33; 95% CI, 0.17-0.62; $P<.001$), together with age, high-risk karyotypes, low platelet, and high blast counts, independently predicted OS. Post-treatment clone size accounting for all drivers significantly correlated with International Working Group (IWG)-response ($P<.001$, trend test), except for that of *DDX41*-mutated clones, which did not predict IWG-response. Combined, IWG-response and post-treatment clone size further improved the prediction of the original model and even that of a recently proposed molecular prediction model, IPSS-M (c-index, 0.653 vs 0.688; $P<.001$, likelihood ratio test). In conclusion, evaluation of post-treatment clone size, together with pre-treatment mutational profile as well as IWG-response have a role in better prognostication of azacitidine-treated myelodysplasia patients.

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IMSUT Hospital

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Founded in 1981, IMSUT hospital started HIV clinic in 1986, and 1308 HIV-infected patients visited us by 2022. Currently, 570 patients in total are actively 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 830 patients admitted to our hospital.

1. Treatment of HIV/AIDS in IMSUT hospital

Statistical characteristics of HIV/AIDS at IMSUT Hospital show that fourteen new patients with HIV infection visited to our hospital this year (from January 1 to December 31, 2022), and 570 patients in total are under medical management in our outpatient clinic. Five hundred fifty-seven people living with HIV (PLWH) are receiving antiretroviral therapy (ART) at the hospital, and most of their plasma HIV viral loads have been well controlled. This is due to the fact that the medication adherence of PLWH visiting our clinic is at an adequate level for HIV suppression. Figure 1 shows the number of PLWH attending IMSUT hospital since 1995.

This year, we completed two international clinical trials: Switch Study to Evaluate Dolutegravir Plus Lamivudine in Virologically Suppressed Human Immunodeficiency Virus Type 1 Positive Adults (TANGO), and Study to Evaluate Efficacy and Safety of Cabotegravir (CAB) Long Acting (LA) Plus (+) Rilpivirine (RPV) LA Versus BIKTARVY® (BIK) in Participants With Human Immunodeficiency Virus (HIV)-1 Who Are Virologically Suppressed (SOLAR), and published the results in the journals and at national and international conferences.

Background factors in people living with HIV in Japan who switch to cabotegravir plus rilpivirine: A pilot study

Eisuke Adachi, Kazuhiko Ikeuchi, Michiko Koga and Hiroshi Yotsuyanagi

Long-acting therapy of cabotegravir and rilpivirine is expected to free people from the negative emotions of living with HIV associated with taking drugs, but problems such as increased number of hospital visits, lack of anti-HBV activity, and limited convenience in people with concomitant drugs have been noted. In this single-center, prospective, cross-sectional study, we investigated background factors of people living with HIV in Japan who chose cabotegravir plus rilpivirine. Forty-seven percent (36 of 76) of individu-

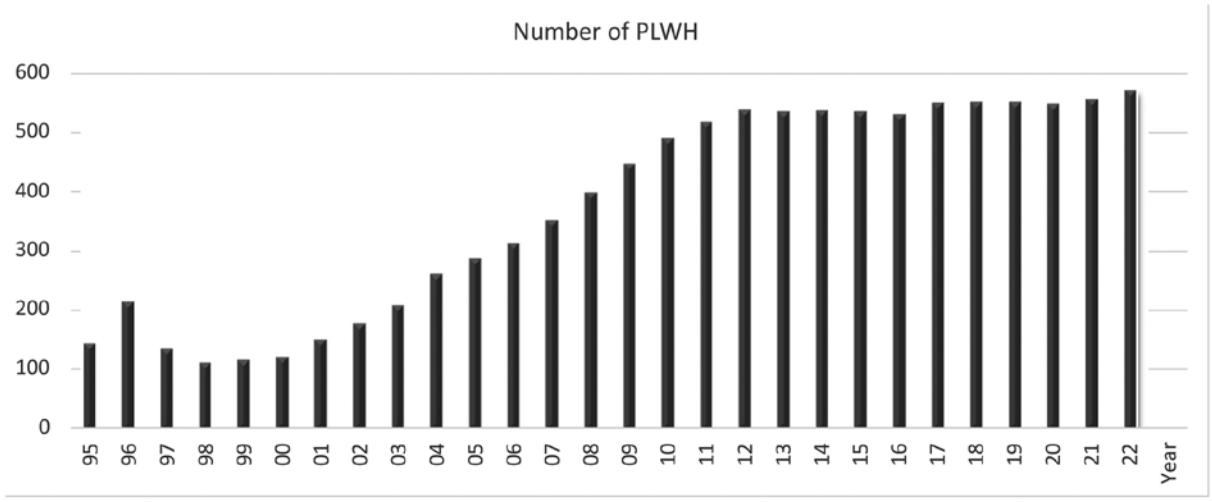

Figure 1. Number of PLWH attending IMSUT Hospital

als chose this regimen, but many people living with HIV who visited the hospital once every 3 months or needed concomitant medications due to complications chose this regimen and there were no significant differences in background factors that could affect convenience between the groups of those who switched and those who did not.

Changes in inflammatory biomarkers when switching from 3-drug regimens to dolutegravir plus lamivudine in people living with HIV

Eisuke Adachi, Kazuhiko Ikeuchi, Michiko Koga and Hiroshi Yotsuyanagi

It is not clear if there is a difference between 3-drug regimens (3DR) and 2-drug regimens (2DR) in terms of suppression of chronic inflammation. We compared CRP, CD4＋/CD8＋ ratio, lipid proliges measured in daily clinical practice before and after the switch to dolutegravir plus lamivudine (DTG/3TC) to examine the difference in the anti-inflammatory effect of 3DR and 2DR.

In this single-center, retrospective, observational study, individuals who were on abacavir/lamivudine/dolutegravir (ABC/3TC/DTG), Tenofovir alafenamide/emtricitabine (TAF/FTC) plus DTG, or bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) prior to switching to DTG/3TC, were eligible. A total of 119 individuals were enrolled in the study. The median (IQR) time since diagnosis of HIV infection was 12 (7-16) years. Overall, inflammation markers such as CD4＋/CD8＋ ratio, CD4＋, CRP and lipid profiles did not change. Analysis of only individuals who switched from ABC/3TC/DTG, TAF-based regimens also showed no significant changes in inflammatory markers.

Since viremia raises inflammatory markers, differences in antiviral efficacy may make a difference in the suppression of chronic inflammation but in conclusion, we did not find any change in inflammatory markers by changing from 3DR to 2DR in daily clinical practice.

2. Treatment of COVID-19 in IMSUT hospital

We started to treat COVID-19 patients at the IMSUT Hospital in February, 2020. To date, the number of patients hospitalized at the request of public health centers, etc., is the second largest among all public university hospitals in Japan. There were 236 hospitalized patients this year, all of whom were infected with the Omicron variant, with the exception of early January. Oxygenation was required for 14.0% of all patients. We participated in several international clinical trials. (e.g., S-217622) and conducted a phase I trial to evaluate the safety, tolerability, and immunogenicity of KM-414 (KM Biologics, Kumamoto, Japan), an inactivated vaccine against SARS-CoV-2 developed by KM biologics and IMSUT (The investigator-initiated clinical trial, phase I trial, investigating the safety and immunogenicity of booster vaccination against COVID-19, jRCT2031210503)

Anti-spike protein antibody titer at the time of breakthrough infection of SARS-CoV-2 omicron

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We investigated here the anti-spike protein antibody titer at the time of breakthrough infection of SARS-CoV-2 omicron. A total of 32 SARS-CoV2 omicron breakthrough infection was included in the study. The median antibody titer at breakthrough infection was 776 AU/mL overall, of which the median antibody titer of BNT162b2 vaccinated was 633 AU/

mL and that of mRNA-1273 vaccinated was 9416 AU/mL. This result suggests that low levels of antibody titers 6 months after vaccination do not provide sufficient antibodies to prevent the omicron variant breakthrough infection, which may occur with a higher anti-spike antibody titer after vaccination with mRNA-1273. However, antibody titers in some patients were comparable to those immediately after the second vaccination with either mRNA vaccine.

3. Pre- and post-travel treatment of imported infectious diseases and tropical diseases at IMSUT Hospital

The pandemic of COVID-19 had unprecedented impact of our life; global transport and travelling was one of the most affected areas. In mid 2022, the number of returnees and travelers consulted gradually increased, and two cases of malaria patients visited the hospital. 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 the medical institution of the research group using these orphan drugs if needed, and colleting clinical data.

4. Treatment of hepatitis in IMSUT hospital:

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 achieved SVR.

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IMSUT Hospital

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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

Satsuki Aochi, Masaaki Uehara, Motohisa Yamamoto

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
- Training of residents and young doctors for rheumatologists
- Clinical trials
- Community medicine

2. Establish of new registry for the patients with IgG4-related disease and development of novel diagnostic and therapeutic approaches for IgG4-related disease

Satsuki Aochi, Masaaki Uehara, Motohisa Yamamoto

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 devel-

op 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

Masaaki Uehara, Motohisa Yamamoto

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

Masaaki Uehara, Motohisa Yamamoto

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.

5. Establishment of pathogenesis and prophylaxis of rheumatic diseases after COVID-19 vaccine

Satsuki Aochi, Masaaki Uehara, Motohisa Yamamoto

The onset of rheumatic diseases after COVID-19 vaccination has attracted much attention in recent years. It has also been experienced that patients with rheumatic diseases suffer exacerbation of the primary condition when receiving the vaccination. For this reason, we are urgently working to elucidate the pathogenesis of this disease and to establish a prophylactic method.

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The department of oncology and general medicine started 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 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., Baba K., Hisjikata Y.

Patients with various, mainly gastrointestinal, cancers were treated by standard therapy including chemotherapy, molecular target agents, immune checkpoint inhibitors, in combination with surgery and radiation therapy. With help of the special patient support team including nurses, pharmacists and nutritionist, the quality and system of patient care during chemotherapy has been improved. The number of chemotherapy cases at outpatient clinic has been increasing (more than 50 patients per month). We are conducting a clinical trial to prevent hand-hoot syndrome in colorectal cancer patients receiving adjuvant chemotherapy with capecitabine plus oxaliplatin, and a prospective observational study to improve our daily activities of patient care. We join in the collaborative study groups (West Japan Oncology Group, Osaka Gastric Cancer Study Group), and we participate in other several multi-center clinical trials and translational research. A phase III trial for approval of ipilimumab plus nivolumab and cytotoxic agents started in our hospital in 2022, and two patients were enrolled. We are now planning an investigator initiated clinical trial based on our previous pharmacokinetic research in collaboration with other hospitals including Tokyo University Hospital. We contribute to the publishing treatment guidelines such as gastric cancer, futility preservation and prevention of chemotherapy induced emesis.

2. Treatment of drug-resistant *Helicobacter pylori* infection and rare gastritis

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 IMSUT, *H. pylori* out-patient clinic, we give eradication therapy for these patients at their own expense, and high rates of successful eradication have been achieved. In addition, we have established effective fourth-line rescue therapy, which is now used for patients who failed with sitafloxacin containing third-line regimen. We also perform clinical studies to unveil the mechanisms of rare 'non-Hp' gastritis, which include autoimmune gastritis and eosinophilic gastroenteritis.

3. Endoscopic examination in IMSUT Hospital (Department of General Medicine)

Matsubara Y., Hirata Y.

About 700 cases of upper gastrointestinal endoscopy and about 700 cases of colonic endoscopy were performed under cooperation with department of surgery from January 1 to December 31, 2022, while examinations were restricted due to covid-19. We have diagnosed relatively rare disease (e.g. infectious disease, malignancy, other disease) in patients with immune dysfunction. We started endoscopic mucosal resection for early gastric and colorectal cancers, and obtained preferable therapeutic results. We also performed a prospective 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.

4. Multicenter clinical and experimental studies of muscular dystrophy.

Koichi Kimura

Multicenter studies, drug interventional study and observational cohort study, in patients with muscular dystrophy have been proceeded in collaboration with NHO (National Hospital Organization) hospitals; Sendai-nishitaga National Hospital (Miyagi), Niigata National Hospital (Niigata), Matsumoto Medical Center (Nagano), Shimoshizu National Hospital (Chiba), Hakone National Hospital (Kanagawa), Osaka-toneyama Medical Center (Osaka), Iou National Hospital (Ishikawa), and Hiroshima-nishi Medical Center (Hiroshima). Also, we performed several animal experiments using CRISPR/CAS9 genome-designed rats in collaboration with department of veterinary physiology (The University of Tokyo), Kobe gakuin university (Hyogo), and National Institute of Advanced Industrial Science and Technology (AIST, Ibaraki). Other animal experiments using dogs, pigs and knockout mice were performed in collaboration with National Center of Neurology and Psychiatry (NCNP, Tokyo). We also contribute a publishing treatment guideline of Duchenne muscular dystrophy.

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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 2022, a total of 551 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 2022, we had a total of 20 counseling cases including Lynch syndrome, hereditary breast and ovarian cancer, myotonic dystrophy, Duchenne muscular dystrophy, multiple system atrophy, spinocerebellar ataxia type 3, Charcot-Marie-Tooth disease, and Huntington's disease. 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
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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, Sap-

poro Medical University. Although liquid-based cytology (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 by target sequencing in a total of 208 LBC samples using Cancer Hotspot Panel comprising of 50 cancer-related genes. We excluded 13 samples with low sequence-coverage and analyzed 195 of the 208 cases. Cytological analysis revealed that 24 of 38 ECs (63.2% of sensitivity) were positive, and genetic analysis identified somatic mutations in 29 of the 38 cases (76.3% of sensitivity). Combination of cytology and genetic analysis increased the detection rate of ECs up to 86.8% (33 of the 38) suggesting that genetic analysis contributes to the enhanced sensitivity of cytological screening for EC. Although all 16 patients with precancerous lesions including endometrial polyps and endometrial hyperplasia were negative for cytology, somatic mutations were found in eight of the 16 (50%) patients, indicating that genetic analysis is useful for the determination of premalignant diseases in the endometrium.

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), Lynch syndrome (LS), and polymerase proofreading-associated polyposis (PPAP). In addition to short read-sequencing, we took advantage of MinION, a long-read sequencer of Oxford Nanopore platform, for the detection of pathogenic structural variants (SVs) because not only single nucleotide variants (SNVs) and short insertions and deletions (indels) but also structural variations (SVs) are responsible for the predisposition of hereditary cancer. Utilizing MinION, we have successfully identified the breakpoint of a pathogenic SV that could not be determined by short-read sequencing technology.

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, gastric, cervical cancer, and pheochromocytoma 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.

Publications

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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.

Motion correction in MR image for analysis of VSRAD using generative adversarial network

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In the present study, we assessed the efficacy of motion correction using a generative adversarial net-

work. Voxel-based specific region analysis systems for Alzheimer's disease (VSRAD) are clinically used to measure the atrophied hippocampus captured by magnetic resonance imaging (MRI). However, motion artifacts during acquisition of images may distort the results of the analysis. We aimed to evaluate the usefulness of the Pix2Pix network in motion correction for the input image of VSRAD analysis. Seventy-three patients examined with MRI were distinguished into the training group (n = 51) and the test group (n = 22). To create artifact images, the k-space images were manipulated. Supervised deep learning was employed to obtain a Pix2Pix that generates motion-corrected images, with artifact images as the input data and original images as the reference data. The results of the VSRAD analysis (severity of voxel of interest (VOI) atrophy, the extent of gray matter (GM) atrophy, and extent of VOI atrophy) were recorded for artifact images and motion-corrected images, and were then compared with the original images. The Bland-Altman analysis showed that the mean of the limits of agreement was smaller for the motion-corrected images compared to the artifact images, suggesting successful motion correction by the Pix2Pix. The Spearman's rank correlation coefficients between original and motion-corrected images were almost perfect for all results (severity of VOI atrophy: 0.87–0.99, extent of GM atrophy: 0.88–00.98, extent of VOI atrophy: 0.90–1.00). Our findings suggest that motion correction using Pix2Pix is a useful method for VSRAD analysis.

Radiological precursor lesions of lung squamous cell carcinoma: Early progression patterns and divergent volume doubling time between hilar and peripheral zones

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The growth patterns of adenocarcinomas on computed tomography (CT) are well documented; however, little is known about the changes of CT findings over time and the CT features of precursor lesions of lung squamous cell carcinoma (SqCC). Therefore, we investigated the early progression patterns of lung SqCC on CT images. 65 patients with SqCC who underwent surgical resection and two CT scans separated by an interval of at least 6 months were enrolled. We categorized the findings of the initial and at-diagnosis CT images into five patterns as previously reported. The volume doubling time (VDT) was calculated for measurable lesions. A single nodule pattern on CT images at-diagnosis was most common in 56 (86.2 %) patients, in line with practical clinical findings. However, the patterns were diverse in the initial images, with 28 (43.1 %) patients displaying atypical findings, including multiple nodules (3.1 %), endobronchial lesions (20.0 %), subsolid nodules (10.8 %), and cyst wall thickening (9.2 %). All endobronchial lesions were located in the central/middle zone of the lung field, whereas lesions presented as multiple nodules, subsolid nodules, and cyst wall thickening were predominantly observed in the peripheral zone. The differences in the developed zones were reflected in the median VDT, and the tumors with an initial endobronchial pattern had a significantly shorter VDT than those with a subsolid nodule pattern (median: 140 days vs 276 days, $p < 0.001$). In conclusion, Lung SqCC initiated with various CT image patterns, although most tumors ultimately developed a single nodule pattern by diagnosis. The initial CT image patterns differed between the hilar and peripheral zones, suggesting a difference in the progression scheme, which was also supported by differences in VDT.

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8. Tajima T, Akai H, Sugawara H, Furuta T, Yasaka K, Kunimatsu A, Yoshioka N, Akahane M, Abe O, Ohtomo K, Kiryu S. Feasibility of accelerated whole-body diffusion-weighted imaging using a deep learning-based noise-reduction technique in patients with prostate cancer. Magn Reson Imaging. 92: 169-179, 2022.
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IMSUT Hospital

Department of Palliative Medicine and Advanced Clinical Oncology

先端緩和医療科

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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], Tetsuya Ito[1], Keishi Mori[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]
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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], Keishi Mori[1], Noriko Fujiwara[1], Aya Watanabe[1], Tomoe Honda[1], Yasuki Hijikata[1]
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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.

Publications

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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. HHV8 negative effusion-based lymphoma has been adopted for the new WHO classification.

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 lym-

phoma-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.

This disease concept has been adopted for the new WHO classification of hematolymphoid tumor.

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 = 1447	
	Endoscopic samples	n = 860
	Surgical resection	n = 304
	Fine-needle aspiration	n = 179
	Intraoperative diagnosis	n = 21
	Consultation	n = 73
	Other	n = 10
Cytological diagnosis	n = 552	
Autopsy	n = 3	

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 = 95).

Publications

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IMSUT Hospital

Department of Gastroenterology
消化器内科

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Senior Assistant Professor	Yasuo Matsubara, M.D., D.M.Sc.	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 2022. 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

1. Matsubara Y, Ota Y, Tanaka Y, Denda T, Hijikata Y, Boku N, Lim LA, Hirata Y, Tsurita G, Adachi E, Yotsuyanagi H. Altered mucosal immunity in HIV-positive colon adenoma: decreased $CD4^+$ T cell infiltration is correlated with nadir but not current $CD4^+$ T cell blood counts. Int J Clin Oncol. 2022 Aug;27(8):1321-1330. doi: 10.1007/s10147-022-02188-8. Epub 2022 May 29. PMID: 35643870.
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IMSUT Hospital

Department of Surgery
外科

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Assistant Professor	Taro Tanabe, M.D.	助　教		田　邊　太　郎
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Assistant Professor	Satoko Monma, M.D.	助　教		門　間　聡　子

The mission of our department is to provide surgical treatment for various gastrointestinal diseases, such as colorectal cancers and gastric cancers. Since the participation of Prof. Shida and Dr. Ahiko in September 2020, we mainly perform laparoscopic surgery instead of open surgery for these diseases. In addition, we started robotic surgery for rectal cancer in April, 2021. This year, we also started robotic surgery for colon cancer in September, 2022.

1. Introduction

This year, Dr Sakuyama N. and Dr. Onoyama H. newly joined in April 2022. And Dr. Monma S. and Dr. Kojima S. also joined in October 2022. 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, Dr. Aiko and Dr. Kojima) as well as qualified console surgeon of robotic surgery (da Vinci system) (Dr. Shida, Dr. Aiko, Dr. Ahiko and Dr. Sakuyama), we are actively performing minimally invasive surgery with less physical burden of patients. In addition, after Dr. Kojima joined us, we also started laparoscopic surgery for inguinal hernia. All the staff do their 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, Dr. Aiko and Dr. Kojima), we are actively performing minimally invasive surgery, that is, laparoscopic surgery and robotic surgery.

3. Surgical treatment for inguinal hernia

We started laparoscopic surgery for inguinal hernia in October, 2022. Our method is totally extra-peritoneal inguinal hernia repair (TEP), which is an effective minimally invasive method for treating hernias that avoids entry into the abdomen.

4. Surgical treatment for other 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 (Prof. Boku N., Dr. Matsubara Y., Dr. Hirata Y. and Dr. Baba K.), 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. This year, we also started robotic surgery for colon cancer in September, 2022.

7.Publications

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Yamaguchi K, Horie C, Takane K, Ikenoue T, Nakagawa S, Isobe Y, Ota Y, Ushiku T, Tanaka M, Fujishiro J, Hoshino N, Arisue A, Nishizuka S, Aikou S, Shida D, Furukawa Y.
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Prognostic Factors of Bone Metastases from Colorectal Cancer in the Era of Targeted Therapy.
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Our clinical practice and clinical studies have been focused on (1) anesthetic management in patients undergoing major surgery including joint arthroplastic surgery for hemophilia patients, variable surgical procedures for translational researches (2) assessment of functional failure of the internal valve of anesthesia machine (3) assessment of reliability of cardiac output measurements (4) risk management of medical electronic devices in Research Hospital. (5) Bioactive lipids and the mechanism of pain

1. 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.

2. 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.

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 and endoscopic surgery support robot for laparoscopic surgery

As a certified proctor of robot-assisted surgery, surgeon actively perform minimally invasive surgery.

We engage in preventive maintenance and care of the life support machines including instruments for mechanical ventilation or blood purification and robot systems for laparoscopic surgery. We also supervise physicians during clinical usage of these instruments. We have promoted dual-directional information system with the Division of Clinical Trial Safety Manage on malfunctions or incidents of the rest of medical electronic devices in this hospital in collaboration.

5. Bioactive lipids and the mechanism of pain

Postoperative incisional pain is characterized by persistent acute pain at the site of tissue injury and is associated with local inflammation.Various bioactive lipids such as Prostaglandins and Leukotrienes were synthesized at injured tissue and involved in acute inflammation and nociceptive pain.

Leukotriene B_4(LTB_4) is a potent lipid mediator of inflammation and its biological effects are mediated primarily through the high affinity receptor BLT1. We investigated the role of LTB_4-BLT1 signaling in inflammatory pain. We defined the transient increase of LTB_4 production in local incisional site in the quite early stage. We found that LTB_4-BLT1 signaling exacerbates pain responses by promoting local infiltration of inflammatory monocytes and cytokine production. LTB_4-BLT1 axis is a potential target for therapeutic intervention of acute pain induced by tissue injury.

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IMSUT Hospital

Department of Joint Surgery
関節外科

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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 2022, more than 265 surgical treatments for hemophilia included other coagulation diseases such as deficiency factor VII, Von Willebrand disease or afibrinogenemia. Some of them have the deficiency factor antibody as well.

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Department of Surgical Neuro-Oncology
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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 in nasal 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 97 operation were carried out in 2022 including 96 gliomas and 1 metastatic brain tumor. Ninety-six 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 are 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.

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Our department of Urology was established in ISMUT hospital on July 1st, 2020 to improve the occupancy rate by introducing advanced medical treatments such as robotic surgery. We successfully performed 550 cases of urological surgery including 87 cases of robotic surgery, 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. Basic research

Molecular targeted therapy using poly (ADP-ribose) polymerase inhibitors has improved survival in patients with castration-resistant prostate cancer (CRPC). However, this approach is only effective in patients with specific genetic mutations, and additional drug discovery targeting epigenetic modulators is required. Here, we evaluated the involvement of the transcriptional coregulator ESS2 in prostate cancer. ESS2-knockdown PC3 cells dramatically inhibited proliferation in tumor xenografts in nude mice. Microarray analysis revealed that ESS2 regulated mRNA levels of chromodomain helicase DNA binding protein 1 (*CHD1*)-related genes and other cancer-related genes, such as *PPAR-γ*, *WNT5A*, and *TGF-β*, in prostate cancer. ESS2 knockdown reduced nuclear factor (NF)-κB/CHD1 recruitment and histone H3K36me3 levels on the promoters of target genes (*TNF* and *CCL2*). In addition, we found that the transcriptional activities of NF-κB, NFAT and SMAD2/3 were enhanced by ESS2. Tamoxifen-inducible Ess2-knockout mice showed delayed prostate development with hypoplasia and disruption of luminal cells in the ventral prostate. Overall, these findings identified ESS2 acts as a transcriptional coregulator in prostate cancer and ESS2 can be novel epigenetic therapeutic target for CRPC.

2. Clinic

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 1,671 patients visited our department in 2022 for the purpose of thorough examinations for diagnosis or surgical treatments.

We totally performed 338 surgical operations in 2022: 46 cases of Robotic-assisted prostatectomy, four Robotic-assisted partial nephrectomy, four Laparoscopic nephrectomy, 10 Laparoscopic nephroureterectomy, one Radical cystectomy with ileal conduit urinary diversion, 16 open surgery, 59 Trans-urethral resection of bladder tumor, two Trans-urethral resection of prostate, 19 Trans-urethral lithotripsy, 24 Ureteroscopy, 37 Ureteral stenting, and six Botox injection for overactive bladder.

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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	Haruomi Yamaguchi, M.D., D.M.Sc.	助 教	博士(医学)	山 口 晴 臣

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, Haruomi Yamaguchi

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, Haruomi Yamaguchi

"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. The latest electronic healthcare record system will help refer patients from hospital to clinic and clinic to hospital in the network. Also, we recently constructed a network with the University of Tokyo Hospital (UTH) that can send medical images of our hospital to the Picture Archiving and Communication System of UTH.

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
Associate Professor	Kazuaki Yokoyama, M.D., Ph.D.		准教授	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 derived mesenchymal stromal cells (UC-MSC). 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. 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, and dendritic cell therapy

Nagamura-Inoue T, Yokoyama K, Takahashi A, Ogami K, , Miharu Y, Kawamata T

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 and other hospitals upon request.

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

This study investigated the safety, efficacy, and immunological influence of allogeneic umbilical cord-derived mesenchymal stromal cells (IMSUT-CORD) processed in serum-free medium and cryoprotectant, for treating steroid-resistant acute graft versus-host disease (aGVHD). In a phase I dose-escalation trial, IMSUT-CORD were infused intravenously twice weekly over two cycles with up to two additional cycles. Four patients received a dose of 1×10^6 cells/kg, while three received 2×10^6/ kg. Of 76 total adverse events, fourteen associated or possibly associated adverse events included 2 cases of a hot flash, headache, and peripheral neuropathy, 1 each of upper abdominal pain, hypoxia, increased γ-GTP, somnolence, peripheral vascular pain at the injection site, thrombocytopenia, hypertension, and decreased fibrinogen. At 16 weeks after the initial IMSUT-CORD infusion, three patients showed complete response (CR), two partial response (PR), one mixed response, and one no response. The overall response rate was 71.4%, and the continuous CR/PR rate was 100% for over 28 days after CR/PR. NK cell count significantly increased and correlated with treatment response, whereas IL-12, IL-17, and IL-33 levelsdecreased, but did not correlate with treatment response. CCL2 and CCL11 levels increased during IMSUT-CORD therapy. IMSUT-CORD are usable in patients with steroid-resistant aGVHD (UMIN000032819: https:// www. umin. ac. jp/ ctr).

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. Research and Development of UC-MSCs (IMSUT-CORD) treatment for new application of UC-MSCs to acute radiation injury, ARDS, cleft palate, and hemorrhagic arthropathy

Nagamura-Inoue T, Yamamoto Y, Takahashi A, Miharu Y, Hori A,

We have been exploring UC-MSCs (IMSUT-CORD) treatment for new application of UC-MSCs to acute radiation injury, ARDS, cleft palate, and hemorrhagic arthropathy in the patients with hemophilia by using mice models.

6. The Research Cord Blood Cell Resource / National BioResource Project (NBRP)

Izawa Y, Sakai R, Miharu Y, Yamamoto Y, Takahashi A, Nagaya N, Nagamura-Inoue T

The Research Cord Blood (CB) 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). This project 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/.

7. 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) CBand UC-MSC banking (IMSUT-CORD; 2012–), 7) aAVC-WT1 cell therapy (2017–), and (8) dendritic cell(DC) therapy using DCs pulsed with neoantigen(2020–).

Visit our website: http: //www.ims.u-tokyo.ac.jp/dcpt/english/

Publications

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3) Nagamura-Inoue T, Kato S, Najima Y, Isobe M, Doki N, Yamamoto H, Uchida N, Takahashi A, Hori A, Nojima M, Ohashi K, Nagamura F, Tojo A., Immunological influence of serum-free manufactured umbilical cord-derived mesenchymal stromal cells for steroid-resistant acute graft-versus-host disease. Int J Hematol. 2022 Nov;116(5):754-769.
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7) Ikeda K, Minakawa K, Yamahara K, Yamada-Fujiwara M, Okuyama Y, Fujiwara SI, Yamazaki R, Kanamori H, Iseki T, Nagamura-Inoue T, Kameda K, Nagai K, Fujii N, Ashida T, Hirose A, Takahashi T, Ohto H, Ueda K, Tanosaki R. Comparison of cryoprotectants in hematopoietic cell infusion-related adverse events. Transfusion. 2022 Jun;62(6):1280-1288.
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IMSUT Hospital

Surgical Center
手術部

Professor Tomoki Todo, M.D., Ph.D.

教　授　医学博士　藤　堂　具　紀

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 2022

Total number of operations	632
Planned operations	614

Emergency operations	18	Epidural	0
		Local	106
General anesthesia	443	Others	0
Spinal	88		

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 supporting many researches conducted in our hospital.

1. Immunological influence of serum-free manufactured umbilical cord-derived mesenchymal stromal cells for steroid-resistant acute graft-versus-host disease.

Tokiko NAGAMURA-INOUE.

This study investigated the safety, efficacy, and immunological influence of allogeneic umbilical cord-derived mesenchymal stromal cells (IMSUT-CORD) processed in serum-free medium and cryoprotectant, for treating steroid-resistant acute graft-versus-host disease. In a phase I dose-escalation trial, the overall response rate was 71.4%, and the continuous CR/PR rate was 100% for over 28 days after CR/PR. NK cell count significantly increased and correlated with treatment response, whereas IL-12, IL-17, and IL-33 levels decreased, but did not correlate with treatment response. CCL2 and CCL11 levels increased during IMSUT-CORD therapy. IMSUT-CORD are usable in patients with steroid-resistant acute GVHD. [Int J Hematol. 2022.] [84th annual meeting of JSH. 2022.]

2. Evaluation of POTELIGEO Test and detailed analysis of CCR4 expression on adult T-cell leukemia/lymphoma (ATL).

Tomohiro ISHIGAKI.

POTELIGEO is a humanized monoclonal antibody (mAb) that targets CC chemokine receptor 4 (CCR4), which is frequently expressed on leukemic cells of adult T-cell leukemia/lymphoma (ATL). POTELIGEO TEST is a companion diagnostic test for POTELIGEO, and is used for evaluating the expression of CCR4 on ATL cells. We retrospectively reviewed the main evaluation items of these clinical

laboratory tests performed for clinical practice purposes: peak channel ratio, CCR4 positivity rate, and its final judgment. The expression pattern of CCR4 could be classified into five types, and the judgment of expression was insufficient in about half of the cases from the stringent point of view of clinical cytometry. Additional multicolor flow cytometry could make the judgment easier and more precise. [Rinsho Kensa. 2022.] [69th annual meeting of JSLM. 2022.]

3. Flow cytometric quantification of HTLV-1-infected cells in HTLV-1 carriers and adult T-cell leukemia/lymphoma patients.

Tomohiro ISHIGAKI.

Quantifying HTLV1-infected cells is important in the treatment of HTLV1 carriers and adult T-cell leukemia/lymphoma (ATL) patients, but conventional methods are inaccurate. Therefore, we developed and validated a new clinical laboratory test. Accuracy and precision were assessed by analyzing HTLV-1-infected cell-line controls and patients' samples. the results were evaluated by comparison with those obtained by conventional methods. Flow cytometric quantification of CD4-positive CADM1-positive cells showed very high accuracy and sensitivity in the quantification of HTLV1-infected cells.

4. Retrospective analysis and search for clinical laboratory parameters associated with cardiac deterioration and shorter survival in Becker Muscular Dystrophy (BMD).

Koichi KIMURA and Tomohiro ISHIGAKI.

Becker muscular dystrophy (BMD) is an X-linked recessive disorder due to a mutation in the dystrophin gene, and is most common in muscular dystrophies. BMD has a later onset and milder symptoms compared to Duchenne muscular dystrophy (DMD), but cardiac diseases are now one of the leading causes of morbidity and mortality in these patients. We have retrospectively reviewed biochemical examination results and echocardiographic findings. We found a clinical laboratory parameter that could be associated with cardiac deterioration and shorter survival.

5. Laboratory contribution as a central medical department and support for many clinical investigations and trials in this hospital.

Hironori SHIMOSAKA, and Clinical laboratory members (clinical hematology, biochemistry/serology, physiology, and microbiology team)

We participate in clinical trials and research led by other hospital departments. Our laboratory members officially contributed to 7 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 other 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	Narikazu Boku, M.D., D.M.Sc.	教　授	博士(医学)	朴　　成　和
Associate Professor	Susumu Aikou, M.D., D.M.Sc.	准教授	博士(医学)	愛　甲　　丞
Associate Professor	Motohisa Yamamoto, M.D., D.M.Sc.	准教授	博士(医学)	山　本　元　久
Nurse Manager	Nozomi Linzbichler	看護師長		リンツビヒラ希
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 is responsible for carrying out medical safety in order to prevent incidents and accidents beforehand and deliver safe medical care to patients. At our hospital, we mainly have focused on hematological malignancies, infectious diseases, immune diseases, but in recent years, robotic surgery and chemotherapy are also increasing. We try to respond appropriately to such medical activities.

Department of Infection Prevention and Control
感染制御部

Head, Senior Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	講　師	博士(医学)	安　達　英　輔
Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Assistant Professor	Ikeuchi Kazuhiko, M.D., D.M.Sc.	助　教	博士(医学)	池　内　和　彦
Nurse Manager	Miya Kogayu	看護師長		小　粥　美　香
Nurse Manager	Fumie Kameda	看護師長		亀　田　史　絵
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.

Nosocomial infection control against prolonged COVID-19 and persistent viral shedding

We reported two cases of COVID-19 with prolonged viral shedding. Each of the two cases was a patient with HIV/AIDS and a patient with malignant lymphoma who was using an anti-CD20 antibody product. Both cases required long-term infection control measures against COVID-19 in private rooms in the hospital.

1. Nagai H, Saito M, Adachi E, Sakai-Tagawa Y, Yamayoshi S, Kiso M, Kawamata T, Koga M, Kawaoka Y, Tsutsumi T, Yotsuyanagi H. Casirivimab/Imdevimab for Active COVID-19 Pneumonia Which Persisted for Nine Months in a Patient with Follicular Lymphoma during Anti-CD20 Therapy. Jpn J Infect Dis. 2022 Nov 22;75(6):608-611
2. Adachi E, Saito M, Koga M, Tsutsumi T, Yotsuyanagi H. Favorable Outcome Following Sotrovimab Monoclonal Antibody in a Patient with Prolonged SARS-CoV-2 Omicron Infection with HIV/AIDS. Intern Med. 2022 Nov 15;61(22):3459-3462.
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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 the acquisition and maintenance of intellectual property rights as well as patent strategies;
3)Providing information necessary in the 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 the 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 2022, we supported four sponsor-investigator clinical trials by site management as well as project management. These four 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, administration of vaccine against the COVID-19 infection. We also supported three non-IND clinical studies.

4. 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 in academia. This program 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 the early stage of clinical trial. In 2022, we supported 32 basic researches (14: other than IMSUT), 22 preclinical studies (14: other than IMSUT), and 12 clinical studies (1: other than IMSUT). The number of studies we assist has been increasing year by year. Efficient operation of the organization is required.

5. 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.

Publications

1. Fujii S, Kawamata T, Shimizu K, Nakabayashi J, Yamasaki S, Iyoda T, Shinga J, Nakazato H, Sanpei A, Kawamura M, Ueda S, Dörrie J, Groß S, Mojsov S, Nojima M, Nagamura F, Yoshida S, Goto T, Tojo A. Reinvigoration of innate and adaptive immunity via therapeutic cellular vaccine for AML patients. Molecular Therapy – Oncolytics. 2022 https://doi.org/10.1016/j.omto.2022.09.001
2. Nagamura-Inoue T, Kato S, Najima Y, Isobe M, Doki N, Yamamoto H, Uchida N, Takahashi A, Hori A, Nojima M, Ohashi K, Nagamura F, Tojo A. Immunological influence of serum-free manufactured umbilical cord-derived mesenchymal stromal cells for steroid-resistant acute graft-versus-host disease. Int J Hematol. 2022 Jul 30. doi: 10.1007/s12185-022-03408-7.
3. Watanabe A, Shimada N, Ishiki H, Fujiwara N, Nojima M, Tojo A. Differences in Fentanyl Requirements in Terminally Ill Cancer Patients. J Pain Palliat Care Pharmacother. 2022 Dec 13:1-8. doi: 10.1080/15360288.2022.2149667. Epub ahead of print. PMID: 36512675.
4. Iimura Y, Furukawa N, Ishibashi M, Ahiko Y, Tanabe T, Aikou S, Shida D, Nojima M, Kuroda S, Boku N. Study protocol of a single-arm phase 2 study evaluating the preventive effect of topical hydrocortisone for capecitabine-induced hand-foot syndrome in colorectal cancer patients receiving adjuvant chemotherapy with capecitabine plus oxaliplatin (T-CRACC study). BMC Gastroenterol. 2022 Jul 14;22(1):341. doi: 10.1186/s12876-022-02411-w. PMID: 35836104; PMCID: PMC9284769.
5. Yuki Y, Nojima M, Kashima K, Sugiura K, Maruyama S, Kurokawa S, Yamanoue T, Nakahashi-Ouchida R, Nakajima H, Hiraizumi T, Kohno H, Goto E, Fujihashi K, Kiyono H. Oral MucoRice-CTB vaccine is safe and immunogenic in healthy US adults. Vaccine. 2022 May 26;40(24):3372-3379. doi: 10.1016/j.vaccine.2022.04.051. Epub 2022 Apr 26. PMID: 35484039.
6. Yamamoto M, Nojima M, Kamekura R, Kuribara-Souta A, Uehara M, Yamazaki H, Yoshikawa N, Aochi S, Mizushima I, Watanabe T, Nishiwaki A, Komai T, Shoda H, Kitagori K, Yoshifuji H, Hamano H, Kawano M, Takano KI, Fujio K, Tanaka H. The differential diagnosis of IgG4-related disease based on machine learning. Arthritis Res Ther. 2022 Mar 19;24(1):71. doi: 10.1186/s13075-022-02752-7. PMID: 35305690; PMCID: PMC8933663.

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.

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. The umbilical cord tissue (UC) also 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. We have supplied UC-MSC products for clinical trials for severe acute graft-versus-host disease (GVHD; 2018– 2020), COVID-19-related ARDS (2020–2022), cerebral palsy (PVL; 2021–), and non-infectious pulmonary disease after allogeneic hematopoietic stem cell transplantation NIPS; 2022–). Main processing facility has been moved from IMSUT cell resource center to new IMSUT-HLC cell processing facility since 2021.

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 (youngest cells!). 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. After receiving them, we 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. 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–), cerebral palsy (PVL; 2021–), and non-infectious pulmonary disease after allogeneic hematopoietic stem cell transplantation NIPS; 2022-), after approval by the review board (PMDA). We are currently preparing for a clinical trial for treating peripheral nerve injury using allograft bio3D conduit made with UC-MSC products with Kyoto University (AMED project 2022-). Since 2021, our main manufacturing location has been moved from the IMSUT-Cell Resource Center (IMSUT-CRC) to a new facility, the IMSUT-HLC Cell Processing Facility (IMSUT-HLC CPF),

Publications

1) Meshitsuka S, Ninomiya R, Nagamura-Inoue T, Okada T, Futami M, Tojo A. CRISPR/Cas9 and AAV mediated insertion of β2 microglobulin-HLA-G fusion gene protects mesenchymal stromal cells from allogeneic rejection and potentiates the use for off-the-shelf cell therapy. Regen Ther. 2022 Oct 14;21:442-452. eCollection 2022 Dec.
2) Nagamura-Inoue T, Kato S, Najima Y, Isobe M, Doki N, Yamamoto H, Uchida N, Takahashi A, Hori A, Nojima M, Ohashi K, Nagamura F, Tojo A., Immunological influence of serum-free manufactured umbilical cord-derived mesenchymal stromal cells for steroid-resistant acute graft-versus-host disease. Int J Hematol. 2022 Nov;116(5):754-769. Epub 2022 Jul 30.
3) Nagamura-Inoue T, You can be a unique female physician scientist with originality to find the raison d'être in diverse career options in medicine, Rinsho Ketsueki. 2022;63(8):934-936.

Visit our website: http://imsutcord.umin.jp

IMSUT Hospital

Department of Nursing
看護部

Director	Eiko Yoshii, RN, CNA.
Deputy Director	Minayo Hisahara, RN.
Deputy Director	Junko Izumi, RN, CNA.
Nurse Manager	Hatsuko Narita, RN.
Nurse Manager	Mika Kogayu, RN. MSN.
Nurse Manager	Tomoko Sato, RN. MSN.
Nurse Manager	Masako Ozawa, RN.
Nurse Manager	Nozomi Linzbichler, RN.
Nurse Manager	Yukari Tsuru, RN.
Nurse Manager	Fumie Kameda, RN.

看護部長	認定看護管理者	吉　井　栄　子
副看護部長		久　原　みな代
副看護部長	認定看護管理者	和　泉　純　子
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看護師長	修士(看護学)	佐　藤　朋　子
看護師長		小　澤　昌　子
看護師長		リンツビヒラ希
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 2022, we focused on responding to the new coronavirus infection (COVID-19) and providing nursing in response to the expansion of medical care. It was a year of perseverance.

Based on the mission of "making a difference in patient outcomes through the power of nursing," we nurses prepare a medical care environment and provide nursing so that patients who choose our medical care and come to our hospital can receive medical care with peace of mind. We would like to respect the social background of each patient and support them so that they can lead a life according to their health condition. To that end, as a member of the medical team, we want to protect the dignity and safety of patients and do our best to improve medical care and quality of life in cooperation with multiple professionals.

2022 began with the sixth wave of COVID-19. As COVID-19 mutations progressed, the Delta variant of the fifth wave shifted to the Omicron variant (BA1 and BA2) in the sixth wave, and the Omicron variant (BA5) spread in the seventh wave in July and August. Then, in December, the eighth wave arrived. During the COVID-19 epidemic, the infectious disease ward will be converted to a dedicated COVID-19 ward, and the surgical ward will be transferred to a ward that accepts all departments. When the epidemic subsides, the infectious disease ward will be mixed with COVID-19 and general beds, and the surgical ward will return to normal operation. Over the past year, we have repeatedly provided medical care and nursing care while responding to changes in the epidemic. However, after three years of the COVID-19 pandemic, nurses were highly exhausted, and the number of sick leave and retirees due to mental disorders increased.

The seventh wave of the Omicron variant (BA5) was highly contagious, with more than 20 nurses becoming infected or close contacts, and an increase in the number of nurses suspended from work. In addition, due to the outbreak of clusters at elderly facilities due to COVID-19, the number of hospitalized patients with elderly people and dementia has increased, and the proportion of patients who need full assis-

tance in their daily lives has increased compared to normal times. As a result, it became difficult to operate the four wards, but the nurses who were able to go to work cooperated to maintain the nursing system and contribute to the operation of the wards. Doctors and other healthcare professionals also helped coordinate hospitalizations to the extent possible. The seventh wave subsided in September, and the infectious disease ward was again mixed with COVID-19 and general beds, and even with the eighth wave in December, the ward management system remained unchanged.

In the surgical departments, robot-assisted surgery has taken root in both surgery and urology, and the number of surgeries has increased due to the expansion of the scope of urology surgery and viral therapy in brain tumor surgery. In the internal medicine department, the acceptance of outpatient chemotherapy patients is progressing, and the Multidisciplinary Collaborated Cancer Support Team (MCST) has started its activities, and nurses are participating as members. The number of hospitalized patients for palliative care also increased. In this way, the expansion of medical care is progressing, and nurses have also prepared to accept patients by holding study sessions in their respective departments and creating manuals, clinical pathways, pamphlets, etc. so that nurses can respond to new treatment methods. In addition, since some patients require stoma care and self-urination due to surgical procedures, guidance to patients by nurses has been emphasized. Nurses work with patients to figure out the best way to help them learn stoma care and self-urination techniques and accept new excretion behaviors.

In 2021, nursing for COVID-19 patients was mainly respiratory nursing due to pneumonia, but this year, in addition to nursing for COVID-19-related symptoms, nursing for the elderly and dementia was required. Even in nursing after viral therapy in brain tumor surgery, temporary decline in consciousness and activities of daily living occurs, so support and monitoring behavior in daily life are required. In addition, there are more opportunities to support patients with palliative care while providing treatment and symptom management. Some patients undergo bone marrow transplantation or hematopoietic cell transplantation due to blood diseases. Nurses grasp daily activities and life patterns, pay attention to preventing fractures and injuries caused by falls while maintaining physical functions, and search for the best nursing care for patients and their families every day. This situation is unfolding in every ward, and it is presumed that the mental burden is great. Although they have not achieved any special results in the field of nursing, it is wonderful that nurses naturally devise ways to support patients' medical treatment lives and make nursing enjoyable as a matter of course.

Publication

鷲尾　彩恵, Impact of the COVID-19 pandemic on the physical and psychological health of female college students in Japan, Nursing and Health Scienses, 2022, Volume 24, Issue 3, p. 634-642　https://doi.org/10.1111/nhs.12962

Conference Presentation

・杉山栄美子, 吉田咲妃, 黒山杏奈, 都留由香里, 和泉純子,　久原みな代,　吉井栄子,　看護ワークショップ「COVID-19禍の移植看護～その後」, COVID-19禍で気づかされた私たちの移植看護,　第44回日本造血・免疫細胞療法学会, 横浜, 2022. 5. 13-14
・宮本幸奈,　砂田純子,都留由香里,宮下英太,杉山栄美子, 急性消化管移植片対宿主病に伴う下痢の客観的色調評価基準の作成, 第44回日本造血・免疫細胞療法学会総会, 横浜, 2022. 5. 13-14
・宮下英太, 小沼貴晶, 杉原望, 田中美穂, 岩崎宏美, 大岩真希, 磯部優理, 加藤せい子, 高橋聡, 南谷泰仁, 都留由香里, 同種造血細胞移植後無病生存者におけるポリファーマシーの実態調査, 第44回日本造血・免疫細胞療法学会総会, 横浜, 2022. 5. 13-14
・塚本公美子, 渡邉由美子, 藤原有沙, 吉田咲妃, 砂田純子, 都留由香里, 造血幹細胞移植の口腔粘膜障害にP-AG導入後のOral Assessment Guideスコアの変動, 第44回日本造血・免疫細胞療法学会総会, 横浜, 2022. 5. 13-14
・亀田　史絵, 小野谷厚子, 小粥美香, COVID-19における高齢入院患者の感染対策への介入, 第37回日本環境感染学会総会・学術集会, 横浜, 2022. 6. 16-18
・小粥美香, 小澤昌子, 和泉純子, 吉井栄子, 周術期看護の充実を図るための取り組み, 第26回日本看護管理学会学術集会, 福岡, 2022. 8. 19-20
・小舘未沙, 小粥美香, 長時間特殊体位による皮膚トラブル予防の実践報告,　第36回　日本手術看護学会, 名古屋, 2022. 11. 04-05
・小林路世, 小粥美香, 周術期看護充実へ向けた多部署間での取り組み, 第36回日本手術看護学会, 名古屋, 2022. 11. 04-05
・上山美香, 古賀道子, 金澤亜由美, 中澤光子, 三浦洋子, 安田真章, 渡辺直子, 安齋英里, 安達英輔, 馬場啓介, 伊藤哲也, 四柳宏, 亀田史絵, 多職種連携と退院支援における当院の取り組み, 第36回日本エイズ学会学術集会, 2022. 11. 18-20

IMSUT Hospital

Department of Pharmacy
薬剤部

Director	Seiichiro Kuroda
Chief	Yohei Iimura
Chief	Sonoe Minegishi-Higashino
Pharmacist	Mika Yamamura
Pharmacist	Mai Yokota

薬剤部長	黒田誠一郎
薬剤主任	飯村洋平
薬剤主任	峰岸園恵
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.

<Publication>

1) Fukushi K, Konuma T, Monna-Oiwa M, Isobe M, Kato S, Kuroda S, Takahashi S, Nannya Y. Long-Term Incidence of Varicella Zoster Virus Disease in Adults Receiving Single-Unit Cord Blood Transplantation. Transplant Cell Ther. 2022 Jun;28(6):339.e1-339.e7. doi: 10.1016/j.jtct.2022.03.022. Epub 2022 Mar 29.
2) Iimura Y, Furukawa N, Ishibashi M, Ahiko Y, Tanabe T, Aikou, Shida D, Nojima M, Kuroda S, Boku N. Study protocol of a single-arm phase 2 study evaluating the preventive effect of topical hydrocortisone for capecitabine-induced hand-foot syndrome in colorectal cancer patients receiving adjuvant chemotherapy with capecitabine plus oxaliplatin (T-CRACC study). BMC Gastroenterol. 2022 Jul 14;22(1):341. doi: 10.1186/s12876-022-02411-w.
3) Higashino S, Yasu T, Momo K, Kuroda S. Effects of raltegravir formulation change on medication adherence and medication errors. Drug Discov Ther. 2022 Sep 17;16(4):198-199. doi: 10.5582/ddt.2022.01054. Epub 2022 Aug 25.
4) Momo K, Yasu T, Kuroda S, Higashino S, Mitsugi E, Ishimaru H, Goto K, Eguchi A, Sato K, Matsumoto M, Shiga T, Kobayashi H, Seki R, Nakano M, Yashiro Y, Nagata T, Yamazaki H, Ishida S, Watanabe N, Tagomori M, Sotoishi N, Sato D, Kuroda K, Harada D, Nagasawa H, Kawakubo T, Miyazawa Y, Aoyagi K, Kanauchi S, Okuyama K, Kohsaka S, Ono K, Terayama Y, Matsuzawa H, Shirota M. A Survey of Near-Miss Dispensing Errors in Hospital Pharmacies in Japan: DEPP-J Study-Multi-Center Prospective Observational Study- Biol Pharm Bull. 2022;45(10):1489-1494. doi: 10.1248/bpb.b22-00355.

<Conference presentation>

1) Iimura Y, Furukawa N, Ishibashi M, Sasuga C, Baba K, Ahiko Y, Onoyama H, Sakuyam N, Aikou

S, Shida D, Nojima M, Kuroda S, Boku N. Study protocol of a single-arm phase 2 study evaluating the preventive effect of topical hydrocortisone for capecitabine-induced hand-foot syndrome in adjuvant chemotherapy (T-CRACC study). The 60th Annual Meeting of Japan Society of Clinical Oncology. 2022, Yokohama.
2) Fukushi K, Konuma T, Oiwa M, Isobe M, Kato, Kuroda S, Takahashi S, Nannya Y. A retrospective analysis of varicella-zoster virus reactivation after cord blood transplantation. 44th JSTCT Annual Meeting (JSTCT2022) Japanese Society for Transplantation and Cellular Therapy. 2022, Yokohama.
3) Ijichi Y, Iimura Y, Ahiko Y, Tanabe T, Tsurita G, Aikou S, Shida D, Kuroda S. Chemotherapy-related dysphonia: similar and differentiating features of six cases. Japanese Society of Pharmaceutical Oncology. 2022, Sendai

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 Neutralizing-antibody-independent SARS-CoV-2 control correlated with intranasal-vaccine-induced CD8+ T-cell responses.

Hiroshi Ishii[1], Takushi Nomura[1], Hiroyuki Yamamoto[1], Masako Nishizawa[1], Trang Thi Thu Hau[1], Shigeyoshi Harada[1], Sayuri Seki[1], Midori Nakamura-Hoshi[1], Midori Okazaki[1], Sachie Daigen[1], Ai Kawana-Tachikawa, Noriyo Nagata[2], Naoko Iwata-Yoshikawa[2], Nozomi Shiwa[2], Tadaki Suzuki[2], Eun-Sil Park[3], Ken Maeda[3], Taishi Onodera[4], Yoshimasa Takahashi[4], Kohji Kusano[5], Ryutaro Shimazaki[5], Yuriko Suzaki[6], Yasushi Ami[6], Tetsuro Matano: [1]AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; [2]Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan; [3]Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan; [4]Research Center for Drug and Vaccine Development, National Institute of Infectious Diseases, Tokyo, Japan; [5]ID Pharma Co., Ltd., Tsukuba, Japan; [6]Management Department of Biosafety, Laboratory Animal, and Pathogen Bank, National Institute of Infectious Diseases, Tokyo, Japan

Effective vaccines are essential for the control of the COVID-19 pandemic. Currently-developed vaccines inducing SARS-CoV-2 spike (S) antigen-specific neutralizing antibodies (NAbs) are effective, but the appearance of NAb-resistant S variant viruses is of great concern. A vaccine inducing S-independent or NAb-independent SARS-CoV-2 control may contribute to containment of these variants. In this study, we investigated the efficacy of an intranasal vaccine expressing viral non-S antigens against intranasal SARS-CoV-2 challenge in cynomolgus macaques. Seven vaccinated macaques exhibited significantly reduced viral load in nasopharyngeal swabs on day 2 post-challenge compared to nine unvaccinated controls. The viral control in the absence of SARS-CoV-2-specific NAbs was significantly correlated with vaccine-induced viral antigen-specific $CD8^+$ T-cell responses. Our results indicate that $CD8^+$ T-cell induction by intranasal vaccination can result in NAb-independent control of SARS-CoV-2 infection, highlighting a potential of vaccine-induced $CD8^+$ T-cell responses to contribute to COVID-19 containment.

2. Sustainable antiviral efficacy of rejuvenated HIV-specific cytotoxic T lymphocytes generated from induced pluripotent stem cells.

Shoji Miki[1], Yohei Kawai[7], Kaori Nakayama-Hosoya[1], Ryutaro Iwabuchi[4], Kazutaka Terahara[4], Yasuko Tsunetsugu-Yokota[4], Michiko Koga[8], Tetsuro

Matano, Shin Kaneko[7], Ai Kawana-Tachikawa: [7]Department of Cell Growth and Differentiation, Center for iPS cell Research and Application, Kyoto University, Kyoto, Japan; [8]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Persistence of HIV latently infected cells is a barrier to HIV cure. The "kick and kill" strategy for cure includes clearance of the viral reservoir by HIV-specific cytotoxic T lymphocytes (CTLs). However, exhaustion and senescence of T cells accelerates during HIV infection, and does not fully recover, despite complete viral suppression under antiretroviral therapy. We previously established an induced pluripotent stem cell (iPSC) from a parental HIV-specific CTL clone and generated an iPSC-derived rejuvenated HIV-specific CTL clone (iPSC-CTL), which exhibited an early memory phenotype, high proliferation capacity and effector functions *in vitro*. In this study, we assessed the antiviral efficacy of the HIV-specific iPSC-CTL by single- and multiple-round viral suppression assays (VSAs). The HIV-specific iPSC-CTL suppressed viral replication in an HLA-dependent manner with equivalent efficacy to the parental CTL clone in single-round VSA. In multiple-round VSA, however, the ability of the iPSC-CTL to suppress viral replication was longer than that of the parental CTL clone. These results indicate that HIV-specific iPSC-CTL can sustainably exert suppressive pressure on viral replication, suggesting a novel approach to facilitate clearance of the HIV reservoir via adoptive transfer of rejuvenated CTLs.

3. Env-independent protection of intrarectal SIV challenge by vaccine induction of Gag/Vif-specific CD8$^+$ T cells but not CD4$^+$ T cells.

Hiroshi Ishii[1], Kazutaka Terahara[4], Takushi Nomura[1], Midori Okazaki[1], Hiroyuki Yamamoto[1], Tsugumine Shu[5], Hiromi Sakawaki[9], Tomoyuki Miura[9], David I. Watkins[10], Tetsuro Matano: [9]Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan; [10]Department of Pathology, George Washington Medical School, Washington DC, USA

Effective T-cell induction is an important strategy in HIV vaccine development. However, it has been indicated that vaccine-induced HIV-specific CD4$^+$ T cells, the preferential targets of HIV infection, might increase viral acquisition after HIV exposure. We have recently developed an immunogen (CaV11), tandemly-connected overlapping 11-mer peptides spanning the SIV Gag capsid and Vif proteins, to selectively induce Gag- and Vif-specific CD8$^+$ T cells but not CD4$^+$ T cells. In this study, we showed protective efficacy of a CaV11-expressing vaccine against repeated intrarectal low-dose SIVmac239 challenge in rhesus macaques. Eight of the twelve vaccinated macaques were protected after eight challenges. Kaplan-Meier analysis indicated significant protection in the vaccinees compared to the unvaccinated macaques. Vaccine-induced Gag-specific CD8$^+$ T-cell responses were significantly higher in the protected than the unprotected vaccinees. These results suggest that classical CD8$^+$ T-cell induction by Env-independent vaccination can confer protection from intrarectal SIV acquisition, highlighting the rationale for this immunogen design to induce virus-specific CD8$^+$ T cells but not CD4$^+$ T cells in HIV vaccine development.

4. Sendai virus particles carrying target virus glycoproteins for antibody induction.

Hiroshi Ishii[1], Midori Nakamura-Hoshi[1], Tsugumine Shu[5], Tetsuro Matano

Induction of antibodies targeting viral glycoproteins is a key for the development of a vaccine against enveloped virus infection. Glycoproteins on the virion exhibiting native multimer structure may be a good immunogen to present antibody epitopes, but it is often difficult to prepare immunogenic inactivated virions. Preparation of soluble glycoprotein multimers has been attempted, while virus-like particles carrying target glycoproteins can be a more immunogenic antigen. In this study, a target glycoprotein-embedded Sendai virus (SeV) particle was developed for induction of anti-virus antibodies. We constructed a chimeric antigen, HIV-1 EnvF, consisting of HIV-1 Env ectodomain and SeV F transmembrane-cytoplasmic domain, which was shown to be efficiently incorporated into the SeV virion. EnvF was recognized by anti-HIV-1 broadly-neutralizing monoclonal antibodies (bnAbs) including 35O22 that targets an Env trimer-dependent epitope. Analysis revealed that HIV-1_{AD8} EnvF can mediate viral entry into the cells, which is inhibited by anti-HIV-1 bnAbs and HIV-1 entry inhibitors, suggesting that the EnvF exhibits an HIV-1 Env native-like functional structure to present bnAb epitopes. Immunization of mice with replication-defective SeVs expressing HIV EnvF and non-infectious SeV particles carrying HIV EnvF efficiently induced anti-HIV Env antibodies. HTLV-1 EnvF also showed the potential to efficiently induce anti-HTLV-1 Env antibodies. These results indicate that SeV particles carrying EnvF can be a promising vaccine platform for induction of antibodies targeting enveloped virus glycoproteins.

5. Super high-resolution single-molecule sequence-based typing of HLA class I alleles in HIV-1 infected individuals in Ghana.

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Polymorphisms in HLA class I loci are known to have a great impact on disease progression in HIV-1 infection. Prevailing HIV-1 subtypes and HLA genotype distribution are different all over the world, and the HIV-1 and host HLA interaction could be specific to individual areas. Data on the HIV-1 and HLA interaction have been accumulated in HIV-1 subtype B- and C-predominant populations but not fully obtained in West Africa where HIV-1 subtype CRF02_AG is predominant. In this study, to obtain accurate HLA typing data for analysis of HLA association with disease progression in HIV-1 infection in West African populations, HLA class I (*HLA-A, -B*, and *-C*) four-digit allele typing was performed in treatment-naïve HIV-1 infected individuals in Ghana by a super high-resolution single-molecule sequence-based typing (SS-SBT) using next-generation sequencing. Comparison of the SS-SBT-based data with those obtained by a conventional sequencing-based typing (SBT) revealed incorrect assignment of several alleles by SBT. Indeed, HLA-A*23:17, HLA-B*07:06, HLA-C*07:18, and HLA-C*18:02 whose allele frequencies were 2.5%, 0.9%, 4.3%, and 3.7%, respectively, were not determined by SBT. Several HLA alleles were associated with clinical markers, viral load and $CD4^+$ T-cell count. Of note, the impact of *HLA-B*57:03* and *HLA-B*58:01*, known as protective alleles against HIV-1 subtype B and C infection, on clinical markers was not observed in our cohort. This study for the first time presents SS-SBT-based four-digit typing data on *HLA-A, -B,* and *-C* alleles in Ghana, describing impact of HLA on viral load and CD4 count in HIV-1 infection. Accumulation of these data would facilitate high-resolution HLA genotyping, contributing to our understanding of the HIV-1 and host HLA interaction in Ghana, West Africa.

6. Association of Envelope-specific B-cell differentiation and viral selective pressure signatures in HIV-1 CRF01_AE infection.

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In HIV-1 infection, virus-specific B-cell and neutralizing antibody (NAb) responses are impaired but exert selective pressure on target viral Env resulting in prominent sequence diversification among geographical areas. The basal induction patterns of HIV Env-specific B cells and their interaction with HIV Env awaits clarification. In this study, we investigated the relationship of Env polymorphisms and Env-specific B-cell responses in treatment-naïve HIV-1 CRF01_AE-infected Vietnamese. Samples of HIV-1 CRF01_AE-infected individuals were divided into acute-phase and chronic-phase by combined criteria of serological recent-infection assay and clinical parameters. We quantified subcloning-based polymorphic residue site numbers in plasma-derived Env variable region 1-5 (V1-V5)-coding regions within each individual, designating their summation as variant index (VI). Peripheral blood Env gp140-specific B-cell responses were examined to analyze correlation between B-cell responses and VIs. HIV-1 CRF01_AE Env gp140-specific total B cell, non-IgG^+ plasmablast ($CD19^+IgD^-CD27^+CD38^{++}CD138^-$) and IgG^+ plasma cell ($CD19^+IgD^-CD27^+CD38^+CD138^+$) responses were determined. Significant correlation of VI in Env V1-V5 with Env-specific PC responses but not with total Env-specific B-cell or PB responses was shown in chronic-phase samples. Results revealed the association between circulating Env-specific PC responses and Env polymorphisms, implicating selective pressure on Env by PC-derived antibodies and conversely suggests that Env-specific B-cell induction alone is insufficient for exerting Env selective pressure in HIV infection..

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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. SARS-CoV-2 Omicron virus causes attenuated disease in mice and hamsters.

Halfmann P[1], Iida S[2], Iwatsuki-Horimoto K, Maemura T[1], Kiso M, Scheaffer S[3], Darling T[3], Joshi A[3], Loeber S[4], Singh G[5,6], Foster S[7], Ying B[3], Case J[3], Chong Z[3], Whitener B[3], Moliva J[8], Floyd K[7], Ujie M, Nakajima N[2], Ito M, Wright R[1], Uraki R, Warang P[5,6], Gagne M[8], Li R[9], Sakai-Tagawa Y, Liu Y[9], Larson D[9], Osorio J[10,11], Hernandez-Ortiz J[11], Henry A[8], Ciouderis K[11], Florek K[12], Patel M[7], Odle A[13], Wong LY[13], Bateman A[12], Wang Z[9], Edara VV[7], Chong Z[3], Franks J[14], Jeevan T[14], Fabrizio T[14], DeBeauchamp J[14], Kercher L[14], Seiler P[14], Gonzalez-Reiche A[15], Sordillo E[16], Chang L[17], Bakel H[15], Simon V[5,15,16,18], PSP study group, Douek D[8], Sullivan N[8], Thackray L[3], Ueki H, Yamayoshi S, Imai M, Perlman S[13], Webby R[14], Seder R[8], Suthar M[7,19], Garcia-Sastre A[5,6,16,18,20], Schotsaert M[5,6], Suzuki T[2], Boon A[3,21,22], Diamond M[3,21-23], KawaokaY: [1]Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA. [2]Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan. [3]Department of Medicine, Washington University School of Medicine, St Louis, MO, USA. [4]Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI, USA. [5]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [6]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [7]Center for Childhood Infections and Vaccines of Children's Healthcare of Atlanta, Department of Pediatrics, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, USA. [8]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. [9]Department of Animal Dairy, and Veterinary Sciences, College of Agriculture and Applied Sciences, Utah State University, Logan, UT, USA. [10]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA. [11]Colombia/Wisconsin One-Health Consortium and One-Health Genomic Laboratory, Universidad Nacional de Colombia, Medellín, Colombia. [12]Wisconsin State Laboratory of Hygiene, Madison, WI, USA. [13]Department of Microbiology and Immunology, University of Iowa, Iowa City, IA, USA. [14]Department of Infectious Diseases, St Jude Children's Research Hospital, Memphis, Tennessee, USA. [15]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York,

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The recent emergence of B.1.1.529, the Omicron variant, has raised concerns of escape from protection by vaccines and therapeutic antibodies. A key test for potential countermeasures against B.1.1.529 is their activity in preclinical rodent models of respiratory tract disease. Here, using the collaborative network of the SARS-CoV-2 Assessment of Viral Evolution (SAVE) programme of the National Institute of Allergy and Infectious Diseases (NIAID), we evaluated the ability of several B.1.1.529 isolates to cause infection and disease in immunocompetent and human ACE2 (hACE2)-expressing mice and hamsters. Despite modelling data indicating that B.1.1.529 spike can bind more avidly to mouse ACE2, we observed less infection by B.1.1.529 in 129, C57BL/6, BALB/c and K18-hACE2 transgenic mice than by previous SARS-CoV-2 variants, with limited weight loss and lower viral burden in the upper and lower respiratory tracts. In wild-type and hACE2 transgenic hamsters, lung infection, clinical disease and pathology with B.1.1.529 were also milder than with historical isolates or other SARS-CoV-2 variants of concern. Overall, experiments from the SAVE/NIAID network with several B.1.1.529 isolates demonstrate attenuated lung disease in rodents, which parallels preliminary human clinical data.

2. Characterization of the SARS-CoV-2 B.1.621 (Mu) variant.

Halfmann PJ[10], Kuroda M[10], Armbrust T[10], Theiler J[24], Balaram A[10], Moreno GK[25], Accola MA[26], Iwatsuki-Horimoto K, Valdez R[27], Stoneman E[28], Braun K[10], Yamayoshi S, Somsen E[25], Baczenas JJ[25,29], Mitamura K[30], Hagihara M[31], Adachi E[32], Koga M[33], McLaughlin M[25], Rehrauer W[26], Imai M, Yamamoto S[33], Tsutsumi T[33], Saito M[33], Friedrich TC[10,29], O'Connor SL[25,29], O'Connor DH[25,29], Gordon A[34], Korber B[35,36], Kawaoka Y: [24]Space Data Science and Systems, Los Alamos National Laboratory, Los Alamos, NM 87545, USA. [25]Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI 53705, USA. [26]UW Health Clinical Laboratories, University of Wisconsin Hospital and Clinics, Madison, WI 53792, USA. [27]Depart-ment of Pathology, University of Michigan, Ann Arbor, MI 48109, USA. [28]Division of Infectious Diseases, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA. [29]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI 53715, USA. [30]Division of Infection Control, Eiju General Hospital, 110-8645 Tokyo, Japan. [31]Department of Hematology, Eiju General Hospital, 110-8645 Tokyo, Japan. [32]Department of Infectious Diseases and Applied Immu-nology, IMSUT Hospital of The Institute of Medical Science, University of Tokyo, 108-8639 Tokyo, Japan. [33]Division of Infectious Diseases, Advanced Clinical Re-search Center, Institute of Medical Science, University of Tokyo, 108-8639 Tokyo, Japan. [34]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI 48109, USA. [35]Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM 87545, USA. [36]New Mexico Consortium, Los Alamos, NM 87545, USA.

The SARS-CoV-2 B.1.621 (Mu) variant emerged in January 2021 and was categorized as a variant of interest by the World Health Organization in August 2021. This designation prompted us to study the sensitivity of this variant to antibody neutralization. In a live virus neutralization assay with serum samples from individuals vaccinated with the Pfizer/BioNTech or Moderna mRNA vaccines, we measured neutralization antibody titers against B.1.621, an early isolate (spike 614D), and a variant of concern (B.1.351, Beta variant). We observed reduced neutralizing antibody titers against the B.1.621 variant (3.4- to 7-fold reduction, depending on the serum sample and time after the second vaccination) compared to the early isolate and a similar reduction when compared to B.1.351. Likewise, convalescent serum from hamsters previously infected with an early isolate neutralized B.1.621 to a lower degree. Despite this antibody titer reduction, hamsters could not be efficiently rechallenged with the B.1.621 variant, suggesting that the immune response to the first infection is adequate to provide protection against a subsequent infection with the B.1.621 variant.

3. Characterization and antiviral susceptibility of SARS-CoV-2 Omicron BA.2.

Uraki R, Kiso M, Iida S[2], Imai M, Takashita E[37], Kuroda M[1], Halfmann PJ[1], Loeber S[4], Maemura T[1], Yamayoshi S, Fujisaki S[37], Wang Z[9], Ito M, Ujie M, Iwatsuki-Horimoto K, Furusawa Y, Wright R[1],

Chong Z[3], Ozono S[2], Yasuhara A, Ueki H, Sakai-Tagawa Y, Li R[9], Liu Y[9], Larson D[9], Koga M[33,38], Tsutsumi T[33,38], Adachi E[38], Saito M[33,38], Yamamoto S[33], Hagihara M[31], Mitamura K[30], Sato T[39], Hojo M[40], Hattori SI[41], Maeda K[41], Valdez R[42]; IASO study team, Okuda M, Murakami J, Duong C, Godbole S[43], Douek DC[43], Maeda K[44], Watanabe S[37], Gordon A[34], Ohmagari N[39], Yotsuyanagi H[33,38], Diamond MS[3,22,23,45], Hasegawa H[37], Mitsuya H[41,46], Suzuki T[2], Kawaoka Y. [37]Center for Influenza and Respiratory Virus Research, National Institute of Infectious Diseases, Tokyo, Japan. [38]Department of Infectious Diseases and Applied Immunology, IMSUT Hospital of The Institute of Medical Science, University of Tokyo, Tokyo, Japan. [39]Disease Control and Prevention Center, National Center for Global Health and Medicine Hospital, Tokyo, Japan. [40]Department of Respiratory Medicine, National Center for Global Health and Medicine Hospital, Tokyo, Japan. [41]Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Tokyo, Japan. [42]Department of Pathology, University of Michigan, Ann Arbor, MI, USA. [43]Human Immunology Section, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. [44]Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan.

The recent emergence of SARS-CoV-2 Omicron (B.1.1.529 lineage) variants possessing numerous mutations has raised concerns of decreased effectiveness of current vaccines, therapeutic monoclonal antibodies and antiviral drugs for COVID-19 against these variants. The original Omicron lineage, BA.1, prevailed in many countries, but more recently, BA.2 has become dominant in at least 68 countries. Here we evaluated the replicative ability and pathogenicity of authentic infectious BA.2 isolates in immunocompetent and human ACE2-expressing mice and hamsters. In contrast to recent data with chimeric, recombinant SARS-CoV-2 strains expressing the spike proteins of BA.1 and BA.2 on an ancestral WK-521 backbone, we observed similar infectivity and pathogenicity in mice and hamsters for BA.2 and BA.1, and less pathogenicity compared with early SARS-CoV-2 strains. We also observed a marked and significant reduction in the neutralizing activity of plasma from individuals who had recovered from COVID-19 and vaccine recipients against BA.2 compared to ancestral and Delta variant strains. In addition, we found that some therapeutic monoclonal antibodies (REGN10987 plus REGN10933, COV2-2196 plus COV2-2130, and S309) and antiviral drugs (molnupiravir, nirmatrelvir and S-217622) can restrict viral infection in the respiratory organs of BA.2-infected hamsters. These findings suggest that the replication and pathogenicity of BA.2 is similar to that of BA.1 in rodents and that several therapeutic monoclonal antibodies and antiviral compounds are effective against Omicron BA.2 variants.

4. Therapeutic efficacy of monoclonal antibodies and antivirals against SARS-CoV-2 Omicron BA.1 in Syrian hamsters.

Uraki R, Kiso M, Imai M, Yamayoshi S, Ito M, Fujisaki S[37], Takashita E[37], Ujie M, Furusawa Y, Yasuhara A, Iwatsuki-Horimoto K, Sakai-Tagawa Y, Watanabe S[37], Hasegawa H[37], Kawaoka Y.

The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the major antigen stimulating the host's protective immune response. Here we assessed the efficacy of therapeutic monoclonal antibodies (mAbs) against Omicron variant (B.1.1.529) sublineage BA.1 variants in Syrian hamsters. Of the FDA-approved therapeutic mAbs tested (that is, REGN10987/REGN10933, COV2-2196/COV2-2130 and S309), only COV2-2196/COV2-2130 efficiently inhibited BA.1 replication in the lungs of hamsters, and this effect was diminished against a BA.1.1 variant possessing the S-R346K substitution. In addition, treatment of BA.1-infected hamsters with molnupiravir (a SARS-CoV-2 RNA-dependent RNA polymerase inhibitor) or S-217622 (a SARS-CoV-2 protease inhibitor) strongly reduced virus replication in the lungs. These findings suggest that the use of therapeutic mAbs in Omicron-infected patients should be carefully considered due to mutations that affect efficacy, and demonstrate that the antiviral compounds molnupiravir and S-217622 are effective against Omicron BA.1 variants.

5. Characterization of SARS-CoV-2 Omicron BA.4 and BA.5 isolates in rodents.

Uraki R, Halfmann PJ[1], Iida S[2], Yamayoshi S, Furusawa Y, Kiso M, Ito M, Iwatsuki-Horimoto K, Mine S[2], Kuroda M[1], Maemura T[1], Sakai-Tagawa Y, Ueki H, Li R[47], Liu Y[47], Larson D[47], Fukushi S, Watanabe S[37], Maeda K[44], Pekosz A[48], Kandeil A[14,49], Webby RJ[14], Wang Z[47], Imai M, Suzuki T[2], Kawaoka Y: [45]Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, USA. [46]Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, NIH, Bethesda, MD, USA. [47]Department of Animal, Dairy, and Veterinary Sciences, College of Agriculture and Applied Sciences, Utah State University, Logan, UT, USA. [48]W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. [49]Center of Scientific Excellence for Influenza Viruses, National Research Centre, Giza, Egypt.

The BA.2 sublineage of the SARS-CoV-2 Omicron

variant has become dominant in most countries around the world; however, the prevalence of BA.4 and BA.5 is increasing rapidly in several regions. BA.2 is less pathogenic in animal models than previously circulating variants of concern. Compared with BA.2, however, BA.4 and BA.5 possess additional substitutions in the spike protein, which play a key role in viral entry, raising concerns that the replication capacity and pathogenicity of BA.4 and BA.5 are higher than those of BA.2. Here we have evaluated the replicative ability and pathogenicity of BA.4 and BA.5 isolates in wild-type Syrian hamsters, human ACE2 (hACE2) transgenic hamsters and hACE2 transgenic mice. We have observed no obvious differences among BA.2, BA.4 and BA.5 isolates in growth ability or pathogenicity in rodent models, and less pathogenicity compared to a previously circulating Delta (B.1.617.2 lineage) isolate. In addition, in vivo competition experiments revealed that BA.5 outcompeted BA.2 in hamsters, whereas BA.4 and BA.2 exhibited similar fitness. These findings suggest that BA.4 and BA.5 clinical isolates have similar pathogenicity to BA.2 in rodents and that BA.5 possesses viral fitness superior to that of BA.2.

6. A broadly protective human monoclonal antibody targeting the sialidase activity of influenza A and B virus neuraminidases.

Yasuhara A, Yamayoshi S, Kiso M, Sakai-Tagawa Y, Okuda M, Kawaoka Y

Improved vaccines and antiviral agents that provide better, broader protection against seasonal and emerging influenza viruses are needed. The viral surface glycoprotein hemagglutinin (HA) is a primary target for the development of universal influenza vaccines and therapeutic antibodies. The other major surface antigen, neuraminidase (NA), has been less well studied as a potential target and fewer broadly reactive anti-NA antibodies have been identified. In this study, we isolate three human monoclonal antibodies that recognize NA from A/H1N1 subtypes, and find that one of them, clone DA03E17, binds to the NA of A/H3N2, A/H5N1, A/H7N9, B/Ancestral-lineage, B/Yamagata-lineage, and B/Victoria-lineage viruses. DA03E17 inhibits the neuraminidase activity by direct binding to the enzyme active site, and provides in vitro and in vivo protection against infection with several types of influenza virus. This clone could, therefore, be useful as a broadly protective therapeutic agent. Moreover, the neutralizing epitope of DA03E17 could be useful in the development of an NA-based universal influenza vaccine.

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Social Cooperation Research Program

Project Division of RNA Medical Science
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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. Anti-TGF-β1 aptamer enhances therapeutic effect of tyrosine kinase inhibitor, gefitinib, on non-small cell lung cancer in xenograft model.

Masaki Takahashi, Yoshifumi Hashimoto, Yoshikazu Nakamura[1]: [1]RIBOMIC Inc., Minato-ku, Tokyo

Transforming growth factor β (TGF-β) is a multifunctional cytokine that plays crucial pathophysiological roles in various diseases, such as cancer and fibrosis. However, the disease modulation by targeting TGF-β1 isoform remains to be established, regardless of several studies employed with limited antibodies. Here, we developed an RNA aptamer to human active TGF-β1, named APT-β1, and characterized its properties in vitro and in vivo. APT-β1 bound to human and mouse active TGF-β1 proteins with high affinity and specificity and strongly inhibited TGF-β1-induced downstream signaling and cell morphology with 50% inhibition concentration (IC50) values at picomolar concentrations. In a xenograft mouse model of non-small cell lung cancer, APT-β1

alone showed no appreciable effect on tumor growth, while it greatly enhanced the anti-tumor effect of gefitinib, an approved tyrosine kinase inhibitor. These findings strongly suggest that the anti-TGF-β1 medication may be a promising cancer therapy to suppress repopulation of lung cancer in combination with certain anti-cancer drugs, such as gefitinib.

2. Nucleic acid aptamers emerging as modulators of G-protein coupled receptors: Challenge to difficult cell-surface proteins

Masaki Takahashi

G-protein-coupled receptors (GPCRs), among various cell surface proteins, are essential targets in the fields of basic science and drug discovery. The discovery and development of modulators for the receptors have provided deep insights into the mechanism of action of receptors and have led to a new therapeutic option for human diseases. Although various modulators against GPCRs have been developed to date, the identification of new modulators for GPCRs remains a challenge due to several technical problems and limitations. To overcome this situation, a variety of strategies have been developed by several modalities, including nucleic acid aptamers, which are emerging as unique molecules isolated by a repetitive selection process against various types of targets from an enormous combinatorial library. I reviewed and summarized the achievements in the development of aptamers targeting GPCRs, and discussed their isolation methods and the diverse functional features of aptamers against GPCRs.

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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.

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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

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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. 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, 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

2. Development of an Outward Proton Pumping Rhodopsin with a New Record in Thermostability by Means of Amino Acid Mutations

Yasuda S, Akiyama T, Kojima K, Ueta T, Hayashi T, Ogasawara S, Nagatoishi S, Tsumoto K, Kunishima N, Sudo Y, Kinoshita M, Murata T.

We have developed a methodology for identifying further thermostabilizing mutations for an intrinsically thermostable membrane protein. The methodology comprises the following steps: (1) identifying thermostabilizing single mutations (TSSMs) for residues in the transmembrane region using our physics-based method; (2) identifying TSSMs for residues in the extracellular and intracellular regions, which are in aqueous environment, using an empirical force field FoldX; and (3) combining the TSSMs identified in steps (1) and (2) to construct multiple mutations. The methodology is illustrated for thermophilic rhodopsin whose apparent midpoint temperature of thermal denaturation Tm is ~91.8 °C. The TSSMs previously identified in step (1) were F90K, F90R, and Y91I with ΔTm ~5.6, ~5.5, and ~2.9 °C, respectively, and those in step (2) were V79K, T114D, A115P, and A116E with ΔTm ~2.7, ~4.2, ~2.6, and ~2.3 °C, respectively (ΔTm denotes the increase in Tm). In this study, we construct triple and quadruple mutants, F90K + Y91I + T114D and F90K + Y91I + V79K + T114D. The values of ΔTm for these multiple mutants are ~11.4 and ~13.5 °C, respectively. Tm of the quadruple mutant (~105.3 °C) establishes a new record in a class of outward proton pumping rhodopsins. It is higher than Tm of Rubrobacter xylanophilus rhodopsin (~100.8 °C) that was the most thermostable in the class before this study.

3. Biophysical Characterization of the Contribution of the Fab Region to the IgG-FcγRIIIa Interaction

Kosuge H, Nagatoishi S, Kiyoshi M, Ishii-Watabe A, Terao Y, Ide T, Tsumoto K.

The cell-surface receptor FcγRIIIa is crucial to the efficacy of therapeutic antibodies as well as the immune response. The interaction of the Fc region of IgG molecules with FcγRIIIa has been characterized, but until recently, it was thought that the Fab regions were not involved in the interaction. To evaluate the influence of the Fab regions in a biophysical context, we carried out surface plasmon resonance analyses using recombinant FcγRIIIa ligands. A van't Hoff analysis revealed that compared to the interaction of the papain-digested Fc fragment with FcγRIIIa, the interaction of commercially available, full-length rituximab with FcγRIIIa had a more favorable binding enthalpy, a less favorable binding entropy, and a slower off rate. Similar results were obtained from analyses of IgG1 molecules and an IgG1-Fc fragment produced by Expi293 cells. For further validation, we also prepared a maltose-binding protein-linked IgG1-Fc fragment (MBP-Fc). The binding enthalpy of MBP-Fc was nearly equal to that of the IgG1-Fc fragment for the interaction with FcγRIIIa, indicating that such alternatives to the Fab domains as MBP do not positively contribute to the IgG-FcγRIIIa interactions. Our investigation strongly suggests that the Fab region directly interacts with FcγRIIIa, resulting in an increase in the binding enthalpy and a decrease in the dissociation rate, at the expense of favorable binding entropy.

4. Experimental Comparison of Bond Lifetime and Viscoelastic Relaxation in Transient Networks with Well-Controlled Structures

Katashima T, Kudo R, Naito M, Nagatoishi S, Miyata K, Chung UI, Tsumoto K, Sakai T.

We demonstrate an experimental comparison of the bond lifetime, estimated using surface plasmon resonance (SPR), and the viscoelastic relaxation time of transient networks with well-controlled structures (dynamically cross-linked Tetra-PEG gel). SPR and viscoelastic measurements revealed that the temperature dependences of the two characteristic times are in agreement, while the viscoelastic response is delayed with respect to the lifetime by a factor of 2-3, dependent on the network strand length. Polymers cross-linked by temporary interactions form transient networks, which show fascinating viscoelasticity with a single relaxation mode. However, the molecular understanding of such simple viscoelasticity has remained incomplete because of the difficulty of experimentally evaluating bond lifetimes and heterogeneous structures in conventional transient networks. Our results suggest that bond dissociation and recombination both contribute to the macromechanical response. This report on direct bond-lifetime-viscoelastic-relaxation time comparison provides important information for the molecular design of transient network materials.

5. Structure and role of the linker domain of the iron surface-determinant protein IsdH in heme transportation in Staphylococcus aureus

Valenciano-Bellido S, Caaveiro JMM, Morante K, Sushko T, Nakakido M, Nagatoishi S, Tsumoto K.

Staphylococcus aureus is a major cause of deadly nosocomial infections, a severe problem fueled by the steady increase of resistant bacteria. The iron surface determinant (Isd) system is a family of proteins that acquire nutritional iron from the host organism, helping the bacterium to proliferate during infection, and therefore represents a promising antibacterial target. In particular, the surface protein IsdH captures hemo-

globin (Hb) and acquires the heme moiety containing the iron atom. Structurally, IsdH comprises three distinctive NEAr-iron Transporter (NEAT) domains connected by linker domains. The objective of this study was to characterize the linker region between NEAT2 and NEAT3 from various biophysical viewpoints and thereby advance our understanding of its role in the molecular mechanism of heme extraction. We demonstrate the linker region contributes to the stability of the bound protein, likely influencing the flexibility and orientation of the NEAT3 domain in its interaction with Hb, but only exerts a modest contribution to the affinity of IsdH for heme. Based on these data, we suggest that the flexible nature of the linker facilitates the precise positioning of NEAT3 to acquire heme. In addition, we also found that residues His45 and His89 of Hb located in the heme transfer route toward IsdH do not play a critical role in the transfer rate-determining step. In conclusion, this study clarifies key elements of the mechanism of heme extraction of human Hb by IsdH, providing key insights into the Isd system and other protein systems containing NEAT domains.

6. Ladder observation of bovine serum albumin by high resolution agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Nagatoishi S, Tsumoto K, Arakawa T, Akuta T.

A commercially available bovine serum albumin (BSA) was examined by agarose native gel electrophoresis using two different agarose sources, UltraPure and MetaPhor agarose. While UltraPure agarose up to 5 % showed no clear separation of BSA oligomers, MetaPhor agarose clearly demonstrated oligomer bands above 4 %, indicating that the latter agarose has greater molecular sieving effects and is hence characterized to have high resolution for size differences, as probed by a greater slope of Ferguson plot. Physical properties are different between two agaroses. In general, UltraPure agarose has physical strength, while MetaPhor agarose is considerably fragile, but MetaPhor agarose solution is less viscous so that even 10 % gel can be made. Cause of oligomers was shown to be not associated with inter-chain disulfide bonds, but is due to association of native or native-like molecules.

7. Analysis of bovine serum albumin unfolding in the absence and presence of ATP by SYPRO Orange staining of agarose native gel electrophoresis

Tomioka Y, Nakagawa M, Sakuma C, Kurosawa Y, Nagatoishi S, Tsumoto K, Arakawa T, Akuta T.

An attempt was made to specifically stain unfolded proteins on agarose native gels. SYPRO Orange is routinely used to detect unfolded protein in differential scanning fluorimetry, which is based on the enhanced fluorescence intensity upon binding to the unfolded protein. We demonstrated that this dye barely bound to the native proteins, resulting in no or faint staining of the native bands, but bound to and stained the unfolded proteins, on agarose native gels. Using bovine serum albumin (BSA), it was shown that staining did not depend on whether BSA was thermally unfolded in the presence of SYPRO Orange or stained after electrophoresis. On the contrary, SYPRO Orange dye stained protein bands in the presence of sodium dodecylsulfate (SDS) due to incorporation of the dye into SDS micelles that bound to the unfolded proteins. This staining resulted in detection of new, intermediately unfolded structure of BSA during thermal unfolding. Such intermediate structure occurred at higher temperature in the presence of ATP.

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Social Cooperation Research Program

Project Division of Cancer Biomolecular Therapy
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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 have found that specific gene contribute to such upregulation. 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, 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. Experiments using immunocompetent mice to examine the anti-tumor immunity induced by the treatment with our RRsyn-oHSV are ongoing. 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
Project Assistant Professor	Tomoko Takahashi, Ph.D.	特任助教	博士(理学)	高橋朋子

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 (extended until March 2024) 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], Tomoko Takahashi[1], Masaru Koido[2], Momoko Horikoshi[3], Takayuki Morisaki[1], 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 project 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 has been extended until March 2024.

Representative publications -

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Ibrahim W, Wilde MJ, Cordell RL, Richardson M, Salman D, Free RC, Zhao B, Singapuri A, Hargadon B, Gaillard EA, Suzuki T, Ng LL, Coats T, Thomas P, Monks PS, Brightling CE, Greening NJ, Siddiqui S; EMBER Consortium.
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Social Cooperation Research Program

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
Project Assistant Professor	Kimihito Kawabata, M.D., D.M.Sc.	特任助教　博士（医学）	川　畑　公　人

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 screening (DSS) 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 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. We have a joint research agreement with Daiichi Sankyo RD Novare Co., Ltd. in 2021 and are pursuing our research activity.

In 2022, we continued to focus on the development of experimental flow for DSS using primary tumor specimens derived from patients with hematologic malignancies. In order to build a novel platform for precision medicine projects combining DSS and comprehensive multi-omics analysis, we aimed to optimize tissue culture methods for leukemia cells obtained from patients with acute myeloid leukemia. Using clinical specimens provided by the Department of Hematology and Oncology, IMSUT Hospital, we evaluated the optimal tissue culture conditions for 3 to 9 days of ex vivo coculture with chemotherapeutic drugs/ epigenetic compounds. As a result, we were able to establish an in-house tissue culture medium that could maintain the hematopoietic stem cell and progenitor cell-like fractions of AML cells throughout the ex vivo drug treatment period. While processing these cells, we also established a high-throughput assay system using automated technologies.

These efforts are highly expected to become a tool for elucidating the pathophysiology of hematologic malignancies and developing further therapeutics.

1. Clinical precision research for hematological diseases by genomic and multi omics analysis

Kimihito Cojin Kawabata[1], Hironobu Komori[1,2], Hayato Tsuji[1,2], Yoshiharu Takama[1,2], Seiko Kato[1], Maiko Morita[1], Kiyoko Izawa[1], Sanae Suzuki[1], Tetsushi Oka[2], Yoshimasa Ono[2], Kenji Wakabayashi[2], Gen Kudo[2], Satoshi Takahashi[1].

[1] IMSUT, [2] Daiichi Sankyo RD Novare Co., Ltd.

Currently, our entire research network is already working within or outside IMSUT on whole genome, RNA expression, transcriptome, DNA methylation, and genome structure analysis of malignant hematopoietic cells, aiming to compare the analysis at the single cell level with the actual clinical course of the

patient. Our group is now focused on installing the DSS part of the project based on good scientific evidence.

In order to validate the tissue culture conditions of primary AML specimens by our group and collaborators, or potentially optimize them for the precision research platform, we repeated the testing of clinical specimens kindly provided by the Department of Hematology and Oncology based on the goodwill of patients. The initial culture conditions for side-by-side comparison were based on previous experiences with ex vivo culture of AML specimens (Kawabata et.al, Blood, 2021, and MS in preparation), and each of the key questions "What is the optimal concentration of serum?" and "Is the use of a serum substitute beneficial for tumor specimens?" were carefully tested. After careful evaluation and further discussion of these data, we were able to find the optimal in-house tissue culture medium for primary AML specimens cultured for up to 9 days while maintaining a certain level of immature components (such as CD34 positive fraction). Those pilot experiments are extremely important because the information obtained from these cultured cells will be used in DSS and omics research. We are now able to treat the specimens for 6 days under these "tentative" optimal culture conditions and have already started this DSS culture system with several AML samples. The next step is to attempt long-term culture and expand the valuable but quantitatively limited primary cultures ex vivo.

Finally, by integrating this information, we plan to advance more precise pathological analysis, search for factors involved in drug sensitivity, and then translate the results back to the clinic to create an analysis system that will assist in real-time decision-making on treatment strategies. In addition to these clinical implications, the project will also shed light on multiple hotspots, including biomarker discovery, the immune environment of hematologic malignancies, detection of minimal residual disease, and even basic biology such as epigenetic regulation.

2. Generation of antigen-specific T cells derived from cord blood

Morita Maiko[1], Kimihito Cojin Kawabata[1], Kiyoko Izawa[1], Satoshi Yamazaki[1,2], Ai Tachikawa-Kawana[1,3], Patrick Hanley[4], Catherin Bollard[4], Seiko Kato[1], Satoshi Takahashi[1]

[1] IMSUT, [2] University of Tsukuba, [3] National Institute of Infectious Diseases, [4] Children's National Research Institute

This project aims to establish a method other than gene transfer to generate and amplify viral antigen-specific T cells from naïve T cells derived from umbilical cord blood.

Previous studies have shown that cGAMP, a type of STING ligand, triggers a type IFN response and promotes cross-priming of antigen-specific CD8 + T cells by mature DCs. It has also been reported that cGAMP induces the transcription factor T-bet, which is required for the development of effector CD8 + T cells. When naïve T cells derived from cord blood were cultured with cGAMP and viral antigen peptides twice for 14 days, a significant production of inflammatory cytokines such as IFN-γ and TNF-α was observed. As the next step, we plan to examine culture conditions, such as cytokines and supplements combined with cGAMP, to explore more effective methods of producing and amplifying viral antigen-specific T cells.

Future clinical studies are planned to advance this research and improve the safety of cord blood transplantation by promoting immune reconstitution against viral infection 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

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.		教　授	博士(医学)	四　柳　　宏
Project Associate Professor	Hiroshi Yasui, M.D., D.M.Sc.		特任准教授	博士(医学)	安　井　　寛
Project Assistant Professor	Muneyoshi Futami, M.D., D.M.Sc.		特任助教	博士(医学)	二　見　宗　孔

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 Yotsuyanagi, Hiroshi Yasui[1], Muneyoshi Futami, Kiyosumi Ochi, Asako Kobayashi, Reika Li, Mikiko Suzuki, Keiji Hirano[2], Yuma Oka[2], Masatoshi Yanagida[2], ArinobuTojo[3]

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[3]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[1], Mikiko Suzuki, Kiyosumi Ochi, Fumitaka Nagamura[2], Arinobu Tojo[3]

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[2]Center for Translational Research, 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[1], Arinobu Tojo[2], Kaoru Uchimaru[3], Toshiki Watanabe[1]
[1]St. Marianna University School of Medicine
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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[1]
[1]St. Marianna University School of Medicine

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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Corporate Sponsored Research Program

Project Division of Oncolytic Virus Development

ウイルス療法開発寄付研究部門

Project Professor	Minoru Tanaka, M.D., Ph.D.	特任教授	博士(医学)	田 中	実
Project Assistant Professor	Seisaku Kanayama, M.D.	助 教		金 山 政	作

We have been conducting basic research and clinical projects to devise oncolytic virus therapies for solid cancers, including glioblastoma, olfactory neuroblastoma, and malignant pleural mesothelioma. We focus on oncolytic virus drug manufacturing processes, including scale-up, purification, filling, quality and stability testing, and characterization, as well as the development of next-generation oncolytic virus drugs to contribute to the prevalence of oncolytic virus therapy in Japan.

Introduction

Our division was established as an endowed division by Denka Company Limited. We work in close conjunction with the laboratory of Innovative Cancer Therapy. Oncolytic viruses are genetically modified to replicate in and kill cancer cells while leaving normal tissues unharmed. The genetic modification of the viruses also grants them the ability to elicit anti-cancer immunity through multiple mechanisms of the patient's immune system. Genetically engineered, conditionally replicating herpes simplex viruses type 1 (HSV-1) are promising therapeutic agents for solid cancers. Our division focuses on process development and scale-up of oncolytic HSV-1 production.

A triple-mutated, third-generation oncolytic HSV-1, G47Δ, teserpaturev.

We developed a triple-mutated, third-generation oncolytic HSV-1, G47Δ, teserpaturev that has triple mutations within the viral genome. A phase II clinical trial of G47Δ was conducted since 2014 in patients with glioblastoma. In June 2021, G47Δ was approved as the world's first oncolytic virus drug for malignant gliomas. Upon commercial distribution, the oncolytic virus therapy using G47Δ (Delytact®) for patients with malignant glioma started at IMSUT hospital in November 2021. The clinical trial is ongoing in patients with olfactory neuroblastoma.

Production of clinical-grade oncolytic HSV-1

We excel at producing master virus seed stocks (MVSS) and subsequent production of working virus seed stocks (WVSS): free of contamination, replication-competent (high titer), identity, purity, and stability. We begin with selecting cell lines for adherent or suspension culture growth, optimization of media and buffers, cell lysis, and purification of oncolytic HSV-1. We performed G47Δ genome structure analysis, stability tests, and preclinical safety evaluation. Clinical-grade G47Δ products were prepared at the Therapeutic Vectors Development Center, IMSUT hospital, with Good Manufacturing Practice (GMP). The Therapeutic Vectors Development Center has been maintained to meet the current GMP standard through regular validation of equipment and production and an ISO9001:2015-certified quality management system. We continue to optimize oncolytic

HSV-1 production to improve their safety, efficacy, and manufacturability for scale-up.

A recombinant herpes simplex type 1 with human IL-12 expression, T-hIL12

One of the advantages of HSV-1 is its capacity to incorporate large or multiple transgenes within the viral genome. Incorporating transgenes encoding immunomodulatory molecules into G47Δ can enhance its ability to trigger anti-cancer immunity. T-hIL12 is a G47Δ-based recombinant HSV-1 that expresses human interleukin-12 (IL-12). This IL-12-mediated anti-tumor immunity is thought to be T cell-mediated. We started a phase 1/2 clinical trial of T-hIL12 in patients with malignant melanoma in January 2020 jointly with Shinshu University.

Publications

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Consortium

Consortium for Gene Therapy and Regenerative Medicine

遺伝子治療・再生医療コンソーシアム

Professor	Atsushi Iwama, M.D., Ph.D.	教　授	博士(医学)	岩　間　厚　志
Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Professor	Kaori Muto, Ph.D.	教　授	博士(保健学)	武　藤　香　織
Professor	Takashi Okada, M.D., Ph.D.	教　授	博士(医学)	岡　田　尚　巳
Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学)	谷　口　英　樹
Associate Professor	Tokiko Nagamura-lnoue, M.D., D.M.Sc.	准教授	博士(医学)	長　村　登紀子

Recent advances in gene therapy, regenerative medicine, and cell therapy have closely linked these fields scientifically as well as in clinical practice. These fields have common target cells, organs, or diseases and utilize similar technologies. Based on these recent trends, we founded a consortium for Gene Therapy and Regenerative Medicine in 2022, in which IMSUT researchers working on gene therapy, regenerative medicine, cell therapy, and Ethical, Legal and Social Issues (ELSI) liaise closely with each other and promote front-line research. Core members belong to the Center for Gene and Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, and Advanced Clinical Research Center, but we recruit all IMSUT researchers interested in these fields and aim to develop this consortium into an international hub for gene therapy and regenerative medicine.

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, Ayako Miyake

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 funds

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 Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教授・室長	博士(医学)	武 川 睦 寛
Advisor and Senior Professor	Jun-ichiro Inoue, Ph.D.	特命教授・アドバイザー	薬学博士	井 上 純一郎

"Platforms for Advanced Technologies and Research Resources" (platform.umin.jp/) was launched in fiscal year (FY) 2022 under the new framework of the Grant-in-Aid for Transformative Research Areas (A) 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 programs "Support Programs for Three Fields in Life Sciences (Cancer, Genome and Brain Sciences) (FY 2010-2015) and "Platforms for Advanced Technologies and Research Resources (FY2016-2021)". "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 more than 80 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 21 members participated: four platform representatives and 17 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, Jun-ichiro Inoue, and Mutsuhiro Takekawa

The following activities have been performed under the management of this office in 2022.

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, 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 aging studies, artificial intelligence (AI) and cancer research in June and December 2022.

3. Language Assistance

Kazuyo Ohara

In 2022, with the COVID-19 pandemic still evolving and impacting on the health and wellbeing of the IMSUT members, the International Affairs Office worked closely with the administrative staff to provide international members of IMSUT with important notices and latest information in English. This includes the Dean's messages and brief outlines of the preventive guidelines which were frequently updated by the UTokyo Novel Coronavirus Task Force. The office also vigorously undertook various other translation works and proofreading, aiming to create 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
Visiting Professor	Takayuki Morisaki, M.D., Ph.D.	客員教授	医学博士	森　崎　隆　幸
Project Professor	Koichi Matsuda, M.D., Ph.D.	特任教授	博士(医学)	松　田　浩　一
Adjunct Professor	Yoichiro Kamatani, M.D., Ph.D.	連携教授	博士(医科学)	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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9: Yukihide Momozawa, Rumi Sasai, Yoshiaki Usui, Kouya Shiraishi, Yusuke Iwasaki, Yukari Taniyama, Michael T. Parsons, Keijiro Mizukami, Yuya Sekine, Makoto Hirata, Yoichiro Kamatani, Mikiko Endo, Chihiro Inai, Sadaaki Takata, Hidemi Ito, Takashi Kohno, Koichi Matsuda, Seigo Nakamura, Kokichi Sugano, Teruhiko Yoshida, Hidewaki Nakagawa, Keitaro Matsuo, Yoshinori Murakami, Amanda B. Spurdle, Michiaki Kubo Expansion of Cancer Risk Profile for BRCA1 and BRCA2 Pathogenic Variants JAMA Oncology 2022 Apr 14.
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SCIENTIFIC MEETINGS & SEMINARS

49th 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「老化医科学の新機軸」というテーマで講演をお願いした。

日　時：令和 4 年 5 月 27 日（金） 13：00 ～ 17：00
会　場：Zoom によるオンライン開催

Toshifumi Inada（Division of RNA and gene regulation, Department of Basic Medical Sciences, IMSUT）
Translation quality control in protein homeostasis and aging

Emi Nishimura（Division of Aging and Regeneration, Department of Cancer Biology , IMSUT）
Stem cell-centric mechanisms of organ aging: Lessons from the skin

Tatsushi Igaki（Kyoto University Graduate School of Biostudies）
Dissecting cellular senescence and aging in Drosophila

Toshiro Sato（Department of Organoid Medicine, Keio University School of Medicine）
Understanding of human gastrointestinal diseases using organoid technology

Makoto Nakanishi（Division of Cancer Cell Biology, Department of Cancer Biology , IMSUT）
Can we control aging process?

学友会セミナー

（令和４年１月～令和４年12月）

１月13日　演題：　免疫グロブリン・スーパーファミリー細胞接着分子群を介するがん細胞の浸潤、転移の分子機構の解析
　　演者：　笠井　優
１月17日　演題：　幹細胞競合ダイナミクスを軸とした皮膚の恒常性維持機構
　　演者：　松村　寛行
２月１日　演題：　造血器腫瘍の発症メカニズム解明と新規治療の開発
　　演者：　福山　朋房
２月９日　演題：　感染症を包括的に理解するためのシステムウイルス学研究とオールジャパンによる最先端の新型コロナウイルス研究
　　演者：　佐藤　　佳
２月10日　演題：　腸内共生病原菌に対する新規制御法の開発
　　演者：　藤本　康介
２月10日　演題：　慢性炎症性疾患としてのHIVとHIV合併COVID-19の臨床と研究
　　演者：　安達　英輔
３月１日　演題：　患者由来血液腫瘍細胞大規模コホートを用いた薬剤感受性プロファイルの有用性
　　演者：　川畑　公人
３月４日　演題：　先進モデル動物作製コアにおける遺伝子改変マウス作製の取り組み
　　演者：　田口　純平
５月11日　演題：　肝臓線維化マウスの細菌叢に与えるリファキシミンの影響
　　演者：　池内　和彦
５月17日　演題：　データ駆動ウイルス学の確立と普及
　　演者：　伊東　潤平
５月19日　演題：　血液疾患における臨床シークエンスの取り組み
　　演者：　横山　和明
５月31日　演題：　乳癌においてDYRK2はCDK14を介して腫瘍増殖を制御する
　　演者：　井廻　良美
６月７日　演題：　ERK経路の活性調節機構と癌および先天性Ras-MAPK症候群（RASopathy）におけるその破綻
　　演者：　久保田　裕二
６月８日　演題：　メタゲノム解析とAIによる病態の総合理解
　　演者：　佐藤　憲明
６月27日　演題：　出生コホートにおける臍帯組織テロメア長と母体のPCB暴露の関連、ゲノム解析のパイロット研究について
　　演者：　髙橋　朋子

6月30日　演題：　深層学習を用いた腹腔鏡下大腸切除術における自律神経のセグメンテーションに関する研究
　　演者：　小島　成浩
8月25日　演題：　大規模バイオバンクを用いた多様な集団における疾患原因変異の同定
　　演者：　金井　仁弘
8月26日　演題：　百万人規模のゲノム情報を医療に役立てるための数理手法を創る
　　演者：　坂上　沙央里
9月15日　演題：　表皮幹細胞に着目した再生と老化に関する研究
　　演者：　浅川 杏祐
10月 3 日　演題：　自然免疫応答の遺伝的多様性を単一細胞分解能で理解する
　　演者：　熊坂　夏彦
10月 7 日　演題：　造血幹細胞制御機構の理解とその制御法の創出
　　演者：　小出　周平
10月11日　演題：　深層生成モデルで腫瘍微小環境の時空間動態を読み解く
　　演者：　島村　徹平
10月21日　演題：　プロリン異性化酵素によるがん細胞の増殖制御機構
　　演者：　島田　緑
10月21日　演題：　細胞膜損傷は細胞老化を誘導する
　　演者：　河野　恵子
11月 4 日　演題：　Innate immune memory induced by vaccines
　　演者：　Anne-Sophie Beignon
11月28日　演題：　乳がん治療耐性機構の解明と治療刷新を目指した革新的創薬開発
　　演者：　片桐　豊雅
11月29日　演題：　Evolution of mechanisms for sensing and responding to ribosome stalling and collision
　　演者：　Claudio Joazeiro
12月 7 日　演題：　シナプス可塑性制御における小胞体カルシウムセンサータンパク STIM1 の機能的役割
　　演者：　小林　静香
12月 7 日　演題：　Structural basis of co-translational quality control
　　演者：　Roland Beckmann
12月14日　演題：　クライオ電子顕微鏡を用いたリボソームのユビキチン化および脱ユビキチン化による翻訳制御機構の解明
　　演者：　池内　健
12月22日　演題：　Dissecting Human Brain Diseases with Organoids and Single-Cell Sequencing
　　演者：　田中 義章
12月23日　演題：　急性ストレス下において過度な恐怖表出を抑制する分子および神経回路の同定
　　演者：　後藤　史子
12月23日　演題：　中国における東大医科研の国際共同研究
　　演者：　林　光江

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。2022 年には、「新規 ＲＮＡ 機能から創薬へ」というテーマの下で次のようなセミナーが行われた。

新規 ＲＮＡ 機能から創薬へ

	月　日	講 師 名		演　　題
1	4 月 4 日	稲田　利文	東京大学医科学研究所 RNA 制御学分野 教授	リボソーム動態制御によるタンパク質恒常性維持機構と疾患
2	4 月 11 日	齊藤　博英	京都大学 iPS 細胞研究所 教授	RNA を基盤とする生命システムの制御と創成
3	4 月 18 日	泊　　幸秀	東京大学 定量生命科学研究所 教授	小さな RNA の大きな世界
4	4 月 25 日	岩崎　信太郎	理化学研究所開拓研究本部 主任研究員	翻訳網羅解析
5	5 月 9 日	竹内　　理	京都大学大学院医学系研究科 教授	免疫疾患における mRNA 制御の役割
6	5 月 16 日	塩見　美喜子	東京大学大学院理学系研究科 生物科学専攻 教授	piRNA を介したトランスポゾン抑制機構は生殖能維持に必須である
7	5 月 23 日	鈴木　　勉	東京大学大学院工学系研究科 化学生命工学専攻 教授	RNA 修飾によるエピトランスクリプトミクス制御と疾患
8	5 月 30 日	Piero CARNINCI	理化学研究所 生命医科学研究センター 副センター長	Transcriptional regulation revealed by high-throughput technologies: towards nucleic acid medicine
9	10 月 3 日	萩原　正敏	京都大学大学院医学研究科 形態形成機構学 教授	RNA スプライシング制御化合物による創薬
10	10 月 17 日	廣瀬　哲郎	大阪大学 大学院生命機能研究科 教授	非コード RNA による細胞内構造構築
11	10 月 24 日	位高　啓史	東京医科歯科大学 生体材料工学研究所 教授	mRNA 創薬の新展開
12	10 月 31 日	中川　真一	北海道大学大学院薬学研究院 RNA 生物学研究室 教授	ノンコーディング RNA による生体制御
13	11 月 7 日	高橋　理貴	東京大学医科学研究所 「RNA 医科学」社会連携研究部門 特任准教授	新規創薬モダリティとして機能性 RNA

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４年 12 月 10 日（土）９：15 ～ 16：40
　　令和４年 12 月 11 日（日）９：30 ～ 16：40
場　所：医科学研究所１号館１階講堂

教員および題目

12 月 10 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
山本　瑞生	アジア感染症研究拠点	新型コロナウイルスの膜融合機構の治療薬探索
山本　元久	附属病院 アレルギー免疫科	臨床から研究、そして臨床へ～ lgG4 関連疾患
村上　善則	癌・細胞増殖部門 人癌病因遺伝子分野	細胞接着とがん

12 月 11 日（日）

講　師　名		題　　目
林　　光江	アジア感染症研究拠点	中国における医科研の国際共同研究プロジェクト
南谷　泰仁	先端医療研究センター 造血病態制御分野	ゲノム研究による疾患の起源の解明
吉見　一人	実験動物研究施設 先進動物ゲノム研究分野	ゲノム編集技術の基礎から応用まで
舘林　和夫	遺伝子解析施設 フロンティア研究領域	細胞が環境ストレスに適切に応答する仕組み

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