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Annual Report

2023

Preface

We are pleased to present the FY2023 Annual Report of the Institute of Medical Science at the University of Tokyo (IMSUT). The predecessor of IMSUT, the Institute of Infectious Diseases (IID), was founded in 1892 by Dr. Shibasaburo Kitasato and incorporated into the University of Tokyo in 1916. At that time, infectious diseases were the greatest threat to public health, and IID was a key center of infectious disease research in Asia and a leading international research center. As this report shows, IMSUT, as the successor to IID, has continued to dedicate itself to tackling infectious diseases such as COVID-19, but has also expanded its mission cancer and other intractable diseases.

In 1967, IMSUT was reorganized as a research institute covering a wider range of medical fields to meet the demands of modern medicine arising after World War II, and adopted its present name. IMSUT's mission has evolved to contribute to the development and welfare of human society through advanced medical research and the practice of cutting-edge medicine, based on the history and traditions of the more than 130 years since its founding. To achieve this mission, IMSUT promotes interdisciplinary research and conducts a wide range of practical research projects, from the establishment of artificial intelligence (AI) and support for state-of-the-art AI medicine, to the development of pharmaceuticals, including gene, virus and vaccine therapies, to cell and organ transplantation using stem cells and iPS cells, to new developments in genomic medicine.

For more than a century, IMSUT has followed the three guiding principles established by Dr. Kitasato: "practical learning" for the benefit of society, "diverse and comprehensive research," and "disease prevention." These three principles are the foundation of medical practice and rapidly developing research at IMSUT, and we are further opening up new intellectual horizons based on them by harnessing vast amounts of information with AI. We are accelerating the efficient use of new technologies by promoting the establishment of medical AI research with our dedicated supercomputer "Shirokane" and have already realized AI-guided medicine for hematopoietic tumors and other diseases at IMSUT hospital.

Importantly, IMSUT was accredited by the Minister of Education, Culture, Sports, Science and Technology in 2018 as the only International Joint Usage/Research Center in Japan in the field of life sciences. In recognition of its activities and achievements, IMSUT was reaccredited last year and is continuing its center project for the next six years. Through this platform, IMSUT is supporting 29 international collaborative research projects in FY2023. As a world-leading medical research institute, we endeavor to further contribute to the development of the basic, translational and clinical research undertaken by the global research community.

This annual report summarizes the scientific achievements of IMSUT in 2023. It is our sincere hope that these results will inspire further research, foster collaboration with scientists around the world, and ultimately contribute to the improvement of global healthcare.

January 2024

Makoto Nakanishi, M.D., Ph.D.
Dean
The Institute of Medical Science
The University of Tokyo

Organization and Faculty Members

機構および職員

〈as of December, 2023〉

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ウイルス病態制御分野

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Project Assistant Professor	Jalal ALSHAWEESH, Ph.D.	特任助教	博士(医学)	アルシャウィシュ　ジャラル

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Project Assistant Professor	Yu Kaku, Ph.D., M.D.	特任助教	博士(医学)	郭　　悠

Department of Cancer Biology　癌・細胞増殖部門

Department of Basic Medical Sciences　基礎医科学部門

Center for Stem Cell Biology and Regenerative Medicine　幹細胞治療研究センター

International Research Center for Infectious Diseases　感染症国際研究センター

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

Medical Proteomics Laboratory ····· 210

疾患プロテオミクスラボラトリー

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		(大学院工学系研究科)		
Assistant Professor	Ryo Matsunaga, Ph.D.	助　教	博士(生命科学)	松　長　　遼
		(大学院工学系研究科)		
Project Assistant Professor	Hiroshi Sagara, Ph.D.	特任助教	博士(医学)	相　良　　洋

Research Center for Asian Infectious Diseases ····· 230

アジア感染症研究拠点

Director/Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	拠点長／教授	博士(獣医学)	川　口　　寧
Project Professor	Mitsue Hayashi, Ph.D.	特任教授	法学博士	林　　光　江
Visiting Professor	Masaki Imai, D.V.M., Ph.D.	客員教授	博士(獣医学)	今　井　正　樹
Visiting Professor	Seiya Yamayoshi, D.V.M., Ph.D.	客員教授	博士(医学)	山　吉　誠　也
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加　藤　哲　久
Project Associate Professor	Jin Gohda, Ph.D.	特任准教授	博士(薬学)	合　田　　仁
Project Senior Assistant Professor	Mizuki Yamamoto, Ph.D.	特任講師	博士(医学)	山　本　瑞　生
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)	丸　鶴　雄　平

Laboratory of Molecular Genetics (Frontier Research Unit) ····· 236

遺伝子解析施設（フロンティア研究領域）

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	理学博士	中　西　　真
Associate Professor	Kazuo Tatebayashi, Ph.D.	准教授	博士(薬学)	舘　林　和　夫

IMSUT Hospital　附属病院

Department of Hematology/Oncology ····· 238

血液腫瘍内科

Professor	Yasuhito Nannya, M.D., D.M.Sc.	教　授	博士(医学)	南　谷　泰　仁
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.	特任准教授	博士(医学)	高　橋　　聡
Associate Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	准教授	博士(医学)	長　村　登紀子
Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授	博士(医学)	小　沼　貴　晶
Associate Professor	Kazuaki Yokoyama, M.D., Ph.D.	准教授	博士(医学)	横　山　和　明
Project Associate Professor	Hiroshi Yasui, M.D., D.M.Sc.	特任准教授	博士(医学)	安　井　　寛
Assistant Professor	Tomofusa Fukuyama, M.D., D.M.Sc.	助　教	博士(医学)	福　山　朋　房
Assistant Professor	Masamichi Isobe, M.D., D.M.Sc.	助　教	博士(医学)	磯　部　優　理
Assistant Professor	Toyotaka Kawamata, M.D., D.M.Sc.	助　教	博士(医学)	川　俣　豊　隆
Assistant Professor	Aki Sato, M.D., D.M.Sc.	助　教	博士(医学)	佐　藤　亜　紀

バイオバンク・ジャパン

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	医学博士	中　西　　真
Professor	Yuji Yamanashi, Ph.D.	教　授	理学博士	山　梨　裕　司
Project Professor	Koichi Matsuda, M.D., Ph.D.	特任教授	博士(医学)	松　田　浩　一
Cooperative Professor	Yoichiro Kamatani, M.D., Ph.D.	連携教授	博士(医科学)	鎌　谷　洋一郎
Visiting Professor	Takayuki Morisaki, M.D., Ph.D.	客員教授	医学博士	森　崎　隆　幸

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放射線管理室

Head	Kensuke Miyake, M.D., Ph.D.	室長	医学博士	三　宅　健　介

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IT サービス室

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Office of Health and Safety

安全衛生管理室

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Office of Intellectual Property

知的財産室

Head	Mutsuhiro Takekawa, M.D., Ph.D.	室長	博士(医学)	武　川　睦　寛

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利益相反アドバイザリー室

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Pathology Core Laboratory

病理コアラボラトリー

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Head of Laboratory II	Yasunori Ota, M.D., Ph.D.	Ⅱ室室長	博士(医学)	大　田　泰　徳

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IMSUT 臨床フローサイトメトリー・ラボ

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IMSUT-HLC Cell Processing Facility

IMSUT-HLC セルプロセッシング施設

Head	Tokiko Nagamura-Inoue, M.D., Ph.D.	施設長	博士（医学） 長　村　登紀子

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事務部

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Manager of Administrative Affairs Division	Yoko Akutsu	管理課長	坏　　陽　子
Manager of Research Support Division	Yuji Takayama	研究支援課長	高　山　勇　二
Manager of Hospital Division	Makoto Jin	病院課長	神　　誠

RESEARCH ACTIVITIES

Department of Microbiology and Immunology

Division of Infectious Genetics
感染遺伝学分野

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

Immune cells express multiple Toll-like receptors (TLRs) which are crucial for recognizing pathogen-derived products from microorganisms and viruses. Recent studies have demonstrated that dysregulation in TLR signaling is a critical factor in the onset of autoimmune and autoinflammatory diseases. Nucleic acid(NA) -sensing TLRs detect not only bacterial and viral NAs, but also host-derived NAs. This raises the possibility of a sophisticated regulatory mechanism that finely tunes NA -sensing TLR expression, subcellular localization, and functional activity to avoid excessive responses to self-derived NAs. This concept suggests that elucidating the regulatory mechanisms of NA-sensing TLRs could advance our understanding of the pathogenesis and process of various TLR-induced 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. We finally aim to unravel the complex pathogenic mechanisms underlying histiocytosis and autoimmune diseases such as Systemic Lupus Erythematosus, which are hypothesized to be mediated by aberrant TLR activation.

1. 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 the lysosomal nucleoside transporter 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 TLR7/8 responses to lysosomal nucleoside stress drive SLC29A3 disorders.

2. Toll-like receptor 7 signaling modifies the subset pattern and functions of monocytes in NZBWF1 mice.

Reika Tanaka[1], Ryutaro Fukui[1], Yusuke Murakami[1,2], Yinga Wu[3], Marie Sekiguchi[3], Shigaru Kakuta[3], Naomi Yamashita[2], Kensuke Miyake[1,4]
: [1]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo. [2]Department of Pharmacotherapy, Research Institute of Pharmaceutical Sciences, Musashino University. [3]Laboratory of Biomedical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo. [4]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production and multiple organ damage. Toll-like receptor 7 (TLR7), an innate immune RNA sensor expressed in monocytes/macrophages, dendritic cells (DCs), and B cells, promotes disease progression. We showed that the lupus nephritis in NZBWF1 mice is ameliorated with anti-mouse TLR7 monoclonal antibody in previous study (Murakami and Fukui, Front. Immunol. 2021). To investigate how TLR7 drives lupus nephritis, we generated *Tlr7*$^{-/-}$ (TLR7 knockout, TLR7-KO) NZBWF1 mice. The phenotype of TLR7-KO NZBWF1 mice is similar to anti-TLR7 antibody treated NZBWF1 mice and more reliable. We compared the immune cells in TLR7-KO NZBWF1 mice and WT by single cell RNA-sequencing, and found that an atypical subset of monocytes expands only in the spleen of WT NZBWF1 mice. We also found that the infiltrating patrolling monocytes in glomeruli expressing specific markers, for example, PD-L2. We are analyzing the function of these monocyte subsets focusing on the cytokine production and the interaction with B cells.

3. Thioredoxin-mediated suppression of NLRP1 inflammasome

Zhikuan Zhang[1], Takuma Shibata[2], Akiko Fujimura[1], Jiro Kitaura[3], Kensuke Miyake[2], Umeharu Ohto[1] and Toshiyuki Shimizu[1]
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Inflammasome sensors detect pathogen- and danger-associated molecular patterns and promote inflammation and pyroptosis. The nucleotide-binding

oligomerization domain-like receptor (NLR) family pyrin-domain containing protein 1 (NLRP1) was the first inflammasome sensor described, and its hyperactivation is linked to autoinflammatory disease and cancer. However, the mechanism underlying the activation and regulation of NLRP1 has not been clearly elucidated and, therefore, in this study, we sought to further investigate this phenomenon. We identified ubiquitously expressed endogenous thioredoxin (TRX) as a binder of NLRP1 and a suppressor of the NLRP1 inflammasome. The cryo-electron microscopy (cryo-EM) structure of a binary complex of NLRP1 and TRX together with mutagenesis studies showed that TRX in the oxidized form was bound to the nucleotide binding domain (NBD) subdomain of NLRP1. This observation highlights the crucial role of redox-active cysteines of TRX in NLRP1 binding. Further cellular assays revealed that TRX suppressed NLRP1 inflammasome activation and, thus, negatively regulated NLRP1. Our data reveal the well-known TRX system as an intrinsic checkpoint for innate immunity and provide opportunities for future therapeutic intervention with NLRP1 inflammasome activation targeting this system.

Publications

Sakaniwa K #, Fujimura A #, Shibata T #, Shigematsu H, Ekimoto T, Yamamoto M, Ikeguchi M, Miyake K, Ohto U, Shimizu T. (#: equally contributed)
TLR3 forms a laterally aligned multimeric complex along double-stranded RNA for efficient signal transduction. Nature Communications. 2023 Jan; 14(1):164. doi: 10.1038/s41467-023-35844-2.

Shibata T, Sato R, Taoka M, Saitoh SI, Komine M, Yamaguchi K, Goyama S, Motoi Y, Kitaura J, Izawa K, Yamauchi Y, Tsukamoto Y, Ichinohe T, Fujita E, Hiranuma R, Fukui R, Furukawa Y, Kitamura T, Takai T, Tojo A, Ohtsuki M, Ohto U, Shimizu T, Ozawa M, Yoshida N, Isobe T, Latz E, Mukai K, Taguchi T, Miyake K.
TLR7/8 stress response drives histiocytosis in SLC29A3 disorders.
Journal of Experimental Medicine. 2023 Sep; 220(9): e20230054. doi: 10.1084/jem.20230054.Zhang Z #, Shibata T #, Fujimura A, Kitaura J, Miyake K, Ohto U, Shimizu T.
Structural basis for thioredoxin-mediated suppression of NLRP1 inflammasome. Nature. 2023 Oct; 622(7981):188-194. doi: 10.1038/s41586-023-06532-4.

Department of Microbiology and Immunology

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

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

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

1. Dual impacts of a glycan shield on the envelope glycoprotein B of HSV-1: evasion from human antibodies in vivo and neurovirulence

Ayano Fukui, Yuhei Maruzuru, Shiho Ohno[1], Moeka Nobe, Shuji Iwata, Kosuke Takeshima, Naoto Koyanagi, Akihisa Kato, Shinobu Kitazume[2], Yoshiki Yamaguchi[1], Yasushi Kawaguchi: [1]Division of Structural Glycobiology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Medical and Pharmaceutical University, Miyagi [2]Department of Clinical Laboratory Sciences, School of Health Sciences, Fukushima Medical University, Fukushima

Identification of the mechanisms of viral evasion from human antibodies is crucial both for understanding viral pathogenesis and for designing effective vaccines. Here we show in cell cultures that an N-glycan shield on the herpes simplex virus 1 (HSV-1) envelope glycoprotein B (gB) mediated evasion from neutralization and antibody-dependent cellular cytotoxicity due to pooled γ-globulins derived from human blood. We also demonstrated that the presence of human γ-globulins in mice and immunity to HSV-1 induced by viral infection in mice significantly reduced replication in their eyes of a mutant virus lacking the glycosylation site but had little effect on the replication of its repaired virus. These results suggest that an N-glycan shield on a specific site of HSV-1 envelope gB mediated evasion from human antibodies in vivo and from HSV-1 immunity induced by viral infection in vivo. Notably, we also found that an N-glycan shield on a specific site of HSV-1 gB was significant for HSV-1 neurovirulence and replication in the central nervous system of naïve mice. Thus, we have identified a critical N-glycan shield on HSV-1 gB that has dual impacts, namely evasion from human antibodies in vivo and viral neurovirulence.

2. Establishment of a system to quantify wild-type herpes simplex virus-induced cell-cell fusion reveals a role of N-glycosylation of HSV-1 envelope glycoprotein B in cell-cell fusion

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Wild-type herpes simplex virus (HSV) strains infrequently mediate cell-cell fusion in cell cultures and barely induce large multinucleated cells. In this study, we established a system to quantify infrequent cell-

cell fusion induced by wild-type HSV strains. The established system clarified that the HSV-1 envelope glycoprotein B and its N-glycosylation at asparagine at position 141 were required for efficient cell-cell fusion. This study provides a link between cell-cell fusion induced by wild-type HSV-1 and viral pathogenesis in vivo.

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

A squalene-based emulsion adjuvant, induces T follicular helper cells and humoral immune responses via α-tocopherol component

Adjuvants are chemical or biological materials that enhance the efficacy of vaccines. A-910823 is a squalene-based emulsion adjuvant used for S-268019-b, a novel vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is currently in clinical development. Published evidence has demonstrated that A-910823 can enhance the induction of neutralizing antibodies against SARS-CoV-2 in humans and animal models. However, the characteristics and mechanisms of the immune responses induced by A-910823 are not yet known. To characterize A-910823, we compared the adaptive immune response profile enhanced by A-910823 with that of other adjuvants (AddaVax, QS21, aluminum salt-based adjuvants, and empty lipid nanoparticle [eLNP]) in a murine model. Compared with other adjuvants, A-910823 enhanced humoral immune responses to an equal or greater extent following potent T follicular helper (Tfh) and germinal center B (GCB) cell induction, without inducing a strong systemic inflammatory cytokine response. Furthermore, S-268019-b containing A-910823 adjuvant produced similar results even when given as a booster dose following primary administration of a lipid nanoparticle-encapsulated messenger RNA (mRNA-LNP) vaccine. Preparation of modified A-910823 adjuvants to identify which components of A-910823 play a role in driving the adjuvant effect and detailed evaluation of the immunological characteristics induced by each adjuvant showed that the induction of humoral immunity and Tfh and GCB cell induction in A-910823 were dependent on α-tocopherol. Finally, we revealed that the recruitment of inflammatory cells to the draining lymph nodes and induction of serum cytokines and chemokines by A-910823 were also dependent on the α-tocopherol component. This study demonstrates that the novel adjuvant A-910823 is capable of robust Tfh cell induction and humoral immune responses, even when given as a booster dose. The findings also emphasize that α-tocopherol drives the potent Tfh-inducing adjuvant function of A-910823. Overall, our data provide key information that may inform the future production of improved adjuvants.

Dendritic cell proliferation by primary cilium in atopic dermatitis

Atopic dermatitis (AD) is a common allergic ecze-

ma that affects up to 10% of adults in developed countries. Immune cells in the epidermis, namely, Langerhans cells (LCs), contribute to the pathogenesis of AD, although their exact role(s) in disease remain unclear. We performed immunostaining on human skin and peripheral blood mononuclear cells (PBMCs) and visualized primary cilium. Result and discussion: We show that human dendritic cells (DCs) and LCs have a previously unknown primary cilium-like structure. The primary cilium was assembled during DC proliferation in response to the Th2 cytokine GM-CSF, and its formation was halted by DC maturation agents. This suggests that the role of primary cilium is to transduce proliferation signaling. The platelet-derived growth factor receptor alpha (PDGFRα) pathway, which is known for transducing proliferation signals in the primary cilium, promoted DC proliferation in a manner dependent on the intraflagellar transport (IFT) system. We also examined the epidermal samples from AD patients, and observed aberrantly ciliated LCs and keratinocytes in immature and proliferating states. Our results identify a potential relationship between the primary cilium and allergic skin barrier disorders, and suggest that targeting the primary cilium may contribute to treating AD.

Challenges in developing personalized neoantigen cancer vaccines

The recent success of cancer immunotherapies has highlighted the benefit of harnessing the immune system for cancer treatment. Vaccines have a long history of promoting immunity to pathogens and, consequently, vaccines targeting cancer neoantigens have been championed as a tool to direct and amplify immune responses against tumours while sparing healthy tissue. In recent years, extensive preclinical research and more than one hundred clinical trials have tested different strategies of neoantigen discovery and vaccine formulations. However, despite the enthusiasm for neoantigen vaccines, proof of unequivocal efficacy has remained beyond reach for the majority of clinical trials. In this Review, we focus on the key obstacles pertaining to vaccine design and tumour environment that remain to be overcome in order to unleash the true potential of neoantigen vaccines in cancer therapy.

The 100 Days Mission: how a new medical-countermeasures network can deliver equity and innovation

As shown during the response to COVID-19, the faster we can develop safe, effective and affordable vaccines, therapeutics and diagnostics for an escalating infectious disease, the more lives we can save. As the scientific advisory member for CEPI as well as IPPS and STEG for G7, Ken Ishii is committed to contribute to the 100 days mission. The goal of the 100 Days Mission is to prepare as much as possible so that within the first 100 days that a pandemic threat is identified, the following interventions can be made available, safe, effective, and affordable:

Accurate and approved rapid point of care diagnostic tests
An initial regimen of therapeutics
Vaccines ready to be produced at scale for global deployment

In June 2021, G7 Leaders welcomed the "100 Days Mission'' (100DM) in Carbis Bay, building on the collaborative scientific efforts which led to the development of Diagnostics, Therapeutics, and Vaccines (DTVs) for COVID-19. The 1st 100 Day mission report was authored by scientific, governmental and industry experts drawn from within and beyond the G7. It proposed 25 recommendations to harness scientific innovation and strengthen public and private collaboration that will reduce the time from discovery to deployment of DTVs within 100 days of the next pandemic threat.

In order to deliver on these ambitions, G7 scientific advisers called for the establishment of an International Pandemic Preparedness Secretariat ('the Secretariat') to support implementation and catalyse scientific exchange on the progress of the 100DM.

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Summary of Activity (Less than 70 words)
Our laboratory focuses on the elucidation of host-pathogen interactions. We mainly work on malaria, but also cover several infectious diseases such as leishmaniasis and respiratory infections. We study both innate and adaptive immune responses against these diverse pathogens in order to develop successful vaccines against them.

1. Adjuvant discovery and development platform

Adjuvants are considered essential vaccine components for enhancing vaccine responses. As a member of IMSUT International Vaccine Design Center (https://vdesc.ims.u-tokyo.ac.jp/en/), we have systematically screened innate and adaptive immune signaling molecules involved in the mode of action (MOA) of adjuvants and vaccines. Recently, we have been involved in the discovery of novel adjuvants as part of the AMED SCARDA project. One of the recent findings is to understand how the combination of TLR9 and STING agonists synergistically induce innate and adaptive responses to generate robust anti-tumor responses (*Temizoz et al., International Immunology, 2022*). Our recent projects have focused on investigating B cell development and the pathways involved in germinal center (GC) formation for the generation of potent antibody responses against infections and during vaccination. We found that TBK1, the well-known innate immune signaling kinase that controls antiviral immune responses and nucleic acid-mediated type I interferon responses, is very important for the generation of GCs that confer sterile immunity to reinfection (*Lee et al., J Exp Medicine, 2022*).

2. Elucidation of host-pathogen interactions

Our laboratory has investigated several aspects of immunopathology caused by *Plasmodium* parasites. We have recently studied the immunopathology of cerebral malaria, the deadliest complication of human malaria infection, in the brain using the CUBIC clearance technique (*Matsuo-Dapaah et al., Int Immunology, 2021*). The 3D reconstruction of malaria-infected brain showed that olfactory bulb is disrupted during experimental cerebral malaria. We have recently made significant progress in understanding new cell types that accumulate/reside in the olfactory bulb and interact with *Plasmodium* parasites. Chronic bone loss is an unforeseen complication of malaria which is mediated via MyD88 adaptor protein (*Lee et al,. Science Immunology, 2017*). We have been studying to address the crucial cell types important for MyD88-mediated bone loss. We also investigate bone marrow niches responsible for malaria-induced loss of memory.

3. Infection and Cancer

Previously, we investigated the role of Lipocalin 2 (LCN2, also known as siderocalin or neutrophil gelatinase-associated lipocalin (NGAL)) in malaria infection that bolsters innate and adaptive immune responses to malaria infection through modulation of iron metabolism (*Zhao et al., Cell Host Microbe, 2012*). LCN2 expression is also increased in cancer. In carcinogenesis, in addition to the accumulation of so-

matic mutations, stroma-associated immunity is an important regulator of tumor growth. Tumor cells create a microenvironment by releasing various mediators to maintain their presence and spread. Due to the infiltration of monocytes and leukocytes against the tumor, it is hypothesized that the iron balance is disrupted by excessive iron consumption, possibly leading to increased expression of LCN2 as an intracellular iron transporter. We recently investigated the expressions of programmed cell death ligand-1 (PD-L1) and LCN2 in breast cancers with various molecular subtypes, along with their correlations with other prognostic indicators, including Ki-67, lymph node metastasis, histological grade, tumor-infiltrating lymphocyte (TILs) accumulation, and necrosis. We found that there is an association of LCN2 with known prognostic factors and molecular subtypes. Moreover, significant elevations of LCN2 and PD-L1 expressions were observed in triple-negative and HER2-positive breast cancers. The findings from this research may contribute to the immunotherapeutic application of LCN2 and its prognostic significance in breast cancer management (*Ekemen et al., Breast Cancer: Targets and Therapy, in press*).

Publications

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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 alone. 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. Understanding the evolution of SARS-CoV-2

Yu Kaku, Keiya Uriu, Yusuke Kosugi, Shigeru Fujita, Luo Chen, Jarel Elgin Tolentino, Lin Pan, Arnon Plianchaisuk, Ziyi Guo, Alfredo Amolong Hinay, Jr., Kaoru Usui, Wilaiporn Saikruang, Wenye Li, Kaho Okumura, Naoko Misawa, Mai Suganami, Adam Patrick Strange, Naomi Ohsumi, Shiho Tanaka, 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. Since then, more than 770 million people have been infected with this virus and more than 7 million people have 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 December 2023, more than 10 principal investigators in Japan and Czech Republic 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. Predicting and understanding virus epidemics and evolution

Jumpei Ito, Arnon Plianchaisuk, Jarel Elgin Tolentino, Mai Suganami, Adam Patrick Strange, Kaho Okumura, Kei Sato

During the COVID-19 pandemic, extensive viral genomic surveillance has been conducted, enabling us to track the epidemic and evolution of SARS-CoV-2 in ultra-high resolution. Leveraging this big data, we are establishing theoretical and technical frameworks to understand and predict the epidemic and evolution of viruses. This year, we have developed a Bayesian hierarchical model to predict the transmissibility of SARS-CoV-2 variants based on mutation patterns in the spike protein (Ito et al., 2023, *Nat. Commun.*). Additionally, we showed that the increased transmissibility observed during the evolution of the Omicron variant can be explained by the acquisition of a small number of mutations in spike protein.

Publications

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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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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. In addition, our members are partly involved in the platform of supporting cohort and biospecimen analysis (CoBiA) project by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and contribute to various scientific projects through advanced technology in biomedical sciences.

1. The biological functions of cell-cell interaction in human oncogenesis and its application to diagnosis and treatment of cancer.

Takeshi Ito, Yutaka Kasai, Yumi Tsuboi, Yue Guo, Marie Kawahara, Jialiang Nie, Tomoko Masuda, Hiromi Ichihara, Motoi Oba, Daisuke Matsubara[1], Goh Tanaka[2], Akihisa Mitani[2], Takahide Nagase[2] and Yoshinori Murakami; [1]Department of Diagnostic Pathology, University of Tsukuba, Tsukuba, [2]Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo.

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 establishing novel diagnostic markers and therapeutic targets for small cell lung cancer (SCLC) using CADM1. 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 a specific protease and released into blood stream, this fragment could provide a novel serum marker of SCLC. We generated monoclonal antibodies against the fragments of CADM1v8/9 and established a sensitive and specific serum marker for diagnosis of SCLC (PCT/JP2019/011201). This detection system of SCLC is 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 drug-conjugated antibodies partly in collaboration with a pharmaceutical company.

In addition, we conducted a comprehensive RT-PCR-based screening for IgSF molecules that promote experimental lung metastasis in mice. By comparing

the expression of 325 genes encoding cell-surface IgSF molecules between mouse melanoma B16 cells and its highly metastatic subline, B16F10 cells, we found that expression of the *Immunoglobulin superfamily member 3* (*Igsf3*) was significantly enhanced in B16F10 cells than in B16 cells. Knockdown of *Igsf3* in B16F10 cells significantly reduced lung metastasis following intravenous injection into C57BL/6 mice. IGSF3 promoted adhesion of B16F10 cells to vascular endothelial cells and functioned as a homophilic cell adhesion molecule between B16F10 cells and vascular endothelial cells. Notably, the knockdown of IGSF3 in either B16F10 cells or vascular endothelial cells suppressed the trans-endothelial migration of B16F10 cells. Moreover, IGSF3 knockdown suppressed the extravasation of B16F10 cells into the lungs after intravenous injection. These results suggest that IGSF3 promotes the metastatic potential of B16F10 cells in the lungs by facilitating their adhesion to vascular endothelial cells (19).

2. Identification of novel immune checkpoint molecules by screening molecular interaction between IgSFs.

Takeshi Ito, Yumi Tsuboi, Mai Mizusawa, Yuki Azuma, Miko Komiya, Shuhei Masuko, Tomoko Masuda and Yoshinori Murakami

We are 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 physico-chemical methods, including the surface plasmon resonance imaging (SPRi) and the amplified luminescence proximity homogenous assay (ALPHA). Significant interaction was then evaluated individually using biological assays between molecules to cells, cells to cells and cells to tissues generated in our laboratory. We have identified several candidate pairs of IgSFs involved in cancer metastasis and tumor immune-checkpoint regulation and their significance in cancer treatment is being investigated partly in collaboration with a pharmaceutical company. This year, we have newly identified Sialic acid-binding Ig-like lectin 7 (Siglec-7) as an inhibitory receptor for V-set and immunoglobulin domain-containing 4 (VSIG4) that regulates an immune checkpoint in NK cells and a small subset of T cells. Siglec-7 recognized sialic acid modification in the VSIG4 protein, and co-culture of cancer cells expressing VSIG4 with NK cell lines inhibited NK cell activity, including IFN-γ secretion and cytotoxicity. Moreover, the blockade of the VSIG4–Siglec-7 interaction by the treatment of anti-VSIG4 or anti-Siglec-7 neutralizing antibodies significantly restored NK cell activity. These results suggest that Siglec-7 functions as an inhibitory receptor for VSIG4 and represents a potential therapeutic target for cancer.

3. 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.

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 (1), colorectal (2) and biliary tract (3) cancer, as well as 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 and Dr. Hidewaki Nakagawa'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 (1).

Furthermore, to understand the mechanisms of complex diseases, including cancer, and various specific human phenotypes, we carried out genome-wide association studies (GWAS) and their downstream analysis for lung adenocarcinoma (4,5), hepatocellular carcinoma (6), peptic ulcer (7), survival time of individuals (8), retinal and renal complications of type 2 diabetes (9) and atrial fibrillation (10). Furthermore, construction of polygenic risk score based on the results of genome-wide association studies of various diseases and phenotypes was investigated from the view point of multi-biobank cooperation (11). 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.

4. Novel therapeutic and diagnostic target discovery for solid cancers

Atsushi Takano, Koji Teramoto[5], Hidetoshi Sumimoto[5], Yataro Daigo[5], Yoshinori Murakami; [5]Department of Medical Oncology and Cancer Center, Center for Advanced Medicine against Cancer, Shiga University of Medical Science

To identify molecules involved in human carcinogenesis and those which could be applied for the development of new molecular therapies and/or biomarkers, we had established a systematic screening

system as follows; i) identification of overexpressed and/or mutated genes in the majority of solid cancers (lung, esophagus, head-and-neck, breast, etc.) by genome-wide screening using the expression microarray as well as next generation sequencer in the combination of enrichment of tumor cell populations from cancer tissues by laser microdissection, ii) verification of no or little expression of each of candidate molecules in normal tissues by expression microarray, iii) validation of the clinicopathological significance of its higher expression with tissue microarray containing thousands of archived solid cancers, iv) verification of a critical role of each target gene in the growth and/or invasiveness of cancer cells by RNAi and cell growth/invasion assays, v) evaluation of their usefulness as targets for passive immunotherapy using specific antibodies and/or as a serum biomarker for solid cancer by high throughput ELISA and proteomics analysis, if they are tumor-specific transmembrane or secretory proteins, vi) screening of the epitope peptides recognized by human histocompatibility leukocyte (HLA)-A*0201- or A*2402-restricted cytotoxic T lymphocyte (CTL) and dendritic cell (DC). This systematic approach identified dozens of molecules that appear to fall into the category of oncoantigens or neoantigens whose overexpression in various solid cancers including lung, esophagus, oral cavity, and breast cancers is an important feature of the malignant nature of cancer cells (12) and that have very high immunogenicity to induce antigen-specific CTLs in cancer patients. We further validated these molecules identified as potential targets for the development of antibodies, small-molecular compounds, growth-suppressive cell-permeable peptides, and cancer vaccines that could have a more specific and strong anti-cancer effect with minimal risk of adverse events. We also identified novel cancer detection and/or prognostic biomarkers (13).

Based on the achievements above, we are developing new immunotherapies as a project of IMSUT International Joint Research Project. We are conducting International Conference on Harmonization (ICH) - Good Clinical Practice (GCP)-based clinical study using the combination of some of these peptides derived from oncoantigens in patients with lung cancer is now being conducted at 6 collaborative cancer centers and university hospitals including IMSUT hospital. In addition, new type of peptides-pulsed DC vaccination therapy is under development.

5. Scientific Platform of Supporting Cohort Study and Biospecimen Analysis (CoBiA)

Yataro Daigo, Atsushi Takano, Rikako Shinozaki, Hiromi Ichihara, Yoshinori Murakami

To support life science researchers in the field of basic life science, cancer diagnostics and therapeutics, we are collecting cancer and corresponding normal tissues, serum, plasma, and peripheral blood mononuclear cell (PBMC) from patients with solid cancers originated from 30 organs. To date, we collected 132,000 clinical materials. We also constructed tissue microarray system covering about 5000 archived clinical cancers. Using these clinical materials, we are validating the clinicopathological significance of various candidate disease biomarkers as requested by researchers and are contributing to their clinical application and publications in international journals (4, 5, 14-18).

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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 and elucidate 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.

Inoue-Yamauchi, A., Eguchi, T.[1], Tokuoka, T., Zhong, Z., Yoda, M., Hwang, J., Ueta, R., Tezuka, T.[2], Weatherbee, SD.[3], Watanabe, Y.[4], Sagara, H.[4], Nagatoishi, S.[4], Tsumoto, K.[4], and Yamanashi, Y.: [1,2] Present affiliation: [1]Brain-Skeletal Muscle Connection in Aging Project Team, Geroscience Research Center, National Center for Geriatrics and Gerontology and [2]Center for the Promotion of Interdisciplinary Education and Research, Kyoto University. [3]Department of Genetics, Yale University. [4]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 transmis-

sion 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 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, implying increased robustness of neuromuscular transmission. We are investigating Dok-7/MuSK-mediated signaling, including downstream effectors, in regulating formation, maintenance and function of NMJs to develop mechanism-based therapies for NMJ disorders. Recently, we have identified the novel downstream gene Cabp7, encoding calcium-binding protein 7, and shown its importance in the maintenance of NMJs in mice (see below).

2. Cabp7 negatively regulates age-related degeneration of NMJs.

Eguchi, T., Tezuka, T., Watanabe, Y., Inoue-Yamauchi, A., Sagara, S., Ozawa, M. [1], and Yamanashi Y.: [1]Core Laboratory for Developing Advanced Animal Models, IMSUT.

As mentioned above, formation and maintenance of NMJs in the central region of the skeletal muscle are governed by MuSK. Interestingly, the transcripts and protein products of AChR subunit and other NMJ-related genes are expressed and accumulated also in the central, synaptic region of myotube in a manner dependent on MuSK. Indeed, we previously reported that midmuscle expression of AChR subunit gene Chrna1 and MuSK transcripts are lost or enhanced in mouse embryos lacking the essential MuSK activator Dok-7 or overexpressing it specifically in skeletal muscle, respectively. Thus, to identify

NMJ-related genes required for the formation and/or maintenance of NMJs, we performed RNA sequencing analysis of the synaptic and extrasynaptic regions of diaphragm muscles in WT mice (3 months old) and found that Cabp7 gene is the most upregulated among significantly upregulated genes in the synaptic region in comparison with the extrasynaptic region. Furthermore, we found that the expression level of the Cabp7 gene is significantly higher in the synaptic region in Dok-7 transgenic (Tg) mice, which overexpress Dok-7 specifically in skeletal muscle, than in wild-type (WT) mice (3 months old). Also, whole-mount in situ hybridization analysis on embryos confirmed that Cabp7 transcripts are specifically expressed in the central region of the diaphragm muscle in Dok-7 Tg and wild-type mice and that the synaptic expression is significantly enhanced in Dok-7 Tg mice compared with that in WT mice. These findings together indicates that Dok-7-MuSK axis regulates expression of Cabp7 gene in skeletal muscle.

To explore the role for Cabp7 in muscle, we generated Cabp7 cKO mice, which lacks Cabp7 protein specifically in skeletal muscle, and found that Cabp7 cKO mice showed a significant reduction in motor function and muscle strength at 12 and 24, but not 3 or 6, months of age in comparison with the controls, as determined by rotarod, forelimb grip, and hindlimb twitch/tetanic force tests. Furthermore, Cabp7 cKO mice showed acceleration of age-related degeneration of NMJs as early as 6 to 12 months of age; namely, axonal swelling, nerve sprouting, denervation and size-reduction of NMJs. Because impaired NMJ function may lead to muscle atrophy and weakness as observed in patients with myasthenia, we investigated if muscle-specific deletion of Cabp7 in mice affects muscle homeostasis and found that tibialis anterior and gastrocnemius muscle masses were significantly reduced in Cabp7 cKO mice at 12 and 24, but not 3 or 6, months of age in comparison with the controls. In addition, the myofiber cross-section area (CSA) of gastrocnemius muscles was significantly reduced in Cabp7 cKO mice at 12 and 24, but not 3, months of age and showed significant shifts in CSA distribution with a higher frequency of small fibers in comparison with the controls. Taken together, these findings indicates that Cabp7 plays a protective role against age-related NMJ degeneration, muscle weakness and atrophy, and motor dysfunction. Indeed, Cabp7 cKO mice showed a lifespan about 8 weeks shorter than the control mice.

In addition, we found that Cabp7 cKO mice showed increased protein expression of p25, a potent activator of Cdk5, in the tibialis anterior muscle at 3 and 12 months of age in comparison with the control mice. Because Cdk5 negatively regulates NMJ formation and maintenance, we generated Adeno-associated virus-based vector (AAV-p25), which expressed p25 in myotubes under the control of the muscle-specific CK8 promoter, and found that AAV-p25 administration induces NMJ degeneration. Moreover, we also generated AAV-CIP, which expressed Cdk5 Inhibitory Peptide (CIP), and found that AAV-CIP administration restores NMJ integrity and muscle strength, and heals muscle atrophy in Cabp7 cKO mice. We are currently investigating how Dok-7-MuSK-Cabp7-p25-Cdk5 axis contributes to NMJ homeostasis and how CIP expression counteracts NMJ degeneration, muscle weakness and atrophy caused by the loss of Cabp7.

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

Inoue-Yamauchi, A., Eguchi, T., Tezuka, T., Mao, Y., Fan, W., Ochiai, C., Ma, WT., 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).

4. Pathophysiological mechanisms underlying *DOK7* myasthenia.

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

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

To investigate pathophysiological mechanisms underlying *DOK7* myasthenia, we established knock-in mice (Dok-7 KI mice) that have a mutation associated with the majority of patients with *DOK7* myasthenia. As expected, Dok-7 KI mice showed characteristic features of severe muscle weakness and died between postnatal day 13 and 20. Furthermore, they showed abnormally small NMJs lacking postsynaptic folding, a pathological feature seen in patients with *DOK7* myasthenia. Consistent with this, Dok-7 KI mice exhibited decreased MuSK activity in skeletal muscle, indicating that the Dok-7 KI mice develop defects similar to those found in patients with *DOK7* myasthenia, although the mice exhibit a more severe phenotype. In collaboration with Prof. David Beeson's group, we examined NMJ 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.

5. *DOK7 gene therapy that enlarges and regenerates NMJs.*

Inoue-Yamauchi, A., Eguchi, T., Ueta, R., Lin, S., 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 reinnervation 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.

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. 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 are conducting transcriptomic and phospho-proteomic analyses related to muscle weakness due to defects of cytoplasmic protein kinases. In addition, we have prepared experimental settings for other omic approaches such as metabolomic analysis.

8. 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, including those in humans, for the hit compounds or protein products of the candidate genes to understand their modes of actions.

Publication

Li D, Johmura Y, Morimoto S, Doi M, Nakanishi K, Ozawa M, Tsunekawa Y, Inoue-Yamauchi A, Naruse H, Matsukawa T, Takeshita Y, Suzuki N, Aoki M, Nishiyama A, Zeng X, Konishi C, Suzuki N, Nishiyama A, Harris AS, Morita M, Yamaguchi K, Furukawa Y, Nakai K, Tsuji S, Yamazaki S, Yamanashi Y, Shimada S, Okada T, Okano H, Toda T, and Nakanishi M. LONRF2 is a protein quality control ubiquitin ligase whose deficiency causes late-onset neurological deficits. ***Nature Aging*** 3: 1001-1019, 2023

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 that senescent cells play an important role in aging and healthy lifespan. However, little is known about the molecular basis of aging-related pathologies. Our research is focused on understanding the common pathologies underlying a variety of aging-related diseases. Currently, we are 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. In addition, we are focusing on the mechanism underlying the 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 are also investigating the molecular link between DNA methylation and the maintenance of genome stability.

1. LONRF family proteins are bona fide protein quality control ubiquitin-ligases

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Many age-related diseases are causally linked to the misfolding of proteins, and certain environmental stresses can trigger the misfolding of mature proteins. To prevent this, all cells have evolved protein quality control (PQC) systems. These include translation control, molecular chaperone activity, and proteolytic degradation by either the proteasome or autophagy. For example, cleaved protein products generated by stalled ribosomes on defective mRNAs have been found to be targets for degradation by molecular pathways initiated at the ribosome. The protein will inevitably be cleaved and is likely to be defective if the ribosome cannot reach the correct termination codon. As a result, it may be advantageous for the cell to degrade such incomplete nascent chains. Protein misfolding is a major factor of neurodegenerative diseases. Post-mitotic neurons are highly susceptible to pro-

tein aggregates that are not diluted by mitosis. Therefore, post-mitotic cells may have a specific protein quality control (PQC) system. The LONRF family of proteins consists of three isozymes, LONRF1-3. 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 (MN) degeneration and cerebellar ataxia. Mouse iPS cell-derived MNs lacking LONRF2 showed reduced survival, shortening of neurites, and accumulation of pTDP-43 and G3BP1 after long-term culture. The shortening of neurites in human ALS patients-derived MNs are rescued by ectopic expression of LONRF2.

Lonrf1 was ubiquitously expressed in different tissues. Its expression in LSEC and Kupffer cells increased with age in the liver. Lonrf1^{high} Kupffer cells showed activation of regulatory pathways of peptidase activity. In normal and NASH liver, the activation of NF-κB and p53 pathways and the suppression of IFNα, IFNγ, and proteasome signaling were detected in LSECs. During wound healing process, Lonrf1^{high}/p16^{high} fibroblasts showed activation of cell growth and suppression of TGFβ and BMP signaling. Lonrf1^{high}/p16^{low} fibroblasts exhibited activation of WNT signaling. These results suggest that although Lonrf1 does not appear to be associated with senescence induction and phenotypes, Lonrf1 may play a key role in linking oxidative damage responses and tissue remodeling during wound healing in different modes in senescent and non-senescent cells.

2. M2 macrophage-derived TGF-β induces age-associated loss of adipogenesis through progenitor cell senescence

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Adipose tissue is an endocrine and energy storage organ composed of several different cell types, including mature adipocytes, stromal cells, endothelial cells, and a variety of immune cells. Adipose tissue aging contributes to the pathogenesis of metabolic dysfunction and is likely induced by crosstalk between adipose progenitor cells (APCs) and immune cells, but the underlying molecular mechanisms remain largely unknown. In this study, we revealed the biological role of p16^{high} senescent APCs, and investigated the crosstalk between each cell types in the aged white adipose tissue. We performed the single-cell RNA sequencing (scRNA-seq) analysis on the p16^{high} adipose cells sorted from aged p16-Cre^{ERT2}/Rosa26-LSL-tdTomato mice. On the other hand, we conducted the time serial analysis on the age-dependent bulk RNA-seq datasets of human and mouse white adipose tissues to infer the transcriptome alteration of adipogenic potential within aging. We show that M2 macrophage-derived TGF-β induces APCs senescence which impairs adipogenesis *in vivo*. p16^{high} senescent APCs increase with age and show loss of adipogenic potential. The ligand-receptor interaction analysis reveals that M2 macrophages are the donors for TGF-β and the senescent APCs are the recipient. Indeed, treatment of APCs with TGF-β1 induces senescent phenotypes through mitochondrial ROS-mediated DNA damage *in vitro*. TGF-β1 injection into gonadal white adipose tissue (gWAT) suppresses adipogenic potential and induces fibrotic genes as well as p16 in APCs. A similar gWAT atrophy is observed in cancer cachexia by APCs senescence. Our results suggest that M2 macrophage-derived TGF-β induces age-related lipodystrophy by APCs senescence. The TGF-β treatment induced the DNA damage, mitochondrial ROS, and finally cellular senescence in APCs.

3. CDCA7 is a hemimethylated DNA adaptor for the nucleosome remodeler HELLS

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Mutations of the SNF2 family ATPase HELLS and its activator CDCA7 cause immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome, characterized by hypomethylation at heterochromatin. The unique zinc-finger domain, zf-4CXXC_R1, of CDCA7 is widely conserved across eukaryotes but is absent from species that lack HELLS and DNA methyltransferases, implying its specialized relation with methylated DNA. Here we demonstrate that zf-4CXXC_R1 acts as a hemimethylated DNA sensor. The zf-4CXXC_R1 domain of CDCA7 selectively binds to DNA with a hemimethylated CpG, but not unmethylated or fully methylated CpG, and ICF disease mutations eliminated this binding. CDCA7 and HELLS interact via their N-terminal alpha helices, through which HELLS is recruited to hemimethylated DNA. While placement of a hemimethylated CpG

within the nucleosome core particle can hinder its recognition by CDCA7, cryo-EM structure analysis of the CDCA7-nucleosome complex suggests that zf-4CXXC_R1 recognizes a hemimethylated CpG in the major groove at linker DNA. Our study provides insights into how the CDCA7-HELLS nucleosome remodeling complex uniquely assists maintenance DNA methylation.

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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 types and cell signaling that cause hair graying and hair thinning

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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. Importantly, DNA damage foci are relatively frequently found in somatic stem cells in the skin during physiological aging. To study the fate and dynamics of DNA-damaged stem cells in tissues and the resultant impact in the expression of aging phenotypes, we first focused on the melanocyte lineage and traced 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. More than that, the fact underlie the fact that cell components in tissues are being replaced through stem cell differentiation and their eventual depletion. We are currently testing whether the selective induction of DNA double strand breaks in melanocyte stem cells similarly causes hair graying and whether it has some beneficial effects in suppressing melanoma development from the skin. Similarly, we are testing whether DNA DSBs in hair follicle stem cells promotes hair thinning and searching for chemicals that can prevent stem cell loss by

DNA DSBs.

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 thining 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

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Maintaining genomic integrity and stability is cru cial 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. We are currently testing the stem cell elimination process is mediated by cell competition mechanisms and also whether the phenomenon has any correlation with systemic aging.

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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. The ER calcium-sensor protein STIM1 presynaptically regulates hippocampal short-term plasticity

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Calcium plays a pivotal role in neurotransmitter release and synaptic plasticity. In the nerve terminals, voltage-gated calcium channels are the main source of calcium influx; however, the endoplasmic reticulum (ER) also serves as an important internal calcium store and regulates calcium levels in the nerve terminal depending on neural activity. Stromal interaction molecule 1 (STIM1) is a transmembrane, calcium-sensor protein that is located primarily on the ER membrane and is a key player in the regulation of store-operated calcium entry. However, the functional roles of presynaptic STIM1 in synaptic transmission and plasticity are not well determined. In this study, to elucidate the roles of STIM1 in presynaptic function, we generated presynaptic site-specific STIM1-deficient mice and examined whether presynaptic STIM1 influenced the release properties and short-term synaptic plasticity. In the electrophysiological analysis with acute hippocampal slices, we found that the loss of presynaptic STIM1 caused an enhancement of short-term synaptic plasticity induced by low-frequency repetitive stimulation. Furthermore, the analysis of synaptic vesicle (SV) dynamics during higher-frequency repetitive train stimulation indicated that the refilling rate of SVs to the readily-releasable pool (RRP) was normal, but that the size of the RRP of SVs significantly increased in mutant mice. These results indicate that the presynaptic STIM1 acts as an important regulator of short-term synaptic plasticity.

Publications

Kobayashi, S., Ogawa, I., Kiyama, Y., Sakimura, K., Mishina, M., Kurosaki, T. and Manabe, T. The ER calcium-sensor protein STIM1 presynaptically regulates hippocampal short-term plasticity (*Japan Neuroscience Society, Annual Meeting in Sendai, Japan; August 3, 2023*) (Abstract: 3Pa-018)

小林静香、真鍋俊也．脳スライス標本　脳科学辞典（https://bsd.neuroinf.jp/wiki/%E8%84%B3%E3%82%B9%E3%83%A9%E3%82%A4%E3%82%B9%E6%A8%99%E6%9C%AC）（2023年9月29日掲載）

小林静香、真鍋俊也．Bienenstock-Cooper-Munro理論　脳科学辞典（https://bsd.neuroinf.jp/wiki/Bienenstock-Cooper-Munro%E7%90%86%E8%AB%96）（2023年11月29日掲載）

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

Daichi Fujikawa[1], Takanori Nakamura[1], Daisuke Yoshioka[1], Zizheng Li[1], Hisashi Miriizumi[1], Mari Taguchi[1], Noriko Nishizumi-Tokai[1], Hiroko Kozuka-Hata[2], Masaaki Oyama[2], Yuji Kubota[1], and Mutsuhiro Takekawa[1,2]:[1]Division of Cell signaling and Molecular Medicine, IMUST, [2]Medical Proteomics Laboratory, IMSUT

In dealing with environmental stresses, human cells either activate defense mechanisms to survive or initiate cell death signaling, 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. Although SGs were initially considered to control RNA metabolism and translation reprograming under stress, their roles in these processes remain obscure. In contrast, increasing evidence shows that SGs function as signaling hubs by concentrating several signaling molecules into the granules, and promote adaptive stress responses such as the protection of cells from apoptosis and pyroptosis. However, the precise function of SGs in the regu-

lation of cell-fate decisions under stress remains ill-defined.

This year, using a proximity-labeling proteomic approach, we comprehensively analyzed SG-resident proteins and identified the executioner caspases, caspase-3 and -7, as SG components. We demonstrated that accumulation of caspase-3/7 into SGs was mediated by evolutionarily conserved amino acid residues within their large catalytic domains of the executioner caspases and inhibits their enzymatic activities and consequent apoptosis induced by various stresses. Expression of a SG-localization-deficient caspase-3 mutant in cells largely counteracted the anti-apoptotic effect of SGs, whereas enforced relocalization of the caspase-3 mutant to SGs restored it. Thus, SG-mediated sequestration of executioner caspases is a mechanism underlying the broad cytoprotective function of SGs. Furthermore, using a mouse xenograft tumor model, we showed that this mechanism prevented cancer cells from apoptosis in tumor tissues, thereby promoting cancer progression. Our results reveal the functional crosstalk between SG-mediated cell survival and caspase-mediated cell death signaling pathways and delineate a molecular mechanism that dictates cell-fate decisions under stress and promotes tumorigenesis.

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

Yuji Kubota, Noriko Nishizumi-Tokai, Junichiro Nashimoto, Hitomi Seki, Jue Wang, Shuri Komai, Yusuke Takagi, and Mutsuhiro Takekawa

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

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.

3. Functional crosstalk between ERK-mediated cell survival and caspase-mediated cell death pathways and its dysregulation in congenital RASopathies.

Yuji Kubota, Hisashi Moriizumi, Tomoyuki Tsuchiya, Ryosuke Naka, Mutsuhiro Takekawa

MEK1 and MEK2, central components of the ERK cascade, are ubiquitously expressed and share a high level of amino acid sequence homology particularly in their kinase domains. However, they possess two regions of lower homology: 1) an N-terminal region, which contains an ERK-docking site and nuclear export sequence, and 2) a proline-rich loop region, which contributes to specific protein–protein interactions important for the regulation of MEKs. The differences in amino acid sequence between MEK1 and MEK2 suggest that these kinases can have unique functions in cells. In fact, previous studies using various cell lines and MEK1/2-null mice demonstrated that these two molecules play overlapping and distinctive roles in the regulation of several biological processes, such as cell growth, survival, embryonic development, and carcinogenesis. However, the functional differences, if any, between MEK1 and MEK2

during apoptotic cell death remain obscure. Besides their critical roles in physiological processes, MEK1/2 also play crucial roles in carcinogenesis. Gain-of-function mutations of MEK1/2 are detected in various sporadic cancers, including colorectal, lung, and ovarian cancers, and melanoma. These MEK mutants hyperactivate the ERK pathway and eventually induce carcinogenesis. Moreover, recent genome-wide sequencing studies identified germline mutations of the MEK1/2 genes in a group of congenital diseases called RASopathies. RASopathies share many overlapping clinical features such as neurocognitive impairment, cranio-facial dysmorphisms, cardiomyopathies, and cutaneous and musculoskeletal abnormalities. Among these diseases, mutations in either MEK1 or MEK2 are detected in approximately 25% of individuals with CFC syndrome, and abnormally enhance their kinase activities. Despite the importance of MEK mutations in the etiology of cancer and RASopathies, the precise roles and regulation of disease-associated MEK mutants during the apoptotic process remain totally unknown.

This year, we identified MEK1, but not MEK2, as a specific substrate for the executioner caspase-3. During apoptosis, MEK1 is cleaved at an evolutionarily conserved Asp282 residue in the kinase domain, and thereby loses its enzymatic activity. Gene knockout experiments showed that MEK1 cleavage was mediated by caspase-3, but not by the other executioner caspases (i.e., caspase-6 or -7). Following exposure of cells to osmotic stress, elevated ERK activity gradually decreased, and this was accompanied by increased cleavage of MEK1. In contrast, the expression of a caspase-uncleavable MEK1 mutant in cells maintained stress-induced ERK activity, thereby attenuating apoptotic cell death. Thus, caspase-3-mediated, proteolytic inhibition of MEK1 sensitizes cells to apoptosis by suppressing pro-survival ERK signaling. Furthermore, we found that a RASopathy-associated MEK1(Y130C) mutation prevented this caspase-3-mediated proteolytic inactivation of MEK1 and efficiently protected cells from stress-induced apoptosis. Our data reveal the functional crosstalk between ERK-mediated cell survival and caspase-mediated cell death pathways and suggest that its dysregulation by a disease-associated MEK1 mutation is at least partly involved in the pathophysiology of congenital RASopathies.

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

Yuji Kubota, Hitomi Seki, and Mutsuhiro Takekawa

The ERK pathway transduces mitogenic signals and plays a pivotal role in a wide array of biological processes, including cell proliferation, differentiation, and carcinogenesis. Upon stimulation of cells with growth factors such as epidermal growth factor (EGF), their respective receptor tyrosine kinases (RTKs) activate Ras and recruit Raf family kinases to the plasma membrane, which promotes Raf activation. Activated Raf phosphorylates and activates MEK1/2, which in turn activate ERK1/2 by phosphorylation. A portion of the activated ERK then translocates to the nucleus where it phosphorylates and activates specific substrate proteins, including several transcription factors (TFs) (e.g., ELK1 and Sp1), and promotes cell growth and tumorigenesis. Since ERK exerts their biological effects through the phosphorylation of their substrate proteins, the identification of which is a prerequisite for the understanding of regulatory mechanisms of critical biological phenomena. By developing a novel screening strategy using yeast Saccharomyces cerevisiae, we have isolated several new human ERK substrate proteins from cDNA libraries, including MCRIP1, NELF-A, and others. These substrates include regulatory molecules for the expression of growth-promoting genes and for centrosome duplication, and several Ser/Thr protein kinases that regulate inflammation and cell death. We confirmed that these molecules were indeed directly phosphorylated by ERK *in vitro* as well as *in vivo* in response to mitogenic stimuli. Thus, these molecules are bona fide substrates of ERK. The biological functions of these novel substrate proteins are currently under investigation in our laboratory.

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

Shunske Fukuta, Natsumi Mikami, Yui Tanishiki, Shuri Komai, Noriko Nishizumi-Tokai, Hisashi Moriizumi, Yuji Kubota, and 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, and uncovered their roles in

DNA-damage response, stress response, inflammation, and cell growth control. Furthermore, using real-time 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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Quality control of translation eliminates aberrant proteins and maintains 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 and Parkinson's diseases. We analyzed the molecular mechanism of quality control mechanisms that suppress abnormal proteins and clarified the molecular basis of drug discovery. We propose that the increase in translation accuracy and enhancement of translation quality control mechanisms are possible strategies to prevent abnormal protein production and prolong healthy life expectancy.

1. Ribosome-associated Quality Control (RQC) for translation abnormalities

Ribosome-associated Quality Control (RQC) monitors aberrant translation and decomposes and removes abnormal proteins during synthesis. RQC plays an important role in maintaining protein homeostasis at a very early stage. We have previously 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 the E3 ubiquitin ligase Hel2 and its mammalian homolog ZNF598 are required for RQC, and that the novel RQT complex is involved in the dissociation of ubiquitinated ribosomes into subunits. We and Hegde lab have reported that E3 ubiquitin ligase recognizes collided ribosomes (Disome/Trisome) and the specific structure of collided ribosomes. We previously reported that the ubiquitination of uS10 by Hel2 was reconstituted. Next, we identified an RQT complex that specifically dissociated the ubiquitinated ribosomes into subunits and reconstituted the reaction in vitro. In yeast, the RQT complex components Cue3 and Rqt4 interact with the K63-linked ubiquitin chain and accelerate the recruitment of the RQT complex to the ubiquitinated colliding ribosomes. The CUE domain of Cue3 and N-terminal domain of Rqt4 bind independently to the K63-linked ubiquitin chain. Their deletion abolished the ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) revealed that the intrinsically disordered regions of Rqt4 enabled expansion of the searchable area for interaction with the ubiquitin chain. These findings provide mechanistic insights into decoding the ubiquitin code for the clearance of colliding ribosomes by the RQT complex.

In mammals, uS10 is polyubiquitinated, whereas eS10 is preferentially mono-ubiquitinated by ZNF598. We characterized the ubiquitination activity of ZNF598 and its importance in human RQT-mediated subunit dissociation using endogenous XBP1u and poly(A) translation stallers. Cryo-EM analysis of a human-collided disome revealed a distinct composite interface, with substantial differences that from of yeast collided disomes. Biochemical analysis of collided ribosomes showed that ZNF598 forms K63-linked polyubiquitin chains on uS10, which are crucial for mammalian RQC initiation. The human RQT (hRQT) complex, composed only of ASCC3, ASCC2,

and TRIP4, dissociates collided ribosomes depending on the ATPase activity of ASCC3 and the ubiquitin-binding capacity of ASCC2. The hRQT-mediated subunit dissociation requires K63-linked polyubiquitination of uS10, while monoubiquitination of eS10 or uS10 is not sufficient. Therefore, we conclude that ZNF598 functionally marks collided mammalian ribosomes via K63-linked polyubiquitination of uS10 for trimeric hRQT complex-mediated subunit dissociation.

The collision of ribosomes also induces No-Go Decay (NGD) 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 the collided ribosome response in RQC and mRNA cleavage independent of RQC in the vicinity of the collided ribosomes. The ubiquitin-binding activity of Cue2 is required for NGDRQC-, but not NGDRQC+, and it involves the first two N-terminal Cue domains. In contrast, Trp122 of Cue2 was crucial for NGDRQC+. Moreover, the colliding ribosome association factor Mbf1 and its interaction with uS3 are crucial for NGDRQC+ via the SDD1-staller. We propose that in Cue2-dependent cleavage upstream of collided ribosomes (NGDRQC-), polyubiquitination of eS7a is recognized by two N-terminal Cue domains of Cue2. In contrast, for cleavage within collided ribosomes (NGDRQC+), the UBA domain, Trp122, and the interaction between Mbf1 and uS3 are critical.

NEMF (Rqc2 in yeast) interacts with 60S RNCs and recruits Ltn1/Listerin, which ubiquitinates peptidyl-tRNA on dissociated 60S subunits. In the 60S subunit, Rqc2 catalyzes 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 an 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 fail-safe mechanism that expands the range of RQC-degradable substrates. However, the physiological functions of CAT-tailing remain elusive. We recently found that Failure to Degrade CAT-Tailed Proteins Disrupts Neuronal Morphogenesis and Cell Survival. NEMF, a mammalian RQC2 homolog, modifies the translation products of nonstop mRNAs, which are major erroneous mRNAs in mammals, with a C-terminal tail mainly composed of alanine and several other amino acids. Overproduction of non-stop mRNAs induces NC aggregation and caspase-3-dependent apoptosis and impairs neuronal morphogenesis, which is ameli

1.1. Decoding of the ubiquitin code for clearance of colliding ribosomes by the RQT complex.

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The collision sensor Hel2 specifically recognizes colliding ribosomes and ubiquitinates the ribosomal protein uS10, leading to noncanonical subunit dissociation by the ribosome-associated quality control trigger (RQT) complex. Although uS10 ubiquitination is essential for rescuing stalled ribosomes, its function and recognition steps are not fully understood. Here, we showed that the RQT complex components Cue3 and Rqt4 interacted with the K63-linked ubiquitin chain and accelerated the recruitment of the RQT complex to the ubiquitinated colliding ribosome. The CUE domain of Cue3 and the N-terminal domain of Rqt4 bound independently to the K63-linked ubiquitin chain. Their deletion abolished ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) reveals that the intrinsically disordered regions of Rqt4 enabled the expansion of the searchable area for interaction with the ubiquitin chain. These findings provide mechanistic insight into the decoding of the ubiquitin code for clearance of colliding ribosomes by the RQT complex.

1.2. Structural basis for clearing of ribosome collisions by the RQT complex.

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Translation of aberrant messenger RNAs can cause stalling of ribosomes resulting in ribosomal collisions. Collided ribosomes are specifically recognized to initiate stress responses and quality control pathways. Ribosome-associated quality control facilitates the degradation of incomplete translation products and requires dissociation of the stalled ribosomes. A central event is therefore the splitting of collided ribosomes by the ribosome quality control trigger complex, RQT, by an unknown mechanism. Here we show that RQT requires accessible mRNA and the presence of a neighboring ribosome. Cryo-

genic electron microscopy of RQT-ribosome complexes reveals that RQT engages the 40S subunit of the lead ribosome and can switch between two conformations. We propose that the Ski2-like helicase 1 (Slh1) subunit of RQT applies a pulling force on the mRNA, causing destabilizing conformational changes of the small ribosomal subunit, ultimately resulting in subunit dissociation. Our findings provide the conceptual framework for a helicase-driven ribosomal splitting mechanism.

1.3. Molecular basis of eIF5A-dependent CAT tailing in eukaryotic ribosome-associated quality control.

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Ribosome-associated quality control (RQC) is a conserved process degrading potentially toxic truncated nascent peptides whose malfunction underlies neurodegeneration and proteostasis decline in aging. During RQC, dissociation of stalled ribosomes is followed by elongation of the nascent peptide with alanine and threonine residues, driven by Rqc2 independently of mRNA, the small ribosomal subunit and guanosine triphosphate (GTP)-hydrolyzing factors. The resulting CAT tails (carboxy-terminal tails) and ubiquitination by Ltn1 mark nascent peptides for proteasomal degradation. Here we present ten cryogenic electron microscopy (cryo-EM) structures, revealing the mechanistic basis of individual steps of the CAT tailing cycle covering initiation, decoding, peptidyl transfer, and tRNA translocation. We discovered eIF5A as a crucial eukaryotic RQC factor enabling peptidyl transfer. Moreover, we observed dynamic behavior of RQC factors and tRNAs allowing for the processivity of the CAT tailing cycle without additional energy input. Together, these results elucidate key differences as well as common principles between CAT tailing and canonical translation.

1.4. Molecular basis for recognition and deubiquitination of 40S ribosomes by Otu2.

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In actively translating 80S ribosomes the ribosomal protein eS7 of the 40S subunit is monoubiquitinated by the E3 ligase Not4 and deubiquitinated by Otu2 upon ribosomal subunit recycling. Despite its importance for translation efficiency the exact role and structural basis for this translational reset is poorly understood. Here, structural analysis by cryo-electron microscopy of native and reconstituted Otu2-bound ribosomal complexes reveals that Otu2 engages 40S subunits mainly between ribosome recycling and initiation stages. Otu2 binds to several sites on the intersubunit surface of the 40S that are not occupied by any other 40S-binding factors. This binding mode explains the discrimination against 80S ribosomes via the largely helical N-terminal domain of Otu2 as well as the specificity for mono-ubiquitinated eS7 on 40S. Collectively, this study reveals mechanistic insights into the Otu2-driven deubiquitination steps for translational reset during ribosome recycling/(re)initiation.

1.5. Mechanistic insights into the roles of the UFM1 E3 ligase complex in ufmylation and ribosome-associated protein quality control.

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Ubiquitin-fold modifier 1 (UFM1) is a ubiquitin-like protein covalently conjugated with intracellular proteins through ufmylation, similar to ubiquitylation. Ufmylation is involved in processes such as endoplasmic reticulum (ER)-associated protein degradation, ribosome-associated protein quality control

(RQC) at the ER (ER-RQC), and ER-phagy. However, it remains unclear how ufmylation regulates such distinct ER-related functions. Here, we provide insights into the mechanism of the UFM1 E3 complex in not only ufmylation but also ER-RQC. The E3 complex consisting of UFL1 and UFBP1 interacted with UFC1, UFM1 E2, and, subsequently, CDK5RAP3, an adaptor for ufmylation of ribosomal subunit RPL26. Upon disome formation, the E3 complex associated with ufmylated RPL26 on the 60*S* subunit through the UFM1-interacting region of UFBP1. Loss of E3 components or disruption of the interaction between UFBP1 and ufmylated RPL26 attenuated ER-RQC. These results provide insights into not only the molecular basis of the ufmylation but also its role in proteostasis.

2. Molecular mechanism of quality control NRD for deficient ribosomes.

The ribosome is the central machinery for protein synthesis and is responsible for accurate codon recognition and highly efficient peptide-bond formation. Ribosomes interact with various factors to perform essential functions in 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, which are essential for accurate codon recognition in 18S rRNA and ubiquitin at the K212 residue of ribosomal protein uS3 in yeast. We identified E3 ubiquitin ligases that are both essential and involved. We identified Fap1 as a stalling sensor that triggers 18S nonfunctional rRNA decay via polyubiquitination of uS3. Ribosome profiling revealed the enrichment of Fap1 at the translation initiation site and an association with elongating individual ribosomes. Cryo-EM structures of Fap1-bound ribosomes revealed that Fap1 probes 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. It was also revealed that the ubiquitinated stagnant 80S ribosome was dissociated into its subunits by Slh1, and then the abnormal 40S was degraded.

3. The function of ribosome dynamic modification in stress response

The synthesis and modification of secretory proteins in the endoplasmic reticulum is essential for cells. The accumulation of abnormal proteins in the endoplasmic reticulum is harmful to cells and therefore responds by inducing the UPR pathway. In Saccharomyces cerevisiae, the membrane protein Ire1, activated by endoplasmic reticulum stress, splices the precursor mRNA of the transcription factor Hac1, and Hac1 is synthesized to induce transcription of chaperones. In higher eukaryotes, PARK phosphorylates eIF2α and suppresses the initiation of cell-wide translation. To elucidate the physiological function of ribosomal ubiquitination, we discovered a novel translational regulator 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 ribosomal protein eS7 by E3 ubiquitin ligase Not4 is essential.

Publication list

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   Structural basis for clearing of ribosome collisions by the RQT complex. ***Nat. Commun***. doi:https://doi.org/10.1038/s41467-023-36230-8. (2023)
3. Tesina, P.#,*, Ebine, S.#, Buschauer, R.#, Thoms, M., Matsuo, Y., Inada, T.* and Beckmann, R.* (#Equal contribution)
   Molecular basis of eIF5A-dependent CAT tailing in eukaryotic ribosome-associated quality control. ***Mol. Cell*** doi.org/10.1016/j.molcel.2023.01.020. (2023)
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   Nat. Commun. doi: 10.1038/s41467-023-38161-w. (2023)
5. Ishimura, R.#, Ito, S.#, Mao, G., Komatsu-Hirota, S., Inada, T.*, Noda, N.*, Komatsu, M.* (#Equal contribution)
   Mechanistic insights into the roles of the UFM1 E3 ligase complex in ufmylation and ribosome-associated protein quality control.
   Sci. Adv. (2023)

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Division of Protein Metabolism
タンパク質代謝制御分野

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The ubiquitin-proteasome system and lysosomes are the major proteolytic pathways and play a fundamental role in cellular protein homeostasis (proteostasis). Their dysregulation causes a plethora of diseases, including neurodegenerative disorders, but the detailed pathogenic mechanisms remain largely unknown. Our goal is to elucidate the basic molecular mechanisms governing proteostasis-related diseases by analyzing the regulation of protein metabolism, thereby providing the basis for therapeutic strategies.

1. Generation and characterization of proteasomopathy model mice

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The proteasome plays a central role in many biological processes, including cell proliferation, transcriptional regulation, inflammation and proteostasis, by selectively degrading ubiquitinated proteins. Nevertheless, proteasome research at the whole-body level has lagged far behind because the proteasome is essential for the viability of all cells and knockout mice of the constitutive subunits are embryonic lethal. Recently, heterozygous mutations in *PSMD12*, a 19S subunit gene of the proteasome, have been identified in patients with developmental disorders and autism. Based on this finding, we generated three lines of heterozygous mutant mice lacking the 3′ end of *Psmd12* at different lengths. The most deficient *Psmd12* mutant mice exhibited growth retardation, impaired liver function, and diaphragmatic hernia. Further histological analysis revealed hepatocyte shedding and accumulation of ubiquitinated substrates in the cerebellum. The other two lines of mutant mice had mild or no phenotypes. Next, we performed a deep proteomic analysis of the liver and unexpectedly found that the mildest *Psmd12* mutant mice showed the largest proteome changes, with accumulation of proteins involved in mitochondrial dysfunction, DNA damage, and oxidative stress responses. Thus, the systemic proteasome mutant mice revealed that proteasome dysfunction exhibits diverse phenotypes, including impaired liver function, and that a slight decrease of the proteasome activity can trigger abnormal proteostasis.

2. A quality control system to monitor the integrity of protein complex

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Approximately half of all proteins form complexes to achieve functional expression in cells. The stoichiometry of the subunits that form a complex must be properly maintained, and failure of assembly or disruption of this balance due to causes such as aneuploidy results in the accumulation of misassembled intermediates and orphan subunits. Recently, multi-

ple protein quality control mechanisms have been discovered that target these incorrectly assembled proteins for degradation. For example, the E3 ligases HUWE1 and STUB1/CHIP are well established as general quality control factors, and some E3 ligases that are dedicated to specific complexes have been identified, such as UBE2O for ribosomal proteins, HERC1 for proteasome intermediates and HERC2 for CCT/TRiC chaperonin. However, it has not been described whether cells have quality control mechanisms that specifically monitors the integrity protein complexes.

We have discovered a quality control system for the disintegrated minichromosome maintenance (MCM) complex generated by depletion of a single MCM subunit using auxin-inducible degron (AID) system. Rapid depletion of MCM2-mAID destabilized the remaining MCM subunits, and we identified the ubiquitin ligase CRL4 (DCAF *X*) for selective removal of MCM5 by the proteasome. Mechanistically, the CRL4 substrate adaptor DCAF *X* recognized the buried region inside the MCM complex and ubiquitinated only after the disintegration. This mechanism appeared to be distinct from the elimination pathway of the orphan subunit during assembly. Thus, CRL4 (DCAF *X*) is an E3 ligase that monitors the integrity of the MCM complex, thus providing insights into the surveillance mechanisms of complex integrity.

3. Liquid-to-solid phase transition of ubiquitylated proteins triggered by ATP depletion

Akinori Endo[1], Chikage Takahashi[1], Hikaru Tsuchiya[1], Keiji Tanaka[1], Yasushi Saeki

Ubiquitin-positive inclusions are a hallmark of almost all the neurodegenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis. Dysregulation of ubiquitin-dependent phase separation has been implicated in the disease pathogenesis, but little is known about the molecular mechanisms involved. We have shown that proteasomes undergo liquid-liquid phase separation (LLPS) together with ubiquitinated proteins in response to changes of in the cellular environments (Yasuda et al, Nature 2020; Iriki et al, Cell Rep 2023). Recently, we found that ATP depletion also induces LLPS of the proteasome and ubiquitinated proteins. The decrease in ATP levels caused a massive remodeling of the ubiquitin proteome, especially in ATP-binding proteins and components of certain protein complexes, and condensation in a RAD23B- and UBQLN-dependent manner. Restoration of ATP levels resolved this condensation, which required p97 activity and proteasome deubiquitination activity, suggesting that the primary function of the condensates is sequestration of the ubiquitinated proteins rather than their degradation. Interestingly, inhibition of p97 or prolonged ATP depletion resulted in insolubilization of the ubiquitinated protein, that is, a phase transition from the liquid-to-solid phase transition. Thus, ubiquitin-dependent LLPS contributes to the correction of impaired proteostasis upon ATP stress and may be involved in the formation of ubiquitin-positive inclusions.

4. USP8 prevents aberrant NF-κB and Nrf2 activation by counteracting ubiquitin signals from endosomes

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K63-linked ubiquitin chains attached to plasma membrane proteins serve as tags for endocytosis and endosome-to-lysosome sorting. USP8 is an essential deubiquitinase for the maintenance of endosomal functions. Prolonged depletion of USP8 leads to cell death, but the major effects on cellular signaling pathways are poorly understood. Here, we show that USP8 depletion causes aberrant accumulation of K63-linked ubiquitin chains on endosomes and induces immune and stress responses. Upon USP8 depletion, two different decoders for K63-linked ubiquitin chains, TAB2/3 and p62, were recruited to endosomes and activated the TAK1–NF-κB and Keap1–Nrf2 pathways, respectively. Oxidative stress, an environmental stimulus that potentially suppresses USP8 activity, induced accumulation of K63-linked ubiquitin chains on endosomes, recruitment of TAB2, and expression of the inflammatory cytokine. The results demonstrate that USP8 is a gatekeeper of misdirected ubiquitin signals and inhibits immune and stress response pathways by removing K63-linked ubiquitin chains from endosomes.

5. Dynamic changes of lysosomal protein degradation in neural stem cells of the postnatal mouse brain

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Lysosomes are intracellular organelles responsible for degrading diverse macromolecules delivered from several pathways, including the endo-lysosomal and autophagic pathways. Recent reports have sug-

gested that lysosomes are essential for regulating neural stem cells in developing, adult, and aged brains. However, the activity of these lysosomes has yet to be monitored in these brain tissues. Here, we report the development of a new probe to measure lysosomal protein degradation in brain tissue by immunostaining. Our results indicate that lysosomal protein degradation fluctuates in neural stem cells of the hippocampal dentate gyrus, depending on age and brain disorders. Neural stem cells increase their lysosomal activity during hippocampal development in the dentate gyrus, but aging and aging-related disease reduce lysosomal activity. In addition, physical exercise increases lysosomal activity in neural stem cells and astrocytes in the dentate gyrus. We therefore propose that three different stages of lysosomal activity exist: the state of increase during development, the stable state during adulthood, and the state of reduction due to damage caused by either age or disease.

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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. Multiancestry comprehensive molecular analysis of gastric cancer

Gastric cancer is among the most common malignancies worldwide, characterized by geographical, epidemiological and histological heterogeneity. Here, we report an extensive, multiancestral landscape of driver events in gastric cancer, involving 1,335 cases. Seventy-seven significantly mutated genes (SMGs) were identified, including *ARHGAP5* and *TRIM49C*. We also identified subtype-specific drivers, including *PIGR* and *SOX9*, which were enriched in the diffuse subtype of the disease. SMGs also varied according to Epstein-Barr virus infection status and ancestry. Non-protein-truncating *CDH1* mutations, which are characterized by in-frame splicing alterations, targeted localized extracellular domains and uniquely occurred in sporadic diffuse-type cases. In patients with gastric cancer with East Asian ancestry, our data suggested a link between alcohol consumption or metabolism and the development of *RHOA* mutations. Moreover, mutations with potential roles in immune evasion were identified. Overall, these data provide comprehensive insights into the molecular landscape of gastric cancer across various subtypes and ancestries.

2. Genomic analysis and evolutionary simulation of MSI-H colorectal cancer

Intratumor heterogeneity (ITH) in microsatellite instability-high (MSI-H) colorectal cancer (CRC) has been poorly studied. We aimed to clarify how the ITH of MSI-H CRCs is generated in cancer evolution and how immune selective pressure affects ITH. We reanalyzed public whole-exome sequencing data on 246 MSI-H CRCs. In addition, we performed a multi-region analysis from 6 MSI-H CRCs. To verify the process of subclonal immune escape accumulation, a novel computational model of cancer evolution under immune pressure was developed. Our analysis presented the enrichment of functional genomic alterations in antigen-presentation machinery (APM). Associative analysis of neoantigens indicated the generation of immune escape mechanisms via HLA alterations. Multiregion analysis revealed the clonal acquisition of driver mutations and subclonal accumulation of APM defects in MSI-H CRCs. Examination of variant allele frequencies demonstrated that subclonal mutations tend to be subjected to selective sweep. Computational simulations of tumour progression with the interaction of immune cells successfully verified the subclonal accumulation of immune escape mutations and suggested the efficacy of early

initiation of an immune checkpoint inhibitor (ICI) -based treatment. Our results demonstrate the heterogeneous acquisition of immune escape mechanisms in MSI-H CRCs by Darwinian selection, providing novel insights into ICI-based treatment strategies.

3. Evaluating selection working on intra-tumor heterogeneity.

A tumor is defined as a population of cells that rapidly expands from a single original cell. It is now widely acknowledged that a tumor is composed of genetically diverse subclones, leading to intra-tumor heterogeneity (ITH). Ongoing discussions center on the impact of selection on the quality and quantity of ITH, as it directly affects the medical response of tumors, resistance to therapy, and the likelihood of recurrence and/or metastasis. The central question revolves around the significance of Darwinian selection within the tumor cell population. Various studies have suggested that the observed ITH patterns could be explained by non-Darwinian or neutral models, while others propose a role for Darwinian selection.We are currently developing a novel and straightforward statistical test to assess the influence of Darwinian selection (or positive selection) on intratumor heterogeneity. Through this approach, we plan to analyze ITH data across different cancer types and investigate the contribution of Darwinian selection, examining its relationship with factors such as cancer type, age, clinical stage, and genomic background. These findings underscore the importance of understanding Darwinian selection in shaping ITH and emphasize its potential implications for improving cancer diagnosis and treatment strategies.

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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 proteome/metabolome analysis, we have isolated a number of biologically and/or medically important genes, and are developing novel diagnostic and therapeutic tools.

1. Functional analysis of p53 signaling pathway Regulation of the innate immune response and gut microbiome by p53

p53 is a key tumor suppressor mutated in half of human cancers. In recent years, p53 was shown to regulate a wide variety of functions. From the transcriptome analysis of 24 tissues of irradiated mice, we identified 553 genes markedly induced by p53. Gene Ontology (GO) enrichment analysis found that the most associated biological process was innate immunity. 16S rRNA-seq analysis revealed that Akkermansia, which has anti-inflammatory properties and is involved in the regulation of intestinal barrier integrity, was decreased in p53-knockout ($p53^{-/-}$) mice after radiation. $p53^{-/-}$ mice were susceptible to radiation-induced GI toxicity and had a significantly shorter survival time than p53-wild-type ($p53^{+/+}$) mice following radiation. However, administration of antibiotics resulted in a significant improvement in survival and protection against GI toxicity. Mbl2 and Lcn2, which have antimicrobial activity, were identified to be directly transactivated by p53 and secreted by liver into the circulatory system. We also found the expression of MBL2 and LCN2 was decreased in liver cancer tissues with p53 mutations compared with those without p53 mutations. These results indicate that p53 is involved in shaping the gut microbiome through its downstream targets related to the innate immune system, thus protecting the intestinal barrier.

2. Analysis of host genetic factors of various diseases
Helicobacter pylori, homologous-recombination genes, and gastric cancer

Background: Helicobacter pylori infection is a well-known risk factor for gastric cancer. However, the contribution of germline pathogenic variants in cancer-predisposing genes and their effect, when combined with H. pylori infection, on the risk of gastric cancer has not been widely evaluated.

Methods: We evaluated the association between germline pathogenic variants in 27 cancer-predisposing genes and the risk of gastric cancer in a sample of 10,426 patients with gastric cancer and 38,153 controls from BioBank Japan. We also assessed the combined effect of pathogenic variants and H. pylori infection status on the risk of gastric cancer and calculated the cumulative risk in 1433 patients with gastric cancer and 5997 controls from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC).

Results: Germline pathogenic variants in nine genes (APC, ATM, BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, and PALB2) were associated with the

risk of gastric cancer. We found an interaction between H. pylori infection and pathogenic variants in homologous-recombination genes with respect to the risk of gastric cancer in the sample from HERPACC (relative excess risk due to the interaction, 16.01; 95% confidence interval [CI], 2.22 to 29.81; P = 0.02). At 85 years of age, persons with H. pylori infection and a pathogenic variant had a higher cumulative risk of gastric cancer than noncarriers infected with H. pylori (45.5% [95% CI, 20.7 to 62.6] vs. 14.4% [95% CI, 12.2 to 16.6]).

Conclusions: H. pylori infection modified the risk of gastric cancer associated with germline pathogenic variants in homologous-recombination genes. (Funded by the Japan Agency for Medical Research and Development and others.).

East Asian-specific and cross-ancestry genome-wide meta-analyses provide mechanistic insights into peptic ulcer disease,

Peptic ulcer disease (PUD) refers to acid-induced injury of the digestive tract, occurring mainly in the stomach (gastric ulcer (GU)) or duodenum (duodenal ulcer (DU)). In the present study, we conducted a large-scale, cross-ancestry meta-analysis of PUD combining genome-wide association studies with Japanese and European studies (52,032 cases and 905,344 controls), and discovered 25 new loci highly concordant across ancestries. An examination of GU and DU genetic architecture demonstrated that GUs shared the same risk loci as DUs, although with smaller genetic effect sizes and higher polygenicity than DUs, indicating higher heterogeneity of GUs. Helicobacter pylori (HP)-stratified analysis found an HP-related host genetic locus. Integrative analyses using bulk and single-cell transcriptome profiles highlighted the genetic factors of PUD being enriched in the highly expressed genes in stomach tissues, especially in somatostatin-producing D cells. Our results provide genetic evidence that gastrointestinal cell differentiations and hormone regulations are critical in PUD etiology.

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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. Epigenetic characterization of housekeeping core promoters and their importance in tumor suppression

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In this research, we elucidate the presence of around 11,000 housekeeping *cis*-regulatory elements (HK-CREs) and describe their main characteristics. Besides the trivial promoters of housekeeping genes, most HK-CREs reside in promoter regions and are involved in a broader role beyond housekeeping gene regulation. HK-CREs are conserved regions rich in unmethylated CpG sites. Their distribution highly correlates with that of protein-coding genes, and they interact with many genes over long distances. We observed reduced activity of a subset of HK-CREs in diverse cancer subtypes due to aberrant methylation, particularly those located in chromosome 19 and associated with zinc finger genes. Further analysis of samples from 17 cancer subtypes showed a significantly increased survival probability of patients with higher expression of these genes, suggesting them as housekeeping tumor suppressor genes. Overall, our work unravels the presence of housekeeping CREs indispensable for the maintenance and stability of cells.

2. A graph-embedding approach dissecting enhancer-cofactor-promoter interactions

Sung-Joon Park and Kenta Nakai

The spatial genome organization mediates the functional impact of distal chromosomal interactions.

Particularly, enhancer-promoter interactions have been intensively studied by cutting-edge computational algorithms. However, we still have a very limited understanding of how enhancer signals transmit to their target promoters through highly intricate regulatory networks. Here, we developed a new computational framework. The method combined a regression modeling that predicts gene expression by inferring important promoter-distal and -proximal gene regulators with a graph-embedding algorithm that detects cell-type specific and conserved regulatory interactions in complex gene regulatory networks. We applied the method to human naïve and germinal center B cells. Consequently, we identified sets of promoter-distal transcription factors and architectural cofactor proteins, which are appropriately co-regulated to prevent malignancy. These results highlight the importance of understanding the cis- and trans-regulatory interactions composed in the transcriptional domain. Our approach offers an alternative method to understanding enhancer biology mediated by protein-protein interactions in the 3D genome organization.

3. HyGAnno: Hybrid graph neural network-based cell type annotation for single-cell ATAC sequencing data

Weihang Zhang, Yang Cui, Bowen Liu, Martin Loza, Sung-Joon Park and Kenta Nakai

Reliable cell type annotations are crucial for investigating cellular heterogeneity in single-cell omics data. Although various computational approaches have been proposed for single-cell RNA sequencing (scRNA-seq) annotation, high-quality cell labels are still lacking in single-cell ATAC sequencing (scATAC-seq) data because of extreme sparsity and inconsistent chromatin accessibility between datasets. Here, we present a novel automated cell annotation method called HyGAnno that transfers cell type information from a well-labeled scRNA-seq reference to an unlabeled scATAC-seq target via a parallel graph neural network in a semi-supervised manner. Unlike existing methods that utilize only gene expression or gene activity features, HyGAnno integrates genome-wide accessibility peak features to facilitate the training process. In addition, HyGAnno reconstructs a reference-target cell graph that can be used to detect cells with low prediction reliability according to their specific graph connectivity patterns. HyGAnno was tested using large datasets and demonstrated the advantages of accurate cell annotation, interpretable cell embedding, robustness to noisy reference data, and adaptability to tumor tissues.

4. Spatial Transcriptomics Analysis via Image-Aided Graph Contrastive Learning for Domain Exploration and Alignment-Free Integration

Yitao Yang, Yang Cui, Xin Zeng, Yubo Zhang, Martin Loza, Sung-Joon Park and Kenta Nakai

Biological tissues, as intricate networks of varied cell types, perform essential life functions through unique spatial configurations. Recent spatial transcriptomics techniques, such as 10x Visium, have greatly enhanced our ability to map genetic data within these configurations, offering deeper insights into the genetic makeup of tissues in health and disease and advancing our grasp of molecular and physiological intricacies. We propose Spatial Transcriptomics and Image-based Graph learning (STAIG), a deep learning framework for advanced spatial region identification in spatial transcriptomics. STAIG integrates gene expression, spatial coordinates, and histological images using graph contrastive learning, excelling in feature extraction and enhancing analysis on datasets with or without histological images. It has outperformed existing methods in recognizing spatial regions within Visium and other platform datasets. STAIG was specifically engineered to counter batch effects and to allow for the integration of tissue slices without pre-alignment. In applications to human breast cancer and zebrafish melanoma, STAIG has identified regions with high precision, uncovering new insights into tumor microenvironments. STAIG thus offers a versatile tool for probing cellular structures and interactions, enriching our understanding of spatial transcriptomics.

5. TF-EPI: An Interpretable Enhancer Promoter Interaction Detection Method Based on Large Language Model

Bowen Liu, Weihang Zhang, Xin Zeng, Martin Loza, Sung-Joon Park and Kenta Nakai

The detection of enhancer-promoter interactions (EPIs) is crucial for understanding gene expression regulation, disease mechanisms, and more. In this study, we developed TF-EPI, a deep learning model based on Transformer, designed to detect these interactions solely from DNA sequences. The performance of TF-EPI surpassed other state-of-the-art methods in multiple benchmark datasets. Importantly, by utilizing the attention mechanism of the Transformer, we identified distinct cell type-specific motifs and sequences in enhancers and promoters, which we validated against databases like Jaspar and UniBind, highlighting the potential of our method in discovering new biological insights. Moreover, our analysis of the transcription factors corresponding to these motif and short sequence pairs revealed the heterogeneity

and commonality of gene regulatory mechanisms and demonstrated the ability to identify TFs relevant to the source information of the cell line. Finally, the introduction of transfer learning can mitigate the challenges posed by cell type-specific gene regulation, yielding enhanced accuracy in cross-cell line EPI detection results. Overall, our work unveils important sequences information for the investigation of enhancer-promoter pairs based on the attention mechanism of Transformer, which provides an important milestone in the investigation of cis-regulatory grammar.

6. Computational Transcriptomic Analysis Identifies a Novel Immune-dysregulated TNBC Subtype Regulated by STAT3

Yang Cui and Kenta Nakai

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and frequently appears to be resistant to treatment due to its extensive intratumoral heterogeneity. The tumor microenvironment (TME) plays a critical role in TNBC immunomodulation and progression. In this study, we leveraged TNBC bulk RNA-seq data and TNBC scRNA-seq data to investigate the TNBC TME. Utilizing CIBERSORTx to deconvolve the bulk RNA-seq data with scRNA-seq as a reference, we identified cell compositions within the TME. Non-negative matrix factorization further revealed three unique clusters, with patients in Cluster 2 (C2) exhibiting significantly shorter survival times. Next, we trained a random forest classifier on TNBC bulk RNA-seq data to identify the C2 patients within the scRNA-seq dataset. We found a reduced abundance of immune cells, including CD4+ T cells, CD8+ T cells, DC, Macrophage, and NK cells, and an increase in breast cancer cells in C2, indicating an immunosuppressive environment. We further conducted cell-cell communication (CCC) analysis and found compromised MHC-I and MHC-II signaling in CD8+ T cells and macrophages, respectively, in C2. Followed SCENIC analysis unveiled the gene regulatory network of the CD8+ T cells in C2, revealing an enrichment of regulon STAT3, which is associated with T cell dysfunction. Our study unveils an immunodysregulated subtype within TNBC, and provides valuable insights into its heterogeneity.

7. Development of the splice site prediction model that can account for long-distance effects using Transformer.

Yuna Miyachi and Kenta Nakai

RNA splicing is an essential process in the regulation of gene expression. Recently, deep learning methods have been applied to predict splice sites and elucidate their complex mechanisms. However, there are no existing methods that account for both long-range sequence effects and high interpretability.

To solve this problem, we created a new splice site prediction model consisting of a convolution layer and an attention mechanism, taking as input sequences up to 100k in length. The combination of the convolutional layer and the attention mechanism allowed the model to learn both local and global features. The results show that the proposed model outperforms the state-of-the-art SpliceAI model in terms of precision and F1 score on a dataset of Gencode annotations. We also performed predictions on an aberrant splicing dataset generated from DBASS and compared the performance of the proposed method with SpliceAI. We found that our model is more sensitive to changes in splice sites than SpliceAI if the threshold is set sufficiently low. We also examined which sequences are important for splicing by analyzing the attention weights. We found several exonic splicing enhancer candidates in addition to the GT-AG rule, which is known as a consensus sequence for donor and acceptor sites of intron. Altogether, our proposed model has high interpretability and achieved high prediction performance, which could be useful for disease diagnosis and mechanistic elucidation, such as the detection of pathogenic mutations that cause aberrant splicing.

8. Comparative Single-cell Analyses of Human and Mouse Dendritic Cell Progenitors

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The 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 as compared to the mouse counterpart. In this study, we compare human and mouse bone marrow cell types in order to find out the progenitors of differentiated DC populations. We integrated multiple public datasets via Mutual Nearest Neighbors (Haghverdi et al., 2018). We also predicted transcription factor (TF) regulons and gene regulatory networks with SCENIC+ (Bravo and De Winter et al., 2023). In the future, we intend to identify the homologous TFs of the same lineage across humans and mouse to understand the genetic mechanisms of DC specification.

9. Discovery of human oncogenes guided by bioinformatics analysis of multi-omics data.

Martin Loza, Alexis Vandenbon[1], Gözde Korkmaz[3], and Kenta Nakai

[3]School of Medicine, Koç University, Turkey

Cis-regulatory elements, as enhancers and promoters, orchestrate the spatial and temporal expression of genes and are indispensable for organismal development and disease protection. In our latest publication (Loza et al., 2023), we uncovered the existence of a subset of housekeeping regulatory elements with potential tumor suppression capabilities.

Functional genomics that is based on functional phenotypic screening has become highly prominent in cancer-focused studies to identify the function of genes and regulatory elements essential for the malignant phenotype. In the last decade, CRISPR-based screening has been stated as a fundamental method for genome-wide loss-of-function (LOF) screening. Pooled library approaches make it possible to uncover novel genes that promote malignancy, and until now, several studies have demonstrated the applicability of CRISPR-based genome-wide LOF screenings to identify novel oncogenic genes.

Altogether, the scope of this study is to validate the functionality of the housekeeping core promoters identified as potential tumor suppressors. Using CRISPR-based screening, we will validate their targets as potential human oncogenes for diverse cancer sub-types.

10. Comparative single-cell transcriptomic analysis reveals key differentiation drivers and potential origin of vertebrate retina

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The retina plays a vital role in capturing and processing visual information in vertebrates, allowing them to adapt to diverse environments. Single-cell expression profiles of the vertebrate retina have been described; however, a deeper understanding of the expression patterns in the context of development and evolution among homologous cell types is lacking. To identify such shared patterns, we examined and compared approximately 230,000 retinal cells from three species: mouse, chicken, and zebrafish. We found significant similarities among homologous cell types, indicating inherent regulatory patterns. To understand these shared patterns, we constructed gene regulatory networks for each developmental stage for the three species. We identified 690 regulons with 381 regulators across the three species. In addition, we identified ten common cell type-specific regulators and 16 preserved regulons. Using RNA velocity analysis, we pinpointed conserved driver genes key to retinal cell differentiation in both mouse and zebrafish, and by intersecting regulators, we extracted the crucial regulators that facilitate these differentiation processes. Finally, investigation of the potential origins of photoreceptors and retinal ganglion cells by examining conserved expression patterns among the three vertebrate species and the invertebrate Ciona intestinalis revealed functional similarities in light transduction mechanisms between Ciona photoreceptor-related cells and vertebrate retinal cells. Our findings offer insights into the conserved regulatory frameworks intrinsic, evolutionarily preserved differentiation programs, and ancestral origins of vertebrate retinal cells.

11. Exploring the role of hysteresis in macrophage lipid metabolism and robustness of repolarization memory

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Macrophages exhibit polarization, leading to functionally distinct phenotypic changes. This process of polarization and subsequent de-/re-polarization results in hysteresis, where the cells retain genetic and epigenetic markers from previous states, impacting their core functions. To probe the role of hysteresis in lipid metabolism, we utilized computational analysis on an extensive public dataset. Our approach involved reanalyzing 154 RNA-seq datasets pertaining to (re)polarized macrophages, including those with lipid accumulation. We applied Weighted Gene Correlation Network Analysis (WGCNA) and conducted functional enrichment analysis. This led to the identification of 10 significant gene modules, including one uniquely associated with polarized macrophages without de-/re-polarization history, linked to cholesterol biosynthesis. Conversely, a module enriched in inflammatory and LPS response was closely related to cells with a history of specific macrophage de-/re-polarization. To further validate the expression trends of hysteresis genes in de-/repolarized macrophages, we are using lab techniques like PCR and Western Blot, aligning these with prior research. We extended the de-/repolarization duration to bolster the credibility of our results. Our research aims to establish the reproducibility of the hysteresis effect and highlight the importance of its inhibition in regulating lipid metabolism. The complex regulation of cellular memory, however, still presents significant unknowns. Herein, we delve into our ongoing efforts to elucidate the role of macrophage hysteresis. This research was presented at InCOB2023.

12. Identification of transcription factors that contribute to enhancer-promoter communication in mESCs by heterogeneous graph neural network

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Transcription factors (TFs) play a critical role in the communication between enhancers and promoters, essential for gene expression in mouse embryonic stem cells (mESCs). Moving beyond the traditional focus on pairwise TF interactions, our research delves into a more sophisticated network involving multiple interactions and transcriptional condensates. We employ state-of-the-art sequencing methods and deep learning techniques to unravel complex TF interactions within enhancer-promoter loops, aiming to uncover crucial, yet hidden, TF pairs that influence mESC identity and differentiation. In this endeavor, we use CAGE-seq to identify promoter and enhancer regions in mESCs precisely and complement this with RADICL-seq and PCHi-C for detecting chromatin loops. By integrating computational analysis of TF binding sites with existing protein-protein interaction data, we create an extensive network graph featuring enhancers, promoters, and TFs. The innovative co-contrastive learning method, HeCo, allows us to produce simplified representations of TF nodes. This methodology aids in predicting missing links in the TF network, which we hypothesize to be critical in enhancer-promoter interactions. These predictions, including 20 high-confidence TF-TF interaction pairs, are currently being validated through experimental wet-lab techniques. Our overarching objective is to lead the development of a detailed graph that maps the Enhancer-TFs-Promoter/Genes regulatory network in mESCs. This project is aimed at pinpointing essential TFs and regulatory components involved in enhancer-promoter communications and loop formations. Through this, we hope to facilitate the prediction and discovery of novel TF interactions, thereby advancing the understanding of complex biological networks in stem cell research.

13. Identification of COPS5 as a novel biomarker of diffuse large B cell lymphoma by a machine-learning algorithm

Yutong Dai, Sung-Joon Park, Jingmei Li[8], Keita Yamamoto[8], Susumu Goyama[8] and Kenta Nakai
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The transition from generalized medicine to precision medicine has been pivotal in cancer research, emphasizing the need for biomarkers. Despite challenges in identifying meaningful biomarkers, machine-learning approaches for biomarker discovery have been widely adopted. Particularly, Deep learning, while powerful, faces criticism for its interpretability and performance issues due to missing data.

To address these challenges, our study employs the Joint Non-Negative Matrix Factorization (JNMF), an unsupervised algorithm, to unravel cancer biomarkers from multi-omics data. Unlike prior pan-cancer studies, our approach integrates JNMF, gene signature and pathway analysis, and patient data validation, ensuring high interpretability and clinical relevance. The JNMF efficiently handles sparse datasets, demonstrated through simulations and the Cancer Cell Line Encyclopedia (CCLE) datasets. The analysis identifies 40 reproducible and robust feature sets, termed Common Pattern Modules (CPMs), revealing cancer-specific patterns. Notably, the hematopoietic and lymphoid-specific CPM highlights genomic features of diffuse large B-cell lymphoma (DLBCL). Pathway analysis associates DLBCL key pathways, including NF-κB, TP53, JAK-STAT, and PI3K, with COPS5 identified as a key upstream regulator of DLBCL-related pathways in this CPM. Survival analysis using The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) data establishes COPS5 overexpression as a predictor of poor patient survival, corroborating its role in DLBCL. Moreover, Consistent with our observation of overexpression of COPS5, MYC, BCL6, TP53, and STAT3 in DLBCL-specific CPM, a positive correlation between COPS5 expression levels and these genes was also observed in DLBCL patients. More significantly, CRISPR/Cas9 knockout experiments further validate COPS5's significance in promoting malignant growth in mature B-cell neoplasms.

In conclusion, the integrated analysis unveils DLBCL genomic characteristics, spotlighting COPS5 as a potential prognostic biomarker. The methodology holds promise for extracting clinically significant biomarkers from diverse omics data, advancing precision medicine in cancer research.

14. GlycoMSParser: an automated pipeline for comprehensive permethylated glycan analysis

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We're trying to establish a pipeline that is applicable to automatically annotate glycans (polysaccharides) extracted from proteins obtained from biological samples, which were generally analyzed and annotated manually. Software for annotating spectra based on in silico calculated glycan fragments and database search still needs man to operate it with proper knowledge and conditions based on the experimental

method. In our research, we developed one non-machine learning algorithm that can annotate glycan assisted with glycosyltransferase gene expression (if it exists and the functions are known) and can predict and annotate similar glycan structures under the same experimental method in unknown samples. We're still improving the whole pipeline and testing it with more data and parameters to get more comprehensive and convincing results for publishing this research.

15. Cross-sample genetic correlations reveal a causal relationship between human complex traits

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Ascertaining causality in human complex traits is crucial for understanding biology and disease prevention. In this research, we introduce a novel concept termed cross-sample genetic correlation (X-rG). It is calculated from GWAS summary statistics of two phenotypes from different cohorts and serves as an indicator to identify potential causal relationships between complex traits. Through mathematical modeling and numerical simulations, we have found that when the difference in genetic correlation between two cohorts is attributable to causal effects, a significant disparity in X-rG values is observed. Conversely, variation in genetic correlations due to pleiotropy leads to symmetric X-rG values. Leveraging this feature, we screened for asymmetry in X-rG among 211 complex traits between male and female cohorts in the UK Biobank, identifying 207 pairs of traits with potential causal relationships. The findings underscore the pivotal roles of education and occupation type in exposure and outcome, respectively, which are beneficial for improving health indicators and well-being. This method provides an effective complement to causal reasoning for complex traits and diseases and has significant implications for the development of precision medicine strategies.

16. Discovery of antibodies with potential therapeutic applications aided by machine learning and artificial intelligence.

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The use of antibodies to improve human health is an emerging area of biotechnology and immunology. They represent a therapeutic strategy with several applications for managing various types of cancer, infectious and inflammatory diseases, and other noninfectious diseases. Several pharmaceutics and research groups have worked in the last years to find new therapeutic applications for antibodies, including antibody modifications to enhance their efficacy and stability and facilitate their administration. One critical step in this area is antibody discovery, which represents the major challenge of the field. The new computational and experimental methodologies offer important advantages for discovering and testing new antibodies. Single-cell RNAseq is the best example of these new tools since it allows isolating antibody sequences from B-cells of individuals immunized with the therapeutic target. However, analyzing a significant number of sequences takes time and effort. The use of computational approaches like deep learning, machine learning, and artificial intelligence can potentially assist in discovering new antibodies, providing a tool for the prediction of neutralizing profiles, thus virtually reducing the time, effort, and resources invested.

In this project, we aim to build a bioinformatics pipeline based on artificial intelligence to discover new neutralizing antibodies against SARS-CoV-2. The final platform could be extended to assist in the discovery of antibodies against other diseases using public libraries or new ones generated by Prof. Hernández´s Laboratory.

17. Identifying pioneer molecular modulating global chromatin accessibility by genome-wide ATAC-see screening and ATAC-seq analysis

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The dynamics of chromatin accessibility play a crucial role in a local environment for accomplishing various biological functions. Here, to understand the molecular mechanisms underlying the control of chromatin accessibility, we conducted a genome-wide CRISPR screening combined with an optimized ATAC-see that covers more than 19,000 human genes in eHAP cells, along with developing a computational analytic pipeline. This examination detected genes previously unknown to modulate global chromatin accessibility, including TFDP1, HNRNPU, EIF3D, and THAP11. Particularly, the transcription factor TFDP1 markedly impacted global chromatin accessibility through transcriptional regulation of canonical histones. That is, the TFDP1-depleted cells showed increased global chromatin accessibility and enhanced the efficiency of DNA-associated applications,

including iPSC reprogramming. These results highlight the potential that the manipulation of chromatin accessibility by altering key molecules is a promising tool for enhancing the efficiency of various cell engineering applications.

18. Resting heart rate and risk of dementia: a Mendelian Randomization Study in the International Genomics of Alzheimer9s Project and UK Biobank

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Observational studies have demonstrated that a higher resting heart rate (RHR) is associated with an increased risk of dementia. However, it is not clear whether the association is causal. This study aimed to determine the causal effects of higher genetically predicted RHR on the risk of dementia. We performed a generalized summary Mendelian randomization (GSMR) analysis to analyze the corresponding effects of higher RHR on the following different outcomes: 1) diagnosis of AD (International Genomics of Alzheimer's Project), 2) family history (maternal and paternal) of AD from UK Biobank, 3) combined meta-analysis including these three GWAS results. Further analyses were conducted to determine the possibility of reverse causal association by adjusting for RHR modifying medication. The results of GSMR showed no significant causal effect of higher genetically predicted RHR on the risk of AD (β_{GSMR} = 0.12, P = 0.30). GSMR applied to the maternal family history of AD (β_{GSMR} = -0.18, P = 0.13) and to the paternal family history of AD (β_{GSMR} = -0.14, P = 0.39) showed the same results. Furthermore, the results were robust after adjusting for RHR modifying drugs (β_{GSMR} = -0.03, P = 0.72). In conclusion, our study did not find any evidence that supports a causal effect of higher RHR on dementia. Previous observational associations between RHR and dementia are likely attributed to the correlation between RHR and other cardiovascular diseases.

19. DeepBIO: An automated and interpretable deep-learning platform for high-throughput biological sequence prediction, functional annotation, and visualization analysis

Ruheng Wang[22], Yi iang[22], Junru Jin[22], Chenglin Yin[22], Haoqing Yu[22], Fengsheng Wang[22], Jiuxin Feng[22], Ran Su[23], Kenta Nakai, Quan Zou[24], & Leyi Wei[22]

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DeepBIO is the first-of-its-kind automated and interpretable deep-learning platform for high-throughput biological sequence functional analysis. It is a one-stop-shop web service that enables researchers to develop new deep-learning architectures to answer any biological question. Specifically, given any biological sequence data, DeepBIO supports a total of 42 state-of-the-art deep-learning algorithms for model training, comparison, optimization, and evaluation in a fully automated pipeline. DeepBIO provides a comprehensive result visualization analysis for predictive models covering several aspects, such as model interpretability, feature analysis, and functional sequential region discovery. We expect DeepBIO to ensure the reproducibility of deep-learning biological sequence analysis, lessen the programming and hardware burden for biologists, and provide meaningful functional insights at both the sequence level and base level from biological sequences alone. DeepBIO is publicly available at https://inner.wei-group.net/DeepBIO.

20. 19n01, a broadly neutralizing antibody against omicron BA.1, BA.2, BA.4/5, and other SARS-CoV2 variants of concern

Melissa Garcia-Vega[12], Edgar A. Melgoza-Gonzalez[12], Sofia Hernandez-Valenzuela[12], Diana Hinojosa-Trujillo[12], Monica Resendiz-Sandoval[12], Mara Anais Llamas-Covarrubias[25], Martin Loza-Lopez, Olivia Valenzuela[26], Alan Soto-Gaxiola[27], Miguel A. Hernandez-Onate[28], Veronica Mata-Haro[28], Irene Cassaniti[39], Jose Camilla Sammartino[29], Alessandro Ferrari[29], Luca Simonelli[30], Mattia Pedotti[30], Rui Sun[11], Fanglei Zuo[11], Fausto Baldanti[29], Luca Varani[30], Harold Marcotte[11], Qiang Pan-Hammarstrom[11], and Jesus Hernandez[12]

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This study reports the isolation and characterization of a human monoclonal antibody (mAb) called 19n01. This mAb was isolated by using single-cell RNAseq of B cells from donors infected with the an-

cestral strain. This mAb possesses a potent and broad capacity to bind and neutralize all previously circulating variants of concern (VOCs), including Omicron sublineages BA.1, BA.2, and BA.4/5. The pseudovirus neutralization assay revealed robust neutralization capacity against the G614 strain, BA.1, BA.2, and BA.4/5, with inhibitory concentration (IC50) values ranging from 0.0035 to 0.0164 mg/mL. The microneutralization assay using the G614 strain and VOCs demonstrated IC50 values of 0.013–0.267 mg/mL. Biophysical and structural analysis showed that 19n01 cross-competes with ACE2 binding to the receptor-binding domain (RBD), and the kinetic parameters confirmed the high affinity against the Omicron sublineages (KD of 61 and 30 nM for BA.2 and BA.4/5, respectively). These results suggest that the 19n01 is a remarkably potent and broadly reactive mAb.

Publication list

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Human Genome Center

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公共政策研究分野

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The Department of Public Policy contributes to accomplishment of the following major missions: research ethics consultation to help scientists to comply with ethical guidelines and build public trust; public policy science studies of translational research and its societal impact; and promotion of patient and public involvement/ engagement in research and health care. Through qualitative and quantitative social science studies and policy analysis, we facilitate discussion of the challenges posed by the advances in medical science.

1. Public attitudes toward cloud computing and willingness to share personal health records (PHRs) and genome data for health care research in Japan.

Japan's government aims to promote the linkage of medical records, including medical genomic testing data and personal health records (PHRs), via cloud computing (the cloud). However, linking national medical records and using them for health care research can be controversial. Additionally, many ethical issues with using cloud networks with health care and genome data have been noted. However, no research has yet explored the Japanese public's opinions about their PHRs, including genome data, being shared for health care research or the use of the cloud for storing and analyzing such data. Therefore, we conducted a survey in March 2021 to clarify the public's attitudes toward sharing their PHRs, including genome data and using the cloud for health care research. We analyzed data to experimentally create digital health basic literacy scores (BLSs). Our results showed that the Japanese public had concerns about data sharing that overlapped with structural cloud computing issues. The effect of incentives on changes in participants' willingness to share data (WTSD) was limited. Instead, there could be a correlation between WTSD and BLSs. Finally, we argue that it is vital to consider not only researchers but also research participants as value cocreators in health care research conducted through the cloud to overcome both parties' vulnerability.

2. Is legislation to prevent genetic discrimination necessary in Japan? An overview of the current policies and public attitudes

Genetic discrimination (GD) has not been discussed in East Asia as extensively as in Europe and North America. Influenced by UNESCO's universal declaration in 1997, the Japanese government took a stringent approach toward GD by releasing the Basic Principles on Human Genome Research in 2000. However, Japanese society has mostly been ignoring the prevention of GD for decades, and the principle of prohibiting GD was never adhered to in any of the Japanese laws. We conducted anonymous surveys among the general adult population in 2017 and 2022 to explore their experiences of GD and attitudes toward laws carrying penalties to prevent GD in Japan. In both years, approximately 3% of the respondents had experienced some unfavorable treatment regarding their genetic information. They showed higher recognition of the benefits of using genetic information and lower recognition of concerns about using genetic information and GD in 2022 than in 2017.

However, the awareness regarding the need for legislation with penalties on GD had increased over the five-year period. In 2022, the framework of a bill to promote genomic medicine and prevent GD without any relevant penalties was released by the Bipartisan Diet Members Caucus. Considering that the absence of regulations may be a barrier to obtaining genomic medicine, as the initial step toward making the prohibition of GD more effective, legislation that no form of GD will be tolerated may stimulate education and awareness regarding respect for the human genome and its diversity.

3. Hope for the best, but prepare for the worst: Social media posted by participants in stem cell clinical trials

This article examines the influence of social media posts on clinical trials involving stem cell–based interventions. Based on the literature review, we identified three potential risks associated with social media posts regarding clinical trials that involve stem cell–based interventions: (1) threats to scientific validity, (2) amplification of excessive expectations, and (3) breaches of confidentiality. Additionally, preliminary recommendations are provided to safeguard the value of stem cell clinical trials for future patients in the age of social media. Our approach aims to safeguard the well-being of forthcoming participants and ensure the scientific validity of stem cell research, as well as possibly aid in the further development of shared guidelines for posting stem cell clinical trial information on social media platforms..

4. Attitudes towards human fetal tissue research: Survey of researchers and the public in Japan

The rules for human fetal tissue (HFT) research in Japan are unclear. We conducted a web survey to examine the attitudes of Japanese researchers (n=535) and the public (n=3,000) toward HFT research. The results demonstrated that 5.8% of researchers and 18.8% of the public explicitly opposed HFT research, and 71.8% of the researchers thought that the rules for HFT research need to be clarified. Even among researchers who intended to consider conducting HFT research, 74.2% responded that the rules should be clarified. Although different from attitudes to make decisions regarding HFT donation, being non-religious and in their reproductive age among women in the public group were factors for accepting attitudes toward HFT research. To establish the rules, it is necessary to develop a system that can adequately protect vulnerable women who are asked to provide HFT.

5. Survey of Japanese researchers and the public regarding the culture of human embryos in vitro beyond 14 days

The International Society for Stem Cell Research (ISSCR) has eliminated its prohibition on research involving the culturing of human embryos beyond 14 days within the updated 2021 guidelines. We conducted a survey of Japanese researchers working in stem cell- or embryo-related research (n = 535) and the public (n=3,000) about their attitudes toward the 14-day rule. Among the researchers, 46.2% agreed that embryos could be cultured beyond 14 days, a result that was slightly lower among the public (37.9%). Among those that disagreed with embryo culturing beyond 14 days, 9.5% of researchers and 5.1% of the public agreed with culturing embryos within 14 days. Among the public, higher comprehension levels correlated with both agreement and disagreement with the culture of embryos beyond 14 days compared with "cannot judge." Further research and public discourse are necessary in order to better understand the factors informing participant decisions regarding the 14-day rule.

6. Current situation of the hospitalization of persons without family in Japan and related medical challenges

This study aims to determine the approximate number of hospitalizations of persons without family and the medical challenges they encounter in hospitals across Japan. Self-administered questionnaires were mailed to 4,000 randomly selected hospitals nationwide to investigate the actual conditions and problems, decision-making processes, and use of the government-recommended Guidelines for the hospitalization of, and decision-making support for, persons without family. To identify the characteristics of each region and role of hospitals, chi-square tests were used to make separate group comparisons by hospital location and type. Responses were received from 1,271 hospitals (31.2% response rate), of which 952 hospitals provided information regarding the number of admissions of persons without family. The mean (SD) and median number of hospitalizations (approximate number per year) of patients without family was 16 (79) and 5, respectively. Approximately 70% of the target hospitals had experienced the hospitalization of a person without family, and 30% of the hospitals did not. The most common difficulties encountered during the hospitalization were collecting emergency contact information, decision-making related to medical care, and discharge support. In the absence of family members and surrogates, the medical team undertook the decision-making process, which was commonly performed according to manuals and guidelines and by consulting an ethics committee. Regarding the use of the government-recommended Guidelines, approximately 70% of the hospitals that were aware of these Guidelines responded that they had never taken any action based on these Guidelines, with significant differences by region and hospital type. To solve the problems related to the hospitalization of persons without family, the public should be made aware of these Guidelines,

and measures should be undertaken to make clinical ethics consultation a sustainable activity within hospitals.

7. Examination of the concept of FPIC (Free, Prior, and Informed Consent) with reference to the draft Ethical Guidelines for Research on the Ainu People

The Ainu Association of Hokkaido and academic anthropology and archaeology societies are developing "Ethical Guidelines for Research on the Ainu People," which position free, prior, and informed consent (FPIC) as their basis. This is the first document that Japanese research ethics regulations introduce FPIC. Based on a literature review, this article examines the expectations and unsolved problems of introducing FPIC in Japanese research ethics. As FPIC was originally advocated to protect lands and resources from exploitation, the challenges of adapting FPIC to research ethics are assessed.

The Ainu are the only officially recognized Indigenous people in Japan. However, after a long history of assimilation, the Ainu do not have representative organizations or an identity certification system. Past excavations of Ainu human remains for the purpose of academic research have been an important topic. The draft guidelines are expected not only to clarify how to handle such human remains but also to cover research about living Ainu people. Against the background of criticism of global genomic projects such as the Human Genome Diversity Project, community engagement is increasingly valued. Therefore, it is appropriate to focus on FPIC, which was advocated to ensure opportunities for Indigenous peoples to be consulted regarding the relevant research project. Canada and Taiwan have governmental structures to ensure such opportunities with guidelines or laws, but some adjustments to their research contexts can be seen. Japanese draft guidelines could also increase opportunities for Ainu people to engage in research projects by introducing FPIC. However, as the draft guidelines are overseen by a limited number of societies, their effectiveness and scope are somewhat limited. To enhance their efficiency, a wider variety of related academic societies should be involved in discussions. Moreover, as the Ainu people have no representative organizations, the problem of how to ensure meaningful consultation is vital. Further evaluation of practices and international comparisons will be required.

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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. (ε, κ)-Randomized Anonymization

Akihito Yamamoto[1], Eizen Kimura[2], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo, [2]Medical school of Ehime University

As the amount of biomedical and healthcare data increases, data mining for medicine becomes more and more important for health improvement. At the same time, 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. In this study, we first present two methods that compose the Randomized Anonymization method [1]. 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.

b. Differentially Private SNPs Ranking Publication of GWAS

i) Compressive Mechanism-based Method with Haar Wavelet Transform

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

To promote the use of personal genome information in medicine, it is important to analyze the relationship between diseases and the human genomes. Therefore, 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. In this study, for the first time, 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.

ii) Enhancement via a Joint Permutate-and-Flip

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

Owing to an increase in the amount of biomedical and healthcare data, privacy concerns regarding the use of genomic data have become well-recognized. Specifically, it is essential to develop personalized medicine to extract significant loci associated with diseases through large-scale genomic statistical analyses while protecting privacy. Although there are several differentially private methods for this purpose, they are too computationally complex to achieve high accuracy, and there is room for improvement in terms of the output error. In this study, we propose a novel mechanism, Joint Permute-and-Flip, that can provide higher-quality outputs than state-of-the-art techniques for top-K selection [8]. We also present an efficient algorithm that can perform Joint Permute-and-Flip in $O(m \log m)$ time when the dataset contains m elements, making it applicable even to large-scale analyses involving 106 elements. Additionally, we propose new score functions suitable for genomic statistical analysis that can be expressed as a single equation and achieve high accuracy. This is expected to facilitate the construction of accurate and efficient scores for a wider variety of genome statistics. Experimental results demonstrate that our Joint Permute-and-Flip method outperforms existing methods in terms of both accuracy and rank error and requires only half the run time of the exponential mechanism.

c. Differentially Private Publication of GWAS Statistics

i) Publication via Local Differential Privacy

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As the amount of personal genomic information and privacy concerns in data publication have been growing, several studies have pointed out that the presence information of a particular individual could be revealed from the statistics obtained in large-scale genomic analyses. Existing methods for releasing genome statistics under differential privacy do not prevent the leakage of personal information by untrusted data collectors. In addition, the existing studies for statistical tests using a contingency table had restrictions on the number of cases and controls. Moreover, the methods for correcting for population stratification cannot protect genotype information. Thus, developing a more general and stronger method is desired. In this study, we present privacy-preserving methods for releasing key genome statistics [6]. Our methods enhance the randomized response technique and guarantee individuals' privacy, even when untrusted data collectors exist. Moreover, our methods do not require any restrictions on the contingency tables, and they also guarantee the privacy of targeted genotype information for the analyses to correct for population stratification. The experimental results indicate that our methods can achieve comparable high accuracy to existing methods while preserving privacy more strictly from any data collectors. Furthermore, for statistical analysis using a contingency table, we consider the case where different privacy budgets are assigned to each of the row and column information, and present optimal methods in terms of privacy assurance for the entire table that outperform the existing method. Overall, this study is the first step toward genomic statistical analysis under local differential privacy.

ii) Publication using Smooth Sensitivity

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With the recent increase in the medical data and health awareness, the use of genomic data to promote personalized medicine has been widely considered. Simultaneously, privacy concerns have arisen with the publication of statistics obtained from large-scale genomic statistical analysis such as GWAS. All existing differentially private methods for GWAS statistics protect privacy by adding noise based on global sensitivity, considering the worst-case scenario of possi-

ble datasets. However, the amount of noise required in practical cases is considerably smaller, and these methods do not achieve the desired accuracy in private statistics. In this study, we propose a privacy-preserving method for publishing much more accurate statistics using smooth sensitivity, which generates tailored noise for each dataset [7]. We first introduce a more rigorous theorem on the properties of the noise distribution than was known previously and propose a new ϵ-differentially private method for publishing GWAS statistics. We also provide theoretical proof of the privacy guarantee. Thereafter, we present novel theorems for computing the smooth sensitivity significantly faster than conventional approaches. This enables the application of smooth sensitivity to GWAS statistics, which would otherwise be impossible because of the exceedingly high computational complexity. Based on these theorems, we performed detailed analyses of key GWAS statistics and developed efficient algorithms to obtain their smooth sensitivities. Experimental results demonstrate that our proposed methods achieve at least 3 times higher accuracy than existing global sensitivity-based methods. Furthermore, the execution time is sufficiently short, and the accuracy increases when the dataset becomes larger, suggesting that our methods are suitable for the publication of statistics in large-scale analysis. Because our method is expected to be applicable to other general statistics, this study is an important step toward highly accurate statistical analysis using smooth sensitivity.

d. Differential Private Publication of Graph Properties

i) Reducing Communication Cost for Publishing Differentially Private Subgraph Counting

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We suggest the use of hash functions to cut down the communication costs when counting subgraphs under edge local differential privacy [5]. While various algorithms exist for computing graph statistics, including the count of subgraphs, under the edge local differential privacy, many suffer with high communication costs, making them less efficient for large graphs. Though data compression is a typical approach in differential privacy, its application in local differential privacy requires a form of compression that every node can reproduce. In our study, we introduce linear congruence hashing. With a sampling rate of s, our method can cut communication costs by a factor of s^2, albeit at the cost of increasing variance in the published graph statistic by a factor of s. The experimental results indicate that, when matched for communication costs, our method achieves a reduction in the l_2-error for triangle counts by up to 1000 times compared to the performance of leading algorithms.

ii) 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 [3]. 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 and 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 those for eigenvector, Katz, and PageRank centralities, hold for planar or planar subcubic classes of 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 $P = NP$.

iii) Hardness of Counting Proper Connected Colorings

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

A k-proper connected 2-coloring for a graph is an edge bipartition which ensures the existence of at least k vertex disjoint simple alternating paths (i.e., paths where no two adjacent edges belong to the same partition) between all pairs of vertices. In this work, for every positive integer k, we show that exactly counting such colorings is #P-hard under many-one counting reductions, as well as #P-complete under many-one counting reductions when $k = 1$. Furthermore, for every positive integer k and every $\varepsilon > 0$, we show that the worst case asymptotic running

time for any algorithm approximating the number of k-proper connected 2-colorings for an order n graph within a multiplicative factor of $1 + \varepsilon$ must be at least the worst case asymptotic running time of an algorithm approximating an n-variable instance of #3-SAT within the same multiplicative factor. Here, assuming the Exponential Time Hypothesis (ETH), for every positive integer k and every $\varepsilon > 0$, we are able to rule out the existence of a $2^{o(nk)}/\varepsilon^2$ algorithm for approximating the number of k-proper connected 2-colorings of an order n graph within a factor of $1 + \varepsilon$. In addition, for every positive integer k, we rule out the existence of a $2^{o(nk)}$ time algorithm for finding a k-proper connected 2-coloring of an order n graph under the ETH, or for exactly counting such colorings assuming the moderated Counting Exponential Time Hypothesis (#ETH) [9].

2. Development of Artificial Intelligence Technologies for Biomedical Research

a. Feature Selection for Cancer Classification

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Microarray data have been widely utilized for cancer classification. The main characteristic of microarray data is "large p and small n" in that data contain a small number of subjects but a large number of genes. It may affect the validity of the classification. Thus, there is a pressing demand of techniques able to select genes relevant to cancer classification. This study proposed a novel feature (gene) selection method, Iso-GA, for cancer classification [10]. Iso-GA hybrids the manifold learning algorithm, Isomap, in the genetic algorithm (GA) to account for the latent nonlinear structure of the gene expression in the microarray data. The Davies–Bouldin index is adopted to evaluate the candidate solutions in Isomap and to avoid the classifier dependency problem. Additionally, a probability-based framework is introduced to reduce the possibility of genes being randomly selected by GA. The performance of Iso-GA was evaluated on eight benchmark microarray datasets of cancers. Iso-GA outperformed other benchmarking gene selection methods, leading to good classification accuracy with fewer critical genes selected. The proposed Iso-GA method can effectively select fewer but critical genes from microarray data to achieve competitive classification performance.

b. KEGG for Taxonomy-based Analysis of Pathways and Genomes

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KEGG is a manually curated database resource integrating various biological objects categorized into systems, genomic, chemical and health information. Each object (database entry) is identified by the KEGG identifier (kid), which generally takes the form of a prefix followed by a five-digit number, and can be retrieved by appending /entry/kid in the URL. The KEGG pathway map viewer, the Brite hierarchy viewer and the newly released KEGG genome browser can be launched by appending /pathway/kid, /brite/kid and /genome/kid, respectively, in the URL. Together with an improved annotation procedure for KO (KEGG Orthology) assignment, an increasing number of eukaryotic genomes have been included in KEGG for better representation of organisms in the taxonomic tree. Multiple taxonomy files are generated for classification of KEGG organisms and viruses, and the Brite hierarchy viewer is used for taxonomy mapping, a variant of Brite mapping in the new KEGG Mapper suite. The taxonomy mapping enables analysis of, for example, how functional links of genes in the pathway and physical links of genes on the chromosome are conserved among organism groups.

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Human Genome Center

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

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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, Sato N, Shimizu E, Kasajima R, Yamaguchi K, Yokoyama K, Yadome M, Hyugaji T, Komura M, Yamamoto M, Saito A, Zhang Y-Z, 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, Miyano S, Yamaguchi R, Uematsu S, Kumasaka N, Takahashi S, Nanya Y, Furukawa Y, 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

Katayama K, Shibuya T, Yamaguchi R, Kumasaka N, Matsuda K, Miyo K[1], Okamura H[2], Ota K[2], Shintani A[2], Shiraishi Y[3], Kohno T[3], Kato M[3], Okada Y[4], Fujimoto A[4], Kasai S[5], Imoto S: [1]National Center for Global Health and Medicine, [2]Osaka Metropolitan University, [3]National Cancer Center, Japan, [4]University of Tokyo School of Medicine, Japan, [5]Information-Technology Promotion Agency, Japan

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

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

2. Metagenome Analysis of Intestinal Microbiota

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

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

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 severe 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

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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 sam-

pling, 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

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. We identified metabolites that may accumulate in the intestine as a result of changes in intestinal bacteria. It has been suggested that this metabolite may produce substances that induce inflammation and contribute to the induction of enteritis. 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 *Enterococcus faecalis* that increase in the gut after bone marrow transplantation. Interestingly, *E. faecalis* isolated from the patients are cytolysin positive virulent strain and may increase the risk of GVHD. Functional analysis of *E. faecalis* 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 10 Crohn's disease patients and 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*. Although vaccination is recommended for protection against invasive pneumococcal disease, the frequency of pneumococcal pneumonia is still high worldwide. In fact, no vaccines are effective for all pneumococcal serotypes. Fusion pneumococcal surface protein A (PspA) has been shown to induce a broad range of cross-reactivity with clinical isolates and afford cross-protection against pneumococcal challenge in mice. Furthermore, we developed prime-boost-type mucosal vaccines that induce both antigen-specific IgG in serum and antigen-specific IgA in targeted mucosal organs in previous studies. We investigated whether our prime-boost-type immunization with a fusion PspA was effective against pneumococcal infection in mice and cynomolgus macaques. C57BL/6 mice were intramuscularly injected with fusion PspA combined with CpG oligodeoxynucleotides and/or curdlan. Six weeks later, PspA was administered intranasally. Blood and bronchoalveolar lavage fluid were collected and antigen-specific IgG and IgA titers were measured. Some mice were given intranasal Streptococcus pneumoniae and the severity of infection was analyzed. Macaques were intramuscularly injected with fusion PspA combined with CpG oligodeoxynucleotides and/or curdlan at week 0 and week 4. Then, 13 or 41 weeks later, PspA was administered intratracheally. Blood and bronchoalveolar lavage fluid were collected and antigen-specific IgG and IgA titers were measured. Some macaques were intranasally administered S. pneumoniae and analyzed for the severity of pneumonia. Serum samples from mice and macaques injected with antigens in combination with CpG oligodeoxynucleotides and/or curdlan contained antigen-specific IgG. Bronchial samples contained antigen-specific IgA after the fusion PspA boosting. This immunization regimen effectively prevented S. pneumoniae infection. Prime-boost-type immunization with a fusion PspA prevented S. pneumoniae infection in mice and macaques.

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Human Genome Center

Division of Digital Genomics
デジタル・ゲノミクス分野

Professor Natsuhiko Kumasaka, Ph.D.

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A genome-wide association study (GWAS) is a powerful approach for identifying genetic variants and related genes involved in the molecular mechanisms of common complex traits, such as diabetes and human height. As of April 2023, the GWAS Catalogue reports 496,341 genetic associations discovered for 56,399 common complex traits. The Division of Digital Genomics aims to identify these genetic associations of common complex traits and uncover their molecular mechanisms using cutting-edge molecular biology assays and integrated mathematical and statistical approaches.

1. Discovery of genetic determinants for child health and development

Natsuhiko Kumasaka

The Japan Environment and Children's Study (JECS) is a nationwide birth cohort study launched in 2011. Its primary objective is to investigate the impact of environmental exposures during pregnancy and childhood on children's health and development. JECS collected a vast amount of data on parents and their children, including information on reproduction and pregnancy complications, congenital anomalies, neuropsychiatric disorders, immune system disorders, and metabolic and endocrine system disorders.

Genome-wide genotyping using SNP microarrays was recently performed to identify common genetic variants in the children of 100,000 participants. The purpose of this genetic screening is to assess which genetic variants affect child health and development outcomes observed in JECS as confounding factors. Furthermore, our aim is to improve child health by mapping gene-environment interactions, particularly for those who belong to a genetically vulnerable group exposed to specific environmental hazards.

My responsibilities involve maintaining whole-genome genotyping data and conducting genome-wide association studies on various child health and developmental outcomes. As of December 2023, a whole-genome imputation has been performed using a reference haplotype panel from the 1000 Genomes Project. I have conducted Genome-Wide Association Studies (GWAS) for various disease states, including Kawasaki disease, food allergies, and congenital anomalies, as well as developmental measures such as BMI and gross motor scores. The aim is to create a comprehensive list of genetic determinants for child health and development. The summary statistics from those GWAS will be publicly available as part of the flagship paper entitled 'Genome-wide association study on longitudinal and cross-sectional traits of child health and development in a Japanese population', which will be prepared in 2024.

2. Development of a novel in-vitro approach to validate and elucidate underlying molecular mechanisms of genetic associations discovered through GWAS

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Although Genome-wide association studies (GWAS) have identified hundreds of thousands of genetic associations in common complex traits, more than 90% of genetic variants discovered by GWAS (referred to as GWAS variants) are located in non-coding regions. This poses a significant challenge in our efforts to identify putative causal variants and functional genes involved in a regulatory cascade of disease onset and progression. In addition, the target cell type(s) and cellular states in which these GWAS variants affect gene expression often remain unknown, limiting our ability to identify molecular mechanisms of GWAS susceptibility loci in follow-up studies.

Expression quantitative trait locus (eQTL) mapping is a powerful approach to gain insight into the role of non-coding variants in gene regulation. It allows us to discover genes that are regulated by these GWAS variants and helps elucidate downstream consequences. In addition, the recent advances in single-cell genomics allow us to identify target cell types and cellular states in which GWAS variants modulate gene expression through eQTLs. However, the study of eQTLs can often prove to be cost-ineffective and labor-intensive, especially when sample collection is challenging, such as in the case of in vivo brain samples collected from hundreds of patients undergoing neurosurgery, or in the case of differentiated neurons derived from hundreds of human pluripotent stem cell (hPSC) lines.

Recently, an alternative approach combining single-cell RNA-seq (scRNA-seq) with massively parallel CRISPR interference (CRISPRi) screening has been proposed to map eQTLs. This approach relies on CRISPR-mediated perturbations instead of natural genetic variation and is theoretically feasible from a single donor's sample. However, even though this approach significantly reduces the cell culture and experimental burden, it has not yet been applied to any brain cell type implicated in neuropsychiatric and neurodegenerative diseases.

Indeed, a combination of a flexible in vitro system and a robust in silico approach is lacking. Although the use of hPSC is a valuable tool to generate different types of mature cells, it is not trivial to maintain a sufficient gRNA repertoire through cell expansion and differentiation processes due to the selection pressure of specific CRISPR-mediated perturbations (*i.e.*, only cells with specific gRNA expand faster and take over other cells). From a data analysis perspective, the identification of dynamic genetic effects that manifest only during specific phases of cellular transition has historically been challenging, due to the absence of robust machine learning approaches until very recently.

We combine the unique expertise of machine learning/bioinformatics and stem cell biology/gene editing to develop a novel CRISPR perturbation system based on midbrain organoids established from hPSC lines, coupled with a state-of-the-art machine learning technique using Gaussian processes. We have already established a comprehensive computational approach, GASPACHO (GAuSsian Processes for Association mapping leveraging Cell HeterOgeneity), for mapping eQTLs along dynamic cellular states, which can be readily applicable to CRISPR-mediated eQTL mapping as if presence/absence of a gRNA in a cell were different genotypes at a natural genetic variant. We also have established several hPSC lines for CRISPR screening with inducible expression of dCas9-KRAB or dCas9-P300 by doxycycline (Dox), which ensures a more stable cell culture and allows to introduce perturbations at any desired time point of cell differentiation. In addition, we have extensive experience in two-dimensional neuronal differentiation and development of brain organoids.

3. Development of novel statistical approaches to map genetic associations using Gaussian Processes

Natsuhiko Kumasaka

Gaussian process (GP) is a powerful approach for modelling nonlinear phenomena in scientific fields such as genomics and genetics. This project focuses on using GPs for genetic association mapping. The goal is to identify genetic variants that affect gene regulation across continuous cellular states at the molecular level, as well as disease susceptibility over time and space at the population level. I am currently developing robust and sensitive methods to map a dynamic genetic effect based on a continuous factor. These methods can be applied to various outcomes, including non-Gaussian outputs, with appropriate statistical calibration.

Publications

Ishitsuka K, Yamamoto-Hanada K, Mezawa H, Yang L, Saito-Abe M, Nishizato M, Sato M, Miyaji Y, Kumasaka N, Ohya Y; Japan Environment, Children's Study Group: Teenage and young adult pregnancy and depression: findings from the Japan environment and children's study. *Arch Womens Ment Health*. 2023 Nov 22. doi: 10.1007/s00737-023-01400-6. Epub ahead of print. PMID: 37989798.

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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.

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

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

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

2. Microbiome ssRNA as an environmental cue to activate TLR13-dependent tissue-protective programs in CD5L^{hi} hepatic macrophages

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Hepatic macrophages maintain liver homeostasis, but little is known about the signals that activate the hepatoprotective programs within macrophages. Here, we show that toll-like receptor 13 (TLR13), a sensor of bacterial 23S ribosomal RNA (rRNA), senses microbiome RNAs to drive tissue-protective responses in CD5L^{hi} hepatic macrophages. Splenomegaly and hepatomegaly developed in the absence of the endosomal RNase, RNaseT2, via TLR13-dependent macrophage proliferation. Furthermore, TLR13 in hepatic Ly6C^{lo} macrophages activated the transcription factors LXRα and MafB, leading to expression of tissue-clearance molecules, such as CD5L, C1qb, and Axl. Consequently, *Rnaset2*$^{-/-}$ mice developed resistance to acute liver injury caused by challenges with acetaminophen and lipopolysaccharide + D-galactosamine. TLR13 responses in *Rnaset2*$^{-/-}$ mice were impaired by antibiotics, suggesting that TLR13 were activated by microbiome rRNAs, which was detected in the sera and hepatic macrophages. Repeated administration of wild-type mice with the TLR13 ligand, rather than other TLR ligands, selectively increased the number of Kupffer cells, which expressed immunoregulatory and tissue-clearance genes as hepatic macrophages in *Rnaset2*$^{-/-}$ mice did. Our results suggest that microbiome ssRNA serves as an environmental cue for initiating tissue-protective TLR13 responses in hepatic macrophages.

3. Aberrant monocytopoiesis drives granuloma development in sarcoidosis

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In sarcoidosis, granulomas develop in multiple organs including the liver and lungs. Although mTORC1 activation in macrophages drives granuloma development in sarcoidosis by enhancing macrophage proliferation, little is known about the macrophage subsets that proliferate and mature into granuloma macrophages. Here, we show that aberrantly increased monocytopoiesis gives rise to granulomas in a sarcoidosis model, in which Tsc2, a negative regulator of mTORC1, is conditionally deleted in CSF1R-expressing macrophages (Tsc2csf1rΔ mice). In Tsc2csf1rΔ mice, common myeloid progenitors (CMPs), granulocyte-monocyte progenitors (GMPs), common monocyte progenitors/monocyte progenitors (cMoPs/MPs), inducible monocyte progenitors (iMoPs), and Ly6Cint CX3CR1low CD14- immature monocytes (iMOs), but not monocyte-dendritic cell progenitors (MDPs) and common dendritic cell progenitors (CDPs), accumulated and proliferated in the spleen. Consistent with this, monocytes, neutrophils, and neutrophil-like monocytes increased in the spleens of Tsc2csf1rΔ mice, whereas dendritic cells did not. The adoptive transfer of splenic iMOs into wild-type mice gave rise to granulomas in the liver and lungs. In these target organs, iMOs matured into Ly6Chi classi-

cal monocytes/macrophages (cMOs). Giant macrophages (gMAs) also accumulated in the liver and lungs, which were similar to granuloma macrophages in expression of cell surface markers such as MerTK and SLAMF7. Furthermore, the gMA-specific genes were expressed in human macrophages from sarcoidosis skin lesions. These results suggest that mTORC1 drives granuloma development by promoting the proliferation of monocyte/neutrophil progenitors and iMOs predominantly in the spleen, and that proliferating iMOs mature into cMOs and then gMAs to give rise to granuloma after migration into the liver and lungs in sarcoidosis.

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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. Age-related decline in spermatogenic activity accompanied with endothelial cell senescence in male mice.

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Male fertility decreases with aging, with spermatogenic decline being one of its causes. Altered testis environment is suggested as a cause of the phenotype; however, the associated mechanisms remain unclear. Herein, we investigated the age-related changes in testicular somatic cells on spermatogenic activity. The number and proliferation of spermatogonia significantly reduced with aging in mice. Interestingly, senescence-associated β-galactosidase-positive cells appeared in testicular endothelial cell (EC) populations, but not in germ cell populations, with aging. Transcriptome analysis of ECs indicated that senescence occurred in the ECs of aged mice. Furthermore, the support capacity of ECs for spermatogonial proliferation significantly decreased with aging; however, the senolytic-induced removal of senescent cells from aged ECs restored their supporting capacity to a comparable level as that of young ECs. Our results suggest that the accumulation of senescent ECs in the testis is a potential factor contributing to the age-related decline in spermatogenic activity.

2. Ptbp1 deletion does not induce astrocyte-to-neuron conversion.

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Although in vivo reprogramming of astrocytes into neurons holds promise for treating neurodegen-

erative disorders, protocols to achieve it are complex and inefficient. A recent study reported that knockdown of *Ptbp1* in the cortex, striatum and substantia nigra of the midbrain efficiently reprogrammed astrocytes into functional neurons that rescued motor defects in a mouse model of Parkinson's disease1, but it did not convincingly demonstrate that astrocyte-to-neuron conversion had actually occurred. Using genetic disruption of *Ptbp1* in combination with cell-lineage analysis, we observe no astrocyte-to-neuron conversion or substantial changes in gene expression in *Ptbp1*-deficient astrocytes. We conclude that the results of astrocyte-to-neuron conversion in the previous study1 most likely reflect leaky neuronal expression of the $Gfap^{cre}$ mouse lines used to label astrocytes, and more-rigorous experimental methods must be used to support their observations of glial reprogramming in vivo.

3. The histone H3K36 demethylase Fbxl11 plays pivotal roles in the development of retinal late-born cell types.

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Histone methylation plays a vital role in retinal development. However, the role of histone H3K36 methylation in retinal development is not clear. We examined the role of H3K36 methylation by loss-of-function analysis of H3K36me1/2 demethylases, Fbxl10, and Fbxl11. We analyzed the effect of knockout of these genes in the developing and mature retina on retinal development. Knockout of Fbxl10 specifically in the developing retina did not result in gross developmental abnormalities. Although adult rod photoreceptor-specific knockout of Fbxl11 in mature retinas did not result in morphological abnormalities, Fbxl11 knockout in developing retinas increased apoptosis, suppressed the proliferation of retinal progenitor cells, and resulted in microphthalmia. Morphological analysis revealed perturbed differentiation of rod photoreceptor and bipolar cells. RNA-seq of retinas at P7 showed markedly decreased expression of genes characterizing rod photoreceptor and bipolar cells in Fbxl11-knockout retinas. In addition, perturbation of alternative splicing increased intron retention in Fbxl11-knockout retinas. Genome-wide evaluation of the H3K36 methylation status revealed that Fbxl11 knockout altered the distribution of H3K36me2/3 in genes important for rod photoreceptor development. Taken together, we show that Fbxl11 plays pivotal roles in the development of retinal late-born cell types and may contribute to tight control of H3K36 methylation during retinal development.

4. CCDC183 is essential for cytoplasmic invagination around the flagellum during spermiogenesis and male fertility.

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Sperm flagellum plays a crucial role in male fertility. Here, we generated Ccdc183 knockout mice using the CRISPR/Cas9 system to reveal the protein function of the testis-specific protein CCDC183 in spermiogenesis. We demonstrated that the absence of CCDC183 causes male infertility with morphological and motility defects in spermatozoa. Owing to the lack of CCDC183, centrioles after elongation of axonemal microtubules do not connect the cell surface and nucleus during spermiogenesis, which causes subsequent loss of cytoplasmic invagination around the flagellum. As a result, the flagellar compartment does not form properly and cytosol-exposed axonemal microtubules collapse during spermiogenesis. In addition, ectopic localization of accessory structures, such as the fibrous sheath and outer dense fibers, and abnormal head shape as a result of abnormal sculpting by the manchette are observed in Ccdc183 knockout spermatids. Our results indicate that CCDC183 plays an essential role in cytoplasmic invagination around the flagellum to form functional spermatozoa during spermiogenesis.

5. A small secreted protein NICOL regulates lumicrine-mediated sperm maturation and male fertility.

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The mammalian spermatozoa produced in the testis require functional maturation in the epididymis for their full competence. Epididymal sperm maturation is regulated by lumicrine signalling pathways in which testis-derived secreted signals relocate to the

epididymis lumen and promote functional differentiation. However, the detailed mechanisms of lumicrine regulation are unclear. Herein, we demonstrate that a small secreted protein, NELL2-interacting cofactor for lumicrine signalling (NICOL), plays a crucial role in lumicrine signalling in mice. NICOL is expressed in male reproductive organs, including the testis, and forms a complex with the testis-secreted protein NELL2, which is transported transluminally from the testis to the epididymis. Males lacking Nicol are sterile due to impaired NELL2-mediated lumicrine signalling, leading to defective epididymal differentiation and deficient sperm maturation but can be restored by NICOL expression in testicular germ cells. Our results demonstrate how lumicrine signalling regulates epididymal function for successful sperm maturation and male fertility.

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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 been widely used in life sciences and medicine. We have developed a novel genome editing tool, CRISPR-Cas3, to overcome the technical and patent limitations of the CRISPR-Cas9 system. We are analyzing the molecular mechanisms underlying Cas3-mediated genome editing in human cells and improving this tool for translational research, such as gene therapy and viral diagnostics. We are also developing some efficient genome editing strategies using these tools in rodents. These technologies enable easy and flexible gene editing in living organisms.

Type I-E CRISPR-Cas3 for large-scale genomic modifications in mice and rats

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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, Cascade, 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 even by using electroporation methods. This work with the Type I CRISPR zygote editing system represents a significant leap forward, offering increased flexibility and broader applications in genetic engineering across multiple species.

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 A (IVA) virus 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 minutes), low-cost, instrument-free detec-

tion method for SARS-CoV-2. This assay, which we have named Cas3-operated nucleic acid detection (CONAN), not only detects SARS-CoV-2 in clinical samples, but also provides specific detection of single base pair mutations in IVA variants. We are also optimizing protocols for cancer detection by liquid biopsy and genetic screening for inherited diseases such as trinucleotide repeat disorders.

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Center for Experimental Medicine and Systems Biology

Division of Cell Regulation
細胞制御研究分野

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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. Understanding genetic heterogeneity in gene-edited hematopoietic stem cell products

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CRISPR/Cas gene editing has transformed genetic research and is poised to drive the next generation of gene therapies targeting hematopoietic stem cells (HSCs). However, the installation of the "desired" edit is most often only achieved in a minor subset of alleles. The array of cellular pathways triggered by gene editing tools produces a broad spectrum of "undesired" editing outcomes, including short insertions and deletions (indels) and chromosome rearrangements, leading to considerable genetic heterogeneity in gene-edited HSC populations. This heterogeneity may undermine the effect of the genetic intervention since only a subset of cells will carry the intended modification. Also, undesired mutations represent a potential safety concern as gene editing advances toward broader clinical use. Here, we will review the different sources of "undesired" edits and will discuss strategies for their mitigation and control.

2. Purging myeloma cell contaminants and simultaneous expansion of peripheral blood mobilised stem cells.

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Human hematopoietic stem cells (HSCs) are widely used as a cellular source for hematopoietic stem cell transplantation (HSCT) in the clinical treatment of hematological malignancies. After transplantation therapy, delays in hematopoietic recovery due to insufficient donor-derived HSCs can lead to increased risks of life-threatening infections and bleeding. Our previous studies developed an efficient ex vivo expansion culture medium (3a medium) for umbilical cord blood-derived HSCs (CBSCs), offering a potential solution to this problem. Nevertheless, the broader applicability of our culture method to alternative cell sources, and, of greater significance, its efficacy in eliminating potentially disease-associated contaminated tumor cells, especially in autologous transplantation, raises critical clinical questions. In this study, we modified the 3a medium by incorporating UM729 to replace UM171, added Flt3 ligand, and adjusted the concentrations of butyzamide, 740Y-P, polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (PCL-PVAc-PEG, Soluplus®) to create the modified 3a medium. This sophistication allowed efficient expansion of not only CBSCs but also peripheral blood mobilized HSCs (PBSCs). Additionally, we successfully removed contaminated myeloma cells by adding bortezomib and TNF-related apoptosis inducing ligand (TRAIL) at appropriate concentrations, while maintaining HSCs through the addition of lenalidomide. Our research findings present the potential for widespread clinical application of the modified 3a medium and suggest a safe ex vivo culture technique for expanding human HSCs within peripheral blood derived donor grafts used for autologous HSCT.

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Center for Experimental Medicine and Systems Biology

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

Professor	Tomoji Mashimo, Ph.D.		教　授	博士(人間・環境学)	真　下　知　士
Visiting Professor	Kimi Araki, Ph.D.		客員教授	博士(理学)	荒　木　喜　美
Associate Professor	Manabu Ozawa, Ph.D.		准教授	博士(農学)	小　沢　　学
Project Assistant Professor	Jumpei Taguchi, Ph.D.		特任助教	博士(医科学)	田　口　純　平

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 mouse or rat models to domestic or international academic institutions. One division, the Division of Genome Engineering, and two laboratories, the Laboratory of Reproductive Systems Biology and the Laboratory of Genetically Engineered Mouse Research, 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 aca-

demic 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 2023

In 2023, our core provided 13 or 11 strains of gene-manipulated mice through the core lab or AdAMS, respectively. In the rat case, 4 strains of gene-manipulated rats have also been provided through AdAMS.

Advanced Clinical Research Center

Division of Infectious Diseases
感染症分野

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Project Senior Assistant Professor	Michiko Koga, M.D., D.M.Sc.	特任講師	博士(医学)	古　賀　道　子
Assistant Professor	Makoto Saito, M.D., D.Phil.	助　教	博士(医学)	齋　藤　　真
Assistant Professor	Aya Ishizaka, Ph.D.	助　教	博士(理学)	石　坂　　彩
Assistant Professor	Yoshiaki Kanno, M.D., D.M.Sc.	助　教	博士(医学)	菅　野　芳　明

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 the 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, to settle down COVID-19.

1. Clinical research of COVID-19

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

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Since the emergence and spread of SARS-CoV-2 in Japan at the beginning of 2020, numerous COVID-19 patients have been admitted to IMSUT Hospital. To date, over 800 patients with COVID-19 have been admitted to our hospital. Through the observation of these patients, we have encountered numerous clinical questions and conducted investigations into various factors, including patients' backgrounds, clinical findings, and laboratory data. Our research has yielded several novel and compelling findings, which we have published in international journals. Moreover, five clinical trials on COVID-19 were conducted at IMSUT Hospital, generating valuable evidence aimed at enhancing both national and global health. In 2023, in Japan, patients with COVID-19 were no longer legally required to be isolated, resulting in a huge reduction in the number of hospitalized patients at our facility. However, towards the end of the year, the number of patients began to increase once more, prompting the need for us to prepare for a potential resurgence or the emergence of a new pandemic.

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 to enhance a better understanding of the protective 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 ran an analysis of changes in the nasal microbiome after COVID-19 vaccination.

3. Analysis of genetic sequence of hepatitis viruses.

Ayako Sedohara, Kazuaki Takahashi, Yoshiaki Kanno, Makoto Saito, Kazuhiko Ikeuchi[1], Eisuke Adachi[1], Michiko Koga, Takeya Tsutsumi, Hiroshi Yotsuyanagi

Of the hepatitis viruses, HAV and HEV are transmitted orally, HAV mainly from marine products and HEV from meat of wild animals such as deer and pork liver. Both HAV and HEV are found in large quantities in stools, but there have been few cases of human-to-human transmission of HEV in Japan. On the other hand, HAV can be sexually transmitted, with epidemics occurring through fecal-oral transmission. Isolation of viral RNA from patient blood and stool, quantification of copy number, and sequencing to determine genotype and mutation are extremely important to understand the characteristics of epidemic strains, such as virulence and drug resistance, and to use them for treatment. In this laboratory, analyses are being conducted using specimens from HAV or HEV-infected patients collected from various regions. At the same time, we are verifying whether HAV or HEV infection is present in specimens of unidentified hepatitis in which infection with HBV or HCV, which also cause hepatitis, has been ruled out.

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

Michiko Koga, Makoto Saito, Megumi Kubota, Tomoe Senkoji, Eisuke Adachi[1], Kazuhiko Ikeuchi[1], Tadashi Kikuchi[1], Amato Otani[1], Kazuaki Takahashi, Takeya Tsutsumi, Hiroshi Yotsuyanagi

Hepatitis A (HA) is a vaccine-preventable disease. In regions with good sanitation, men who have sex with men (MSM) are the key affected populations. During the 2018–2019 HA outbreak among MSM in Japan, we actively vaccinated MSM living with human immunodeficiency virus (MSM-LWHIV) with Aimmugen®. As previously reported, their antibody seroconversion rate due to vaccination was lower than that of healthy individuals. We evaluated attenuation after the one-series vaccination (comprising three inoculations) and the factors associated with attenuation. We retrospectively examined anti-HA-IgG titers and other clinical data from our hospital's medical records. Fifty-one MSM-LWHIV were included. All were seropositive after the third dose with a median HA-IgG titer of 10.1 (interquartile range: 7.2–12.2) s/co. In 45 (40–49) months, seropositivity decreased to 90% (46/51) and was attenuated to a median of 4.4 (2.3–6.5) s/co. Lower baseline B cell counts (p=0.049), lower anti-HA-IgG levels after the second dose (p=0.002), and lower anti-HA-IgG levels after the third dose (p=0.003) were associated with seronegativity. Anti-HA-IgG titers of vaccinated MSM-LWHIV may be attenuated; thus, additional immunizations should be considered.

Now, we have started to evaluate NAFLD and MAFLD of LWHIV. Of the 102 LWHIV, 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 the prevalence of transmitted drug-resistant (TDR) HIV in Japan from 2003.

Drug-resistance tests had been performed on nationwide HIV-1-infected cases newly diagnosed. The overall prevalence of TDR was about 10.4% in 2022.

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

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

It is speculated that hemophilia who were infected with HIV through contaminated blood products produced in the US before 1986 are at a higher risk of developing malignancies because of aging and immune dysfunction. Since April 2021, we have initiated

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. Characterization of mutations in HBV isolated from HBsAg-positive blood donors in Japan.

Ayako Sedohara, Kazuaki Takahashi, Yoshiaki Kanno, Kazuhiko Ikeuchi[1], Eisuke Adachi[1], Takeya Tsutsumi, Hiroshi Yotsuyanagi

In Japan, routine HBV vaccination began in 2016 for children aged 0 years. The HBV vaccine targets the hydrophilic amino acids in the S-region of the HBV epitope. Mutations in the DNA sequence of this S-region can cause amino acid substitutions. The amino acid substitution causes a drop in the titer of antibodies to HBV produced by the vaccine, which is called a vaccine escape mutation. In Japan, the rate of infection with HBV is low and few people over the age of 8 have antibodies against HBV because few people have been vaccinated against it. However, if the number of people with antibodies against HBV increases in the future due to vaccination, the number of HBV with antibodies resistant to HBV may increase. It is very important to regularly monitor the prevalence of HBV in Japan. In this study, blood donations that tested positive for HBsAg are collected, HBV DNA is isolated, HBV copy number is examined, and genotypes and mutations are analyzed by whole genome sequence. As a result, we are detecting vaccine escape mutations and mutations that confer resistance to therapeutic drugs for hepatitis B. Drug resistance mutations are important for the treatment of acute and chronic hepatitis and should continue to be monitored closely. This study provides a baseline for future HBV epidemic strain trends.

8. Identification of HIV-associated neurocognitive disorder (HAND) biomarker from HIV-infected individuals presenting with neuropathy.

Ayako Sedohara, Kotaro Arizono, Hiroshi Yotsuyanagi

Antiviral drugs suppress the growth of HIV, allowing HIV-infected people to live the same life as healthy people. Because HIV is a retrovirus with reverse transcriptase, the HIV gene is incorporated into the host genome. Even though antiviral drugs can suppress viral production, the HIV provirus incorporated into the genome cannot be eliminated, and chronic inflammation is known to persist in HIV patients. Various complications are known to result from this chronic inflammation, among which HIV-associated neurocognitive disorder (HAND) significantly affects the quality of life of patients. On the other hand, HAND cannot be detected by common dementia tests, so a diagnosis requires a combination of several psychological tests. It is against this background that testing for HAND is not performed unless the physician determines that HAND is suspected or the patient does not want to be tested. A biomarker that would allow HAND to be diagnosed with a minimally invasive blood test is desired. In this study, we identified several candidate HAND biomarkers from plasma of HIV patients. In the future, we will develop a method to test them as HAND biomarkers in clinical tests.

9. 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 (H3K27me3) marks the gene silencing region and is mainly observed around the 5′LTR, the transcriptional regulatory region, in latently infected HIV-1 cells. EZH2 is involved in the maintenance of HIV-1 latency. Treatment of HIV-1 latently infected cells with valemetostat reversed latency *in vitro* and *ex vivo* in a dose- and duration-dependent manner at levels similar to SAHA. Furthermore, co-administration of SAHA with valemetostat showed an additive effect on latency reversal.

10. Analysis of the HIV-associated gut microbiome

Aya Ishizaka, Michiko Koga, Taketoshi Mizutani[2], 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). We are currently analyzing gut microbiota alterations longitudinally to further elucidate the causal relationship between HIV-specific gut microbiota dysbiosis and the incidence of age-related diseases in PLWH.

11. Clinical epidemiology of malaria in pregnancy

Makoto Saito

Malaria stands as the primary cause of mortality in tropical regions, with pregnant women being especially vulnerable. Malaria during pregnancy has detrimental effects on both the mother and fetus. As part of a global collaboration with colleagues in sub-Saharan Africa and Asia, we conducted a pooled meta-analysis of clinical data to evaluate the safety of antimalarials during pregnancy. This research supported the revision of malaria guidelines by the World Health Organization and by the US CDC. Currently, we are engaged in new projects focusing on the pharmacoepidemiology of antimalarials during pregnancy.

12. 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.

13. 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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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 six projects: 1) Identification of novel molecular targets for the treatment of colorectal cancer, 2) understanding the role of Wnt/β-catenin signaling pathway in human carcinogenesis, 3) discovery of Wnt inhibitors through a screening of large-scale chemical libraries, 4) establishment of intrahepatic cholangiocarcinoma mouse model by orthotopic transplantation of syngeneic tumor cells, 5) elucidation of the genetic features of rare cancers and the mechanisms of their development, and 6) clinical sequencing for the implementation of genomic medicine.

1. Identification of novel molecular targets for the treatment of colorectal cancer

Kiyoshi Yamaguchi, Yoichi Furukawa

Epigenetic modifications such as DNA methylation and histone modification change in gene expression with global dynamics of chromatin structure. Accumulated evidence has demonstrated that aberrant epigenetic modifications are involved in carcinogenesis. Bromodomain has been known as a protein-interaction module that recognizes acetylated lysine residues. Through this interaction, protein containing a bromodomain(s) directs the assembly of nuclear factor complexes to their target sites on chromatin, resulting in the transcriptional activation. Recently, we found that bromodomain containing 8 (BRD8) was frequently accumulated in colorectal cancer. Transcriptome analysis coupled with genome-wide mapping of BRD8-binding sites disclosed that BRD8 regulates the expression of multiple subunits of the pre-replicative complex in concert with the activator protein-1. Depletion of BRD8 induced cell-cycle arrest at the G1 phase and suppressed cell proliferation. We also showed that the bromodomain of BRD8 is indispensable for not only the interaction with histone H4 or transcriptional regulation but also its own protein stability. These findings highlight the importance of bromodomain as a therapeutic target.

2. 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 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 analysis using β-catenin-depleted colorectal cancer cells identified a total of 64 candidate genes whose expression was regulated by the Wnt/β-catenin signaling. One of

these genes was visinin-like 1 (*VSNL1*), which belongs to the neuronal calcium-sensor gene family. The expression of *VSNL1* was regulated by β-catenin/TCF7L2 via two Wnt-responsive elements in intron 1. Knockdown of VSNL1 induced apoptosis in colorectal cancer cells. In contrast, forced expression of wild-type VSNL1, but not a myristoylation, Ca^{2+}-binding, or dimerization-defective mutant, suppressed the apoptosis induced by camptothecin and doxorubicin. Our data suggest that VSNL1, a novel target gene of the Wnt/β-catenin signaling pathway, is associated with apoptosis resistance in colorectal cancer cells.

3. Discovery of Wnt inhibitors through a screening of large-scale chemical libraries

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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. Using this assay, we established a high-throughput screening system, and performed a screening of small molecule and natural product libraries. As a result, several compounds that inhibit Wnt/β-catenin signaling activity were identified, and their target(s) are currently under investigation. To elucidate the detailed mode of action of these hit compounds, we have adopted an approach based on the estimation of gene network from gene expression data.

4. Establishment of intrahepatic cholangiocarcinoma mouse model by orthotopic transplantation of syngeneic tumor cells

Kiyoko Takane, Yoichi Furukawa

Genetically engineered mice are useful tools for studying human diseases including cancer. In this project, we previously generated a novel mouse model of intrahepatic cholangiocarcinoma (ICC) using liver-specific expression of oncogenic *Kras* and homozygous *Pten* deletion (AKPP: *Alb-Cre*$^{+}$; *LSL-Kras*$^{G12D/+}$; *Pten*$^{flox/flox}$). Subsequently, a cell line was established from the ICC of AKPP mice and named AKPP cells. First, we confirmed that conventional ICC markers such as cytokeratin-19 and pan-cytokeratin were abundantly expressed in AKPP cells. In contrast, the cells expressed low levels of hepatocyte nuclear factor 4 alpha (HNF4α), a marker of hepatocellular carcinoma. To investigate the downstream effects expected from the loss of Pten and activation of Kras, the phosphorylation levels of Akt (Ser473) and Erk1/2 were analyzed by immunoblotting. As a result, increased phosphorylation of these effectors was observed in AKPP cells, compared to mouse fibroblast L-cells. We are currently investigating the tumorigenic potential of AKPP cells using syngeneic immunocompetent mice.

5. Elucidation of the genetic features of rare cancers and the mechanisms of their development

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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 may confer malignant properties to PMP.

We further performed genome-wide DNA methylome analysis of 15 appendiceal PMP samples using Infinium 850K BeadChip. As a result, the 15 PMPs were classified into at least two epigenotypes, unique methylation epigenotype and normal-like methylation epigenotype. In addition, we have found that *HOXD1* and *TSPYL5* showed high methylation and low expression in all PMP samples tested. We are currently investigating the molecular mechanisms of PMP involved in the low expression of *HOXD1* and *TSPYL5*. These data may contribute to the better understanding of molecular mechanisms underlying PMP, and help in the diagnosis, treatment, and prevention of this life-threatening disease.

6. Clinical sequencing for the implementation of genomic medicine

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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). Long-read sequencing has technical advantages over short-read sequencing for the detection of structural variants. Therefore, we tested the ability of nanopore sequencing and adaptive sampling for the enrichment of 91 genes involved in hereditary cancer predisposition using DNA from a patient with LS carrying a characterized SV in our previous study. Application of the adaptive sampling using peripheral blood DNA resulted in approximately six times higher target composition than the nonadaptive sampling. Consequently, we successfully identified the breakpoints of a pathogenic SV that was difficult to identify by short-read sequencing technology.

In the second project, we have been working on the implementation of genomic data in clinics. We offered consultation of genetic analysis to patients with rare or intractable cancer as an outpatient clinic service in IMSUT hospital. Patients with colorectal, esophageal, cervical, pancreatic, and breast cancer 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. The Tumor Board is composed of members from a variety of specialties, including physicians, medical oncologists, genetic counsellors, geneticists, bioinformaticians, and experts of ethics, and meets online every two weeks.

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先端がん治療分野

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Project Professor	Minoru Tanaka, M.D., Ph.D.	特任教授	博士(医学)	田　中　　実
Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	博士(医学)	伊　藤　博　崇
Assistant Professor	Yoshinori Sakata, M.D., Ph.D.	助　教	博士(医学)	坂　田　義　詞
Assistant Professor	Yuta Takeshima, M.D., Ph.D.	助　教	博士(医学)	竹　島　雄　太
Assistant Professor	Seisaku Kanayama, M.D.	助　教		金　山　政　作

Our Laboratory is focused on developing oncolytic virus therapies for various malignant tumors. Oncolytic viruses are engineered to kill tumor cells without affecting normal tissues. 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 serum 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, a triple-mutated oncolytic HSV-1 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 studies, clinical lot manufacturing and clinical trials. G47Δ was approved as the world's first oncolytic virus product for malignant glioma 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 malignant mesothelioma, olfactory neuroblastoma and prostate cancer, and that of human IL-12-expressing G47Δ (T-hIL12) for malignant melanoma have been steadily proceeding.

Publications

1. 伊藤博崇、藤堂具紀：第2章がん免疫療法 7. ウイルス療法。In 先進医療フォーラム（編）：先進医療NAVIGATORがん免疫療法最前線。東京、日本医学出版、2023, pp.59-61.
2. 田中実、藤堂具紀：Ⅳ章 遺伝子治療 8. がんのウイルス療法。日本医師会雑誌（特別号(1) 遺伝を考える）152: 283-286, 2023.
3. 田中実、藤堂具紀：III.各種疾患 3.脳腫瘍 2)悪性神経膠腫に対するウイルス療法。In 鈴木則宏、荒木信夫、宇川義一、桑原聡、塩川芳昭（編）：Annual Review 神経2023。東京、中外医学社、2023, pp.180-187.
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5. Nomura T, Endo S, Kuwano T, Fukasawa K, Takashima S, Todo T, Furuta K, Yamamoto T, Hinoi E, Koyama H, Honda R: ARL-17477 is a dual inhibitor of NOS1 and the autophagic-lysosomal system that prevents tumor growth in vitro and in vivo. Sci Rep 13(1):10757, 2023.
6. Fukasawa K, Lyu J, Kubo T, Tanaka Y, Suzuki A, Horie T, Tomizawa A, Osumi R, Iwahashi S, Tokumura K, Murata M, Kobayashi M, Todo T, Hirao A, Hinoi E: MEK5-ERK5 axis promotes self-renewal and tumorigenicity of glioma stem cells. Cancer Res Commun 3(1):148-159, 2023.
7. Fukuhara H, Sato YT, Hou J, Iwai M, Todo T: Fusion peptide is superior to co-expressing subunits for arming oncolytic herpes virus with interleukin 12. Commun Med 3: 40, 2023.
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Advanced Clinical Research Center

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

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

Our mission is to assist the development of translational research. For this purpose, it is critical to discover new "seeds" and to eradicate blockades until the clinical utilization. We also assist the conduct of clinical trials at IMSUT Hospital. At IMSUT Hospital, we work together with 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 2023, we supported 4 sponsor-investigator clinical trials.

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, 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, 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.

6. 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.

Publications

1. Motoki Amai, Masanori Nojima, Yoshikazu Yuki, Hiroshi Kiyono, Fumitaka Nagamura. A review of criteria strictness in "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Vaccine. 2023 Aug 31;41(38):5622-5629. doi: 10.1016/j.vaccine.2023.07.072. Epub 2023 Aug 1.
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6. Adachi Y, Nojima M, Mori M, Yamano HO, Sasaki Y, Nakase H, Lin Y, Wakai K, Tamakoshi A; for JACC study. Association of serum superoxide dismutase activity and the incidence of colorectal cancer in a nested case-control study. Cancer Epidemiol. 2023 Sep 23;87:102455. doi: 10.1016/j.canep.2023.102455. Epub ahead of print. PMID: 37748209.
7. Kazama T, Ando K, Ueno N, Fujiya M, Ito T, Maemoto A, Ishigami K, Nojima M, Nakase H. Long-term effectiveness and safety of infliximab-biosimilar: A multicenter Phoenix retrospective cohort study. PLoS One. 2023 Sep 12;18(9):e0288393. doi: 10.1371/journal.pone.0288393. PMID: 37699041; PMCID:
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In-Person Visits During Video-Based Telemedicine Among Patients With Hypertension: Cluster Randomized Controlled Trial. JMIR Cardio. 2023 Jun 8;7:e45230. doi: 10.2196/45230. PMID: 37161483; PMCID: PMC10288346.

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, Nobumi Suzuki[1], Yoku Hayakawa[1]. [1]Department of Gastroenterology, The University of Tokyo

Using several lines of gastric IL-33 overexpression mice (TFF1-cre-LSL-IL33, Mist1creERT-LSL-IL33, TFF1pro-IL33), we found IL33 is involved in the pathogenesis of gastritis, especially recruitment of specific immune cells into the stomach. Currently, we try to visualize in vivo immune cells using new EGFP transgenic line to elucidate the dynamics of gastritis after the IL33 stimuli.

2. Role of Sox9 in the gastric carcinogenesis

Hu Ke, Kazuya Koyanagi, 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. Now we investigate the characteristics of antral epithelium of Sox9 especially focused on mucin phenotype.

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

Ru Lin, 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.

4. Role of acetylcholine signaling in the inflammatory bowel diseases

Aya Yamashita[1], Yoshihiro Hirata, Sozaburo Ihara[1].

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.

5. Analysis of primary biliary cholangitis mouse model

Jiaqi Zhang[4], Ryo Nakagawa[4], Naoya Kato[4], Hayato Nakagawa[3], Yoshihiro Hirata[4], Department of Gastroenterology, Chiba University

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.

Publications

1. Arai J, Niikura R, Hayakawa Y, Hirata Y, Ushiku T, Fujishiro M. Autoimmune gastritis may be less susceptible to cancer development than *Helicobacter pylori*-related gastritis based on histological analysis. Gut. 2023 May 17:gutjnl-2023-330052. doi: 10.1136/gutjnl-2023-330052. Epub ahead of print. PMID: 37197906.
2. Kato H, Tateishi K, Iwadate D, Yamamoto K, Fujiwara H, Nakatsuka T, Kudo Y, Hayakawa Y, Ijichi H, Otsuka M, Kishikawa T, Takahashi R, Miyabayashi K, Nakai Y, Hirata Y, Toyoda A, Morishita S, Fujishiro M. HNF1B-driven three-dimensional chromatin structure for molecular classification in pancreatic cancers. Cancer Sci. 2023 Apr;114(4): 1672-1685. doi: 10.1111/cas.15690. Epub 2022 Dec 25. PMID: 36511816; PMCID: PMC10067390.

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. 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.

1. Survey on Public Awareness of Medical Research Terminology and the Accuracy of Physicians' Predictions regarding that Awareness in Japan

Ayako Kamisato, Hong Hyunsoo

One of the ethical principles of medical research involving human subjects is obtaining proper informed consent (IC). However, if the participants' actual awareness of medical research terminology is lower than the researchers' prediction of that awareness, it may cause difficulty obtaining proper IC. Therefore, this study aims to clarify the presence of "perception gaps" and then discuss IC-related issues and measures based on the insights obtained. We conducted two online surveys: a "public survey" to understand the Japanese public's awareness of 11 medical research terms and a "physicians' survey" to investigate physicians' predictions regarding public awareness. In the "public survey," for each term, respondents were instructed to select their situation from "understand," "have heard," or "have never heard." In the "physicians' survey," respondents were asked to estimate the proportions of the general public who would "have understood," "have heard," or "have never heard" by using an 11-step scale. We analyzed separately in two age groups to understand the age-related difference. We received 1002 valid responses for the "public survey" and 275 for the "physicians' survey." Of the public respondents, more than 80% had never heard of terms such as *interventional study*, *prospective clinical study*, *cohort study*, *Phase I clinical trial*, or *double-blind study*. Concurrently, physicians overestimated general public awareness of the terms *placebo*, *cohort study*, *double-blind study*, and *randomized clinical trial* (in the *group of people under 60*). The results revealed the perception gap between the general public and physicians which raises serious concerns about obtaining proper IC from clinical research participants.

2. Research on the Japan's clinical research review system

Ayako Kamisato

As of the end of 2023, clinical research is being conducted under four laws, orders based on them, and eight guidelines. All of these laws and guidelines have in common that they require researchers to create a research protocol and get it reviewed by a review body established at the research institution (hereinafter referred to as a local committee). However, on the other hand, there are many differences on the review system between laws and guidelines, such as whether additional review is required by a review body set up by the government or whether there is a

local committee certification system. Therefore, we examined the examination system of all laws and guidelines and considered the issues that emerged from this and the future system design.

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

Ayako Kamisato, Kazuyo Arisawa, Yoshiko Takahashi

"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. As part of our support, in 2023, we established an ethics review committee dedicated to this project. We began reviewing it to promote the use of health checkup data for research and development.

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

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo, Yoshiko Takahashi

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. 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.

In 2023, we revised the video programs following revisions of the "Ethical Guidelines for Medical and Biological Research Involving Human Subjects."

出版物

- 神里彩子「第8章　個人情報の取扱いに関する基礎知識」飯嶋久志、氏原淳、内田直樹、神里彩子、佐藤愛美『超簡単！！研究倫理審査と申請　第2版～適正な臨床・疫学研究の推進に向けて～ 』（薬事日報社、2023年）
- 神里彩子「第5章　臨床研究の審査体制－現状と今後の在り方」甲斐克則編『臨床研究と医事法（医事法講座 第13巻）』（信山社、2023年）
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- 神里彩子「生殖技術に関する倫理的・法的・社会的課題（ELSI）の全体」医事法研究第8号（甲斐克則責任編集、信山社、2023年12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. On October 1th, 2022, 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. We also perform laparoscopic surgery for inguinal hernias.

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

Refractory Intestinal Behçet-Like Disease Associated with Trisomy 8 Myelodysplastic Syndrome Resolved by Parenteral Nutrition.

Takahashi R, Matsubara Y, Takahashi S, Yokoyama K, Ahyoung LL, Koga M, Sakamoto H, Boku N, Shida D, Yotsuyanagi H.

Case Rep Gastroenterol. 2023 Oct 11;17(1):287-293.

Prognostic factors associated with the transition in treatment methods for brain metastases from colorectal cancer.

Imaizumi J, Shida D, Boku N, Igaki H, Itami J, Miyakita Y, Narita Y, Takashima A, Kanemitsu Y.

Int J Clin Oncol. 2023 Aug;28(8):1043-1053.

Genomic landscape and its prognostic significance in stage III colorectal cancer: JCOG1506A1, an ancil-

lary of JCOG0910.

Shida D, Kuchiba A, Shibata T, Hamaguchi T, Yamasaki S, Ito M, Kobatake T, Tonooka T, Masaki T, Shiozawa M, Takii Y, Uetake H, Okamura S, Ojima H, Kazama S, Takeyama H, Kanato K, Shimada Y, Murakami Y, Kanemitsu Y.

Cancer Sci. 2023 Aug;114(8):3352-3363.

Bromodomain protein BRD8 regulates cell cycle progression in colorectal cancer cells through a TIP60-independent regulation of the pre-RC complex.

Yamaguchi K, Nakagawa S, Saku A, Isobe Y, Yamaguchi R, Sheridan P, Takane K, Ikenoue T, Zhu C, Miura M, Okawara Y, Nagatoishi S, Kozuka-Hata H, Oyama M, Aikou S, Ahiko Y, Shida D, Tsumoto K, Miyano S, Imoto S, Furukawa Y.

iScience. 2023 Apr 1;26(4):106563.

Comparison of clinicopathological and genomic profiles in anal squamous cell carcinoma between Japanese and Caucasian cohorts.

Ito T, Takayanagi D, Sekine S, Hashimoto T, Shimada Y, Matsuda M, Yamada M, Hamamoto R, Kato T, Shida D, Kanemitsu Y, Boku N, Kohno T, Takashima A, Shiraishi K.

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Advanced Clinical Research Center

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

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Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授	博士(医学)	小　沼　貴　晶
Assistant Professor	Seiko Kato, M.D., Ph.D.	助　教	博士(医学)	加　藤　せい子

The main goal of our research is to elucidate the pathogenesis of hematopoietic diseases and to study the development of therapeutic methods for these diseases. For those therapeutic targets that have already been identified, we will continue to develop them and move them to the next stage of clinical application. In particular, in terms of elucidating the pathophysiology through genome analysis, we are collaborating with various research groups in Japan and overseas, and have made interesting findings on myeloproliferative tumors and the clonal evolution of PIGA. We are also leading a whole-genome sequencing project of more than 1,400 leukemia samples collected from major hematopoietic disease centers in Japan, and will begin analyzing genomic data from these samples this fiscal year. The project aims to comprehensively search for abnormalities that occur in non-genetic coding regions and other regions of the genome, and to elucidate previously undiscovered pathomechanisms. We also aim to identify the genetic predisposition to leukemia.

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

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4 Department of Pathology and Tumor Biology, Kyoto University

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 cases 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. Insights from Trajectories of PIGA-mutated clones in Paroxysmal Nocturnal Hemoglobinuria

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6)Immunoglycobiology, WPI Immunology Frontier Research Center, Osaka University
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8)M&D Data Science Center, Tokyo Medical and Dental University
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The pathophysiology of clonal expansion with paroxysmal nocturnal hemoglobinuria (PNH) remains unclear. To elucidate the trajectories of PNH clones, we constructed phylogenetic trees using whole genome sequencing data of single cell-derived colonies of bone marrow samples from two PNH patients (52 colonies for case 1 and 77 for case 2). In case 1, we identified three PIGA-mutated and one BCOR-mutated clone with no shared common somatic variants, showing independent origin. In case 2, one PIGA-mutated clone was detected, in which HMGA2 mutations (Inoue et al. Blood. 2006) were accompanied in all the colonies examined. Bayesian estimation revealed that these PIGA mutations are assumed to be acquired around 14 years before the clinical PNH onset in both cases. PIGA-mutated clones exhibited a higher mutation acquisition rate than unmutated ones (16 vs. 11 mutations/year for case 1 and 14 vs. 12 for case 2). We estimated the effective population size of PIGA-mutated clones using coalesce principles. PIGA-mutated clones grew more slowly than unmutated normal clones of early embryonic development. In case 1, the onset of the significant clonal expansion of two PIGA-mutated clones seems to coincide with diagnosis of the aplastic anemia (AA), which supports the theory that immune attack associated with AA facilitated the expansion of PNH clones. This technique helps to infer the disease development mechanism by depicting the diseased clone's evolutionary history, which cannot be directly observed.

3. A forward-looking analytical study of minimal residual disease after allogeneic hematopoietic stem cell transplantation using circulating tumor DNA in acute myeloid leukemia: KSGCT1702

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Medicine, Jikei University School of Medicine

Post-allogeneic relapse of AML/MDS is an important clinical issue, and there is a need to develop a non-invasive method to detect relapse at an early stage. To evaluate the usefulness of the minimal residual disease assay, which measures driver mutations identified by comprehensive genetic analysis in post-transplant AML/MDS patients by VAF of circulating tumor DNA in post-transplant patients' serum, in transplant patients in the Kanto Hematopoietic Stem Cell Group.

Patients diagnosed as AML/MDS cases according to WHO Classification 2008, aged 20 to 65 years, who may undergo allogeneic hematopoietic stem cell transplantation with myeloablative pretreatment at KSGCT participating centers, and whose written consent has been obtained from the patient. Target cases will be enrolled and next-generation sequencing will be performed on tumor and control (oral mucosa) specimens. Driver gene mutations will be identified and a Droplet Digital PCR (ddPCR) assay will be designed. Cell free DNA will be extracted from serum samples before and after bone marrow transplantation, and driver gene mutations will be quantitatively measured using ddPCR. 70 cases were enrolled from 12 centers, of which 12 cases dropped out before transplantation. Transplantation was performed in 58 cases, recurrence within 1 year after transplantation in 15 cases, and death within 1 year after transplantation in 10 cases (preimplantation death in 1 case, recurrence death in 4 cases). Of the transplant recipients, 57 underwent NGS to identify driver mutations. 52 were identified as MRD-eligible driver mutations. 48 completed MRD measurement by ddPCR.

4. A forward-looking analytical study of minimal residual disease after allogeneic hematopoietic stem cell transplantation using circulating tumor DNA in acute lymphoid leukemia: KSGCT1901

Ogawa M1, Yokoyama K2, Ishii H3, Nannya Y12,

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3 Division of Clinical Oncology and Hematology, Department of Internal

Post-allogeneic relapse of ALL is an important clinical issue, and there is a need to develop a non-invasive method to detect relapse at an early stage. To evaluate the usefulness of the minimal residual disease assay, which measures driver mutations identified by comprehensive genetic analysis in post-transplant ALL patients by VAF of circulating tumor DNA in post-transplant patients' serum, in transplant patients in the Kanto Hematopoietic Stem Cell Group.

Patients 16 years of age or older, acute lymphoblastic leukemia according to WHO classification 2016, any history of chemotherapy at the time of transplantation, specimens with tumor volume of at least 20% available, potential for allogeneic transplantation, and written consent obtained from the patient. Target cases will be enrolled and next-generation sequencing will be performed on tumor and control (oral mucosa) specimens. Driver gene mutations will be identified and a Droplet Digital PCR (ddPCR) assay will be designed. Cell free DNA will be extracted from serum samples before and after bone marrow transplantation, and driver gene mutations will be quantitatively measured using ddPCR. There were 54 cases enrolled, 5 cases of pre-transplant dropout, and 43 cases of transplantation performed. NGS of tumor samples was performed in 40 cases and driver gene mutations were identified in 24 cases. A Droplet Digital PCR assay is being designed for each case.

5. Novel recurrent structural variants in hematopoietic malignancies and construction of Japanese-SV-specific panel of normal (PoN)

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3 Department of Pathology and Tumor Biology, Kyoto University

Structural Variation analysis was performed with GRIDSS/MANTA/GenomonSV on a total of 1453 hematological paired whole genome sequencing(WGS) data. We found that the intersection of calls from GRIDSS, MANTA, GenomonSV, Delly and SVABA was less than 1%, and the vast majority of them are presumed to be false positive calls. This is mainly due to the inability of every caller to remove alignment errors at repetitive or low-complexity sequences. We, therefore, constructed Japanese specific Structural Variant Panel of Normal (PoN) with recurrent calls from samples of healthy japanese donors, aiming to provide a set of false-positive calls that should be subtracted from the call results of Japanese tumour samples. We confirmed a significant (>80%) reduction in the number of calls using this panel, and currently figuring out novel recurrent pathogenic structural variants.

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Advanced Clinical Research Center

Division of Advanced Gastroenterology and Endoscopy
先端消化器内視鏡学分野

Professor Hiroaki Ikematsu, M.D., Ph.D.
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A new field, "Division of Advanced Gastroenterology and Endoscopy," was established at the Institute of Medical Science of the University of Tokyo from October 2023.
Our mission is to conduct cutting-edge endoscopy-related research and develop novel endoscopic equipment, with a focus on gastrointestinal cancer. In addition, we will conduct translational research with other research fields at the institute to create new diagnoses and treatments.

1. Introduction

Advances in endoscopic diagnosis and treatment have been remarkable. Our objective is to continue advancing, spanning from basic research in endoscopy to clinical investigations, and the creation of innovative endoscopic devices for practical clinical applications. In addition, our objective is to create novel diagnostic and therapeutic approaches through translational research in collaboration with various fundamental disciplines.

2. Research Activities

The device development in 2023 was as follows: (1) Report on the usefulness of highly quantitative imaging of fecal occult blood, (2) Development of automatic colon insertion endoscope, and (3) Research targeting submucosal tumors and gastric cancer by irradiating with near-infrared light in the wavelength range of 1,000 nm or more, as well as the development of a flexible endoscope for this purpose. All of these are highly novel seeds and systems that have never existed before, and can be expected to be clinically useful.

3. Future Prospects

We will continue basic and clinical research to elucidate, detect, and diagnose lesions with the aim of preventing gastrointestinal cancers.

We will continue to develop innovative medical devices desired by the next generation that reflect the needs of clinical practice. In the future, we will continue to develop endoscopic equipment and attempt innovative approaches to produce all-new endoscopy approaches/devices in collaboration with academia and corporations.

4. Publications

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Center for Stem Cell Biology and Regenerative Medicine

Division of Regenerative Medicine
再生医学分野

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Associate Professor	Naoki Tanimizu, Ph.D.	准教授	博士(農学)	谷　水　直　樹
Assistant Professor	Yun-Zhong Nie, Ph.D.	助　教	博士(医学)	聶　　運　中
Project Assistant Professor	Yasuharu Ueno, Ph.D.	特任助教	博士(医学)	上　野　康　晴
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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 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 organoids (LOs) 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 iPS cell derived 3D-organoids.

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The liver plays a crucial role in maintaining homeostasis in the living organism by performing various metabolic functions such as glucose metabolism, lipid metabolism, and ammonia metabolism. On the other hand, abnormalities in these metabolic functions can lead to a variety of diseases of the liver. Liver transplantation is the only curative therapy for end-stage liver disease, but the absolute shortage and rapid availability of donor organs is a serious challenge and alternative treatments are clinically highly demanded. We established a technique to produce human pluripotent stem cell (hiPSC) derived liver organoids (hiPSC-LO) by inducing differentiation of hepatic endodermal cells, vascular endothelial cells, and mesenchymal cells from hiPSCs and co-culturing them in a 3D manner (***Nature*** 2013, ***Nature*** 2017, ***Cell Reports*** 2017, ***Sci Rep*** 2020). Currently, we are developing a novel therapeutic method using hiPSC-LO transplantation for urea cycle disorders, a serious liver disease,

and metabolic insufficiency steatohepatitis (MASH), for which there is a huge number of patients.

Urea cycle disorders is a severe metabolic liver disease resulting from the dysfunction of ornithine carbamoylase (OTC) and other enzymes, which is associated with hyperammonemia. We have optimized the ECM coating on the culture dishes and further optimized the organoid culture method to establish a robust production method for hiPSC-LO with appropriate ammonia metabolic capacity (***Biol Methods Protoc*** 2022). In addition, to evaluate the safety of the hiPSC-LOs, we established a highly sensitive method detecting undifferentiated cells after induction of LO component cells from hiPSCs (***Stem Cell Rev Rep*** 2022). Next, we evaluated the efficacy of hiPSC-LO transplantation in a severe OTCD animal model with a background of immunodeficiency. Subadrenocortical transplantation of hiPSC-LOs into immunocompromised OTCD mice significantly improved their hyperammonemic state, suggesting that iPSC-LO transplantation is effective as the treatment of OTCD.

Liver cirrhosis is the end stage pathological condition of chronic liver diseases such MASH. MASH is characterized by reduced liver function and regenerative capacity and is expected to explode in the number of patients worldwide. With the support of AMED, we are currently developing a novel treatment for MASH cirrhosis by transplantation of a newly developed fused-type hiPSC-LO based on hiPSC-LO production technology. To this end, we have established a stable method for creating fused-type hiPSC-LOs and now we are examining its efficacy as MASH treatment by transplanting them into MASH liver cirrhosis model animals. Given that no effective treatment has been developed for MASH cirrhosis, there is great hope for fused-type hiPSC-LOs transplantation.

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. hiPSCs have shown immense potential for cell replacement therapy for disease treatment. However, hiPSC-derived cells that can effectively repopulate in the damaged tissues such as liver have not been reported. Here, we present the generation of expandable hiPSC-derived hepatoblast (hiPSC-HB) with robust repopulation capacity after transplantation. These hiPSC-HB exhibited an impressive expansion capability and displayed bipotential differentiation abilities both *in vitro* and *in vivo*. Moreover, we noticed that the repopulation capacity of hiPSC-HB could be enhanced with specific conditions, leading to engraftment levels comparable to primary human hepatocytes (PHHs) in liver failure mice. Further, the engrafted hiPSC-HB matured into functional human hepatocytes with tissue-specific structural features. This study marks a breakthrough as the first successful generation of lineages from pluripotent stem cells capable of in vivo repopulating and restoring tissue function, offering promising prospects for innovative disease treatment through regenerative medicine.

3. Modeling liver diseases with hiPSC-derived organoid

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The developed human cell-based liver *ex vivo* models still could not accurately recapitulate liver physiology and disease progression due to lack of non-parenchymal cell, including Kupffer cell, hepatic stellate cells and sinusoidal endothelial cells. Currently, we generated LOs containing Kupffer cells (KuLOs) by recapitulating fetal liver hematopoiesis using hiPSC-derived erythro-myeloid progenitors (EMPs), origin of tissue-resident macrophages, and hiPSC-derived LOs. Exposing KuLOs to sepsis-like endotoxins led to significant organoid dysfunction that closely resembled the pathological characteristics of the human septic liver. Furthermore, we observed a notable functional recovery in KuLOs upon endotoxin elimination, which was accelerated by using Toll-like receptor 4-directed endotoxin antagonist. Moreover, we are trying to establish a mini liver with hepatocytes, Kupffer cells, hepatic stellate cells, and sinusoidal endothelial cells, which will allow a more precise understanding of the cell-to-cell interactions during liver disease progression, and provide 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 organs with structures and functions is urgently needed. Although hiPSC-organoid is an innovative technology to reconstitute tissue structure and function, an alternative for organ transplantation. Blood perfusion is a critical event for organ growth by supplying nutrients and oxygen. However, blood perfusion is still lacking in the present organoid culture system. We are developing perfusion culture systems using two approaches; hiPSC-liver buds (LBs) connected with reconstructed hiPSC-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. Under an optimized culture condition, we successfully induced the connection between the hiPSC-derived macrovessel with the microvessels within hiPSC-LBs. Therefore, we recently tried to establish the organoid perfusion system. The established perfusion system enabled us to culture the hiPSC-LBs by 14 days from co-culture and increase $CD31^+$ blood vessels in LBs. Optimizing the perfusion culture system, we are trying to enhance hiPSC-LB growth.

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 limited hepatocyte functions in the recellularized liver. 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 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. To recapitulate tumor microenvironment (TME), which is thought to be crucial for the poor prognosis of PDAC, we generated multicellular spheroids consisting of primary PDAC cells isolated from Japanese pancreatic cancer patients with hiPSC-mesenchymal cells (MCs) and -endothelial cells (ECs), and then fused them to construct fused pancreatic cancer organoid (FPCO). Our FPCO resembles the tissue structure of clinical tissue including PDAC ductal structures and dense deposition of extracellular matrix components compared to conventional organoids. Single cell RNA sequence analysis demonstrated that FPCO contains various types of cancer-related fibroblasts (CAFs) namely immunological CAF (iCAF), myofibroblastic (myCAF), and antigen presenting ones (apCAFs). 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. We are currently making FPCO containing tumor associated macrophages (TAMs) to recapitulate immunosuppressive TME. We will apply this new cancer organoid in drug screening and biological analysis to develop effective therapies against PDAC.

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

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ty

Microgravity in orbit does not cause subsidence or convection and is considered advantageous in expanding cells in three-dimension. By utilizing this microgravity environment, we aim to develop a novel method for generating human iPSCs-derived liver tissue in collaboration with Japan Aerospace Exploration Agency (JAXA). In 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 around 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 samples 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. Next, we upgraded our system by using a large blood vessel composed of hiPSC-derived smooth muscle cells and endothelial cells which resulted in the improvement of hiPSC-LB functions. The second space experiment is planned to be conducted in March 2024. 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 (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 hiPSC-liver organoids associated with the bile drainage system consisting of IHBD and EHBD has not been reported so far.

First, to generate liver organoid containing IHBD-like structures, we developed a new co-culture system in which the hiPSC-liver progenitors are located in next to the hiPSC-blood vessel (BV) to recapitulate the fetal portal vein-IHBD tissue interaction. In this condition, hiPSC-liver progenitors differentiated into cholangiocytes and formed duct structures. We named this organoid as blood vessel incorporated liver organoid (BVLO). hiPSC-cholangiocytes in BVLO showed secretory functions in vitro and formed duct structures within the recipient liver after organoid transplantation to immunodeficient mice. We applied BLVO technology to modelling a human congenital biliary disease for understating underlying mechanisms.

Second, to generate EHBDs, we induced EHBD progenitor cells from hiPSC- definitive endoderm cells and generated 3D cystic structures. We are currently analyzing transcriptome data of mouse fetal EHBD epithelial cells to acquire information about proliferation signal for EHBD progenitors and their functional maturation process. 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*.

8. Generation of a novel treatment for pediatric craniofacial deformity using human auricular perichondrium-derived elastic cartilage devices

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Auto-transplantation of rib cartilage segments is the current most popular treatment for patients with craniofacial deformity. However, major disadvantages such as limited harvestable amounts and post-operative pain of the donor site remain to be solved. To this end, a none-invasive, morphologically stable scaffold-free elastic cartilage implantation treatment for patients with craniofacial deformity is essential.

Our previous study showed the world's first technology of separating and identifying chondroprogenitor cells from the human auricular perichondrium (Kobayashi S et al. PNAS 2011, Patent registration no. 4748222; PCT/JP2008/051327). We succeeded in developing non-scaffold elastic cartilage, which is obtainable in vitro, by using three-dimensional rotation culture and U–bottomed micropatterned plate culture (Enomura M et al. Int J Mol Sci 2020, Oba T et al. J Tissue Eng 2022, Patent application no. 2021-141210; PCT/JP2022/25582). Furthermore, the size and elasticity of the tissue were maintained after craniofacial transplantation in immunodeficient mice, indicating the tissue to be morphologically stable.

Our major goal is to establish a non-invasive novel promising treatment for pediatric patients with nasal deformity by transplanting morphologically stable non-scaffold elastic cartilage. To obtain the clinical POC of the novel treatment,we are currently establishing the manufacturing system, quality control methods, product specification, evaluation of non-clinical safeness and determination of clinical protocol.

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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. Polycomb repressive complex 1.1 coordinates homeostatic and emergency myelopoiesis

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Polycomb repressive complex (PRC) 1 regulates stem cell fate by mediating mono-ubiquitination of histone H2A at lysine 119. While canonical PRC1 is critical for hematopoietic stem and progenitor cell (HSPC) maintenance, the role of non-canonical PRC1 in hematopoiesis remains elusive. PRC1.1, a non-canonical PRC1, consists of PCGF1, RING1B, KDM2B,

and BCOR. We recently showed that PRC1.1 insufficiency induced by the loss of PCGF1 or BCOR causes myeloid-biased hematopoiesis and promotes transformation of hematopoietic cells in mice. Here we show that PRC1.1 serves as an epigenetic switch that coordinates homeostatic and emergency hematopoiesis. PRC1.1 maintains balanced output of steady-state hematopoiesis by restricting C/EBPαa-dependent precocious myeloid differentiation of HSPCs and the HOXA9- and β-catenin-driven self-renewing network in myeloid progenitors. Upon regeneration, PRC1.1 is transiently inhibited to facilitate formation of granulocyte-macrophage progenitor (GMP) clusters, thereby promoting emergency myelopoiesis. Moreover, constitutive inactivation of PRC1.1 results in unchecked expansion of GMPs and eventual transformation. Collectively, our results define PRC1.1 as a novel critical regulator of emergency myelopoiesis, dysregulation of which leads to myeloid transformation.

2. UTX inactivation in germinal center B cells promotes the development of multiple myeloma with extramedullary disease

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UTX/KDM6A, a histone H3K27 demethylase and a key component of the COMPASS complex, is frequently lost or mutated in cancer; however, its tumor suppressor function remains largely uncharacterized in multiple myeloma (MM). Here, we show that the conditional deletion of the X-linked *Utx* in germinal center (GC) derived cells collaborates with the activating $Braf^{V600E}$ mutation and promotes induction of lethal GC/post-GC B cell malignancies with MM-like plasma cell neoplasms being the most frequent. Mice that developed MM-like neoplasms showed expansion of clonal plasma cells in the bone marrow and extramedullary organs, serum M proteins, and anemia. Add-back of either wild-type *UTX* or a series of mutants revealed that cIDR domain, that forms phase-separated liquid condensates, is largely responsible for the catalytic activity-independent tumor suppressor function of UTX in MM cells. *Utx* loss in concert with $Braf^{V600E}$ only slightly induced MM-like profiles of transcriptome, chromatin accessibility, and H3K27 acetylation, however, it allowed plasma cells to gradually undergo full transformation through activation of transcriptional networks specific to MM that induce high levels of *Myc* expression. Our results reveal a tumor suppressor function of UTX in MM and implicate its insufficiency in the transcriptional reprogramming of plasma cells in the pathogenesis of MM.

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We are studying the clinical promotion and medical development of hematopoietic stem cell transplantation with a focus on cord blood transplantation. In addition to data on hematopoietic stem cell transplantation performed at the Department of Hematology/Oncology, we are analyzing various issues related to clinical transplantation using the Japanese transplant database. Basic studies include the development of efficient in vitro amplification of patient-derived primary cells and preclinical studies on the use of virus-specific CTLs in post-transplant and other immunocompromised patients. Our goal is to make allogeneic transplantation a safer treatment option and extend it to older patients.

1. Should a matched sibling donor still be considered the primary option for allogeneic hematopoietic cell transplantation in patients over 50 years of age with myelodysplastic syndrome?

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Human leukocyte antigen (HLA)-matched sibling donors (MSDs) are the preferred choice for allogeneic hematopoietic cell transplantation (HCT). However, as myelodysplastic syndrome (MDS) is most frequently diagnosed in the elderly, MSDs are also likely to be of advanced age. It is unclear whether an MSD should be considered the primary choice for allogeneic HCT in elderly patients with MDS. We retrospectively compared survival and other outcomes in 1787 patients with MDS over 50 years of age and receiving allogeneic HCT between 2014 and 2020, using either MSD (n=214), 8/8 allele-matched unrelated donor (MUD) (n=562), 7/8 allele-MUD (n=334), or unrelated cord blood (UCB) (n=677) in Japan. In multivariate analysis, compared to MSD transplants, the risk of relapse was significantly lower following 8/8MUD transplants (hazard ratio [HR], 0.74; P=0.047), whereas non-relapse mortality was significantly higher following UCB transplants (HR, 1.43; P=0.041). However, donor type did not determine overall survival, disease-free survival, or graft-versus-host disease (GVHD)-free, relapse-free survival, but chronic GVHD-free, relapse-free survival was better after UCB (HR, 0.80; P=0.025) and 8/8MUD (HR, 0.81; P=0.032) compared to MSD transplants. Our study demonstrated that MSDs are not superior to alternative HCT methods, such as 8/8MUD, 7/8MUD, or UCB, in this population.

2. Comparison of allogeneic transplant outcomes between matched sibling donors and alternative donors in patients over 50 years of age with acute myeloid leukemia: 8/8 allele-matched unrelated donors and unrelated cord blood provide better leukemia-free survival compared with matched sibling donors during non-remission status

Takaaki Konuma 1, Satoshi Yamasaki 2, et al., rep-

resenting for: the Adult Acute Myeloid Leukemia and Donor/source Working Groups of the Japanese Society for Transplantation and Cellular Therapy
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Acute myeloid leukemia (AML) is the most common indication for allogeneic hematopoietic cell transplantation (HCT). The increased availability of alternative donor sources has broadened donor types for older patients without HLA-matched sibling donors (MSD). It is uncertain if an MSD should be the first option for allogeneic HCT in patients with AML over 50 years of age. The objective of this study was to compare survival and other posttransplant outcomes between MSDs, 8/8 allele-matched unrelated donors (MUDs), 7/8 allele-MUDs, unrelated cord blood (UCB), and haploidentical donors for patients with AML over 50 years of age. We conducted a retrospective study to compare outcomes in 5,704 patients with AML over 50 years of age and receiving allogeneic HCT between 2013 and 2021, using either MSD, 8/8 allele-MUD, 7/8 allele-MUD, UCB, or haploidentical donors in Japan. Complete remission (CR) and non-remission at HCT were analyzed separately for all analyses. In total, 3041 patients were CR, and 2663 patients were non-remission at the time of HCT. In multivariate analysis, donor type did not determine overall survival, irrespective of disease status at HCT. Leukemia-free survival (LFS) was significantly better for 8/8 allele-MUD (hazard ratio [HR], 0.77; 95% confidence interval [CI], 0.64 to 0.93; P = 0.005) and UCB (HR, 0.76; 95% CI, 0.65 to 0.88; P = 0.001), but not for 7/8 allele-MUD (HR, 0.97; 95% CI, 0.79 to 1.19; P=0.794), and haploidentical donor (HR, 0.86; 95% CI, 0.70 to 1.05; P=0.146) compared to the MSD group in non-remission status. However, donor type did not determine LFS among CR status. Relapse rates were significantly lower for 8/8 allele-MUD and UCB, whereas non-relapse mortality was higher for UCB compared to the MSD group among both CR and non-remission status. Our registry-based study demonstrated that MSDs do not lead to superior survival compared to alternative donors for patients with AML over 50 years of age. Furthermore, 8/8 allele-MUDs and UCB provide better LFS compared with MSDs during non-remission status. Therefore, MSD is not necessarily the best donor option for allogeneic HCT in this population.

3. Development of ex vivo culture methods for primary tumor specimens derived from patients of myeloid malignancies.

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High throughput drug sensitivity screening (DSS) using patient-derived primary tumor specimens (PTS) are one of the most updated methods in current precision medicine. Recently, we have developed an experimental platform in which completely robotized handling of PTS is installed. They provide us with capability of conducting high throughput drug screening equipped with multicolor flow cytometry analyses upon ex vivo cultured PTS prepared on miniaturized 384-well-plate system. Even with those extremely modernized system, paucity of available primary cells is a yet-to-be-solved issue, considering numbers of candidate drugs, and types of clinical questions. The issue is more valid for PTS of myelodysplastic syndrome (MDS) , since the disease is characterized by its lower ratios of blast cells, and relatively lower cell numbers than highly proliferative acute myeloid leukemia (AML) cells. In order to expand possibility of those AML/ MDS PTS for more advanced applications, we have performed experiments to side-by-side compare different ex vivo culture methods. At Clinical Precision Research Platform, we have already collected 159 different PTS aliquoted into 1495 cryovials. They are ongoingly used either in the automated DSS experiments, OMICS-analyses, or in this ex vivo culture assays. We have discovered that 1. Short-term culture protocols (up to 7 days) optimized for the current DSS assays make a few differences regardless of changed concentrations of serum or other supplements for culture media. 2. Use of stromal feeder cells prevails other conventional stroma-free culture methods that are currently utilized for normal hematopoietic stem cells. Moreover, 3. Recently, we installed the most updated culture methods to challenge for stroma-free, serum-free ex vivo culture system optimized for PTS. The idea for the updated culture media is derived from ongoing research projects by collaborators regarding normal hematopoietic stem cell culture. Initial data on this topic is continuously fueling our research motivations, day by day. Details of those findings will be followingly updated.

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Center for Stem Cell Biology and Regenerative Medicine

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

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

教 授 博士(医学) 谷 口 英 樹

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 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

Our lab aims to understand mechanisms underlying the cell fate decisions in early mammalian embryos and to apply their principle for future reproductive and regenerative medicine. In particular, we use pluripotent stem cells and early embryos from various mammals, which will enable us to investigate conserved mechanisms among the mammals and to develop novel technology by the use of species-specific features.

1. Rat post-implantation epiblast-derived pluripotent stem cells produce functional germ cells

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In mammals, pluripotent cells transit through a continuum of distinct molecular and functional states en route to initiating lineage specification. Capturing pluripotent stem cells (PSCs) mirroring in vivo pluripotent states provides accessible in vitro models to study the pluripotency program and mechanisms underlying lineage restriction. Here, we develop optimal culture conditions to derive and propagate post-implantation epiblast-derived PSCs (EpiSCs) in rats, a valuable model for biomedical research. We show that rat EpiSCs can be reset toward the naïve

pluripotent state with exogenous Klf4, albeit not with the other five candidate genes (Nanog, Klf2, Esrrb, Tfcp2l1, and Tbx3) effective in mice. Finally, we demonstrate that rat EpiSCs retain competency to produce authentic primordial germ cell-like cells that undergo functional gametogenesis leading to the birth of viable offspring. Our findings in the rat model uncover conserved principles underpinning pluripotency and germline competency across species.

2. Origin and segregation of the human germline

Aracely Castillo-Venzor [7,14], Christopher A Penfold, Michael D Morgan [15,16], Walfred Wc Tang [7], Toshihiro Kobayashi, Frederick Ck Wong [7], Sophie Bergmann [5, 6], Erin Slatery [5,6], Thorsten E Boroviak [5,6], John C Marioni [15,16], M Azim Surani [7]

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Human germline-soma segregation occurs during weeks 2-3 in gastrulating embryos. Although direct studies are hindered, here, we investigate the dynamics of human primordial germ cell (PGCs) specification using in vitro models with temporally resolved single-cell transcriptomics and in-depth characterization using in vivo datasets from human and nonhuman primates, including a 3D marmoset reference atlas. We elucidate the molecular signature for the transient gain of competence for germ cell fate during peri-implantation epiblast development. Furthermore, we show that both the PGCs and amnion arise from transcriptionally similar TFAP2A-positive progenitors at the posterior end of the embryo. Notably, genetic loss of function experiments shows that TFAP2A is crucial for initiating the PGC fate without detectably affecting the amnion and is subsequently replaced by TFAP2C as an essential component of the genetic network for PGC fate. Accordingly, amniotic cells continue to emerge from the progenitors in the posterior epiblast, but importantly, this is also a source of nascent PGCs.

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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 types and cell signaling that cause hair graying and hair thinning

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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. Importantly, DNA damage foci are relatively frequently found in somatic stem cells in the skin during physiological aging. To study the fate and dynamics of DNA-damaged stem cells in tissues and the resultant impact in the expression of aging phenotypes, we first focused on the melanocyte lineage and traced 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. More than that, the fact underlie the fact that cell components in tissues are being replaced through stem cell differentiation and their eventual depletion. We are currently testing whether the selective induction of DNA double strand breaks in melanocyte stem cells similarly causes hair graying and whether it has some beneficial effects in suppressing melanoma development from the skin. Similarly, we are testing whether DNA DSBs in hair follicle stem cells promotes hair thinning and searching for chemicals that can prevent stem cell loss by DNA DSBs.

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 thining 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 nor p16 expression 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. We are currently testing the stem cell elimination process is mediated by cell competition mechanisms and also whether the phenomenon has any correlation with systemic aging.

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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) and we explored new immune and regenerative gene/cell therapies using CB and UC-MSCs. Another mission is to manage the IMSUT-HLC cell processing facility (IMSUT-HLC-CPF) for translational research. To achieve the high-quality processing and tests for UC-MSCs therapy, IMSUT-HLC-CPF obtained manufacturing license as the first national University in 2023.

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

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

We explored new immune and regenerative gene/cell therapies using umbilical cord blood (CB) and umbilical cord-derived MSCs (UC-MSCs) with high quality and safety standards. For the high quality and safety standards

In addition, it is our mission to keep the IMSUT-HLC cell processing facility clean and functional to enable high-quality manufacturing for translational gene and cell therapy. To achieve this mission IMSUT-HLC-CPF obtained manufacturing license for UC-MSCs therapy as the first national University in 2023.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Cell Engineering
幹細胞基盤技術研究分野

Professor Satoshi Yamazaki, Ph.D.

教 授 博士(生命科学) 山 﨑 聡

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

1. Understanding genetic heterogeneity in gene-edited hematopoietic stem cell products

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CRISPR/Cas gene editing has transformed genetic research and is poised to drive the next generation of gene therapies targeting hematopoietic stem cells (HSCs). However, the installation of the "desired" edit is most often only achieved in a minor subset of alleles. The array of cellular pathways triggered by gene editing tools produces a broad spectrum of "undesired" editing outcomes, including short insertions and deletions (indels) and chromosome rearrangements, leading to considerable genetic heterogeneity in gene-edited HSC populations. This heterogeneity may undermine the effect of the genetic intervention since only a subset of cells will carry the intended modification. Also, undesired mutations represent a potential safety concern as gene editing advances toward broader clinical use. Here, we will review the different sources of "undesired" edits and will discuss strategies for their mitigation and control.

2. Purging myeloma cell contaminants and simultaneous expansion of peripheral blood mobilised stem cells.

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Human hematopoietic stem cells (HSCs) are widely used as a cellular source for hematopoietic stem cell transplantation (HSCT) in the clinical treatment of hematological malignancies. After transplantation therapy, delays in hematopoietic recovery due to insufficient donor-derived HSCs can lead to increased risks of life-threatening infections and bleeding. Our previous studies developed an efficient ex vivo expansion culture medium (3a medium) for umbilical cord blood-derived HSCs (CBSCs), offering a potential solution to this problem. Nevertheless, the broader applicability of our culture method to alternative cell sources, and, of greater significance, its efficacy in eliminating potentially disease-associated contaminated tumor cells, especially in autologous transplantation, raises critical clinical questions. In this study, we modified the 3a medium by incorporating UM729 to replace UM171, added Flt3 ligand, and adjusted the concentrations of butyzamide, 740Y-P, polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (PCL-PVAc-PEG, Soluplus®) to create the modified 3a medium. This sophistication allowed efficient expansion of not only CBSCs but also peripheral blood mobilized HSCs (PBSCs). Additionally, we successfully removed contaminated myeloma cells by adding bortezomib and TNF-related apoptosis inducing ligand (TRAIL) at appropriate concentrations, while maintaining HSCs through the addition of lenalidomide. Our research findings present the potential for widespread clinical application of the modified 3a medium and suggest a safe ex vivo culture technique for expanding human HSCs within peripheral blood derived donor grafts used for autologous HSCT.

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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 four BD FACS Aria Cell sorters (SORPAria 1, Aria1, AriaIII 2) from BD Biosciences. For cell analysis, the FACS Core Laboratory is equipped with four bench-top analyzers (Verse, Calibur, CantoII from BD Biosciences and CytoFLEX LX from Beckman Coulter).

FCM usage performance in 2023

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 2023.

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
Visiting Professor	Masaki Imai, D.V.M., Ph.D.	客員教授	博士(獣医学)	今　井　正　樹
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Associate Professor	Takeshi Ichinohe, Ph.D.	准教授	博士(工学)	一　戸　猛　志
Associate Professor	Jumpei Ito, Ph.D., D.V.M.	准教授	博士(理学)	伊　東　潤　平

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.

1. Virological characteristics of the SARS-CoV-2 XBB variant derived from recombination of two Omicron subvariants

Tomokazu Tamura[1], Jumpei Ito, Keiya Uriu, Jiri Zahradnik[2], Izumi Kida[1], Yuki Anraku[1], Hesham Nasser[3], Maya Shofa[4], Yoshitaka Oda[1], Spyros Lytras[5], Naganori Nao[1], Yukari Itakura[1], Sayaka Deguchi[6], Rigel Suzuki[1], Lei Wang[1], Mst Monira Begum[3], Shunsuke Kita[1], Hisano Yajima[6], Jiei Sasaki[6], Kaori Sasaki-Tabata[7], Ryo Shimizu[3], Masumi Tsuda[1], Yusuke Kosugi, Shigeru Fujita, Lin Pan, Daniel Sauter, Kumiko Yoshimatsu[1], Saori Suzuki[1], Hiroyuki Asakura[8], Mami Nagashima[8], Kenji Sadamasu[8], Kazuhisa Yoshimura[8], Yuki Yamamoto[9], Tetsuharu Nagamoto[9], Gideon Schreiber[2], Katsumi Maenaka[1], Genotype to Phenotype Japan (G2P-Japan) Consortium, Takao Hashiguchi[6], Terumasa Ikeda[3], Takasuke Fukuhara[1], Akatsuki Saito[4], Shinya Tanaka[1], Keita Matsuno[1], Kazuo Takayama[6], Kei Sato:

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In late 2022, SARS-CoV-2 Omicron subvariants have become highly diversified, and XBB is spreading rapidly around the world. Our phylogenetic analyses suggested that XBB emerged through the recombination of two cocirculating BA.2 lineages, BJ.1 and BM.1.1.1 (a progeny of BA.2.75), during the summer of 2022. XBB.1 is the variant most profoundly resistant to BA.2/5 breakthrough infection sera to date and is more fusogenic than BA.2.75. The recombination

breakpoint is located in the receptor-binding domain of spike, and each region of the recombinant spike confers immune evasion and increases fusogenicity. We further provide the structural basis for the interaction between XBB.1 spike and human ACE2. Finally, the intrinsic pathogenicity of XBB.1 in male hamsters is comparable to or even lower than that of BA.2.75. Our multiscale investigation provides evidence suggesting that XBB is the first observed SARS-CoV-2 variant to increase its fitness through recombination rather than substitutions.

Publications

Keiya Uriu, Jumpei Ito, Jiri Zahradnik, Shigeru Fujita, Yusuke Kosugi, Gideon Schreiber, Genotype to Phenotype Japan (G2P-Japan) Consortium, Kei Sato. Enhanced Transmissibility, Infectivity, and Immune Resistance of the SARS-CoV-2 Omicron XBB.1.5 Variant. **Lancet Infectious Diseases** 23(3):280–81 (2023).

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International Research Center for Infectious Diseases

Department of Infectious Disease Control
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Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加　藤　哲　久
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)	丸　鶴　雄　平

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

1. Dual impacts of a glycan shield on the envelope glycoprotein B of HSV-1: evasion from human antibodies in vivo and neurovirulence

Ayano Fukui, Yuhei Maruzuru, Shiho Ohno[1], Moeka Nobe, Shuji Iwata, Kosuke Takeshima, Naoto Koyanagi, Akihisa Kato, Shinobu Kitazume[2], Yoshiki Yamaguchi[1], Yasushi Kawaguchi: [1]Division of Structural Glycobiology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Medical and Pharmaceutical University, Miyagi [2]Department of Clinical Laboratory Sciences, School of Health Sciences, Fukushima Medical University, Fukushima

Identification of the mechanisms of viral evasion from human antibodies is crucial both for understanding viral pathogenesis and for designing effective vaccines. Here we show in cell cultures that an N-glycan shield on the herpes simplex virus 1 (HSV-1) envelope glycoprotein B (gB) mediated evasion from neutralization and antibody-dependent cellular cytotoxicity due to pooled γ-globulins derived from human blood. We also demonstrated that the presence of human γ-globulins in mice and immunity to HSV-1 induced by viral infection in mice significantly reduced replication in their eyes of a mutant virus lacking the glycosylation site but had little effect on the replication of its repaired virus. These results suggest that an N-glycan shield on a specific site of HSV-1 envelope gB mediated evasion from human antibodies in vivo and from HSV-1 immunity induced by viral infection in vivo. Notably, we also found that an N-glycan shield on a specific site of HSV-1 gB was significant for HSV-1 neurovirulence and replication in the central nervous system of naïve mice. Thus, we have identified a critical N-glycan shield on HSV-1 gB that has dual impacts, namely evasion from human antibodies in vivo and viral neurovirulence.

2. Establishment of a system to quantify wild-type herpes simplex virus-induced cell-cell fusion reveals a role of N-glycosylation of HSV-1 envelope glycoprotein B in cell-cell fusion

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

Wild-type herpes simplex virus (HSV) strains infrequently mediate cell-cell fusion in cell cultures and barely induce large multinucleated cells. In this study, we established a system to quantify infrequent cell-

cell fusion induced by wild-type HSV strains. The established system clarified that the HSV-1 envelope glycoprotein B and its N-glycosylation at asparagine at position 141 were required for efficient cell-cell fusion. This study provides a link between cell-cell fusion induced by wild-type HSV-1 and viral pathogenesis in vivo.

Publications

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Viral Infection

感染制御系・ウイルス学分野

Associate Professor Takeshi Ichinohe, Ph.D.

准教授 博士(工学) 一 戸 猛 志

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

1. 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.

Fever is a common symptom of influenza and coronavirus disease 2019 (COVID-19), yet its physiological role in host resistance to viral infection remains less clear. Here, we demonstrate that exposure of mice to the high ambient temperature of 36 °C increases 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 hamsters from lethal SARS-CoV-2 infection. Moreover, we demonstrate that certain bile acids are reduced in the plasma of COVID-19 patients who develop moderate I/II disease compared with the minor severity of illness group. These findings implicate a 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.

Publications

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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.

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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.

1. Understanding the evolution of SARS-CoV-2

Yu Kaku, Keiya Uriu, Yusuke Kosugi, Shigeru Fujita, Luo Chen, Jarel Elgin Tolentino, Lin Pan, Arnon Plianchaisuk, Ziyi Guo, Alfredo Amolong Hinay, Jr., Kaoru Usui, Wilaiporn Saikruang, Wenye Li, Kaho Okumura, Naoko Misawa, Mai Suganami, Adam Patrick Strange, Naomi Ohsumi, Shiho Tanaka, 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. Since then, more than 770 million people have been infected with this virus and more than 7 million people have 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 December 2023, more than 10 principal investigators participate in this consortium and proceed fruitful collaboration. We aim to elucidate the virological characteristics of the SARS-CoV-2 variants continuously emerging in the world.

Publications

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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

Professor	Ken Ishii, M.D., Ph.D.	教　授	医学博士	石　井　　健
Visiting Professor	Noriko Toyama-Sorimachi, Ph.D.	客員教授	博士(医学)	反　町　典　子
Project Associate 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 Toyama-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.

1. *Establishment of a platform for rapid development of vaccines and therapeutics in the event of an infectious disease pandemic.*

Background: After the experience of the COVID-19 pandemic, various research systems for infectious disease pandemics have been in place around the world. IMSUT is the central hub of SCARDA, and the University of Tokyo has set up UTOPIA, where researchers have begun to work together. However, much work remains to be done to overcome the various obstacles that may arise during a disaster. To address issues such as how to rapidly obtain research materials including purified proteins as antigens and antibodies as reagents to test vaccine efficacy, and how to rapidly prepare various tools and technologies for therapeutic drug discovery in parallel with vaccine development, further collaboration among researchers across disciplines, organizations, and institutions, which is unique to emergency situations, is essential. Dr. Sorimachi of the International Vaccine Design Center is the program officer in the BINDS project of AMED. BINDS is a platform consisting of a group of academic researchers with world-class analytical technologies and supports academic drug discovery research with advanced technologies ranging from structural analysis, hit discovery, protein expression analysis, and various omics analyses to in silico drug discovery.

Methods: In response to the spread of M-pox, we worked on an interproject collaboration between SCARDA and BINDS, using the SCARDA Ishii team's project to test smallpox vaccine efficacy against M-pox as a practical problem. In addition, the BINDS platform has begun to centralize plasmid information to expedite the process of applying for recombinant DNA experiments.

Conclusions: The collaboration between the SCARDA Ishii team and the BINDS researchers has enabled rapid progress in the immunological analysis of the effects of smallpox vaccine. This successful case is the first inter-project collaboration within AMED, and demonstrates that cooperation between SCARDA and BINDS can create a strong research impetus in times of emergency. We will continue to work on the establishment of a system of research collaboration to address specific issues anticipated in an emergency. Collaboration with other researchers on hu-

man immunology using human samples and cell lines are underway and some of them have resulted in the publications in 2023.

2. *Initiatives to out-license lead compounds to a company for the development of therapeutic agents for autoimmune diseases.*

We have been conducting exploratory drug discovery research to develop novel therapeutics for autoimmune diseases by targeting amino acid transporters that are preferentially expressed in immune cells, and has succeeded in obtaining lead compounds that suppress inflammatory cytokine and type I interferon production at sub-μM levels. Since a foreign pharmaceutical company have been expressing strong interest in our lead compounds, we had ongoing meetings under a confidentiality agreement regarding licensing negotiations and a third-party evaluation of the compound's potential. This was followed by discussions on the content of a term sheet for out-licensing and an agreement was reached. We are in the process of drafting an agreement. We expect to be able to out-license the compounds in 2024. In parallel, with the same pharmaceutical company, we have been preparing to enter into a broad joint research agreement.

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PCT application

1. WO2023/008402 A1: THERAPEUTIC AGENT FOR PULMONARY FIBROSIS

2. WO2023/008451 A1: TREATMENT AND REHABILITATION METHOD FOR PULMONARY FIBROSIS.

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

Professor	Cevayir COBAN, M.D., Ph.D. (Clinical Microbiology)	教　授	博士(医学)(臨床微生物学位)	チョバン　ジェヴァイア
Visiting Professor	Anavaj SAKUNTABHAI, M.D., Ph.D.	客員教授	博士(医学)	サクンタバイ　アナヴァジ
Project Assistant Professor	Michelle S.J. LEE, Ph.D.	特任助教	博士(医学)	リー　ミシェル

As a member of the International Vaccine Design Center of IMSUT, we focus on elucidating host-pathogen interactions in the context of emerging infectious diseases such as dengue and Covid19 and the neglected tropical diseases (i.e. malaria, leishmaniasis), to understand the virulence factors of these pathogens and ultimately to design successful vaccines against them.

1. Adjuvant discovery and development platform

Adjuvants are considered essential vaccine components for enhancing vaccine responses. As a member of IMSUT International Vaccine Design Center (https://vdesc.ims.u-tokyo.ac.jp/en/), we have systematically screened innate and adaptive immune signaling molecules involved in the mode of action (MOA) of adjuvants and vaccines. Recently, we have been involved in the discovery of novel adjuvants as part of the AMED SCARDA project. Our recent projects have focused on investigating B cell development and the pathways involved in germinal center (GC) formation for the generation of potent antibody responses against infections and during vaccination. We found that TBK1, the well-known innate immune signaling kinase that controls antiviral immune responses and nucleic acid-mediated type I interferon responses, is very important for the generation of GCs that confer sterile immunity to reinfection (*Lee et al., J Exp Medicine, 2022*).

2. Elucidation of host-pathogen interactions

Our laboratory has investigated several aspects of immunopathology caused by *Plasmodium* parasites. We have recently studied the immunopathology of cerebral malaria, the deadliest complication of human malaria infection, in the brain using the CUBIC clearance technique (*Matsuo-Dapaah et al., Int Immunology, 2021*). The 3D reconstruction of malaria-infected brain showed that olfactory bulb is disrupted during experimental cerebral malaria. We have recently made significant progress in understanding new cell types that accumulate/reside in the olfactory bulb and interact with *Plasmodium* parasites. Chronic bone loss is an unforeseen complication of malaria which is mediated via MyD88 adaptor protein (*Lee et al,. Science Immunology, 2017*). We have been studying to address the crucial cell types important for MyD88-mediated bone loss. We also investigate bone marrow niches responsible for malaria-induced loss of memory.

3. Infection and cancer

Previously, we investigated the role of Lipocalin 2 (LCN2, also known as siderocalin or neutrophil ge-

latinase-associated lipocalin (NGAL)) in malaria infection that bolsters innate and adaptive immune responses to malaria infection through modulation of iron metabolism (*Zhao et al., Cell Host Microbe, 2012*). LCN2 expression is also increased in cancer. In carcinogenesis stroma-associated immunity is an important regulator of tumor growth. Tumor cells create a microenvironment by releasing various mediators to maintain their presence and spread. Due to the infiltration of monocytes and leukocytes in tumor microenvironment, it is hypothesized that the iron balance is disrupted by excessive iron consumption, possibly leading to increased expression of LCN2 as an intracellular iron transporter. We recently investigated the expressions of programmed cell death ligand-1 (PD-L1) and LCN2 in breast cancers with various molecular subtypes, along with their correlations with other prognostic indicators, including Ki-67, lymph node metastasis, histological grade, tumor-infiltrating lymphocyte (TILs) accumulation, and necrosis. We found that there is an association of LCN2 with known prognostic factors and molecular subtypes. Moreover, significant elevations of LCN2 and PD-L1 expressions were observed in triple-negative and HER2-positive breast cancers. The findings from this research may contribute to the immunotherapeutic application of LCN2 and its prognostic significance in breast cancer management (*Ekemen et al., Breast Cancer: Targets and Therapy, in press*).

4. Infection and host genetics

The genetics of an individual contributes to the susceptibility and response to viral infections. International groups including Prof. Sakuntabhai's group formed a global network of researchers to investigate the role of human genetics in SARS-CoV-2 infection and COVID-19 severity (*COVID-19 Host Genetics Initiative. Mapping the human genetic architecture of COVID-19. Nature 600, 472–477, 2021*). Investigating the role of host genetic factors in COVID-19 severity and susceptibility can inform our understanding of the underlying biological mechanisms that influence adverse outcomes and drug development. Recently, they published a second update on mapping the human genetic architecture of COVID-19 (*The COVID-19 Host Genetics Initiative. A second update on mapping the human genetic architecture of COVID-19. Nature 621, E7–E26, 2023*). They performed a meta-analysis of up to 219,692 cases and over 3 million controls. They expanded the current knowledge of host genetics for COVID-19 susceptibility and severity by further doubling the case numbers from the previous data release and identifying 28 additional loci. Notably, they observed severity loci mapped to type I interferon pathway, while susceptibility loci mapped to viral entry and airway defense pathways, with notable exceptions for severity-classified TMPRSS2 and MUC5B loci.

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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. Analytical Method for Experimental Validation of Computer-Designed Antibody

Tanabe A, Tsumoto K.

In the computational design of antibodies, the interaction analysis between target antigen and antibody is an essential process to obtain feedback for validation and optimization of the design. Kinetic and thermodynamic parameters as well as binding affinity (KD) allow for a more detailed evaluation and understanding of the molecular recognition. In this chapter, we summarize the conventional experimental methods which can calculate KD value (ELISA, FP), analyze a binding activity to actual cells (FCM), and evaluate the kinetic and thermodynamic parameters (ITC, SPR, BLI), including high-throughput analysis and a recently developed experimental technique.

2. Structural Classification of CDR-H3 in Single-Domain VHH Antibodies

Kuroda D, Tsumoto K.

The immune systems protect vertebrates from foreign molecules or antigens, and antibodies are important mediators of this system. The sequences and structural features of antibodies vary depending on species. Many of antibodies from vertebrates, including camelids, have both heavy and light chain variable domains, but camelids also have antibodies that lack the light chains. In antibodies that lack light chains, the C-terminal variable region is called the VHH domain. Antibodies recognize antigens through six complementarity-determining regions (CDRs). The third CDR of the heavy chain (CDR-H3) is at the center of the antigen-binding site and is diverse in terms of sequence and structure. Due to the importance of antibodies in basic science as well as in medical applications, there have been many studies of CDR-H3s of antibodies that possess both light and

heavy chains. However, nature of CDR-H3s of single-domain VHH antibodies is less well studied. In this chapter, we describe current knowledge of sequence-structure-function correlations of single-domain VHH antibodies with emphasis on CDR-H3. Based on the 370 crystal structures in the Protein Data Bank, we also attempt structural classification of CDR-H3 in single-domain VHH antibodies and discuss lessons learned from the ever-increasing number of the structures.

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. 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, 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 malignancies.

5. Structural insights into 3Fe-4S ferredoxins diversity in M. tuberculosis highlighted by a first redox complex with P450.

Gilep A, Varaksa T, Bukhdruker S, Kavaleuski A, Ryzhykau Y, Smolskaya S, Sushko T, Tsumoto K, Grabovec I, Kapranov I, Okhrimenko I, Marin E, Shevtsov M, Mishin A, Kovalev K, Kuklin A, Gordeliy V, Kaluzhskiy L, Gnedenko O, Yablokov E, Ivanov A, Borshchevskiy V, Strushkevich N.

Ferredoxins are small iron-sulfur proteins and key players in essential metabolic pathways. Among all types, 3Fe-4S ferredoxins are less studied mostly due to anaerobic requirements. Their complexes with cytochrome P450 redox partners have not been structurally characterized. In the present work, we solved the structures of both 3Fe-4S ferredoxins from M. tuberculosis-Fdx alone and the fusion FdxE-CYP143. Our SPR analysis demonstrated a high-affinity binding of FdxE to CYP143. According to SAXS data, the same complex is present in solution. The structure reveals extended multipoint interactions and the shape/charge complementarity of redox partners. Furthermore, FdxE binding induced conformational changes in CYP143 as evident from the solved CYP143 structure alone. The comparison of FdxE-CYP143 and modeled Fdx-CYP51 complexes further revealed the specificity of ferredoxins. Our results illuminate the diversity of electron transfer complexes for the production of different secondary metabolites.

6. Peripheral administration of nanomicelle-encapsulated anti-Aβ oligomer fragment antibody reduces various toxic Aβ species in the brain

Amano A, Sanjo N, Araki W, Anraku Y, Nakakido M, Matsubara E, Tomiyama T, Nagata T, Tsumoto K, Kataoka K, Yokota

Background: Although a large amount of evidence has revealed that amyloid β (Aβ), especially Aβ oligomers, protofibrils, and pyroglutamated Aβs, participate primarily in the pathophysiological processes of Alzheimer's disease, most clinical trials of anti-Aβ antibody therapy have never acquired successful efficacy in human clinical trials, partly because peripheral administration of antibody medications was unable to deliver sufficient amounts of the molecules to the brain. Recently, we developed polymeric nanomicelles capable of passing through the blood-brain barrier that function as chaperones to deliver larger amounts of heavy molecules to the brain. Herein, we aimed to evaluate the efficacy of newly developed antibody 6H4 fragments specific to Aβ oligomers encapsulated in polymeric nanomicelles on the development of Alzheimer's disease pathology in Alzheimer's disease model mice at the age of emergence of early Alzheimer's disease pathology.

Results: During the 10-week administration of 6H4 antibody fragments in polymeric nanomicelles, a significant reduction in the amounts of various toxic Aβ species, such as Aβ oligomers, toxic Aβ conformers, and pyroglutamated Aβs in the brain was observed. In addition, immunohistochemistry indicated inhibition of diameters of Aβ plaques, Aβ-antibody immunoreactive areas, and also plaque core formation. Behavioral analysis of the mice model revealed that the 6H4 fragments-polymeric nanomicelle group was significantly better at maintaining long-term spatial reference memory in the probe and platform tests of the water maze, thereby indicating inhibition of the pathophysiological process of Alzheimer's disease.

Conclusions: The results indicated that the strategy of reducing toxic Aβ species in early dementia owing to Alzheimer's disease by providing sufficient antibodies in the brain may modify Alzheimer's disease progression.

7. Non-Affinity Purification of Antibodies

Arakawa T, Tomioka Y, Nakagawa M, Sakuma C, Kurosawa Y, Ejima D, Tsumoto K, Akuta T.

Currently, purification of antibodies is mainly carried out using a platform technology composed primarily of Protein A chromatography as a capture step, regardless of the scale. However, Protein A chromatography has a number of drawbacks, which are summarized in this review. As an alternative, we propose a simple small-scale purification protocol without Protein A that uses novel agarose native gel electrophoresis and protein extraction. For large-scale antibody purification, we suggest mixed-mode chromatography that can in part mimic the properties of Protein A resin, focusing on 4-Mercapto-ethyl-pyridine (MEP) column chromatography.

8. Inactivation and spike protein denaturation of novel coronavirus variants by CuxO/TiO2 nano-photocatalysts

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

In order to reduce infection risk of novel coronavirus (SARS-CoV-2), we developed nano-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. 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 the original and alpha variant of novel coronavirus under irradiation with fluorescent light for 3 h. The photocatalysts greatly suppressed binding ability of the receptor binding domain (RBD) of coronavirus (the original, alpha and delta variants) spike protein to the receptor of human cells. The coating also exhibited antivirus effects on influenza A virus, feline calicivirus, bacteriophage Qβ and bacteriophage M13. The photocatalysts would be applied to practical coatings and lower the risk of coronavirus infection via solid surfaces.

9. Ligand Installation to Polymeric Micelles for Pediatric Brain Tumor Targeting

Watanabe T, Mizuno HL, Norimatsu J, Obara T, Cabral H, Tsumoto K, Nakakido M, Kawauchi D, Anraku Y

Medulloblastoma is a life-threatening disease with poor therapeutic outcomes. In chemotherapy, low drug accumulation has been a cause of these outcomes. Such inadequate response to treatments has been associated with low drug accumulation, particularly with a limited cellular uptake of drugs. Recently, the conjugation of drugs to ligand molecules with high affinity to tumor cells has attracted much attention for enhancing drug internalization into target cells. Moreover, combining tumor-targeting ligands with nano-scaled drug carriers can potentially improve drug loading capacity and the versatility of the

delivery. Herein, we focused on the possibility of targeting CD276/B7-H3, which is highly expressed on the medulloblastoma cell membrane, as a strategy for enhancing the cellular uptake of ligand-installed nanocarriers. Thus, anti-CD276 antibodies were conjugated on the surface of model nanocarriers based on polyion complex micelles (PIC/m) via click chemistry. The results showed that the anti-CD276 antibody-installed PIC/m improved intracellular delivery into CD276-expressing medulloblastoma cells in a CD276-dependent manner. Moreover, increasing the number of antibodies on the surface of micelles improved the cellular uptake efficiency. These observations indicate the potential of anti-CD276 antibody-installed nanocarriers for promoting drug delivery in medulloblastoma.

10. Bromodomain protein BRD8 regulates cell cycle progression in colorectal cancer cells through a TIP60-independent regulation of the pre-RC complex

Yamaguchi K, Nakagawa S, Saku A, Isobe Y, Yamaguchi R, Sheridan P, Takane K, Ikenoue T, Zhu C, Miura M, Okawara Y, Nagatoishi S, Kozuka-Hata H, Oyama M, Aikou S, Ahiko Y, Shida D, Tsumoto K, Miyano S, Imoto S, Furukawa Y.

Bromodomain-containing protein 8 (BRD8) is a subunit of the NuA4/TIP60-histone acetyltransferase complex. Although BRD8 has been considered to act as a co-activator of the complex, its biological role remains to be elucidated. Here, we uncovered that BRD8 accumulates in colorectal cancer cells through the inhibition of ubiquitin-dependent protein degradation by the interaction with MRG domain binding protein. Transcriptome analysis coupled with genome-wide mapping of BRD8-binding sites disclosed that BRD8 transactivates a set of genes independently of TIP60, and that BRD8 regulates the expression of multiple subunits of the pre-replicative complex in concert with the activator protein-1. Depletion of BRD8 induced cell-cycle arrest at the G1 phase and suppressed cell proliferation. We have also shown that the bromodomain of BRD8 is indispensable for not only the interaction with histone H4 or transcriptional regulation but also its own protein stability. These findings highlight the importance of bromodomain as a therapeutic target.

11. Molecular mechanism underlying the increased risk of colorectal cancer metastasis caused by single nucleotide polymorphisms in LI-cadherin gene

Yui A, Kuroda D, Maruno T, Nakakido M, Nagatoishi S, Uchiyama S, Tsumoto K.

LI-cadherin is a member of the cadherin superfamily. LI-cadherin mediates Ca2+-dependent cell-cell adhesion through homodimerization. A previous study reported two single nucleotide polymorphisms (SNPs) in the LI-cadherin-coding gene (CDH17). These SNPs correspond to the amino acid changes of Lys115 to Glu and Glu739 to Ala. Patients with colorectal cancer carrying these SNPs are reported to have a higher risk of lymph node metastasis than patients without the SNPs. Although proteins associated with metastasis have been identified, the molecular mechanisms underlying the functions of these proteins remain unclear, making it difficult to develop effective strategies to prevent metastasis. In this study, we employed biochemical assays and molecular dynamics (MD) simulations to elucidate the molecular mechanisms by which the amino acid changes caused by the SNPs in the LI-cadherin-coding gene increase the risk of metastasis. Cell aggregation assays showed that the amino acid changes weakened the LI-cadherin-dependent cell-cell adhesion. In vitro assays demonstrated a decrease in homodimerization tendency and MD simulations suggested an alteration in the intramolecular hydrogen bond network by the mutation of Lys115. Taken together, our results indicate that the increased risk of lymph node metastasis is due to weakened cell-cell adhesion caused by the decrease in homodimerization tendency.

12. An Engineered Synthetic Receptor-Aptamer Pair for an Artificial Signal Transduction System.

Liu H, Baeumler TA, Nakamura K, Okada Y, Cho S, Eguchi A, Kuroda D, Tsumoto K, Ueki R, Sando S.

Cell membrane receptors regulate cellular responses through sensing extracellular environmental signals and subsequently transducing them. Receptor engineering provides a means of directing cells to react to a designated external cue and exert programmed functions. However, rational design and precise modulation of receptor signaling activity remain challenging. Here, we report an aptamer-based signal transduction system and its applications in controlling and customizing the functions of engineered receptors. A previously reported membrane receptor-aptamer pair was used to design a synthetic receptor system that transduces cell signaling depending on exogenous aptamer input. To eliminate the cross-reactivity of the receptor with its native ligand, the extracellular domain of the receptor was engineered to ensure that the receptor was solely activated by the DNA aptamer. The present system features tunability in the signaling output level using aptamer ligands with different receptor dimerization propensities. In addition, the functional programmability of DNA aptamers enables the modular sensing of extracellular molecules without the need for genetic engineering of the receptor.

13. Agarose native gel electrophoresis analysis of thermal aggregation controlled by Hofmeister series

Tomioka Y, Sato R, Takahashi R, Nagatoishi S, Shiba K, Tsumoto K, Arakawa T, Akuta T.

The effects of salting-in and salting-out salts defined by Hofmeister series on the solution state of bovine serum albumin (BSA) in 50 mM Tris-HCl buffer at pH 7.4 before and after thermal unfolding at 80 °C for 5 min were examined using agarose native gel electrophoresis and mass photometry. Gel electrophoresis showed that salting-in MgCl2, CaCl2 and NaSCN resulted in formation of intermediate structures of BSA upon heating on native gel, while heating in buffer alone resulted in aggregated bands. Mass photometry showed large loss of monomer and oligomers when heated in this buffer, but retaining these structures in the presence of 1 M MgCl2 and NaSCN. To our surprise, salting-out MgSO4 also showed a similar effect on gel electrophoresis and mass photometry. Salting-out NaCl and (NH4)2SO4 resulted in smearing and aggregated bands, which were supported by mass photometry. Aggregation-suppressive ArgHCl also showed oligomer aggregates upon gel electrophoresis and mass photometry.

14. Elucidating Conformational Dynamics and Thermostability of Designed Aromatic Clusters by Using Protein Cages

Hishikawa Y, Noya H, Nagatoishi S, Yoshidome T, Maity B, Tsumoto K, Abe S, Ueno T.

Multiple aromatic residues assemble to form higher ordered structures known as “aromatic clusters” in proteins and play essential roles in biological systems. However, the stabilization mechanism and dynamic behavior of aromatic clusters remain unclear. This study describes designed aromatic interactions confined within a protein cage to reveal how aromatic clusters affect protein stability. The crystal structures and calorimetric measurements indicate that the formation of inter-subunit phenylalanine clusters enhance the interhelix interactions and increase the melting temperature. Theoretical calculations suggest that this is caused by the transformation of the T-shaped geometry into π-π stacking at high temperatures, and the hydration entropic gain. Thus, the isolated nanoenvironment in a protein cage allows reconstruction and detailed analysis of multiple clustering residues for elucidating the mechanisms of various biomolecular interactions in nature which can be applied to design of bionanomaterials.

15. Design of single-domain VHH antibodies to increase the binding activity in SPR amine coupling

Hirao A, Nagatoishi S, Ikeuchi E, Yamawaki T, Mori C, Nakakido M, Tsumoto K.

Single-domain antibodies, or VHH, nanobodies, are attractive tools in biotechnology and pharmaceuticals due to their favorable biophysical properties. Single-domain antibodies have potential for use in sensing materials to detect antigens, and in this paper, we propose a generic design strategy of single-domain antibodies for the highly efficient use of immobilized antibodies on a sensing substrate. Amine coupling was used to immobilize the single-domain antibodies on the substrate through a robust covalent bond. First, for two model single-domain antibodies with lysines at four highly conserved positions (K48, K72, K84, and K95), we mutated the lysines to alanine and measured the binding activity of the mutants (the percentage of immobilized antibodies that can bind antigen) using surface plasmon resonance. The two model single-domain antibodies tended to have higher binding activities when K72, which is close to the antigen binding site, was mutated. Adding a Lys-tag to the C-terminus of single-domain antibodies also increased the binding activity. We also mutated the lysine for another model single-domain antibodies with the lysine in a different position than the four residues mentioned above and measured the binding activity. Thus, single-domain antibodies immobilized in an orientation accessible to the antigen tended to have a high binding activity, provided that the physical properties of the single-domain antibodies themselves (affinity and structural stability) were not significantly reduced. Specifically, the design strategy of single-domain antibodies with high binding activity included mutating the lysine at or near the antigen binding site, adding a Lys-tag to the C-terminus, and mutating a residue away from the antigen binding site to lysine. It is noteworthy that mutating K72 close to the antigen binding site was more effective in increasing the binding activity than Lys-tag addition, and immobilization at the N-terminus close to the antigen binding site did not have such a negative effect on the binding activity compared to immobilization at the K72.

16. Real-Time Search-Assisted Multiplexed Quantitative Proteomics Reveals System-Wide Translational Regulation of Non-Canonical Short Open Reading Frames

Kozuka-Hata H, Hiroki T, Miyamura N, Kitamura A, Tsumoto K, Inoue JI, Oyama M.

Abnormal expression of histone deacetylases (HDACs) is reported to be associated with angiogen-

esis, metastasis and chemotherapy resistance regarding cancer in a wide range of previous studies. Suberoylanilide hydroxamic acid (SAHA) is well known to function as a pan-inhibitor for HDACs and recognized as one of the therapeutic drug candidates to epigenetically coordinate cancer cell fate regulation on a genomic scale. Here, we established a Real-Time Search (RTS)-assisted mass spectrometric platform for system-wide quantification of translated products encoded by non-canonical short open reading frames (ORFs) as well as already annotated protein coding sequences (CDSs) on the human transciptome and applied this methodology to quantitative proteomic analyses of suberoylanilide hydroxamic acid (SAHA)-treated human HeLa cells to evaluate proteome-wide regulation in response to drug perturbation. Very intriguingly, our RTS-based in-depth proteomic analysis enabled us to identify approximately 5000 novel peptides from the ribosome profiling-based short ORFs encoded in the diversified regions on presumed 'non-coding' nucleotide sequences of mRNAs as well as lncRNAs and nonsense mediated decay (NMD) transcripts. Furthermore, TMT-based multiplex large-scale quantification of the whole proteome changes upon differential SAHA treatment unveiled dose-dependent selective translational regulation of a limited fraction of the non-canonical short ORFs in addition to key cell cycle/proliferation-related molecules such as UBE2C, CENPF and PRC1. Our study provided the first system-wide landscape of drug-perturbed translational modulation on both canonical and non-canonical proteome dynamics in human cancer cells.

17. Targeting hemoglobin receptors IsdH and IsdB of Staphylococcus aureus with a single VHH antibody inhibits bacterial growth

Valenciano-Bellido S, Caaveiro JMM, Nakakido M, Kuroda D, Aikawa C, Nakagawa I, Tsumoto K.

Methicillin-resistant Staphylococcus aureus, or MRSA, is one of the major causative agents of hospital-acquired infections worldwide. Novel antimicrobial strategies efficient against antibiotic-resistant strains are necessary and not only against S. aureus. Among those, strategies that aim at blocking or dismantling proteins involved in the acquisition of essential nutrients, helping the bacteria to colonize the host, are intensively studied. A major route for S. aureus to acquire iron from the host organism is the Isd (iron surface determinant) system. In particular, the hemoglobin receptors IsdH and IsdB located on the surface of the bacterium are necessary to acquire the heme moiety containing iron, making them a plausible antibacterial target. Herein, we obtained an antibody of camelid origin that blocked heme acquisition. We determined that the antibody recognized the heme-binding pocket of both IsdH and IsdB with nanomolar order affinity through its second and third complementary-determining regions. The mechanism explaining the inhibition of acquisition of heme in vitro could be described as a competitive process in which the complementary-determining region 3 from the antibody blocked the acquisition of heme by the bacterial receptor. Moreover, this antibody markedly reduced the growth of three different pathogenic strains of MRSA. Collectively, our results highlight a mechanism for inhibiting nutrient uptake as an antibacterial strategy against MRSA.

18. A Proximity-Induced Fluorogenic Reaction Triggered by Antibody-Antigen Interactions with Adjacent Epitopes

Nishiyama K, Akiba H, Nagata S, Tsumoto K, Kamada H, Ohno H.

Proximity-induced chemical reactions are site-specific and rapid by taking advantage of their high affinity and highly selective interactions with the template. However, reactions induced solely by antibody-antigen interactions have not been developed. Herein, we propose a biepitopic antigen-templated chemical reaction (BATER) as a novel template reaction. In BATER, reactive functional groups are conjugated to two antibodies that interact with two epitopes of the same antigen to accelerate the reaction. We developed a method for visualizing the progress of BATER using fluorogenic click chemistry for optimal antibody selection and linker design. The reaction is accelerated in the presence of a specific antigen in a linker length-dependent manner. The choice of the antibody epitope is important for a rapid reaction. This design will lead to various applications of BATER in living systems.

19. Raman Spectroscopic Analysis of Highly-Concentrated Antibodies under the Acid-Treated Conditions

Sato Y, Nagatoishi S, Noguchi S, Tsumoto K.

Purpose: Antibody drugs are usually formulated as highly-concentrated solutions, which would easily generate aggregates, resulting in loss of efficacy. Although low pH increases the colloidal dispersion of antibodies, acid denaturation can be an issue. Therefore, knowing the physical properties at low pH under high concentration conditions is important.

Methods: Raman spectroscopy was used to investigate pH-induced conformational changes of antibodies at 50 mg/ml. Experiments in pH 3 to 7 were performed for human serum IgG and recombinant rituximab.

Results: We detected the evident changes at pH 3

in Tyr and Trp bands, which are the sensitive markers of intermolecular interactions. Thermal transition analysis over the pH range demonstrated that the thermal transition temperature (Tm) was highest at pH 3. Acid-treated and neutralized one showed higher Tm than that of pH 7, indicating that their extent of intermolecular interactions correlated with the Tm values. Onset temperature was clearly different between concentrated and diluted samples. Colloidal analyses confirmed the findings of the Raman analysis.

Conclusion: Our studies demonstrated the positive correlation between Raman analysis and colloidal information, validating as a method for evaluating antibody conformation associated with aggregation propensities.

20. Histone H3 lysine 27 crotonylation mediates gene transcriptional repression in chromatin

Liu N, Konuma T, Sharma R, Wang D, Zhao N, Cao L, Ju Y, Liu D, Wang S, Bosch A, Sun Y, Zhang S, Ji D, Nagatoishi S, Suzuki N, Kikuchi M, Wakamori M, Zhao C, Ren C, Zhou TJ, Xu Y, Meslamani J, Fu S, Umehara T, Tsumoto K, Akashi S, Zeng L, Roeder RG, Walsh MJ, Zhang Q, Zhou MM.

Histone lysine acylation, including acetylation and crotonylation, plays a pivotal role in gene transcription in health and diseases. However, our understanding of histone lysine acylation has been limited to gene transcriptional activation. Here, we report that histone H3 lysine 27 crotonylation (H3K27cr) directs gene transcriptional repression rather than activation. Specifically, H3K27cr in chromatin is selectively recognized by the YEATS domain of GAS41 in complex with SIN3A-HDAC1 co-repressors. Proto-oncogenic transcription factor MYC recruits GAS41/SIN3A-HDAC1 complex to repress genes in chromatin, including cell-cycle inhibitor p21. GAS41 knockout or H3K27cr-binding depletion results in p21 de-repression, cell-cycle arrest, and tumor growth inhibition in mice, explaining a causal relationship between GAS41 and MYC gene amplification and p21 downregulation in colorectal cancer. Our study suggests that H3K27 crotonylation signifies a previously unrecognized, distinct chromatin state for gene transcriptional repression in contrast to H3K27 trimethylation for transcriptional silencing and H3K27 acetylation for transcriptional activation.

21. Dispersion Function of a Protein, DP-1, Identified in Collimonas sp. D-25, for the Synthesis of Gold Nanoparticles

Tang D, Kato Y, Zhang D, Negishi L, Kurumizaka H, Hirata T, Nakakido M, Tsumoto K, Shuji F, Tsuguyuki S, Okumura T, Nagata K, Suzuki M.

Collimonas sp. (D-25), found in the soil of Akita Prefecture, is a gram-negative bacterium with the ability to synthesize gold nanoparticles (AuNPs). During the synthesis of AuNPs, one specific protein (DP-1) was found to have disappeared in the sonicated solution of the bacterium. Recombinant DP-1 (rDP-1) from Escherichia coli BL21 (DE3) was used to study the effect of DP-1 on the synthesis of AuNPs. AuNPs synthesized with rDP-1 result in small, stabilized nanoparticles. AuNPs synthesized by DP-1 retained the stability of both the dispersion and nano-size particles under high salt concentrations. Isothermal titration calorimetry was employed to investigate the bonding ratio of rDP-1 to AuNPs. Several thousand rDP-1 proteins are attached to the surface of an AuNP to form a protein corona containing multiple layers. These results suggest that DP-1 obtained from D-25 has a size and stability control function during AuNP synthesis.

22. Development of a 1:1-binding biparatopic anti-TNFR2 antagonist by reducing signaling activity through epitope selection

Akiba H, Fujita J, Ise T, Nishiyama K, Miyata T, Kato T, Namba K, Ohno H, Kamada H, Nagata S, Tsumoto K

Conventional bivalent antibodies against cell surface receptors often initiate unwanted signal transduction by crosslinking two antigen molecules. Biparatopic antibodies (BpAbs) bind to two different epitopes on the same antigen, thus altering crosslinking ability. In this study, we develop BpAbs against tumor necrosis factor receptor 2 (TNFR2), which is an attractive immune checkpoint target. Using different pairs of antibody variable regions specific to topographically distinct TNFR2 epitopes, we successfully regulate the size of BpAb-TNFR2 immunocomplexes to result in controlled agonistic activities. Our series of results indicate that the relative positions of the two epitopes recognized by the BpAb are critical for controlling its signaling activity. One particular antagonist, Bp109-92, binds TNFR2 in a 1:1 manner without unwanted signal transduction, and its structural basis is determined using cryo-electron microscopy. This antagonist suppresses the proliferation of regulatory T cells expressing TNFR2. Therefore, the BpAb format would be useful in designing specific and distinct antibody functions.

23. Discovery of a cystathionine γ-lyase (CSE) selective inhibitor targeting active-site pyridoxal 5'-phosphate (PLP) via Schiff base formation

Echizen H, Hanaoka K, Shimamoto K, Hibi R, Toma-Fukai S, Ohno H, Sasaki E, Komatsu T, Ueno T, Tsuchiya Y, Watanabe Y, Otsuka T, Saito H, Nagat-

oishi S, Tsumoto K, Kojima H, Okabe T, Shimizu T, Urano Y.

D,L-Propargylglycine (PAG) has been widely used as a selective inhibitor to investigate the biological functions of cystathionine γ-lyase (CSE), which catalyzes the formation of reactive sulfur species (RSS). However, PAG also inhibits other PLP (pyridoxal-5′-phosphate)-dependent enzymes such as methionine γ-lyase (MGL) and L-alanine transaminase (ALT), so highly selective CSE inhibitors are still required. Here, we performed high-throughput screening (HTS) of a large chemical library and identified oxamic hydrazide 1 as a potent inhibitor of CSE (IC50 = 13 ± 1 μM (mean ± S.E.)) with high selectivity over other PLP-dependent enzymes and RSS-generating enzymes. Inhibitor 1 inhibited the enzymatic activity of human CSE in living cells, indicating that it is sufficiently membrane-permeable. X-Ray crystal structure analysis of the complex of rat CSE (rCSE) with 1 revealed that 1 forms a Schiff base linkage with the cofactor PLP in the active site of rCSE. PLP in the active site may be a promising target for development of selective inhibitors of PLP-dependent enzymes, including RSS-generating enzymes such as cystathionine β-synthase (CBS) and cysteinyl-tRNA synthetase 2 (CARS2), which have unique substrate binding pocket structures.

24. Modulation of a conformational ensemble by a small molecule that inhibits key protein-protein interactions involved in cell adhesion

Senoo A, Nagatoishi S, Kuroda D, Ito S, Ueno G, Caaveiro JMM, Tsumoto K.

Small molecules that regulate protein-protein interactions can be valuable drugs; however, the development of such small molecules is challenging as the molecule must interfere with an interaction that often involves a large surface area. Herein, we propose that modulating the conformational ensemble of the proteins participating in a given interaction, rather than blocking the interaction by directly binding to the interface, is a relevant strategy for interfering with a protein-protein interaction. In this study, we applied this concept to P-cadherin, a cell surface protein forming homodimers that are essential for cell-cell adhesion in various biological contexts. We first determined the crystal structure of P-cadherin with a small molecule inhibitor whose inhibitory mechanism was unknown. Molecular dynamics simulations suggest that the inhibition of cell adhesion by this small molecule results from modulation of the conformational ensemble of P-cadherin. Our study demonstrates the potential of small molecules altering the conformation ensemble of a protein as inhibitors of biological relevant protein-protein interactions.

25. Arginine cluster introduction on framework region in anti-lysozyme antibody improved association rate constant by changing conformational diversity of CDR loops

Maeta S, Nakakido M, Matsuura H, Sakai N, Hirata K, Kuroda D, Fukunaga A, Tsumoto K.

Antibodies are used for many therapeutic and biotechnological purposes. Because the affinity of an antibody to the antigen is critical for clinical efficacy of pharmaceuticals, many affinity maturation strategies have been developed. Although we previously reported an affinity maturation strategy in which the association rate of the antibody toward its antigen is improved by introducing a cluster of arginine residues into the framework region of the antibody, the detailed molecular mechanism responsible for this improvement has been unknown. In this study, we introduced five arginine residues into an anti-hen egg white lysozyme antibody (HyHEL10) Fab fragment to create the R5-mutant and comprehensively characterized the interaction between antibody and antigen using thermodynamic analysis, X-ray crystallography, and molecular dynamics (MD) simulations. Our results indicate that introduction of charged residues strongly enhanced the association rate, as previously reported, and the antibody-antigen complex structure was almost the same for the R5-mutant and wild-type Fabs. The MD simulations indicate that the mutation increased conformational diversity in complementarity-determining region loops and thereby enhanced the association rate. These observations provide the molecular basis of affinity maturation by R5 mutation.

26. Group A Streptococcus cation diffusion facilitator proteins contribute to immune evasion by regulating intracellular metal concentrations

Aikawa C, Shimizu A, Nakakido M, Murase K, Nozawa T, Tsumoto K, Nakagawa I.

Cation diffusion facilitators (CDFs) are a large family of divalent metal transporters with broad specificities that contribute to intracellular metal homeostasis and toxicity in bacterial pathogens. Streptococcus pyogenes (Group A Streptococcus [GAS]) expresses two homologous CDF efflux transporters, MntE and CzcD, which selectively transport Mn and Zn, respectively. We discovered that the MntE- and CzcD-deficient strains exhibited a marked decrease in the viability of macrophage-differentiated THP-1 cells and neutrophils. In addition, the viability of mice infected with both deficient strains markedly increased. Consistent with a previous study, our results suggest that MntE regulates the PerR-dependent oxidative stress response by maintaining intracellular

Mn levels and contributing to the growth of GAS. The maturation and proteolytic activity of streptococcal cysteine protease (SpeB), an important virulence factor in GAS, has been reported to be abrogated by zinc and copper. Zn inhibited the maturation and proteolytic activity of SpeB in the culture supernatant of the CzcD-deficient strain. Furthermore, Mn inhibited SpeB maturation and proteolytic activity in a MntE-deficient strain. Since the host pathogenicity of the SpeB-deficient strain was significantly reduced, maintenance of intracellular manganese and zinc levels in the GAS via MntE and CzcD may not only confer metal resistance to the bacterium, but may also play an essential role in its virulence. These findings provide new insights into the molecular mechanisms of pathogenicity, which allow pathogens to survive under stressful conditions associated with elevated metal ion concentrations during host infection.

27. Ferguson plot analysis of multiple intermediate species of thermally unfolded bovine serum albumin

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

Ferguson plot was used to characterize the multiple intermediate species of bovine serum albumin (BSA) upon thermal unfolding. Differential scanning calorimetry showed an irreversible melting of BSA in Tris-HCl and phosphate buffers with a mid-transition temperature, Tm, of ~68 °C. Thermally unfolded BSA was analyzed by agarose native gel electrophoresis stained by Coomassie blue and SYPRO Orange staining as a function of pH or protein concentration. SYPRO Orange was used to stain unfolded proteins. BSA heated at 70 and 80 °C, i.e., above the Tm, formed multiple intermediate species, which depended on the pH between 7.0 and 8.0, protein concentration and which buffer was used. These intermediate species were analyzed by Ferguson plot, which showed that BSA heated at 60 °C had a similar size to the native BSA, indicating that they are either native or native-like state consistent with no SYPRO Orange staining. The intermediate species observed at higher temperatures with the mobility less than that of the native BSA showed a steeper Ferguson plot and were stained by SYPRO Orange, indicating that these species had a larger hydrodynamic size than the native BSA and were unfolded.

28. Anti-InlA single-domain antibodies that inhibit the cell invasion of Listeria monocytogenes

Yamazaki T, Nagatoishi S, Yamawaki T, Nozawa T, Matsunaga R, Nakakido M, Caaveiro JMM, Nakagawa I, Tsumoto K.

Listeriosis, caused by infection with Listeria monocytogenes, is a severe disease with a high mortality rate. The L. monocytogenes virulence factor, internalin family protein InlA, which binds to the host receptor E-cadherin, is necessary to invade host cells. Here, we isolated two single-domain antibodies (VHHs) that bind to InlA with picomolar affinities from an alpaca immune library using the phage display method. These InlA-specific VHHs inhibited the binding of InlA to the extracellular domains of E-cadherin in vitro as shown by biophysical interaction analysis. Furthermore, we determined that the VHHs inhibited the invasion of L. monocytogenes into host cells in culture. High-resolution X-ray structure analyses of the complexes of VHHs with InlA revealed that the VHHs bind to the same binding site as E-cadherin against InlA. We conclude that these VHHs have the potential for use as drugs to treat listeriosis.

29. PRELP secreted from mural cells protects the function of blood brain barrier through regulation of endothelial cell-cell integrity

Davaapil H, Hopkins J, Bonnin N, Papadaki V, Leung A, Kosuge H, Tashima T, Nakakido M, Sekido R, Tsumoto K, Sagoo MS, Ohnuma SI.

Introduction: Proline/arginine-rich end leucine-rich repeat protein (PRELP), is a small secreted proteoglycan expressed by pericytes and vascular smooth muscle cells surrounding the brain vasculature of adult mouse. Methods: We utilised a Prelp knockout (Prelp -/-) mouse model to interrogate vasculature integrity in the brain alongside performing in vitro assays to characterise PRELP application to endothelial cells lines. Our findings were supplemented with RNA expression profiling to elucidate the mechanism of how PRELP maintains neurovasculature function. Results: Prelp -/- mice presented with neuroinflammation and reducedneurovasculature integrity, resulting in IgG and dextran leakage in the cerebellum and cortex. Histological analysis of Prelp -/- mice revealed reducedcell-cell integrity of the blood brain barrier, capillary attachment of pericytes andastrocyte end-feet. RNA-sequencing analysis found that cell-cell adhesion andinflammation are affected in Prelp -/- mice and gene ontology analysis as well as gene set enrichment analysis demonstrated that inflammation related processes and adhesion related processes such as epithelial-mesenchymal transition and apical junctions were significantly affected, suggesting PRELP is a regulator of cell-cell adhesion. Immunofluorescence analysis showed that adhesion junction protein expression levels of cadherin, claudin-5, and ZO-1, was suppressed in Prelp -/- mice neurovasculature. Additionally, in vitro studies revealed that PRELP application to endothelial cells enhances cell-cell integrity, induces mesenchymal-endothelial transition and inhibits TGF-β mediated damage to cell-cell adhesion. Discussion: Our study

indicates that PRELP is a novel endogenous secreted regulator of neurovasculature integrity and that PRELP application may be a potential treatment for diseases associated with neurovascular damage.

30. Specific peptide conjugation to a therapeutic antibody leads to enhanced therapeutic potency and thermal stability by reduced Fc dynamics

Kiyoshi M, Nakakido M, Rafique A, Tada M, Aoyama M, Terao Y, Nagatoishi S, Shibata H, Ide T, Tsumoto K, Ito Y, Ishii-Watabe A.

Antibody-drug conjugates are powerful tools for combatting a wide array of cancers. Drug conjugation to a therapeutic antibody often alters molecular characteristics, such as hydrophobicity and effector function, resulting in quality deterioration. To develop a drug conjugation methodology that maintains the molecular characteristics of the antibody, we engineered a specific peptide for conjugation to the Fc region. We used trastuzumab and the chelator (DOTA) as model antibody and payload, respectively. Interestingly, peptide/DOTA-conjugated trastuzumab exhibited enhanced antibody-dependent cellular cytotoxicity (ADCC) and increased thermal stability. Detailed structural and thermodynamic analysis clarified that the conjugated peptide blocks the Fc dynamics like a "wedge." We revealed that (1) decreased molecular entropy results in enhanced ADCC, and (2) blockade of Fc denaturation results in increased thermal stability. Thus, we believe that our methodology is superior not only for drug conjugation but also as for reinforcing therapeutic antibodies to enhance ADCC and thermal stability.

31. One-pot preparation of mannan-coated antigen nanoparticles using human serum albumin as a matrix for tolerance induction

Li S, Murakami D, Nagatoishi S, Liu Y, Tsumoto K, Katayama Y, Mori T.

Nanoparticles (NPs) for allergen immunotherapy have garnered attention for their high efficiency and safety compared with naked antigen proteins. In this work, we present mannan-coated protein NPs, incorporating antigen proteins for antigen-specific tolerance induction. The heat-induced formation of protein NPs is a one-pot preparation method and can be applied to various proteins. Here, the NPs were formed spontaneously via heat denaturation of three component proteins: an antigen protein, human serum albumin (HSA) as a matrix protein, and mannoprotein (MAN) as a targeting ligand for dendritic cells (DCs). HSA is non-immunogenic, therefore suitable as a matrix protein, while MAN coats the surface of the NP. We applied this method to various antigen proteins and found that the self-disperse after heat denaturation was a requirement for incorporation into the NPs. We also established that the NPs could target DCs, and the incorporation of rapamycin into the NPs enhanced the induction of a tolerogenic phenotype of DC. The MAN coating provided steric hindrance and heat denaturation destroyed recognition structures, successfully preventing anti-antigen antibody binding, indicating the NPs may avoid anaphylaxis induction. The MAN-coated NPs proposed here, prepared by a simple method, have the potential for effective and safe allergies treatment for various antigens.

32. Biophysical insight into protein-protein interactions in the Interleukin-11/Interleukin-11Rα/glycoprotein 130 signaling complex

Mori C, Nagatoishi S, Matsunaga R, Kuroda D, Nakakido M, Tsumoto K.

Interleukin-11 (IL-11) is a member of the interleukin-6 (IL-6) family of cytokines. IL-11 is a regulator of multiple events in hematopoiesis, and IL-11-mediated signaling is implicated in inflammatory disease, cancer, and fibrosis. All IL-6 family cytokines signal through the signal-transducing receptor, glycoprotein 130 (gp130), but these cytokines have distinct as well as overlapping biological functions. To understand IL-11 signaling at the molecular level, we performed a comprehensive interaction analysis of the IL-11 signaling complex, comparing it with the IL-6 complex, one of the best-characterized cytokine complexes. Our thermodynamic analysis revealed a clear difference between IL-11 and IL-6. Surface plasmon resonance analysis showed that the interaction between IL-11 and IL-11 receptor α (IL-11Rα) is entropy driven, whereas that between IL-6 and IL-6 receptor α (IL-6Rα) is enthalpy driven. Our analysis using isothermal titration calorimetry revealed that the binding of gp130 to the IL-11/IL-11Rα complex results in entropy loss, but that the interaction of gp130 with the IL-6/IL-6Rα complex results in entropy gain. Our hydrogen-deuterium exchange mass spectrometry experiments suggested that the D2 domain of gp130 was not involved in IL-6-like interactions in the IL-11/IL-11Rα complex. It has been reported that IL-6 interaction with gp130 in the signaling complex was characterized through the hydrophobic interface located in its D2 domain of gp130. Our findings suggest that unique interactions of the IL-11 signaling complex with gp130 are responsible for the distinct biological activities of IL-11 compared to IL-6.

33. High-throughput analysis system of interaction kinetics for data-driven antibody design

Matsunaga R, Ujiie K, Inagaki M, Fernández Pérez J, Yasuda Y, Mimasu S, Soga S, Tsumoto K.

Surface plasmon resonance (SPR) is widely used for antigen-antibody interaction kinetics analysis. However, it has not been used in the screening phase because of the low throughput of measurement and analysis. Herein, we proposed a high-throughput SPR analysis system named "BreviA" using the Brevibacillus expression system. Brevibacillus was transformed using a plasmid library containing various antibody sequences, and single colonies were cultured in 96-well plates. Sequence analysis was performed using bacterial cells, and recombinant antibodies secreted in the supernatant were immobilized on a sensor chip to analyze their interactions with antigens using high-throughput SPR. Using this system, the process from the transformation to 384 interaction analyses can be performed within a week. This system utility was tested using an interspecies specificity design of an anti-human programmed cell death protein 1 (PD-1) antibody. A plasmid library containing alanine and tyrosine mutants of all complementarity-determining region residues was generated. A high-throughput SPR analysis was performed against human and mouse PD-1, showing that the mutation in the specific region enhanced the affinity for mouse PD-1. Furthermore, deep mutational scanning of the region revealed two mutants with > 100-fold increased affinity for mouse PD-1, demonstrating the potential efficacy of antibody design using data-driven approach.

34. Experimental modification in thermal stability of oligomers by alanine substitution and site saturation mutagenesis of interfacial residues

Hoya M, Matsunaga R, Nagatoishi S, Tsumoto K.

For certain industrial applications, the stability of protein oligomers is important. In this study, we demonstrated an efficient method to improve the thermal stability of oligomers using the trimeric protein chloramphenicol acetyltransferase (CAT) as the model. We substituted all interfacial residues of CAT with alanine to detect residues critical for oligomer stability. Mutation of six of the forty-nine interfacial residues enhanced oligomer thermal stability. Site saturation mutagenesis was performed on these six residues to optimize the side chains. About 15% of mutations enhanced thermal stability by more than 0.5 °C and most did not disrupt activity of CAT. Certain combinations of mutations further improved thermal stability and resistance against heat treatment. The quadruple mutant, H17V/N34S/F134A/D157C, retained the same activity as the wild-type after heat treatment at 9 °C higher temperature than the wild-type CAT. Furthermore, combinations with only alanine substitutions also improved thermal stability, suggesting the method we developed can be used for rapid modification of industrially important proteins.

35. Quantitative analysis of antibody aggregates by combination of pinched-flow fractionation and coulter method

Nagatoishi S, Toyoshima T, Furukawa K, Tsumoto K.

For the pharmaceutical development of proteins, multiple methods of analysis are recommended for evaluating aggregation, and the development of more quantitative and simpler analytical techniques for subvisible particles is expected. This study introduces the Pinched-Flow Fractionation (PFF)-Coulter method, which combines the Pinched-flow fractionation (PFF) and Coulter methods to analyze the concentration of submicron-sized particles. The PFF method separates the particles by size. Separated particles were individually detected using the Coulter method. We have utilized the PFF-Coulter method to quantitatively analyze particle concentrations using standard particles, evaluate detection limits, variability, and correlation between theoretical and measured values, and analyze mixtures of different particle sizes. The PFF-Coulter method allows for quantitatively analyzing of particle sizes from 0.2 to 2.0 μm. The quantifiable weight concentration range was 2.5 × 10-2 - 50 μg/mL, and the number concentration range was 104-1010 particles/mL. The sample volume was small (<10 μL). The PFF-Coulter method is capable of quantitative analysis that complements data from conventional measurement techniques, and when used in conjunction with existing submicron-size particle analysis techniques, will enable more accurate particle analysis.

36. Megalin is involved in angiotensinogen-induced, angiotensin II-mediated ERK1/2 signaling to activate Na + -H + exchanger 3 in proximal tubules

Goto S, Yoshida Y, Hosojima M, Kuwahara S, Kabasawa H, Aoki H, Iida T, Sawada R, Ugamura D, Yoshizawa Y, Takemoto K, Komochi K, Kobayashi R, Kaseda R, Yaoita E, Nagatoishi S, Narita I, Tsumoto K, Saito A.

Background: Kidney angiotensin (Ang) II is produced mainly from liver-derived, glomerular-filtered angiotensinogen (AGT). Podocyte injury has been reported to increase the kidney Ang II content and induce Na + retention depending on the function of megalin, a proximal tubular endocytosis receptor. However, how megalin regulates the renal content and action of Ang II remains elusive.

Methods: We used a mass spectrometry-based, parallel reaction-monitoring assay to quantitate Ang

II in plasma, urine, and kidney homogenate of kidney-specific conditional megalin knockout (MegKO) and control (Ctl) mice. We also evaluated the pathophysiological changes in both mouse genotypes under the basal condition and under the condition of increased glomerular filtration of AGT induced by administration of recombinant mouse AGT (rec-mAGT).

Results: Under the basal condition, plasma and kidney Ang II levels were comparable in the two mouse groups. Ang II was detected abundantly in fresh spot urine in conditional MegKO mice. Megalin was also found to mediate the uptake of intravenously administered fluorescent Ang II by PTECs. Administration of rec-mAGT increased kidney Ang II, exerted renal extracellular signal-regulated kinase 1/2 (ERK1/2) signaling, activated proximal tubular Na + -H + exchanger 3 (NHE3), and decreased urinary Na + excretion in Ctl mice, whereas these changes were suppressed but urinary Ang II was increased in conditional MegKO mice.

Conclusion: Increased glomerular filtration of AGT is likely to augment Ang II production in the proximal tubular lumen. Thus, megalin-dependent Ang II uptake should be involved in the ERK1/2 signaling that activates proximal tubular NHE3 in vivo , thereby causing Na + retention.

37. Safe and efficient oral allergy immunotherapy using one-pot-prepared mannan-coated allergen nanoparticles

Li S, Toriumi H, Takahashi D, Kamasaki T, Fujioka Y, Nagatoishi S, Li J, Liu Y, Hosokawa T, Tsumoto K, Ohba Y, Katayama Y, Murakami D, Hase K, Mori T.

Allergen immunotherapy (AIT) is the only curative treatment for allergic diseases. However, AIT has many disadvantages related to efficiency, safety, long-term duration, and patient compliance. Dendritic cells (DCs) have an important role in antigen-specific tolerance induction; thus, DC-targeting strategies to treat allergies such as glutaraldehyde crosslinked antigen to mannoprotein (MAN) have been established. However, glutaraldehyde crosslinking may reduce the antigen presentation efficiency of DCs. To overcome this, we developed a MAN-coated ovalbumin (OVA) nanoparticle (MDO), which uses intermolecular disulfide bond to crosslink OVA and MAN. MDO effectively targeted DCs resulting in tolerogenic DCs, and promoted higher antigen presentation efficiency by DCs compared with OVA or glutaraldehyde crosslinked nanoparticles. In vitro and in vivo experiments showed that DCs exposed to MDO induced Treg cells. Moreover, MDO had low reactivity with anti-OVA antibodies and did not induce anaphylaxis in allergic mice, demonstrating its high safety profile. In a mouse model of allergic asthma, MDO had significant preventative and therapeutic effects when administered orally or subcutaneously. Therefore, MDO represents a promising new approach for the efficient and safe treatment of allergies.

38. Enhancing thermal stability in the CH2 domain to suppress aggregation through the introduction of simultaneous disulfide bonds in Pichia pastoris

Oyama K, Nakakido M, Ohkuri T, Nakamura H, Tsumoto K, Ueda T.

Protein aggregations decrease production yields and impair the efficacy of therapeutics. The CH2 domain is a crucial part of the constant region of human IgG. But, it is also the least stable domain in IgG, which can result in antibody instability and aggregation problems. We created a novel mutant of the CH2 domain (T250C/L314C, mut10) by introducing a disulfide bond and expressed it using Pichia pastoris. The mut10 variant exhibited enhanced thermal stability, resistance to enzymatic degradation, and reduced aggregation in comparison to the original CH2 domain. However, it was less stable than mut20 (L242C/K334C), which is the variant prepared in a previous study (Gong et al., J. Biol. Chem., 2009). A more advanced mutant, mut25, was created by combining mut10 and mut20. Mut25 artificially contains two disulfide bonds. The new mutant, mut25, showed enhanced thermal stability, increased resistance to enzymatic digestion, and reduced aggregation in comparison to mut20. According to our knowledge, mut25 achieves an unprecedented level of stability among the humanized whole CH2 domains that have been reported so far. Mut25 has the potential to serve as a new platform for antibody therapeutics due to its ability to reduce immunogenicity by decreasing aggregation.

39. Conformational features and interaction mechanisms of VH H antibodies with β-hairpin CDR3: A case of Nb8-HigB2 interaction

Yamamoto K, Nagatoishi S, Matsunaga R, Nakakido M, Kuroda D, Tsumoto K.

The β-hairpin conformation is regarded as an important basic motif to form and regulate protein-protein interactions. Single-domain VH H antibodies are potential therapeutic and diagnostic tools, and the third complementarity-determining regions of the heavy chains (CDR3s) of these antibodies are critical for antigen recognition. Although the sequences and conformations of the CDR3s are diverse, CDR3s sometimes adopt β-hairpin conformations. However,

characteristic features and interaction mechanisms of β-hairpin CDR3s remain to be fully elucidated. In this study, we investigated the molecular recognition of the anti-HigB2 VH H antibody Nb8, which has a CDR3 that forms a β-hairpin conformation. The interaction was analyzed by evaluation of alanine-scanning mutants, molecular dynamics simulations, and hydrogen/deuterium exchange mass spectrometry. These experiments demonstrated that positions 93 and 94 (Chothia numbering) in framework region 3, which is right outside CDR3 by definition, play pivotal roles in maintaining structural stability and binding properties of Nb8. These findings will facilitate the design and optimization of single-domain antibodies.

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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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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 2023, we reported various papers related to immunology on vaccine and adjuvant R&D.

1. *TLR9 plus STING Agonist Adjuvant Combination Induces Potent Neopeptide T Cell Immunity and Improves Immune Checkpoint Blockade Efficacy in a Tumor Model.*

Immune checkpoint blockade (ICB) immunotherapies have emerged as promising strategies for the treatment of cancer; however, there remains a need to improve their efficacy. Determinants of ICB efficacy are the frequency of tumor mutations, the associated neoantigens, and the T cell response against them. Therefore, it is expected that neoantigen vaccinations that boost the antitumor T cell response would improve ICB therapy efficacy. The aim of this study was to develop a highly immunogenic vaccine using pattern recognition receptor agonists in combination with synthetic long peptides to induce potent neoantigen-specific T cell responses. We determined that the combination of the TLR9 agonist K-type CpG oligodeoxynucleotides (K3 CpG) with the STING agonist c-di-AMP (K3/c-di-AMP combination) significantly increased dendritic cell activation. We found that immunizing mice with 20-mer of either an OVA peptide, low-affinity OVA peptides, or neopeptides identified from mouse melanoma or lung mesothelioma, together with K3/c-di-AMP, induced potent Ag-specific T cell responses. The combined K3/c-di-AMP adjuvant formulation induced 10 times higher T cell responses against neopeptides than the TLR3 agonist polyinosinic:polycytidylic acid, a derivative of which is the leading adjuvant in clinical trials of neoantigen peptide vaccines. Moreover, we demonstrated that our K3/c-di-AMP vaccine formulation with 20-mer OVA peptide was capable of controlling tumor growth and improving survival in B16-F10-OVA tumor-bearing C57BL/6 mice and synergized with anti-PD-1 treatment. Together, our findings demonstrate that the K3/c-di-AMP vaccine formulation induces potent T cell immunity against synthetic long peptides and is a promising candidate to improve neoantigen vaccine platform.

2. *TLR4 agonist activity of Alcaligenes lipid a utilizes MyD88 and TRIF signaling pathways for efficient antigen presentation and T cell differentiation by dendritic cells.*

Alcaligenes faecalis was previously identified as

an intestinal lymphoid tissue-resident commensal bacteria, and our subsequent studies showed that lipopolysaccharide and its core active element (i.e., lipid A) have a potent adjuvant activity to promote preferentially antigen-specific Th17 response and antibody production. Here, we compared A. faecalis lipid A (ALA) with monophosphoryl lipid A, a licensed lipid A-based adjuvant, to elucidate the immunological mechanism underlying the adjuvant properties of ALA. Compared with monophosphoryl lipid A, ALA induced higher levels of MHC class II molecules and costimulatory CD40, CD80, and CD86 on dendritic cells (DCs), which in turn resulted in strong T cell activation. Moreover, ALA more effectively promoted the production of IL-6 and IL-23 from DCs than did monophosphoryl lipid A, thus leading to preferential induction of Th17 and Th1 cells. As underlying mechanisms, we found that the ALA-TLR4 axis stimulated both MyD88- and TRIF-mediated signaling pathways, whereas monophosphoryl lipid A was biased toward TRIF signaling. These findings revealed the effects of ALA on DCs and T cells and its induction pattern on signaling pathways.

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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

Project Professor　Kohtaro Fujihashi, D.D.S., Ph.D.　特任教授　博士（歯学）　藤　橋　浩太郎

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 showed that mice deficient with this molecule resulted in reduced levels of antigen-specific IgA antibody responses in the vaginal washes despite intact levels of serum IgG titers. Further, we are currently confirming these results at the cellular level. When we have assessed chemokine receptor expression which involved for the regulation of antigen-specific IgA responses, none of these receptors are essential for the regulation of mucosal IgA antibody responses.

2. Cationic-nanogel nasal vaccine containing the ectodomain of RSV-small hydrophobic protein induces protective immunity in rodents

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Respiratory syncytial virus (RSV) is a leading cause of upper and lower respiratory tract infection, especially in children and the elderly. Various vaccines containing the major transmembrane surface proteins of RSV (proteins F and G) have been tested; however, they either afford inadequate protection or are associated with the risk of vaccine-enhanced disease (VED). Here, we examined the potential of using the ectodomain of small hydrophobic protein (SHe), also an RSV transmembrane surface protein, as a nasal vaccine antigen. A vaccine was formulated using our previously developed cationic cholesteryl-group-bearing pullulan nanogel as the delivery system, and SHe was linked in triplicate to pneumococcal surface protein A as a carrier protein. Nasal immunization of mice and rats induced both SHe-specific serum IgG and mucosal IgA antibodies, preventing viral invasion in both the upper and lower respiratory tracts without inducing VED. Moreover, nasal immunization induced greater protective immunity against RSV in the upper respiratory tract than did systemic immunization, suggesting a critical role for mucosal RSV-specific IgA responses in viral elimination at the airway epithelium. Thus, our nasal vaccine induced effective protection against RSV infection in the airway mucosa and is therefore a promising vaccine candidate for further development.

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Center for Gene & Cell Therapy

Division of Molecular and Medical Genetics
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In the Division of Molecular and Medical Genetics (DMMG), we focus on the development of virus vector-related technology, development of treatments for rare intractable diseases, development of next-generation AAV vaccines, and identification of biomarkers for Duchenne muscular dystrophy (DMD). A large-scale short-term purification method for functional full-genome AAV particles was established using two-step cesium chloride density-gradient ultracentrifugation with a zonal rotor. In addition, amnion-derived messenchymal stromal cells were suggested to be promising tools for the treatment of DMD due to their immunomodulatory properties. Furthermore, the biomarkers we found were suggested to be valuable for evaluating disease progression and monitoring treatment response.

Virus vector-related technology development

With a final goal of commercializing improved adeno-associated virus (AAV) vectors, we are developing functionally enhanced host cells for vector production, investigating expression culture methods involving bioreactors, and analyzing the efficient secretion of virus particles into the cell culture supernatant. While AAV vectors have attracted attention for their clinical utility, issues remain regarding their safety, particularly their immunogenicity. Therefore, it is desirable to establish a method for manufacturing high-quality and safe AAVs in large quantities, quickly, and at low cost. To resolve these issues, a large-scale short-term purification method for functional full-genome AAV particles was established using two-step cesium chloride density-gradient ultracentrifugation with a zonal rotor. Another difficulty is that viruses are generally stored at freezing temperatures reaching -80°C after production, which poses challenges such as difficulty in quality control and preserving their biological activity due to repeated freezing and thawing. To address this, we succeeded in maintaining the activity of AAV by spraying the liquid condition purified AAV in a vacuum and immediately freeze-drying it into a powder, which does not revert even after returning to room temperature, making quality control easier. In addition, tangential flow filtration (TFF) is a widely used method for the purification of adeno-associated virus (AAV) vectors. The use of TFF for AAV vector purification has been shown to be rapid, scalable, and cost-effective, making it a valuable technique in gene therapy and vaccine manufacturing.

Development of treatment methods for rare intractable diseases

The unique characteristics of amnion-derived mesenchymal stromal cells (AMSCs) make them a

clinically viable cell source for various applications. These features include non-invasive cell isolation, mitotic stability, ethical acceptability, low immunogenicity, and low risk of tumorigenesis. AMSCs have been studied for their potential in the treatment of conditions such as Duchenne muscular dystrophy (DMD) due to their immunomodulatory properties and therapeutic benefits. Additionally, the immunosuppressive effects of human AMSCs have been already demonstrated, further supporting their potential for therapeutic applications.

Development of next-generation AAV vaccine

The development of a vaccine can be a lengthy process, typically taking several years to complete. However, due to the urgent need for COVID-19 vaccines, unprecedented financial investments and scientific collaborations are changing how vaccines are developed, for example with some clinical trials evaluating multiple vaccines simultaneously. The establishment of the Strategic Center of Biomedical Advanced Vaccine Research and Development for Preparedness and Response (SCARDA) is part of Japan's efforts to modernize and improve the government's pandemic preparedness and response capabilities. Research has shown that AAV-vectored vaccines have demonstrated advantages such as thermostability, high efficiency, safety, and the ability to induce robust immune responses. These vaccines have been designed to avoid biased immune responses and mutational immune escape, making them a promising avenue for future vaccine development. Extracellular vesicle (EV)-based vaccines have also recently received attention in vaccine development. Accordingly, we are also conducting research on extracellular vesicle-based AAV-vectored vaccines against viruses, including SARS-CoV-2. In addition, the "Japan Initiative for World-leading Vaccine Research and Development Center" has been launched, and the University of Tokyo Pandemic preparedness, infection and Advanced research center (UTOPIA) has been selected as the flagship center. At UTOPIA, we are working to develop the use of empty particles as viral vectors.

Search for biomarkers in Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration and weakness due to the alterations of a protein called dystrophin. Unfortunately, there is currently no known treatment that halts the progression of the disease and available treatments are only palliative. Patients usually die in their twenties due to respiratory muscle weakness or cardiomyopathy. Biomarkers play a crucial role in the clinical management of Duchenne muscular dystrophy (DMD). We have been working to identify proteomic and metabolomic biomarkers for DMD, to monitor the disease progression and the effects of novel treatment strategies. These biomarkers are valuable for evaluating disease progression, monitoring treatment response, and improving the success of clinical trials in DMD. In addition, we have identified several microRNAs (miRNAs) as potential biomarkers for Duchenne muscular dystrophy (DMD). These small non-coding RNAs have shown promise as diagnostic and prognostic markers for DMD. Dysregulation of serum circulating miRNAs has been observed in DMD, suggesting their potential utility in monitoring the disease progression and response to therapy. While further research is needed to validate the clinical utility of these miRNAs as biomarkers, current evidence supports their potential use in the management of Duchenne muscular dystrophy.

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To improve the safety and effectiveness of gene and cell therapy, we are developing GMP-based mass production of high-quality viral vectors and methods for their tissue targeted use, also new treatments capitalizing on the anti-inflammatory and regenerative effects of mesenchymal stromal cells (MSC) and their immunoregulatory functions. To support research and development we are establishing manufacturing equipment that can quickly produce viral vectors economically at the scale necessary for academic research.

Construction of a high-performance purification system for viral vectors and application to gene and cell therapy

While AAV vectors have attracted attention for their clinical utility, issues remain regarding their safety, particularly their immunogenicity. Therefore, it is desirable to establish a method to manufacture high-quality, safe AAVs in large quantities, quickly, and at low cost. To resolve these issues, a large-scale short-term purification method for functional full-genome AAV particles was established using two-step cesium chloride density-gradient ultracentrifugation with a zonal rotor. The advantages of this new method are improved separation between empty and full-genome AAV particles, high-throughput of ultracentrifugation time, and increased AAV volume for purification. Furthermore, a new cell therapy method for Duchenne muscular dystrophy (DMD) using MSCs and details on the pathological mechanism of DMD were reported. In this method, systemic administration of amnion-derived MSCs to DMD model mice resulted in recovery of skeletal muscle locomotor function and cardiac function through improvement of inflammatory pathology in muscle tissue. To promote the practical application of cell and gene therapy research, we will support the production and provision of GMP-compliant viral vectors, including AAV and Lentivirus-based therapeutic vectors. For the advancement of translational research (TR) in gene therapy, it is necessary to collaborate with researchers with experience in therapeutic development, as this expertise is required to design and specify vectors for therapeutic targets, plan nonclinical studies with appropriate treatment evaluation items and endpoints, assessment of effectiveness and safety, and advice on PMDA strategies. In addition, clinical development requires a large amount of funding. If pharmaceutical companies cannot be expected to participate early, many therapeutic research projects initiated by academia will not reach clinical development even after obtaining an effective nonclinical PoC. Given this current situation, it is essential to promote therapeutic research by initiating information matching on corporate needs at an early stage and establishing a collaborative system by matching basic researchers with experts in TR promotion. Furthermore, it is also necessary to collaborate with gene therapy researchers and to establish standards and quality controls together with an outsourced manufacturing company choosing from various national and international candidates the one that best meets

the requirements, with the goal of prompt vector procurement. Therefore, we aim to accelerate the advancement of the gene therapy field by supporting the development and social implementation of effective gene therapy technology.

Development of an oncolytic virus therapy using third-generation herpesvirus G47Δ

Viral oncotherapy is a method that directly destroys cancer cells using viruses that infect them and is expected to be an innovative method for cancer treatment. Viral therapy for cancer is a treatment that consists of infecting cancer cells with a virus that can only multiply and directly destroys these cells. G47 Δ is a third-generation herpes virus for cancer treatment that is made by artificially modifying three viral genes of herpes simplex virus type 1 (HSV-1). The main feature of HSV-1 is that it is highly infectious and cytolytic while also less susceptible to neutralizing antibodies. By removing viral genes from the HSV-1 genome that are necessary for proliferation in normal cells but unnecessary in cancer cells, it is possible to create a virus that grows only in cancer cells. A characteristic of G47D is that it induces anti-tumor immunity in the process of destroying cancer cells, so it is expected to be effective against cancer cells not only at the site where G47D is administered, but also through the immune system in areas where the virus is not present. Animal experiments have shown that G47D is effective against not only brain tumors but solid cancers in general; in particular, the usefulness of G47D for tongue cancer was demonstrated using a mouse model, in which the use of G47Δ as a neoadjuvant was proved as beneficial in the prevention of local recurrence after tongue cancer surgery. In addition, interleukin-12 (IL-12) is one of the oldest known proinflammatory cytokines, and its strong ability to induce immune cells makes it suitable for use in cancer immunotherapy. After optimizing a methodology for expressing IL-12 as a payload for the G47Δ-based oncolytic HSV-1, a significantly higher intratumoral expression of functional IL-12 was found, resulting in stronger stimulation of specific anti-tumor immune responses.

Improvement of CAR-T cell therapy for solid cancers by using interleukin (IL)-7 and chemokine (C-C motif) ligand 19 (CCL19) production

CAR-T cell therapy is attracting attention as a treatment for intractable cancers that are difficult to kill completely with normal immune function alone. Therefore, it is desirable to develop next-generation technologies using CAR-T therapy for solid tumors, which account for more than 90% of all cancer patients. However, due to the heterogenous nature of solid tumors, it is difficult to identify solid tumor-specific molecular targets. In addition, immunological barriers due to immunosuppressive mechanisms in the solid tumor microenvironment impede the efficacy of CAR-T therapy. To overcome this problem, modified CAR-T cells co-expressing IL-7 and CCL19 were developed. The IL-7 enhances T cell proliferation and memory formation, and CCL19 induces active migration of T cells and dendritic cells. This modification was shown to promote the therapeutic effect of effector T cells on solid tumors.

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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 HSCs and IPSCs. 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

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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.

Efficient and precise gene disruption with CRISPR-Cas3 in human T cells

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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

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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 29,733 mice are housed for research of IMSUT, and strictly maintained in the SPF condition. The Animal Center building of LARC was improved in 1998 to perform genome engineering in animals, to make infectious experiments (P2A, P3A), and to house bigger animals, such as rats and rabbits. Techniques of mouse embryo manipulation and generating genetically modified mice, including genome editing technologies, have been introduced into the LARC.

Animal Husbandry and Housing

The Animal Center building is a centralized facility designed, constructed and maintained to meet regulatory standards required for the operation of research animal facilities. We provide barrier housing rooms to support the production and use of genetically-engineered mice, biohazardous experiments area and equipment room which has X-ray Irradiator, MRI, CT and IVIS imaging system. In 2023, 439 researchers from 44 laboratories are engaged in this facility with about 29,733 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 2023, 60 strains of embryos and 39 strains of sperm were stored, and 91 tubes of frozen embryo were used for rederivation.

Amami Laboratory of Injurious Animals
奄美病害動物研究施設

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The Amami Laboratory of Injurious Animals (since 1966) 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. Two newborns were given in reproductive groups of squirrel monkeys in 2023. On the other hand, owl monkeys have become male-only colonies, and breeding has stopped at present. In this year, the installation of the new large cage unit was completed in our squirrel monkey room, improving the breeding environment for the monkeys.

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. In this year, the renovation work of the 3rd building equipped with BSL3 animal experimental rooms, which allows for experiments on mosquito-borne infectious diseases in primates has completed, and its use is about to begin. 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. We are conducting research with collaborators 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

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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 cel-

lular 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. Real-Time Search-Assisted Multiplexed Quantitative Proteomics Reveals System-Wide Translational Regulation of Non-Canonical Short Open Reading Frames

Hiroko Kozuka-Hata, Tomoko Hiroki, Naoaki Miyamura, Aya Kitamura, Kouhei Tsumoto, Jun-ichiro Inoue and Masaaki Oyama.

Abnormal expression of histone deacetylases (HDACs) is reported to be associated with angiogenesis, metastasis and chemotherapy resistance regarding cancer in a wide range of previous studies. Suberoylanilide hydroxamic acid (SAHA) is well known to function as a pan-inhibitor for HDACs and recognized as one of the therapeutic drug candidates to epigenetically coordinate cancer cell fate regulation on a genomic scale. Here, we established a Real-Time Search-assisted mass spectrometric platform for system-wide quantification of translated products encoded by non-canonical short open reading frames (ORFs) as well as already annotated protein coding sequences (CDSs) on the human transcriptome and applied this methodology to quantitative proteomic analyses of SAHA-treated human HeLa cells to evaluate proteome-wide regulation in response to drug perturbation. Very intriguingly, our RTS-based in-depth proteomic analysis enabled us to identify approximately 5000 novel peptides from the ribosome profiling-based short ORFs encoded in the diversified regions on presumed 'non-coding' nucleotide sequences of mRNAs as well as lncRNAs and nonsense mediated decay (NMD) transcripts. Furthermore, TMT-based multiplex large-scale quantification of the whole proteome changes upon differential SAHA treatment unveiled dose-dependent selective translational regulation of a limited fraction of the non-canonical short ORFs in addition to key cell cycle/proliferation-related molecules such as UBE2C, CENPF and PRC1. Our study provided the first system-wide landscape of drug-perturbed translational modulation on both canonical and non-canonical proteome dynamics in human cancer cells.

<Group II>

Biomolecular recognition is based on collective and specific non-covalent interactions between discrete biological molecules. Our laboratory studies various protein systems, for instance, antibody-antigen and protein-ligand complexes, to understand quantitatively how these coordinated non-covalent interactions contribute to their specific recognition in biological and artificial systems. We seek to elucidate the molecular mechanisms by which biological molecules obtain high-specificity and affinity from multiple angles using advanced instrumentation. To produce functional molecules with higher performance and better properties, we aim to build a solid foundation from which to develop drugs that modulate specific interactions between biomolecules and ultimately to understand the principles of molecular interactions in our lives.

1. Analytical Method for Experimental Validation of Computer-Designed Antibody

Tanabe A and Tsumoto K.

In the computational design of antibodies, the interaction analysis between target antigen and antibody is an essential process to obtain feedback for validation and optimization of the design. Kinetic and thermodynamic parameters as well as binding affinity (KD) allow for a more detailed evaluation and understanding of the molecular recognition. In this chapter, we summarize the conventional experimental methods which can calculate KD value (ELISA, FP), analyze a binding activity to actual cells (FCM), and evaluate the kinetic and thermodynamic parameters (ITC, SPR, BLI), including high-throughput analysis and a recently developed experimental tech-

nique.

2. Structural Classification of CDR-H3 in Single-Domain VHH Antibodies

Kuroda D and Tsumoto K.

The immune systems protect vertebrates from foreign molecules or antigens, and antibodies are important mediators of this system. The sequences and structural features of antibodies vary depending on species. Many of antibodies from vertebrates, including camelids, have both heavy and light chain variable domains, but camelids also have antibodies that lack the light chains. In antibodies that lack light chains, the C-terminal variable region is called the VHH domain. Antibodies recognize antigens through six complementarity-determining regions (CDRs). The third CDR of the heavy chain (CDR-H3) is at the center of the antigen-binding site and is diverse in terms of sequence and structure. Due to the importance of antibodies in basic science as well as in medical applications, there have been many studies of CDR-H3s of antibodies that possess both light and heavy chains. However, nature of CDR-H3s of single-domain VHH antibodies is less well studied. In this chapter, we describe current knowledge of sequence-structure-function correlations of single-domain VHH antibodies with emphasis on CDR-H3. Based on the 370 crystal structures in the Protein Data Bank, we also attempt structural classification of CDR-H3 in single-domain VHH antibodies and discuss lessons learned from the ever-increasing number of the structures.

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 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.

4. 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 malignancies.

5. Structural insights into 3Fe-4S ferredoxins diversity in M. tuberculosis highlighted by a first redox complex with P450.

Gilep A, Varaksa T, Bukhdruker S, Kavaleuski A, Ryzhykau Y, Smolskaya S, Sushko T, Tsumoto K, Grabovec I, Kapranov I, Okhrimenko I, Marin E, Shevtsov M, Mishin A, Kovalev K, Kuklin A, Gordeliy V, Kaluzhskiy L, Gnedenko O, Yablokov E, Ivanov A, Borshchevskiy V and Strushkevich N.

Ferredoxins are small iron-sulfur proteins and key players in essential metabolic pathways. Among all types, 3Fe-4S ferredoxins are less studied mostly due to anaerobic requirements. Their complexes with cytochrome P450 redox partners have not been structurally characterized. In the present work, we solved the structures of both 3Fe-4S ferredoxins from M. tuberculosis-Fdx alone and the fusion FdxE-CYP143. Our SPR analysis demonstrated a high-affinity binding of FdxE to CYP143. According to SAXS data, the same complex is present in solution. The structure reveals extended multipoint interactions and the shape/charge complementarity of redox partners. Furthermore, FdxE binding induced conformational changes in CYP143 as evident from the solved CYP143 structure alone. The comparison of FdxE-CYP143 and modeled Fdx-CYP51 complexes further revealed the specificity of ferredoxins. Our results illuminate the diversity of electron transfer complexes for the production of different secondary metabolites.

6. Peripheral administration of nanomicelle-encapsulated anti-Aβ oligomer fragment antibody reduces various toxic Aβ species in the brain

Amano A, Sanjo N, Araki W, Anraku Y, Nakakido M, Matsubara E, Tomiyama T, Nagata T, Tsumoto K, Kataoka K and Yokota T.

Although a large amount of evidence has revealed that amyloid β (Aβ), especially Aβ oligomers, protofibrils, and pyroglutamated Aβs, participate primarily in the pathophysiological processes of Alzheimer's disease, most clinical trials of anti-Aβ antibody therapy have never acquired successful efficacy in human clinical trials, partly because peripheral administration of antibody medications was unable to deliver sufficient amounts of the molecules to the brain. Recently, we developed polymeric nanomicelles capable of passing through the blood-brain barrier that function as chaperones to deliver larger amounts of heavy molecules to the brain. Herein, we aimed to evaluate the efficacy of newly developed antibody 6H4 fragments specific to Aβ oligomers encapsulated in polymeric nanomicelles on the development of Alzheimer's disease pathology in Alzheimer's disease model mice at the age of emergence of early Alzheimer's disease pathology. During the 10-week administration of 6H4 antibody fragments in polymeric nanomicelles, a significant reduction in the amounts of various toxic Aβ species, such as Aβ oligomers, toxic Aβ conformers, and pyroglutamated Aβs in the brain was observed. In addition, immunohistochemistry indicated inhibition of diameters of Aβ plaques, Aβ-antibody immunoreactive areas, and also plaque core formation. Behavioral analysis of the mice model revealed that the 6H4 fragments-polymeric nanomicelle group was significantly better at maintaining long-term spatial reference memory in the probe and platform tests of the water maze, thereby indicating inhibition of the pathophysiological process of Alzheimer's disease. The results indicated that the strategy of reducing toxic Aβ species in early dementia owing to Alzheimer's disease by providing sufficient antibodies in the brain may modify Alzheimer's disease progression.

7. Non-Affinity Purification of Antibodies

Arakawa T, Tomioka Y, Nakagawa M, Sakuma C, Kurosawa Y, Ejima D, Tsumoto K and Akuta T.

Currently, purification of antibodies is mainly carried out using a platform technology composed primarily of Protein A chromatography as a capture step, regardless of the scale. However, Protein A chromatography has a number of drawbacks, which are summarized in this review. As an alternative, we propose a simple small-scale purification protocol without Protein A that uses novel agarose native gel electrophoresis and protein extraction. For large-scale antibody purification, we suggest mixed-mode chromatography that can in part mimic the properties of Protein A resin, focusing on 4-Mercapto-ethyl-pyridine (MEP) column chromatography.

8. Inactivation and spike protein denaturation of novel coronavirus variants by CuxO/TiO2 nano-photocatalysts

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 nano-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. 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 the original and alpha variant of novel coronavirus under irradiation with fluorescent light for 3 h. The photocatalysts greatly suppressed binding ability of the receptor binding domain (RBD) of coronavirus (the original, alpha and delta variants) spike protein to the receptor of human cells. The coating also exhibited antivirus effects on influenza A virus, feline calicivirus, bacteriophage Qβ and bacteriophage M13. The photocatalysts would be applied to practical coatings and lower the risk of coronavirus infection via solid surfaces.

9. Ligand Installation to Polymeric Micelles for Pediatric Brain Tumor Targeting

Watanabe T, Mizuno HL, Norimatsu J, Obara T, Cabral H, Tsumoto K, Nakakido M, Kawauchi D and Anraku Y

Medulloblastoma is a life-threatening disease with poor therapeutic outcomes. In chemotherapy, low drug accumulation has been a cause of these outcomes. Such inadequate response to treatments has been associated with low drug accumulation, particularly with a limited cellular uptake of drugs. Recently, the conjugation of drugs to ligand molecules with high affinity to tumor cells has attracted much attention for enhancing drug internalization into target cells. Moreover, combining tumor-targeting ligands with nano-scaled drug carriers can potentially improve drug loading capacity and the versatility of the delivery. Herein, we focused on the possibility of targeting CD276/B7-H3, which is highly expressed on the medulloblastoma cell membrane, as a strategy for enhancing the cellular uptake of ligand-installed nanocarriers. Thus, anti-CD276 antibodies were conjugated on the surface of model nanocarriers based on polyion complex micelles (PIC/m) via click chemistry. The results showed that the anti-CD276 antibody-installed PIC/m improved intracellular delivery into CD276-expressing medulloblastoma cells in a CD276-dependent manner. Moreover, increasing the number of antibodies on the surface of micelles improved the cellular uptake efficiency. These observations indicate the potential of anti-CD276 antibody-installed nanocarriers for promoting drug delivery in medulloblastoma.

10. Bromodomain protein BRD8 regulates cell cycle progression in colorectal cancer cells through a TIP60-independent regulation of the pre-RC complex

Yamaguchi K, Nakagawa S, Saku A, Isobe Y, Yamaguchi R, Sheridan P, Takane K, Ikenoue T, Zhu C, Miura M, Okawara Y, Nagatoishi S, Kozuka-Hata H, Oyama M, Aikou S, Ahiko Y, Shida D, Tsumoto K, Miyano S, Imoto S and Furukawa Y.

Bromodomain-containing protein 8 (BRD8) is a subunit of the NuA4/TIP60-histone acetyltransferase complex. Although BRD8 has been considered to act as a co-activator of the complex, its biological role remains to be elucidated. Here, we uncovered that BRD8 accumulates in colorectal cancer cells through the inhibition of ubiquitin-dependent protein degradation by the interaction with MRG domain binding protein. Transcriptome analysis coupled with genome-wide mapping of BRD8-binding sites disclosed that BRD8 transactivates a set of genes independently of TIP60, and that BRD8 regulates the expression of multiple subunits of the pre-replicative complex in concert with the activator protein-1. Depletion of BRD8 induced cell-cycle arrest at the G1 phase and suppressed cell proliferation. We have also shown that the bromodomain of BRD8 is indispensable for not only the interaction with histone H4 or transcriptional regulation but also its own protein stability. These findings highlight the importance of bromodomain as a therapeutic target.

11. Molecular mechanism underlying the increased risk of colorectal cancer metastasis caused by single nucleotide polymorphisms in LI-cadherin gene

Yui A, Kuroda D, Maruno T, Nakakido M, Nagatoishi S, Uchiyama S and Tsumoto K.

LI-cadherin is a member of the cadherin superfamily. LI-cadherin mediates Ca2 + -dependent cell-cell adhesion through homodimerization. A previous study reported two single nucleotide polymorphisms (SNPs) in the LI-cadherin-coding gene (CDH17). These SNPs correspond to the amino acid changes of Lys115 to Glu and Glu739 to Ala. Patients with colorectal cancer carrying these SNPs are reported to have a higher risk of lymph node metastasis than patients without the SNPs. Although proteins associated with metastasis have been identified, the molecular mechanisms underlying the functions of these proteins remain unclear, making it difficult to develop effective strategies to prevent metastasis. In this study, we employed biochemical assays and molecular dynamics (MD) simulations to elucidate the molecular mechanisms by which the amino acid changes caused by the SNPs in the LI-cadherin-coding gene increase the risk of metastasis. Cell aggregation assays showed that the amino acid changes weakened the LI-cadherin-dependent cell-cell adhesion. In vitro assays demonstrated a decrease in homodimerization tendency and MD simulations suggested an alteration in the intramolecular hydrogen bond network by the mutation of Lys115. Taken together, our results indicate that the increased risk of lymph node metastasis is due to weakened cell-cell adhesion caused by the decrease in homodimerization tendency.

12. An Engineered Synthetic Receptor-Aptamer Pair for an Artificial Signal Transduction System.

Liu H, Baeumler TA, Nakamura K, Okada Y, Cho S, Eguchi A, Kuroda D, Tsumoto K, Ueki R and Sando S.

Cell membrane receptors regulate cellular responses through sensing extracellular environmental signals and subsequently transducing them. Receptor engineering provides a means of directing cells to re-

act to a designated external cue and exert programmed functions. However, rational design and precise modulation of receptor signaling activity remain challenging. Here, we report an aptamer-based signal transduction system and its applications in controlling and customizing the functions of engineered receptors. A previously reported membrane receptor-aptamer pair was used to design a synthetic receptor system that transduces cell signaling depending on exogenous aptamer input. To eliminate the cross-reactivity of the receptor with its native ligand, the extracellular domain of the receptor was engineered to ensure that the receptor was solely activated by the DNA aptamer. The present system features tunability in the signaling output level using aptamer ligands with different receptor dimerization propensities. In addition, the functional programmability of DNA aptamers enables the modular sensing of extracellular molecules without the need for genetic engineering of the receptor.

13. Agarose native gel electrophoresis analysis of thermal aggregation controlled by Hofmeister series

Tomioka Y, Sato R, Takahashi R, Nagatoishi S, Shiba K, Tsumoto K, Arakawa T and Akuta T.

The effects of salting-in and salting-out salts defined by Hofmeister series on the solution state of bovine serum albumin (BSA) in 50 mM Tris-HCl buffer at pH 7.4 before and after thermal unfolding at 80 °C for 5 min were examined using agarose native gel electrophoresis and mass photometry. Gel electrophoresis showed that salting-in MgCl2, CaCl2 and NaSCN resulted in formation of intermediate structures of BSA upon heating on native gel, while heating in buffer alone resulted in aggregated bands. Mass photometry showed large loss of monomer and oligomers when heated in this buffer, but retaining these structures in the presence of 1 M MgCl2 and NaSCN. To our surprise, salting-out MgSO4 also showed a similar effect on gel electrophoresis and mass photometry. Salting-out NaCl and (NH4)2SO4 resulted in smearing and aggregated bands, which were supported by mass photometry. Aggregation-suppressive ArgHCl also showed oligomer aggregates upon gel electrophoresis and mass photometry.

14. Elucidating Conformational Dynamics and Thermostability of Designed Aromatic Clusters by Using Protein Cages

Hishikawa Y, Noya H, Nagatoishi S, Yoshidome T, Maity B, Tsumoto K, Abe S and Ueno T.

Multiple aromatic residues assemble to form higher ordered structures known as “aromatic clusters” in proteins and play essential roles in biological systems. However, the stabilization mechanism and dynamic behavior of aromatic clusters remain unclear. This study describes designed aromatic interactions confined within a protein cage to reveal how aromatic clusters affect protein stability. The crystal structures and calorimetric measurements indicate that the formation of inter-subunit phenylalanine clusters enhance the interhelix interactions and increase the melting temperature. Theoretical calculations suggest that this is caused by the transformation of the T-shaped geometry into π-π stacking at high temperatures, and the hydration entropic gain. Thus, the isolated nanoenvironment in a protein cage allows reconstruction and detailed analysis of multiple clustering residues for elucidating the mechanisms of various biomolecular interactions in nature which can be applied to design of bionanomaterials.

15. Design of single-domain VHH antibodies to increase the binding activity in SPR amine coupling

Hirao A, Nagatoishi S, Ikeuchi E, Yamawaki T, Mori C, Nakakido M and Tsumoto K.

Single-domain antibodies, or VHH, nanobodies, are attractive tools in biotechnology and pharmaceuticals due to their favorable biophysical properties. Single-domain antibodies have potential for use in sensing materials to detect antigens, and in this paper, we propose a generic design strategy of single-domain antibodies for the highly efficient use of immobilized antibodies on a sensing substrate. Amine coupling was used to immobilize the single-domain antibodies on the substrate through a robust covalent bond. First, for two model single-domain antibodies with lysines at four highly conserved positions (K48, K72, K84, and K95), we mutated the lysines to alanine and measured the binding activity of the mutants (the percentage of immobilized antibodies that can bind antigen) using surface plasmon resonance. The two model single-domain antibodies tended to have higher binding activities when K72, which is close to the antigen binding site, was mutated. Adding a Lys-tag to the C-terminus of single-domain antibodies also increased the binding activity. We also mutated the lysine for another model single-domain antibodies with the lysine in a different position than the four residues mentioned above and measured the binding activity. Thus, single-domain antibodies immobilized in an orientation accessible to the antigen tended to have a high binding activity, provided that the physical properties of the single-domain antibodies themselves (affinity and structural stability) were not significantly reduced. Specifically, the design strategy of single-domain antibodies with high binding activity included mutating the lysine at or near the antigen binding site, adding a Lys-tag to the C-terminus, and

mutating a residue away from the antigen binding site to lysine. It is noteworthy that mutating K72 close to the antigen binding site was more effective in increasing the binding activity than Lys-tag addition, and immobilization at the N-terminus close to the antigen binding site did not have such a negative effect on the binding activity compared to immobilization at the K72.

16. Real-Time Search-Assisted Multiplexed Quantitative Proteomics Reveals System-Wide Translational Regulation of Non-Canonical Short Open Reading Frames

Kozuka-Hata H, Hiroki T, Miyamura N, Kitamura A, Tsumoto K, Inoue JI and Oyama M.

Abnormal expression of histone deacetylases (HDACs) is reported to be associated with angiogenesis, metastasis and chemotherapy resistance regarding cancer in a wide range of previous studies. Suberoylanilide hydroxamic acid (SAHA) is well known to function as a pan-inhibitor for HDACs and recognized as one of the therapeutic drug candidates to epigenetically coordinate cancer cell fate regulation on a genomic scale. Here, we established a Real-Time Search (RTS)-assisted mass spectrometric platform for system-wide quantification of translated products encoded by non-canonical short open reading frames (ORFs) as well as already annotated protein coding sequences (CDSs) on the human transciptome and applied this methodology to quantitative proteomic analyses of suberoylanilide hydroxamic acid (SAHA)-treated human HeLa cells to evaluate proteome-wide regulation in response to drug perturbation. Very intriguingly, our RTS-based in-depth proteomic analysis enabled us to identify approximately 5000 novel peptides from the ribosome profiling-based short ORFs encoded in the diversified regions on presumed 'non-coding' nucleotide sequences of mRNAs as well as lncRNAs and nonsense mediated decay (NMD) transcripts. Furthermore, TMT-based multiplex large-scale quantification of the whole proteome changes upon differential SAHA treatment unveiled dose-dependent selective translational regulation of a limited fraction of the non-canonical short ORFs in addition to key cell cycle/proliferation-related molecules such as UBE2C, CENPF and PRC1. Our study provided the first system-wide landscape of drug-perturbed translational modulation on both canonical and non-canonical proteome dynamics in human cancer cells.

17. Targeting hemoglobin receptors IsdH and IsdB of Staphylococcus aureus with a single VHH antibody inhibits bacterial growth

Valenciano-Bellido S, Caaveiro JMM, Nakakido M, Kuroda D, Aikawa C, Nakagawa I and Tsumoto K.

Methicillin-resistant Staphylococcus aureus, or MRSA, is one of the major causative agents of hospital-acquired infections worldwide. Novel antimicrobial strategies efficient against antibiotic-resistant strains are necessary and not only against S. aureus. Among those, strategies that aim at blocking or dismantling proteins involved in the acquisition of essential nutrients, helping the bacteria to colonize the host, are intensively studied. A major route for S. aureus to acquire iron from the host organism is the Isd (iron surface determinant) system. In particular, the hemoglobin receptors IsdH and IsdB located on the surface of the bacterium are necessary to acquire the heme moiety containing iron, making them a plausible antibacterial target. Herein, we obtained an antibody of camelid origin that blocked heme acquisition. We determined that the antibody recognized the heme-binding pocket of both IsdH and IsdB with nanomolar order affinity through its second and third complementary-determining regions. The mechanism explaining the inhibition of acquisition of heme in vitro could be described as a competitive process in which the complementary-determining region 3 from the antibody blocked the acquisition of heme by the bacterial receptor. Moreover, this antibody markedly reduced the growth of three different pathogenic strains of MRSA. Collectively, our results highlight a mechanism for inhibiting nutrient uptake as an antibacterial strategy against MRSA.

18. A Proximity-Induced Fluorogenic Reaction Triggered by Antibody-Antigen Interactions with Adjacent Epitopes

Nishiyama K, Akiba H, Nagata S, Tsumoto K, Kamada H and Ohno H.

Proximity-induced chemical reactions are site-specific and rapid by taking advantage of their high affinity and highly selective interactions with the template. However, reactions induced solely by antibody-antigen interactions have not been developed. Herein, we propose a biepitopic antigen-templated chemical reaction (BATER) as a novel template reaction. In BATER, reactive functional groups are conjugated to two antibodies that interact with two epitopes of the same antigen to accelerate the reaction. We developed a method for visualizing the progress of BATER using fluorogenic click chemistry for optimal antibody selection and linker design. The reaction is accelerated in the presence of a specific antigen in a linker length-dependent manner. The choice of the antibody epitope is important for a rapid reaction. This design will lead to various applications of BATER in living systems.

19. Raman Spectroscopic Analysis of Highly-Concentrated Antibodies under the Acid-Treated Conditions

Sato Y, Nagatoishi S, Noguchi S and Tsumoto K.

Antibody drugs are usually formulated as highly-concentrated solutions, which would easily generate aggregates, resulting in loss of efficacy. Although low pH increases the colloidal dispersion of antibodies, acid denaturation can be an issue. Therefore, knowing the physical properties at low pH under high concentration conditions is important. Raman spectroscopy was used to investigate pH-induced conformational changes of antibodies at 50 mg/ml. Experiments in pH 3 to 7 were performed for human serum IgG and recombinant rituximab. We detected the evident changes at pH 3 in Tyr and Trp bands, which are the sensitive markers of intermolecular interactions. Thermal transition analysis over the pH range demonstrated that the thermal transition temperature (Tm) was highest at pH 3. Acid-treated and neutralized one showed higher Tm than that of pH 7, indicating that their extent of intermolecular interactions correlated with the Tm values. Onset temperature was clearly different between concentrated and diluted samples. Colloidal analyses confirmed the findings of the Raman analysis. Our studies demonstrated the positive correlation between Raman analysis and colloidal information, validating as a method for evaluating antibody conformation associated with aggregation propensities.

20. Histone H3 lysine 27 crotonylation mediates gene transcriptional repression in chromatin

Liu N, Konuma T, Sharma R, Wang D, Zhao N, Cao L, Ju Y, Liu D, Wang S, Bosch A, Sun Y, Zhang S, Ji D, Nagatoishi S, Suzuki N, Kikuchi M, Wakamori M, Zhao C, Ren C, Zhou TJ, Xu Y, Meslamani J, Fu S, Umehara T, Tsumoto K, Akashi S, Zeng L, Roeder RG, Walsh MJ, Zhang Q and Zhou MM.

Histone lysine acylation, including acetylation and crotonylation, plays a pivotal role in gene transcription in health and diseases. However, our understanding of histone lysine acylation has been limited to gene transcriptional activation. Here, we report that histone H3 lysine 27 crotonylation (H3K27cr) directs gene transcriptional repression rather than activation. Specifically, H3K27cr in chromatin is selectively recognized by the YEATS domain of GAS41 in complex with SIN3A-HDAC1 co-repressors. Proto-oncogenic transcription factor MYC recruits GAS41/SIN3A-HDAC1 complex to repress genes in chromatin, including cell-cycle inhibitor p21. GAS41 knockout or H3K27cr-binding depletion results in p21 de-repression, cell-cycle arrest, and tumor growth inhibition in mice, explaining a causal relationship between GAS41 and MYC gene amplification and p21 downregulation in colorectal cancer. Our study suggests that H3K27 crotonylation signifies a previously unrecognized, distinct chromatin state for gene transcriptional repression in contrast to H3K27 trimethylation for transcriptional silencing and H3K27 acetylation for transcriptional activation.

21. Dispersion Function of a Protein, DP-1, Identified in Collimonas sp. D-25, for the Synthesis of Gold Nanoparticles

Tang D, Kato Y, Zhang D, Negishi L, Kurumizaka H, Hirata T, Nakakido M, Tsumoto K, Shuji F, Tsuguyuki S, Okumura T, Nagata K and Suzuki M.

Collimonas sp. (D-25), found in the soil of Akita Prefecture, is a gram-negative bacterium with the ability to synthesize gold nanoparticles (AuNPs). During the synthesis of AuNPs, one specific protein (DP-1) was found to have disappeared in the sonicated solution of the bacterium. Recombinant DP-1 (rDP-1) from Escherichia coli BL21 (DE3) was used to study the effect of DP-1 on the synthesis of AuNPs. AuNPs synthesized with rDP-1 result in small, stabilized nanoparticles. AuNPs synthesized by DP-1 retained the stability of both the dispersion and nano-size particles under high salt concentrations. Isothermal titration calorimetry was employed to investigate the bonding ratio of rDP-1 to AuNPs. Several thousand rDP-1 proteins are attached to the surface of an AuNP to form a protein corona containing multiple layers. These results suggest that DP-1 obtained from D-25 has a size and stability control function during AuNP synthesis.

22. Development of a 1:1-binding biparatopic anti-TNFR2 antagonist by reducing signaling activity through epitope selection

Akiba H, Fujita J, Ise T, Nishiyama K, Miyata T, Kato T, Namba K, Ohno H, Kamada H, Nagata S and Tsumoto K

Conventional bivalent antibodies against cell surface receptors often initiate unwanted signal transduction by crosslinking two antigen molecules. Biparatopic antibodies (BpAbs) bind to two different epitopes on the same antigen, thus altering crosslinking ability. In this study, we develop BpAbs against tumor necrosis factor receptor 2 (TNFR2), which is an attractive immune checkpoint target. Using different pairs of antibody variable regions specific to topographically distinct TNFR2 epitopes, we successfully regulate the size of BpAb-TNFR2 immunocomplexes to result in controlled agonistic activities. Our series of results indicate that the relative positions of the two epitopes recognized by the BpAb are critical for controlling its signaling activity. One particular an-

tagonist, Bp109-92, binds TNFR2 in a 1:1 manner without unwanted signal transduction, and its structural basis is determined using cryo-electron microscopy. This antagonist suppresses the proliferation of regulatory T cells expressing TNFR2. Therefore, the BpAb format would be useful in designing specific and distinct antibody functions.

23. Discovery of a cystathionine γ-lyase (CSE) selective inhibitor targeting active-site pyridoxal 5'-phosphate (PLP) via Schiff base formation

Echizen H, Hanaoka K, Shimamoto K, Hibi R, Toma-Fukai S, Ohno H, Sasaki E, Komatsu T, Ueno T, Tsuchiya Y, Watanabe Y, Otsuka T, Saito H, Nagatoishi S, Tsumoto K, Kojima H, Okabe T, Shimizu T and Urano Y.

D,L-Propargylglycine (PAG) has been widely used as a selective inhibitor to investigate the biological functions of cystathionine γ-lyase (CSE), which catalyzes the formation of reactive sulfur species (RSS). However, PAG also inhibits other PLP (pyridoxal-5'-phosphate)-dependent enzymes such as methionine γ-lyase (MGL) and L-alanine transaminase (ALT), so highly selective CSE inhibitors are still required. Here, we performed high-throughput screening (HTS) of a large chemical library and identified oxamic hydrazide 1 as a potent inhibitor of CSE (IC50 = 13 ± 1 μM (mean ± S.E.)) with high selectivity over other PLP-dependent enzymes and RSS-generating enzymes. Inhibitor 1 inhibited the enzymatic activity of human CSE in living cells, indicating that it is sufficiently membrane-permeable. X-Ray crystal structure analysis of the complex of rat CSE (rCSE) with 1 revealed that 1 forms a Schiff base linkage with the cofactor PLP in the active site of rCSE. PLP in the active site may be a promising target for development of selective inhibitors of PLP-dependent enzymes, including RSS-generating enzymes such as cystathionine β-synthase (CBS) and cysteinyl-tRNA synthetase 2 (CARS2), which have unique substrate binding pocket structures.

24. Modulation of a conformational ensemble by a small molecule that inhibits key protein-protein interactions involved in cell adhesion

Senoo A, Nagatoishi S, Kuroda D, Ito S, Ueno G, Caaveiro JMM and Tsumoto K.

Small molecules that regulate protein-protein interactions can be valuable drugs; however, the development of such small molecules is challenging as the molecule must interfere with an interaction that often involves a large surface area. Herein, we propose that modulating the conformational ensemble of the proteins participating in a given interaction, rather than blocking the interaction by directly binding to the interface, is a relevant strategy for interfering with a protein-protein interaction. In this study, we applied this concept to P-cadherin, a cell surface protein forming homodimers that are essential for cell-cell adhesion in various biological contexts. We first determined the crystal structure of P-cadherin with a small molecule inhibitor whose inhibitory mechanism was unknown. Molecular dynamics simulations suggest that the inhibition of cell adhesion by this small molecule results from modulation of the conformational ensemble of P-cadherin. Our study demonstrates the potential of small molecules altering the conformation ensemble of a protein as inhibitors of biological relevant protein-protein interactions.

25. Arginine cluster introduction on framework region in anti-lysozyme antibody improved association rate constant by changing conformational diversity of CDR loops

Maeta S, Nakakido M, Matsuura H, Sakai N, Hirata K, Kuroda D, Fukunaga A and Tsumoto K.

Antibodies are used for many therapeutic and biotechnological purposes. Because the affinity of an antibody to the antigen is critical for clinical efficacy of pharmaceuticals, many affinity maturation strategies have been developed. Although we previously reported an affinity maturation strategy in which the association rate of the antibody toward its antigen is improved by introducing a cluster of arginine residues into the framework region of the antibody, the detailed molecular mechanism responsible for this improvement has been unknown. In this study, we introduced five arginine residues into an anti-hen egg white lysozyme antibody (HyHEL10) Fab fragment to create the R5-mutant and comprehensively characterized the interaction between antibody and antigen using thermodynamic analysis, X-ray crystallography, and molecular dynamics (MD) simulations. Our results indicate that introduction of charged residues strongly enhanced the association rate, as previously reported, and the antibody-antigen complex structure was almost the same for the R5-mutant and wild-type Fabs. The MD simulations indicate that the mutation increased conformational diversity in complementarity-determining region loops and thereby enhanced the association rate. These observations provide the molecular basis of affinity maturation by R5 mutation.

26. Group A Streptococcus cation diffusion facilitator proteins contribute to immune evasion by regulating intracellular metal concentrations

Aikawa C, Shimizu A, Nakakido M, Murase K, Nozawa T, Tsumoto K and Nakagawa I.

Cation diffusion facilitators (CDFs) are a large family of divalent metal transporters with broad specificities that contribute to intracellular metal homeostasis and toxicity in bacterial pathogens. Streptococcus pyogenes (Group A Streptococcus [GAS]) expresses two homologous CDF efflux transporters, MntE and CzcD, which selectively transport Mn and Zn, respectively. We discovered that the MntE- and CzcD-deficient strains exhibited a marked decrease in the viability of macrophage-differentiated THP-1 cells and neutrophils. In addition, the viability of mice infected with both deficient strains markedly increased. Consistent with a previous study, our results suggest that MntE regulates the PerR-dependent oxidative stress response by maintaining intracellular Mn levels and contributing to the growth of GAS. The maturation and proteolytic activity of streptococcal cysteine protease (SpeB), an important virulence factor in GAS, has been reported to be abrogated by zinc and copper. Zn inhibited the maturation and proteolytic activity of SpeB in the culture supernatant of the CzcD-deficient strain. Furthermore, Mn inhibited SpeB maturation and proteolytic activity in a MntE-deficient strain. Since the host pathogenicity of the SpeB-deficient strain was significantly reduced, maintenance of intracellular manganese and zinc levels in the GAS via MntE and CzcD may not only confer metal resistance to the bacterium, but may also play an essential role in its virulence. These findings provide new insights into the molecular mechanisms of pathogenicity, which allow pathogens to survive under stressful conditions associated with elevated metal ion concentrations during host infection.

27. Ferguson plot analysis of multiple intermediate species of thermally unfolded bovine serum albumin

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

Ferguson plot was used to characterize the multiple intermediate species of bovine serum albumin (BSA) upon thermal unfolding. Differential scanning calorimetry showed an irreversible melting of BSA in Tris-HCl and phosphate buffers with a mid-transition temperature, Tm, of ~68 °C. Thermally unfolded BSA was analyzed by agarose native gel electrophoresis stained by Coomassie blue and SYPRO Orange staining as a function of pH or protein concentration. SYPRO Orange was used to stain unfolded proteins. BSA heated at 70 and 80 °C, i.e., above the Tm, formed multiple intermediate species, which depended on the pH between 7.0 and 8.0, protein concentration and which buffer was used. These intermediate species were analyzed by Ferguson plot, which showed that BSA heated at 60 °C had a similar size to the native BSA, indicating that they are either native or native-like state consistent with no SYPRO Orange staining. The intermediate species observed at higher temperatures with the mobility less than that of the native BSA showed a steeper Ferguson plot and were stained by SYPRO Orange, indicating that these species had a larger hydrodynamic size than the native BSA and were unfolded.

28. Anti-InlA single-domain antibodies that inhibit the cell invasion of Listeria monocytogenes

Yamazaki T, Nagatoishi S, Yamawaki T, Nozawa T, Matsunaga R, Nakakido M, Caaveiro JMM, Nakagawa I and Tsumoto K.

Listeriosis, caused by infection with Listeria monocytogenes, is a severe disease with a high mortality rate. The L. monocytogenes virulence factor, internalin family protein InlA, which binds to the host receptor E-cadherin, is necessary to invade host cells. Here, we isolated two single-domain antibodies (VHHs) that bind to InlA with picomolar affinities from an alpaca immune library using the phage display method. These InlA-specific VHHs inhibited the binding of InlA to the extracellular domains of E-cadherin in vitro as shown by biophysical interaction analysis. Furthermore, we determined that the VHHs inhibited the invasion of L. monocytogenes into host cells in culture. High-resolution X-ray structure analyses of the complexes of VHHs with InlA revealed that the VHHs bind to the same binding site as E-cadherin against InlA. We conclude that these VHHs have the potential for use as drugs to treat listeriosis.

29. PRELP secreted from mural cells protects the function of blood brain barrier through regulation of endothelial cell-cell integrity

Davaapil H, Hopkins J, Bonnin N, Papadaki V, Leung A, Kosuge H, Tashima T, Nakakido M, Sekido R, Tsumoto K, Sagoo MS and Ohnuma SI.

Introduction: Proline/arginine-rich end leucine-rich repeat protein (PRELP), is a small secreted proteoglycan expressed by pericytes and vascular smooth muscle cells surrounding the brain vasculature of adult mouse. Methods: We utilised a Prelp knockout (Prelp -/-) mouse model to interrogate vasculature integrity in the brain alongside performing in vitro assays to characterise PRELP application to endothelial cells lines. Our findings were supplemented with RNA expression profiling to elucidate the mechanism of how PRELP maintains neurovasculature function. Results: Prelp -/- mice presented with neuroinflammation and reducedneurovasculature integrity, resulting in IgG and dextran leakage in the cerebellum and cortex. Histological analysis of Prelp -/- mice revealed reducedcell-cell integrity of the blood brain barrier, capillary attachment of pericytes

andastrocyte end-feet. RNA-sequencing analysis found that cell-cell adhesion andinflammation are affected in Prelp -/- mice and gene ontology analysis as well as gene set enrichment analysis demonstrated that inflammation related processes and adhesion related processes such as epithelial-mesenchymal transition and apical junctions were significantly affected, suggesting PRELP is a regulator of cell-cell adhesion. Immunofluorescence analysis showed that adhesion junction protein expression levels of cadherin, claudin-5, and ZO-1, was suppressed in Prelp -/- mice neurovasculature. Additionally, in vitro studies revealed that PRELP application to endothelial cells enhances cell-cell integrity, induces mesenchymal-endothelial transition and inhibits TGF-β mediated damage to cell-cell adhesion. Discussion: Our study indicates that PRELP is a novel endogenous secreted regulator of neurovasculature integrity and that PRELP application may be a potential treatment for diseases associated with neurovascular damage.

30. Specific peptide conjugation to a therapeutic antibody leads to enhanced therapeutic potency and thermal stability by reduced Fc dynamics

Kiyoshi M, Nakakido M, Rafique A, Tada M, Aoyama M, Terao Y, Nagatoishi S, Shibata H, Ide T, Tsumoto K, Ito Y and Ishii-Watabe A.

Antibody-drug conjugates are powerful tools for combatting a wide array of cancers. Drug conjugation to a therapeutic antibody often alters molecular characteristics, such as hydrophobicity and effector function, resulting in quality deterioration. To develop a drug conjugation methodology that maintains the molecular characteristics of the antibody, we engineered a specific peptide for conjugation to the Fc region. We used trastuzumab and the chelator (DOTA) as model antibody and payload, respectively. Interestingly, peptide/DOTA-conjugated trastuzumab exhibited enhanced antibody-dependent cellular cytotoxicity (ADCC) and increased thermal stability. Detailed structural and thermodynamic analysis clarified that the conjugated peptide blocks the Fc dynamics like a "wedge." We revealed that (1) decreased molecular entropy results in enhanced ADCC, and (2) blockade of Fc denaturation results in increased thermal stability. Thus, we believe that our methodology is superior not only for drug conjugation but also as for reinforcing therapeutic antibodies to enhance ADCC and thermal stability.

31. One-pot preparation of mannan-coated antigen nanoparticles using human serum albumin as a matrix for tolerance induction

Li S, Murakami D, Nagatoishi S, Liu Y, Tsumoto K, Katayama Y and Mori T.

Nanoparticles (NPs) for allergen immunotherapy have garnered attention for their high efficiency and safety compared with naked antigen proteins. In this work, we present mannan-coated protein NPs, incorporating antigen proteins for antigen-specific tolerance induction. The heat-induced formation of protein NPs is a one-pot preparation method and can be applied to various proteins. Here, the NPs were formed spontaneously via heat denaturation of three component proteins: an antigen protein, human serum albumin (HSA) as a matrix protein, and mannoprotein (MAN) as a targeting ligand for dendritic cells (DCs). HSA is non-immunogenic, therefore suitable as a matrix protein, while MAN coats the surface of the NP. We applied this method to various antigen proteins and found that the self-disperse after heat denaturation was a requirement for incorporation into the NPs. We also established that the NPs could target DCs, and the incorporation of rapamycin into the NPs enhanced the induction of a tolerogenic phenotype of DC. The MAN coating provided steric hindrance and heat denaturation destroyed recognition structures, successfully preventing anti-antigen antibody binding, indicating the NPs may avoid anaphylaxis induction. The MAN-coated NPs proposed here, prepared by a simple method, have the potential for effective and safe allergies treatment for various antigens.

32. Biophysical insight into protein-protein interactions in the Interleukin-11/Interleukin-11Rα/glycoprotein 130 signaling complex

Mori C, Nagatoishi S, Matsunaga R, Kuroda D, Nakakido M and Tsumoto K.

Interleukin-11 (IL-11) is a member of the interleukin-6 (IL-6) family of cytokines. IL-11 is a regulator of multiple events in hematopoiesis, and IL-11-mediated signaling is implicated in inflammatory disease, cancer, and fibrosis. All IL-6 family cytokines signal through the signal-transducing receptor, glycoprotein 130 (gp130), but these cytokines have distinct as well as overlapping biological functions. To understand IL-11 signaling at the molecular level, we performed a comprehensive interaction analysis of the IL-11 signaling complex, comparing it with the IL-6 complex, one of the best-characterized cytokine complexes. Our thermodynamic analysis revealed a clear difference between IL-11 and IL-6. Surface plasmon resonance analysis showed that the interaction between IL-11 and IL-11 receptor α (IL-11Rα) is entropy driven, whereas that between IL-6 and IL-6 receptor α (IL-6Rα) is enthalpy driven. Our analysis using isothermal titration calorimetry revealed that the binding of gp130 to the IL-11/IL-11Rα complex results in entropy loss, but that the interaction of gp130 with the IL-6/IL-6Rα complex results in entropy gain. Our

hydrogen-deuterium exchange mass spectrometry experiments suggested that the D2 domain of gp130 was not involved in IL-6-like interactions in the IL-11/IL-11Rα complex. It has been reported that IL-6 interaction with gp130 in the signaling complex was characterized through the hydrophobic interface located in its D2 domain of gp130. Our findings suggest that unique interactions of the IL-11 signaling complex with gp130 are responsible for the distinct biological activities of IL-11 compared to IL-6.

33. High-throughput analysis system of interaction kinetics for data-driven antibody design

Matsunaga R, Ujiie K, Inagaki M, Fernández Pérez J, Yasuda Y, Mimasu S, Soga S and Tsumoto K.

Surface plasmon resonance (SPR) is widely used for antigen-antibody interaction kinetics analysis. However, it has not been used in the screening phase because of the low throughput of measurement and analysis. Herein, we proposed a high-throughput SPR analysis system named "BreviA" using the Brevibacillus expression system. Brevibacillus was transformed using a plasmid library containing various antibody sequences, and single colonies were cultured in 96-well plates. Sequence analysis was performed using bacterial cells, and recombinant antibodies secreted in the supernatant were immobilized on a sensor chip to analyze their interactions with antigens using high-throughput SPR. Using this system, the process from the transformation to 384 interaction analyses can be performed within a week. This system utility was tested using an interspecies specificity design of an anti-human programmed cell death protein 1 (PD-1) antibody. A plasmid library containing alanine and tyrosine mutants of all complementarity-determining region residues was generated. A high-throughput SPR analysis was performed against human and mouse PD-1, showing that the mutation in the specific region enhanced the affinity for mouse PD-1. Furthermore, deep mutational scanning of the region revealed two mutants with > 100-fold increased affinity for mouse PD-1, demonstrating the potential efficacy of antibody design using data-driven approach.

34. Experimental modification in thermal stability of oligomers by alanine substitution and site saturation mutagenesis of interfacial residues

Hoya M, Matsunaga R, Nagatoishi S and Tsumoto K.

For certain industrial applications, the stability of protein oligomers is important. In this study, we demonstrated an efficient method to improve the thermal stability of oligomers using the trimeric protein chloramphenicol acetyltransferase (CAT) as the model. We substituted all interfacial residues of CAT with alanine to detect residues critical for oligomer stability. Mutation of six of the forty-nine interfacial residues enhanced oligomer thermal stability. Site saturation mutagenesis was performed on these six residues to optimize the side chains. About 15% of mutations enhanced thermal stability by more than 0.5 °C and most did not disrupt activity of CAT. Certain combinations of mutations further improved thermal stability and resistance against heat treatment. The quadruple mutant, H17V/N34S/F134A/D157C, retained the same activity as the wild-type after heat treatment at 9 °C higher temperature than the wild-type CAT. Furthermore, combinations with only alanine substitutions also improved thermal stability, suggesting the method we developed can be used for rapid modification of industrially important proteins.

35. Quantitative analysis of antibody aggregates by combination of pinched-flow fractionation and coulter method

Nagatoishi S, Toyoshima T, Furukawa K and Tsumoto K.

For the pharmaceutical development of proteins, multiple methods of analysis are recommended for evaluating aggregation, and the development of more quantitative and simpler analytical techniques for subvisible particles is expected. This study introduces the Pinched-Flow Fractionation (PFF)-Coulter method, which combines the Pinched-flow fractionation (PFF) and Coulter methods to analyze the concentration of submicron-sized particles. The PFF method separates the particles by size. Separated particles were individually detected using the Coulter method. We have utilized the PFF-Coulter method to quantitatively analyze particle concentrations using standard particles, evaluate detection limits, variability, and correlation between theoretical and measured values, and analyze mixtures of different particle sizes. The PFF-Coulter method allows for quantitatively analyzing of particle sizes from 0.2 to 2.0 μm. The quantifiable weight concentration range was 2.5 × 10-2 - 50 μg/mL, and the number concentration range was 104-1010 particles/mL. The sample volume was small (<10 μL). The PFF-Coulter method is capable of quantitative analysis that complements data from conventional measurement techniques, and when used in conjunction with existing submicron-size particle analysis techniques, will enable more accurate particle analysis.

36. Megalin is involved in angiotensinogen-induced, angiotensin II-mediated ERK1/2 signaling to activate Na + -H + exchanger 3 in proximal tubules

Goto S, Yoshida Y, Hosojima M, Kuwahara S, Kabasawa H, Aoki H, Iida T, Sawada R, Ugamura D, Yoshizawa Y, Takemoto K, Komochi K, Kobayashi R, Kaseda R, Yaoita E, Nagatoishi S, Narita I, Tsumoto K and Saito A.

Kidney angiotensin (Ang) II is produced mainly from liver-derived, glomerular-filtered angiotensinogen (AGT). Podocyte injury has been reported to increase the kidney Ang II content and induce Na + retention depending on the function of megalin, a proximal tubular endocytosis receptor. However, how megalin regulates the renal content and action of Ang II remains elusive. We used a mass spectrometry-based, parallel reaction-monitoring assay to quantitate Ang II in plasma, urine, and kidney homogenate of kidney-specific conditional megalin knockout (MegKO) and control (Ctl) mice. We also evaluated the pathophysiological changes in both mouse genotypes under the basal condition and under the condition of increased glomerular filtration of AGT induced by administration of recombinant mouse AGT (rec-mAGT). Under the basal condition, plasma and kidney Ang II levels were comparable in the two mouse groups. Ang II was detected abundantly in fresh spot urine in conditional MegKO mice. Megalin was also found to mediate the uptake of intravenously administered fluorescent Ang II by PTECs. Administration of rec-mAGT increased kidney Ang II, exerted renal extracellular signal-regulated kinase 1/2 (ERK1/2) signaling, activated proximal tubular Na + -H + exchanger 3 (NHE3), and decreased urinary Na + excretion in Ctl mice, whereas these changes were suppressed but urinary Ang II was increased in conditional MegKO mice. Increased glomerular filtration of AGT is likely to augment Ang II production in the proximal tubular lumen. Thus, megalin-dependent Ang II uptake should be involved in the ERK1/2 signaling that activates proximal tubular NHE3 in vivo , thereby causing Na + retention.

37. Safe and efficient oral allergy immunotherapy using one-pot-prepared mannan-coated allergen nanoparticles

Li S, Toriumi H, Takahashi D, Kamasaki T, Fujioka Y, Nagatoishi S, Li J, Liu Y, Hosokawa T, Tsumoto K, Ohba Y, Katayama Y, Murakami D, Hase K and Mori T.

Allergen immunotherapy (AIT) is the only curative treatment for allergic diseases. However, AIT has many disadvantages related to efficiency, safety, long-term duration, and patient compliance. Dendritic cells (DCs) have an important role in antigen-specific tolerance induction; thus, DC-targeting strategies to treat allergies such as glutaraldehyde crosslinked antigen to mannoprotein (MAN) have been established. However, glutaraldehyde crosslinking may reduce the antigen presentation efficiency of DCs. To overcome this, we developed a MAN-coated ovalbumin (OVA) nanoparticle (MDO), which uses intermolecular disulfide bond to crosslink OVA and MAN. MDO effectively targeted DCs resulting in tolerogenic DCs, and promoted higher antigen presentation efficiency by DCs compared with OVA or glutaraldehyde crosslinked nanoparticles. In vitro and in vivo experiments showed that DCs exposed to MDO induced Treg cells. Moreover, MDO had low reactivity with anti-OVA antibodies and did not induce anaphylaxis in allergic mice, demonstrating its high safety profile. In a mouse model of allergic asthma, MDO had significant preventative and therapeutic effects when administered orally or subcutaneously. Therefore, MDO represents a promising new approach for the efficient and safe treatment of allergies.

38. Enhancing thermal stability in the CH2 domain to suppress aggregation through the introduction of simultaneous disulfide bonds in Pichia pastoris

Oyama K, Nakakido M, Ohkuri T, Nakamura H, Tsumoto K and Ueda T.

Protein aggregations decrease production yields and impair the efficacy of therapeutics. The CH2 domain is a crucial part of the constant region of human IgG. But, it is also the least stable domain in IgG, which can result in antibody instability and aggregation problems. We created a novel mutant of the CH2 domain (T250C/L314C, mut10) by introducing a disulfide bond and expressed it using Pichia pastoris. The mut10 variant exhibited enhanced thermal stability, resistance to enzymatic degradation, and reduced aggregation in comparison to the original CH2 domain. However, it was less stable than mut20 (L242C/K334C), which is the variant prepared in a previous study (Gong et al., J. Biol. Chem., 2009). A more advanced mutant, mut25, was created by combining mut10 and mut20. Mut25 artificially contains two disulfide bonds. The new mutant, mut25, showed enhanced thermal stability, increased resistance to enzymatic digestion, and reduced aggregation in comparison to mut20. According to our knowledge, mut25 achieves an unprecedented level of stability among the humanized whole CH2 domains that have been reported so far. Mut25 has the potential to serve as a new platform for antibody therapeutics due to its ability to reduce immunogenicity by decreasing aggregation.

39. Conformational features and interaction mechanisms of VH H antibodies with β-hairpin CDR3: A case of Nb8-HigB2 interaction

Yamamoto K, Nagatoishi S, Matsunaga R, Nakakido M, Kuroda D and Tsumoto K.

The β-hairpin conformation is regarded as an important basic motif to form and regulate protein-protein interactions. Single-domain VH H antibodies are potential therapeutic and diagnostic tools, and the third complementarity-determining regions of the heavy chains (CDR3s) of these antibodies are critical for antigen recognition. Although the sequences and conformations of the CDR3s are diverse, CDR3s sometimes adopt β-hairpin conformations. However, characteristic features and interaction mechanisms of β-hairpin CDR3s remain to be fully elucidated. In this study, we investigated the molecular recognition of the anti-HigB2 VH H antibody Nb8, which has a CDR3 that forms a β-hairpin conformation. The interaction was analyzed by evaluation of alanine-scanning mutants, molecular dynamics simulations, and hydrogen/deuterium exchange mass spectrometry. These experiments demonstrated that positions 93 and 94 (Chothia numbering) in framework region 3, which is right outside CDR3 by definition, play pivotal roles in maintaining structural stability and binding properties of Nb8. These findings will facilitate the design and optimization of single-domain antibodies.

<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 can provide us precise information of much wider areas of thin sections more effectively and more easily than transmission electron microscopy. 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 individual technique or combination of some of these, we can offer direct visual evidence that cannot be acquired by other methods. This year, 21 projects in 10 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. Analysis of sodium iodate effects on retinal degeneration

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 effects of sodium iodate ($NaIO_3$) administration on retinal degeneration. $NaIO_3$ is widely used to induce retinal degeneration in model animals such as mice, rats and rabbits. With precise morphological analysis of $NaIO_3$ administered mouse retina, we found that $NaIO_3$ specifically damages retinal choriocapillaris and this leads to the disfunction of the retinal pigment epithelial (RPE) cells and father to the retinal degeneration. With biochemical analysis, retinal degeneration is supposed to be induced by rapid decrease of VEGF in RPE cells. (ref. Iwagawa *et al*)

Some other collaborative research works using thin section electron microscopy and/or immuno-electron microscopy were performed with Dr. Eguchi[3], in [3]Division of Genetics, about the roles of muscle specific kinase on formation of neuromuscular junction. (ref. Eguchi *et al*) Dr. Kamioka[4], in [4]Division of Malaria Immunology, about the function of Paneth cells on Malaria infection, 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. Isoo[7], in [7]Division of Infectious Diseases.

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 and also about the morphology of the sperm and spermatocyte. 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

Enveloped viruses, including HIV-1, Flaviviruses, Herpes Simplex Viruses, and Coronaviruses, exhibit pathogenicity and are clinically significant. The J. Gohda and Y. Kawaguchi research groups are conducting studies aimed at the development of antiviral molecules targeting enveloped viruses, including SARS-CoV-2, as well as the exploration of molecules for the reactivation of HIV-1 reservoirs.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative virus for Coronavirus Disease 2019 (COVID-19) and has globally expanded since the first reported patient in December 2019 in China. To bring an end to the ongoing COVID-19 pandemic, development of antiviral drugs and vaccines targeting SARS-CoV-2 infection is imperative. We have established a dual split protein-based cell fusion assay utilizing the SARS-CoV-2 spike protein to evaluate the antiviral activity of several molecules, advancing the screening of antibodies and small molecular compounds. This year, we have established novel neutralizing antibodies that exhibit neutralizing activity without binding to the conventional antibody pharmaceutical target, the Receptor Binding Domain (RBD). These antibodies target conserved regions, even in related coronaviruses and SARS-CoV-2 variants, suggesting potential long-term utility. Moreover, these conserved regions may serve as candidates for inducing effective neutralizing antibodies through vaccination. From the analysis of small molecular compounds, multiple inhibitors against TMPRSS2, a crucial host protease for virus infection, were identified from synthetic compounds. Additionally, two compounds inhibiting virus entry and proliferation within cells were identified from subtropical plant extracts. These inhibitory molecules not only hold promise for future therapeutic development but also play a crucial role in elucidating the infection mechanisms of SARS-CoV-2. The elucidation of the target molecules of these agents is expected to contribute to the discovery of new infection mechanisms and therapeutic targets.

The utilization of combination antiretroviral therapy (cART) has significantly contributed to impeding the progression to acquired immunodeficiency syndrome (AIDS) in individuals infected with human immunodeficiency virus type 1 (HIV-1). Nevertheless, the presence of latent reservoirs of HIV-1, housing silenced yet replication-competent provirus, constitutes a formidable barrier to viral eradication in affected individuals. The "shock and kill" strategy, a promising approach toward curing HIV-1 infection, seeks to reactivate latent provirus through treatment with latency reversing agents (LRAs), denoted as "shock," in conjunction with antiretroviral drugs. While several drugs have been identified as LRAs, no drug has been clinically applied to date. We have identified multiple existing drugs as novel LRA candidates. In this year, our efforts have focused on elucidating the molecular mechanism of reactivation of latent HIV-1 provirus by these drugs. Our findings suggest that one of the candidate drugs may reactivate HIV-1 proviral transcription through a distinct mechanism from the established LRAs that induce proviral reactivation. We are currently engaged in identifying target proteins and advancing their functional analysis. Furthermore, we are analyzing the synergistic interactions between novel drugs and existing LRAs, aiming to establish methods that induce more effective "shock". Through these analyses, there is a prospect for the future depletion of HIV reservoirs in patients, thereby offering the potential for a complete treatment of AIDS.

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 vi-

rus 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) We isolated 77 HPAI viruses during routine surveillance in live poultry markets in northern provinces of Vietnam from 2018–2021. These viruses were genetically different from those in other parts of the world. These viruses do not encode major determinants of mammalian adaptation but possess amino acid substitutions that may affect viral receptor-binding, replication, or responses to human antiviral factors. Our ongoing surveillance of HPAI viruses in several parts of the world is important to monitor the evolution of these viruses. (2) We analyzed the efficacy of antiviral drugs and antibodies against Omicron variants. The susceptibilities of CH.1.1 and XBB.1.5 to remdesivir, molnupiravir, nirmatrelvir, and ensitrelvir were similar to those of the ancestral strain and other variants of concern. The effectiveness of monoclonal antibodies (i.e., sotrovimab, bebtelovimab, casirivimab/imdevimab, and tixagevimab/cilgavimab) varied with the omicron strain tested. None of these monoclonal antibodies was effective against CH.1.1 or XBB.1.5. In addition, we found that a bivalent vaccine (ancestral and BA.4/5) can improve humoral responses to both viruses.

IMSUT PROJECT OFFICE

The office (M. Hayashi) supports the activities of the joint laboratory in Beijing and the joint research program in Harbin. It serves as Secretariat for Steering Committee Meeting and files MOU and Minutes. It helps scientists visiting the joint laboratory/program for collaborative research. It has been gathering the information about emerging infections in China from the Chinese mass media and official announcements, and the gathered information (in Japanese) has been presented and updated on the website of the Project (http://www.rcaid.jp/).

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Laboratory of Molecular Genetics (Frontier Research Unit)
遺伝子解析施設（フロンティア研究領域）

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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. Negative regulation of Pbs2 MAP2K by PP2C phosphatases in the yeast HOG pathway.

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 *sln1*Δ 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 effects of inactivating the genes of type 2C phosphatases upon the phosphorylation status of Pbs2 at the activating phosphorylation sites Ser-514 and Thr-518 (S514 and T518) by directly measuring the levels of Pbs2 phosphorylation at S514 and T518 using the assay method we developed recently. We found that S514 and T518 are differentially dephosphorylated by different type 2C serine/threonine phosphatases. 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 is more evenly dephosphorylated by Ptc1, Ptc2, Ptc3, and Ptc4. We also show that Pbs2 dephosphorylation by Ptc1 requires the adaptor protein Nbp2 that recruits Ptc1 to Pbs2.

2. Osmostress enhances Pbs2 MAP2K phosphorylation by Ste11 MAP3K in the yeast osmo-regulatory HOG pathway

Kazuo Tatebayashi

The yeast MAPK Hog1 is activated by hyperosmotic stress through the High Osmolarity Glycerol (HOG) pathway, and orchestrates an array of osmo-adaptive changes in transcription, translation, cell cycle, and metabolism. The current widely held model of the HOG pathway is as follows. The upstream portion of the HOG pathway is comprised of the functionally redundant SHO1 and SLN1 branches. In the SHO1 branch, osmosensing complexes composed of Sho1, Opy2, Hkr1, and Msb2 activate the MAP3K Ste11. In the SLN1 branch, the Sln1-Ypd1-Ssk1 phospho-relay mechanism is involved in activation of the functionally redundant MAP3Ks Ssk2 and Ssk22 (Ssk2/22). Ste11 and Ssk2/22 phosphorylate the MAP2K Pbs2 at Ser-514 and/or Thr-518. Phosphorylated Pbs2 then activates Hog1. Recently, we found that osmostress not only activates membrane-associated osmosensors, but also enhances Hog1 phosphorylation by mono-phosphorylated Pbs2. The lack of the osmotic enhancement of the Pbs2-Hog1 reaction suppresses Hog1 activation by basal MAP3K activities and prevents pheromone-to-Hog1 crosstalk in the absence of osmostress, which ensures the appropriate Hog1 activation only under high osmolarity. It has been unclear if osmostress acts on other phosphorylation steps in the MAP kinase cascade of the HOG pathway.

This year, we examined if osmostress enhances Pbs2 MAP2K phosphorylation by Ste11 MAP3K as well as Hog1 phosphorylation. Ste11 is phosphorylated and activated by the Ste20/Cla4 kinases upon high osmolarity. The constitutively-active Ste11-DDD mutant carries the substitution mutations of all activating phosphorylation sites for Ste20/Cla4 to phosphomimic Asp (S281D, S285D, and T286D), which circumvent the need for Ste20/Cla4 for Ste11 activation. The endogenous-level expression of Ste11-DDD did not induce the phosphorylation of Pbs2 under unstimulated condition, but induced it upon high osmolarity. These results strongly suggest that Pbs2 phosphorylation by activated Ste11 is osmotically-enhanced as Hog1 is. The underlying mechanism is under study.

Publication

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The Division conducts clinical, pathologic, and therapeutic research on hematopoietic tumors and other hematologic diseases. In the field of genomic medicine, which has been developing in recent years, research for clinical implementation is also underway. In collaboration with HGC, our laboratory is conducting research on clinical sequencing, as well as research on artificial intelligence curation, automation and efficiency of clinical implementation, and the clinical significance of clinical sequencing. We are also working on elucidating the pathogenesis of genomic abnormalities revealed by clinical sequencing, such as hairy cell leukemia. In addition, the Center serves as a clinical hub for adult-onset histiocytosis and is working to bring novel therapies for refractory adult-onset histiocytosis into the clinic. The division is also working to improve the clinical practice of transplantation through the analysis of hematopoietic stem cell transplantation data.

1. Pretransplant EASIX score predicts non-relapse and overall mortality of adult patients undergoing single-unit unrelated cord blood transplantation

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The Endothelial Activation and Stress Index (EASIX) is a laboratory-based score to estimate endothelial damage occurring after hematopoietic cell transplantation (HCT). EASIX score exhibits dynamic changes during the transplantation course and has been shown as a predictor of non-relapse mortality (NRM) and worse overall survival (OS) in studies mainly focused on patients who received matched related and unrelated donor allogeneic HCT. However, the role of EASIX score is unclear in the setting of cord blood transplantation (CBT). The objective of this study was to examine the association between pretransplant EASIX score and post-transplant outcomes in adult patients undergoing single-unit CBT. We ret-

rospectively evaluated the impact of EASIX score at different time points on post-transplant outcomes in adults following single-unit unrelated CBT between 1998 and 2022 at our institute. EASIX scores were calculated at the start of conditioning (EASIX-PRE), at day 30 post-CBT (EASIX-d30), at day 100 post-CBT (EASIX-d100), and at the onset of grade II to IV acute graft-versus-host disease (GVHD) (EASIX-GVHD II–IV). 317 patients were included in this study. In the multivariate analysis, log2-EASIX-PRE (continuous variable) was significantly associated with lower risks of neutrophil (hazard ratio [HR] 0.87; 95% confidence interval [CI] 0.80-0.94; P<0.001) and platelet (HR 0.91; 95%CI 0.83-0.99; P = 0.047) engraftment, lower risk of grade II–IV acute GVHD (HR 0.85; 95%CI 0.76-0.94; P = 0.003), and higher risk of veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) (HR 1.44; 95%CI 1.03-2.02; P = 0.032). Log2-EASIX-PRE was also significantly associated with higher NRM (HR 1.42; 95%CI 1.08-1.86; P = 0.011) and worse OS (HR 1.26; 95%CI 1.08-1.46; P = 0.003), but not relapse (HR 1.02; 95%CI 0.88-1.18; P = 0.780). Similarly, log2-EASIX-d30 (HR 1.60; 95%CI 1.26-2.05; P<0.001), and log2-EASIX-d100 (HR 2.01; 95%CI 1.63-2.48; P<0.001) were also significantly associated with higher NRM, but not log2-EASIX-GVHD II–IV (HR 1.15; 95%CI 0.85-1.55; P = 0.360). Pretransplant EASIX score is a powerful predictor of engraftment, VOS/SOS, NRM, and OS in adult patients undergoing single-unit unrelated CBT who mainly received intensified conditioning regimens. EASIX is an easily evaluable and dynamic prognostic score to accurately predict post-transplant outcomes in patients at any time during allogeneic HCT, particularly for CBT.

2. Development of Explainable AI for Genomic Medicine

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【Background and Objectives】

In the ever-evolving field of genomic medicine, identifying disease-causing genetic variants is crucial for effective treatment. Given the vast number of these variants, the application of Artificial Intelligence (AI) becomes essential. Our research, in collaboration with Fujitsu Corporation, focuses on leveraging AI to address these challenges in two significant ways.

【Method】 We introduced an explainable AI (XAI) model, which combines high estimation accuracy with explainability, utilizing a knowledge graph. This graph integrates databases relevant to genomic medicine, enabling the XAI to assist physicians in identifying disease-causing variants. By comparing our XAI with traditional methods like random forests and decision trees, we demonstrated its superior estimation performance and explainability. This advancement is a step forward in promoting genomic medicine by providing a tool that not only predicts accurately but also explains its predictions. Next, we extend our XAI system to the analysis of structural variants (SV). This involves studying patterns in pathogenic SV registered in databases like COSMIC and Mitelman. The AI model is trained to not only estimate the pathogenicity of unknown SV but also to provide summaries of significant papers on known abnormalities. This approach aims to enhance explainability and reduce the research burden for scientists in the field.

【Conclusion】

These studies underscore our commitment to integrating AI into genomic medicine. By developing AI that are both accurate and explainable, we aim to enhance the identification of genetic variants and SV, thereby supporting the advancement of personalized medicine.

3. Deciphering the Genetic Landscape of Hairy Cell Leukemia: The Japanese Variant Perspective

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7 Prospective observation study of chronic lymphocytic leukemia, hairy cell leukemia and related diseases in Japan (CLLRSG-01)

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Background: Hairy Cell Leukemia (HCL), a rare form of mature B-cell tumor, presents in various subtypes, including the BRAF p.V600E mutation-bearing

classic type (HCLc), and variant forms. Among these, the Western variant (HCLv) is well characterized, with established molecular pathologies and treatment including targeted therapies. In contrast, the Japanese variant (HCLjv), which shows distinct clinical features, remains less understood..

Key Findings: In this context, we performed Whole Genome Sequencing (WGS) on two cases of HCLjv and identified three novel genetic variants. Of these variants, the most notable variant involved a cancer-related oncogene X, which was positioned near the immunoglobulin heavy chain enhancer (Eμ) due to a structural variant (SV). Loss-of-function mutations were also identified in genes Y and Z, which are an inhibitor of the NFκB pathway and a key gene involved in epigenetic control through histone modification, respectively. Additionally, we investigated the overexpression of oncogene X and the deregulation of the NF-κB pathway due to the loss of gene Y. These findings were confirmed using patient samples through flow cytometry and Western blotting (WB).

Progress & Collaborative Research: In a joint research effort with the Chronic Lymphocytic Leukemia Research Study Group (CLLRSG-01), Saga University, Jikei University, and Kyoto University Biobank, we compiled cases of HCLjv and classic HCL (HCLc, n = 7; HCLv + HCLjv, n = 24). WGS revealed that 20% of HCLv + HCLjv cases had the X-related SV, strongly suggesting its involvement in the pathogenesis of HCLjv.

Future Directions: Currently, we are developing a conditional knock-in mouse model that recapitulate the X mutation to explore its functional significance in vivo. Simultaneously, we are evaluating an in vivo model involving the transplantation of HCL cell lines into immunodeficient mice. These cell lines are characterized by the overexpression of gene X and the knockdown of gene Y. These studies aim to deepen our understanding of the molecular mechanisms underlying HCLjv and pave the way for targeted therapeutic approaches.

Conclusion: This research marks a significant step in unraveling the complex genomic landscape of HCLjv, potentially leading to more effective treatments and a better understanding of this rare leukemia variant.

4. Flow cytometric profiles with CD7 and CADM1 reflect clinical course and predict prognosis of ATL

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Flow cytometric plots of CD7 versus CADM1 in CD4 + T cells have been shown to be indicators of clonal evolution from HTLV-1 carriers to aggressive ATL. However, the role of flow cytometric profile on predicting clinical courses of ATL has not been elucidated. We retrospectively analyzed the clinical and flow cytometric data (n = 497) of 92 HTLV-1 infected patients from June 2012 to January 2023. We focused on the ratio of CD7 + CADM1- fraction (P fraction; normal cells) and CD7- CADM1 + fraction (N fraction; infected/tumor cells) in CD4 + cells and referred to this as N/P ratio. N/P ratio significantly correlated with laboratory data reflecting the disease severity including sIL-2R (U/ml), LDH (U/L), WBC count (/μL), and morphologically defined ATL cells (%, /μL). In addition, changes of N/P ratio before and after treatment was significantly correlated with clinical response (CR/PR vs SD: P<0.001, SD vs PD: P = 0.022). Furthermore, N/P ratio predicted outcomes of aggressive ATL cases. Pre-treatment N/P ratio stratified ATL cases who received chemotherapy in terms of OS (n = 57; HR 2.60, 95%CI 1.15-5.87, P = 0.017). N/P ratio after 1 course of treatment identified favorable cases with higher precision (n = 57; HR 2.84, 95%CI 1.29-6.21, P = 0.006 for OS; HR 2.57, 95%CI 1.21-5.43, P = 0.011 for PFS). Pre-allo-HSCT N/P values also predicted outcomes (n = 33; HR 3.32, 95%CI 1.15-9.58, P = 0.019 for OS; HR 3.72, 95%CI 1.26-11.01, P = 0.011 for PFS). These results suggest that flow cytometric profiles with CD7 and CADM1 could be a useful indicator to predict disease status and outcomes of aggressive ATL.

5. Clinical and prognostic features of Langerhans cell histiocytosis in adults

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Langerhans cell histiocytosis (LCH) is a rare disease characterized by clonal expansion of CD1a + CD207 + myeloid dendritic cells. The features of LCH are mainly described in children and remain poorly defined in adults; therefore, we conducted a nationwide survey to collect clinical data from 148 adult patients with LCH. The median age at diagnosis was 46.5 (range: 20–87) years with male predominance (60.8%). Among the 86 patients with detailed treatment information, 40 (46.5%) had single system LCH, whereas 46 (53.5%) had multisystem LCH. Moreover, 19 patients (22.1%) had an additional malignancy. BRAF V600E in plasma cell-free DNA was associated with a low overall survival (OS) rate and the risk of the pituitary gland and central nervous system involvement. At a median follow-up of 55 months from diagnosis, six patients (7.0%) had died, and the four patients with LCH-related death did not respond to initial chemotherapy. The OS probability at 5 years post-diagnosis was 90.6% (95% confidence interval: 79.8–95.8). Multivariate analysis showed that patients aged ≥60 years at diagnosis had a relatively poor prognosis. The probability of event-free survival at 5 years was 52.1% (95% confidence interval: 36.6–65.5), with 57 patients requiring chemotherapy. In this study, we first revealed the high rate of relapse after chemotherapy and mortality of poor responders in adults as well as children. Therefore, prospective therapeutic studies of adults with LCH using targeted therapies are needed to improve outcomes in adults with LCH.

6. A clonal relationship between histiocytic neoplasms and additional malignancies.

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Background: Histiocytic neoplasms (HN) in adults were reported that they were associated with a high prevalence of hematologic and solid malignancies.

Purpose: We identified the cases with different histiocytic neoplasms and additional malignancies bearing identical oncogenic mutations.

Methods: We investigated associations between histiocytic disorders and additional malignancies bearing the same genetic alteration(s) using whole-exome sequencing of the lesion tissue.

Results: We report three cases in which mutational analysis elucidated the clonal relationship and evolution.

Conclusion: These cases help to unravel the underlying clinicopathological mechanisms of increased association of malignancies in histiocytic neoplasms.

7. Antibody response after SARS-CoV-2 mRNA vaccination after single-unit cord blood transplantation.

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Allogeneic hematopoietic cell transplantation is a risk for COVID-19 severity and vaccination is recommended. On the other hand, immune reconstitution is more likely to be delayed after cord blood transplantation compared to adult donors. Since there was a lack of previous reports on the efficacy of mRNA vaccination after cord blood transplantation, we tested the efficacy of mRNA vaccination using our cohort, which includes a large number of post-cord blood transplant patients.

Between September 2021 and March 2022, we analyzed 138 patients aged 16 years and older in our department who received two doses of BNT162b2 or mRNA-1273 during or after treatment for hematopoi-

etic diseases. 2 weeks after the second dose, serum anti-S protein IgG antibody titer was measured using Abbott SARS-CoV-2 IgG II Quant. Patients were divided into two groups: after cord blood transplantation and after allogeneic bone marrow/peripheral blood stem cell transplantation, as well as those who did not undergo allogeneic transplantation, and were compared retrospectively.

The number of patients was 51, 16, and 71, respectively. Median age was 54 (range, 22-74), 51 (29-68), and 60 (26-89) years, and the most common diseases were acute myeloid leukemia in 23 (45%), B-cell malignant lymphoma in 4 (25%), and B-cell malignant lymphoma in 22 (31%), respectively. Median time from last treatment to vaccination was 3.2 (range, 0.0-21.0), 7.6 (0.0-33.4), and 0.0 (0.0-10.7) years, respectively. When antibody titer of 7.1 BAU/ml or higher was defined as antibody acquisition, antibody acquisition was observed in 94.1% of patients after cord blood transplantation, 93.8% after allogeneic bone marrow/peripheral blood stem cell transplantation, and 91.5% of non-transplant cases after 2-dose immunization. After cord blood transplantation, systemic steroid administration at the time of initial immunization ($p = 0.014$) and low total lymphocyte count ($p = 0.023$) affected failure to acquire antibodies. The lower cumulative incidence of extensive chronic graft-versus-host disease after cord blood transplantation and the superior post-transplant B lymphocyte engraftment compared to other grafts may have contributed to the relatively good antibody acquisition after cord blood transplantation.

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Founded in 1981, IMSUT Hospital started its HIV clinic in 1986, and as of 2023, 1328 HIV-infected patients have visited us. Currently, a total of 572 patients are actively under our clinical management. In addition to HIV infection, we also provide treatment for other infections such as hepatitis and malaria. Furthermore, we have addressed cases of mpox, an emerging and re-emerging infection prevalent among HIV-MSM.

1. Treatment of HIV/AIDS and sexually-transmitted diseases 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 572 patients in total are under medical management in our outpatient clinic. Five hundred fifty-seven people living with HIV (PLWH) are receiving antiretroviral therapy [1] 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, the results of the SOLAR study, Study to Evaluate Efficacy and Safety of Cabotegravir (CAB) Long Acting [1] Plus (+) Rilpivirine (RPV) LA Versus BIKTARVY® (BIK) in Participants With Human Immunodeficiency Virus (HIV)-1 Who Are Virologically Suppressed were presented at national and international conferences, and real-world data from our hospital on CAB plus RPV were presented at conferences and in papers. We also published a paper on the treatment and clinical findings of mpox, an emerging infectious disease of HIV-MSM.

2. Changes in inflammatory biomarkers and lipid profiles after switching to long-acting cabotegravir plus rilpivirine

Eisuke Adachi, Makoto Saito, Amato Otani, Michiko Koga, Hiroshi Yotsuyanagi

We assessed whether the impact of cabotegravir plus rilpivirine on inflammation reduction differs from that of oral antiretrovirals, using real-world data. Inflammatory biomarkers and lipid profiles were followed from baseline to 8 months after switching. Seventy-eight participants were analyzed. The CD4/CD8 ratio and C-reactive protein did not change. There were transient decreases in CD8 and CD4 counts in the group that switched from the dolutegra-

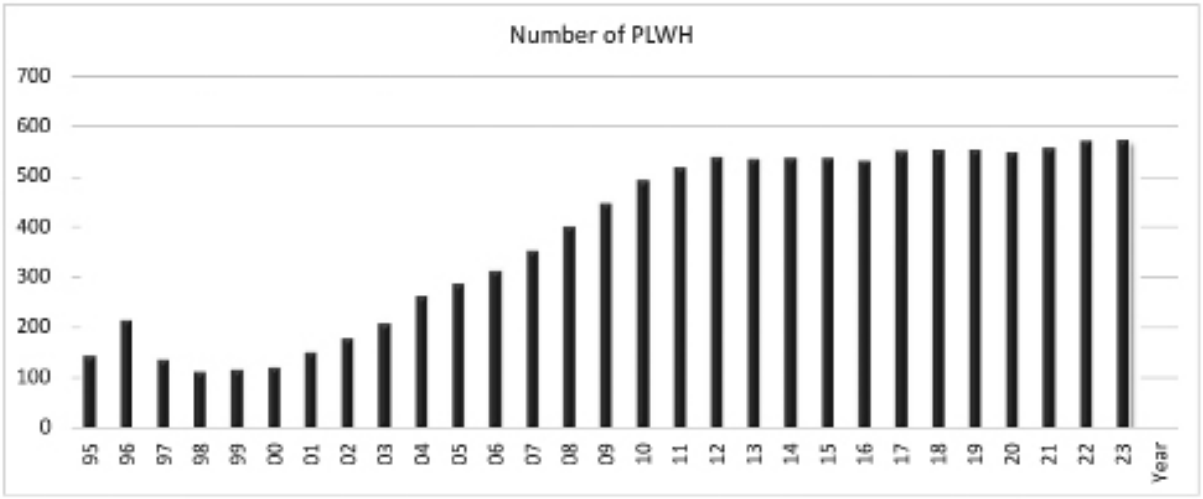

Figure 1. Number of PLWH attending IMSUT Hospital

vir-based regimen, but not in the tenofovir alafenamide-based regimen group. High-density lipoprotein (HDL) cholesterol increased, resulting in a decrease in the total-cholesterol to HDL cholesterol ratio, whereas there was no significant change in low-density lipoprotein cholesterol.

3. Mpox associated with Panton-Valentin leucocidin-producing methicillin-resistant Staphylococcus aureus among people with HIV

Eisuke Adachi, Kazuhiko Ikeuchi, Amato Otani, Makoto Saito, Michiko Koga, Hiroshi Yotsuyanagi

We report three cases of mpox (disease caused by the monkeypox virus) that developed in people with HIV co-infected with Panton-Valentin leucocidin-producing methicillin-resistant Staphylococcus aureus (PVL-MRSA), diagnosed in mid-February 2023. All three cases had preserved HIV immune status, and their mpox was mild and resolved without antiviral medications, but the trigger for their visit was the presence and history of skin and soft tissue infections. Our cases suggest that mpox is already prevalent among sexually active MSM in Tokyo, Japan. PVL-MRSA has been extremely rare in the general population of Japan, but several literatures reported widespread prevalence of PVL-MRSA among sexually active MSM-HIV. Mpox will become prevalent in the future in a population of sexually active MSM at high risk for PVL-MRSA infection, requiring an understanding of the interaction and pathogenesis of the two diseases.

4. 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. 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).

5. Bioterrorism-related information website management and crisis management

“バイオテロ対応ホームページ” (a website providing information on bio-terrorism in Japanese), which was developed in 2008 to provide information on clinical diagnosis and testing procedures for bioterrorism-related diseases for medical institutions, and was opened to the public in 2016 in anticipation of international situations and mass gathering events in Japan.

6. Crisis management for the future: Building a platform to provide information on emerging and re-emerging infectious diseases from normal times in Japan

Eisuke Adachi, Amato Otani, Hiroshi Yotsuyanagi, Masayuki Saijo[1], Tomoya Saito[2]

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At the beginning of the mpox (disease caused by monkey pox) epidemic, there was no platform in Japan to provide appropriate information on emerging and reemerging infectious diseases, and the number of accesses to bioterrorism-related information sites increased rapidly. Even though the interest in mpox

was much smaller than in coronavirus infectious disease, emerged in late 2019 (COVID-19), the increase in the number of views were much greater than during the COVID-19 epidemic. This may not be because mpox is bioterrorism-related as an analog of smallpox, but rather because there were no other websites providing information on mpox. For future crisis management, there should be a platform for providing information on emerging and reemerging infectious diseases from normal times.

7. 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.

8. 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.

9. Incidence of sexually transmitted hepatitis C virus infection among men who have sex with men in Japan from 2009 to 2023

Eisuke Adachi, Makoto Saito, Tadashi Kikuchi, Kazuhiko Ikeuchi, Michiko Koga, Takeya Tsutsumi, Hiroshi Yotsuyanagi

Although the prevalence of hepatitis C virus (HCV) infection has decreased significantly with the advent of direct-acting antiviral agents, HCV is known to spread as a sexually transmitted disease among men who have sex with men (MSM), and this study aims to provide a perspective on the future prevalence of HCV in Japan. We examined incidence in two groups of MSM with HIV attending our institution in this retrospective cohort study, from 2009 to 2019 and from 2020 to May 2023 and investigated their background factors. Twenty-two cases were newly confirmed to be HCV infection in 2009-2019 and a total of 9 cases in 2020-2023, with an incidence rate of 5.04 per 1000 person-years in 2009-2019 and 5.55 per 1000 person-years in 2020-2023. All of them were diagnosed at routine outpatient visits for HIV, and few cases were considered to have symptoms of suspected hepatitis that led to a visit to the hospital and a diagnosis of HCV. Although HCV is still prevalent among MSM in Japan, it is possible that it would not have been diagnosed without testing at regular visits as in the case of people with HIV, and that the true prevalence rate among MSM, including non-HIV-infected persons, may be much higher.

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IMSUT Hospital

Department of Rheumatology and Allergy
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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

Tomonao Tanaka, Satsuki Aochi, Masaaki Uehara, Motohisa Yamamoto

Rheumatologists at our division provide state-of-the-art diagnosis and treatment for systemic autoimmune diseases (the total number of patients was approximately 3,000 per year). Our physicians have active basic and clinical research projects and also are involved in the 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 patients with IgG4-related disease and develop novel diagnostic and therapeutic approaches for IgG4-related disease

Tomonao Tanaka, 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 (TOMORROW registry), and started to enroll IgG4-related disease patients. We cooperate with national policies and also provide the data to the Rare Disease Data Registry of Japan (RADDAR-J), which was established by AMED. We will organize the clin-

ical figures of IgG4-related disease and develop a more accurate diagnostic and therapeutic approach by a TOMORROW registry.

Furthermore, using the obtained blood and tissue samples, we will carry out a 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. 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

Tomonao Tanaka, 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 reflects 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 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 prevention 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.

Patients with various, mainly gastrointestinal, cancers were treated by standard therapy including cytotoxic chemotherapy, molecular target agents, immune checkpoint inhibitors, in combination with surgery and radiation therapy. The number of chemotherapy cases has been increasing: 116 chemotherapy regimens for 90 patients in 2023. 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. Patient enrollment to the clinical trial to prevent hand-hoot syndrome in colorectal cancer patients receiving adjuvant chemotherapy with capecitabine plus oxaliplatin has been completed. We are conducting a prospective observational study to improve our daily activities of patient care. In 2023, we started an investigator initiated phase I/II clinical trial of S-1 at an elevated dose according to the BBT formula developed by us in combination with oxaliplatin and nivolumab for advanced gastric cancer in collaboration with other hospitals. For new drug development, we enrolled two patients to the phase III trial of ipilimumab plus nivolumab and cytotoxic agents, and one clinical trial for gastric cancer has been initiated in 2023: a phase III trial of ONO-4578 in combination with nivolumab and chemotherapy and a phase I trial of recombinant SLAM-blind measles virus, which was developed in our institute, for nectin-4-positive solid tumors. Furthermore, 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. 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 2023, 94 patients (18 with drug allergies, 34 with drug-resistant *H. pylori*) visited our hospital and received eradication treatment. Of these patients, 86% were successfully eradicated.

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

Matsubara Y., Hirata Y.

About 760 cases of upper gastrointestinal endoscopy and about 770 cases of colonic endoscopy were performed under cooperation with department of surgery from January 1 to December 31, 2023. 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.

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 tokiwa 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 to the publication of Japanese clinical guideline of Duchenne muscular dystrophy.

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IMSUT Hospital

Department of Applied Genomics
ゲノム診療科

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Associate Professor	Tsuneo Ikenoue, M.D., Ph.D.	准教授　博士(医学)	池　上　恒　雄

Our department has been working on the application of human genome information in clinics. As clinical services in IMSUT Hospital, we provide genetic counseling, genetic tests for human diseases, 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

Yoichi Furukawa

As a part of clinical service, we have performed genetic analysis of human neoplasms including colorectal cancer. A total of 25 cases were analyzed by WGS in 2023. 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 2023, we had a total of 23 counseling cases for various hereditary disease such as myotonic dystrophy, Duchenne muscular dystrophy, Huntington's disease, familial thoracic aortic aneurysm and dissection, Hirschsprung disease, and Lynch syndrome. In the counseling, we provided appropriate information about hereditary diseases to the clients and took their psychological care in collaboration with a clinical psychologist. Genetic testing was performed in cases with informed consent after thoughtful discussion about its merit and demerit.

Systematic surveillance programs are provided for the patients susceptible for hereditary tumors.

3. Application of liquid-based genetic diagnosis for the screening of endometrial cancer

Kiyoko Takane[1], Kiyoshi Yamaguchi[1], Tsuneo Ikenoue, Yoichi Furukawa
[1]Division of Clinical Genome Research, Advanced Clinical Research Center

We have conducted a study to elucidate the usefulness of liquid-based genetic diagnosis (LBGDx) for screening of endometrial cancer (EC) in collaboration with Department of Obstetrics and Gynecology, Sapporo Medical University. Although liquid-based cy-

tology (LBC) has increased the sensitivity of cytological diagnosis of EC compared with conventional smear cytology, the sensitivity of LBC for the detection of EC 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. Importantly, driver alterations were identified in 32 out of 125 women who did not have malignant or premalignant conditions. *PTEN* mutations were identified in two (1.6%) of the 125 women without malignant or premalignant diseases but were found in 20 (51.3%) of the 39 patients with endometrial malignancies. Additionally, *CTNNB1* and *TP53* mutations were identified in single cases (0.8%) of the 125 individuals but were found in eight (20.5%) and five (12.8%), respectively, in the 39 women with endometrial malignancy. These data suggest that caution is warranted for the interpretation of genetic alterations detected in endometrial samples. Although further studies are necessary, *PTEN*, *CTNNB1* and *TP53* mutations may be useful for the biomarkers to predict endometrial malignancies.

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. Patients with colorectal, gastric, cervical cancer, and pheochromocytoma who 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 our tumor board. This board consisted of physicians, medical oncologists, genetic counsellors, geneticists, bioinformaticians, and experts of ethics, and was held online every two weeks.

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Department of Radiological Technology
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Head Radiologic Technologist	Kenji Ino, RT	放射線技師長		井 野 賢 二

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.

Feasibility study of direct CT lymphangiography in mice: comparison with interstitial CT/MR lymphangiography.

Sugawara H, Furuta T, Sumiyoshi A[1], Iiyama M[1], Kamitani M, Suzuki A[2], Murakami A[2], Abe O[3], Aoki I[1], Akai H.

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In the present study, we aimed to establish a CT lymphangiography method in mice via direct lymph node puncture. We injected healthy mice (n = 8) with 50 μl of water-soluble iodine contrast agent (iomeprol; iodine concentration, 350 mg/mL) subcutaneously into the left-rear foot pad (interstitial injection) and 20 μl of the same contrast agent directly into the popliteal lymph node (direct puncture) 2 days later. Additionally, we performed interstitial MR lymphangiography on eight mice as a control group. We calculated the contrast ratio for each lymph node and visually assessed the depiction of lymph nodes and lymphatic vessels on a three-point scale. As a result, the contrast ratios of 2-min post-injection images of sacral and lumbar-aortic lymph nodes were 20.7 ± 16.6 (average ± standard deviation) and 17.1 ± 12.0 in the direct puncture group, which were significantly higher than those detected in the CT or MR interstitial lymphangiography groups (average, 1.8-3.6; p = 0.008-0.019). The visual assessment scores for sacral lymph nodes, lumbar-aortic lymph nodes, and cisterna chyli were significantly better in the direct puncture group than in the CT interstitial injection group (p = 0.036, 0.009 and 0.001, respectively). The lymphatic vessels between these structures were significantly better scored in direct puncture group than in the CT or MR interstitial lymphangiography groups at 2 min after injection (all p ≤ 0.05). To sum up, in CT lymphangiography in mice, the direct lymph node puncture provides a better delineation of the lymphatic pathways than the CT/MR interstitial injection method, which we believe that direct CT lymphangiography will be the first choice for the evaluation of lymphatic pathways in mice.

Early detection of hypervascularization in hepatocellular carcinoma (≤ 2 cm) on hepatic arterial phase with virtual monochromatic imaging: Comparison with low-tube voltage CT.

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This study aims to assess the diagnostic value of virtual monochromatic image (VMI) at low keV energy for early detection of small hepatocellular carcinoma (HCC) in hepatic arterial phase compared with low-tube voltage (80 kVp) CT generated from dual-energy CT (DE-CT). A total of 107 patients with 114 hypervascular HCCs (≤2 cm) underwent DE-CT, 140 kVp, blended 120 kVp, and 80 kVp images were generated, as well as 40 and 50 keV. CT numbers of HCCs and the standard deviation as image noise on psoas muscle were measured. The contrast-to-noise ratios (CNR) of HCC were compared among all techniques. Overall image quality and sensitivity for detecting HCC hypervascularity were qualitatively assessed by three readers. The mean CT numbers, CNR, and image noise were highest at 40 keV followed by 50 keV, 80 kVp, blended 120 kVp, and 140 kVp. Significant differences were found in all evaluating endpoints except for mean image noise of 50 keV and 80 kVp. Image quality of 40 keV was the lowest, but still it was considered acceptable for diagnostic purposes. The mean sensitivity for detecting lesion hypervascularity with 40 keV (92%) and 50 keV (84%) was higher than those with 80 kVp (56%). Low keV energy images were superior to 80 kVp in detecting hypervascularization of early HCC.

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6. Akai H, Yasaka K, Sugawara H, Tajima T, Akahane M, Yoshioka N, Ohtomo K, Abe O, Kiryu S. Commercially Available Deep-learning-reconstruction of MR Imaging of the Knee at 1.5T Has Higher Im-

age Quality Than Conventionally-reconstructed Imaging at 3T: A Normal Volunteer Study. Magn Reson Med Sci. 22:353-360 2023.
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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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特任講師 博士(医学) 土方康基
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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

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. 2023 Mar;37(1):26-33.

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IMSUT Hospital

Department of Diagnostic Pathology
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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 = 1540
Biopsy	n = 959
Surgical resection	n = 294
Bone marrow aspiration	n = 176
Intraoperative diagnosis	n = 22
Consultation	n = 78
Other	n = 11
Cytological diagnosis	n = 445
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 = 71).

Publications

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IMSUT Hospital

Department of Gastroenterology
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The department of Gastroenterology, which focuses on endoscopy and treatment, started in October 2023. We are committed to quality endoscopy, and our mission is to provide careful observation, accurate diagnosis using magnifying endoscopes, and advanced treatments such as endoscopic submucosal dissection (ESD). In addition, collaborating with various departments for comprehensive, minimally burdensome patient care.

1. Introduction

We primarily focus on endoscopic examinations for the early detection and treatment of esophageal, gastric, and colorectal cancers. Our mission is to provide painless endoscopic procedures, thorough and minimally overlook observations, precise diagnoses using magnification endoscopy, and advanced endoscopic treatments, particularly Endoscopic Submucosal Dissection (ESD). In addition, we aim to tailor our approach to each specific lesion, choosing the most suitable treatment options and collaborate with Oncology, Surgery, Radiology, and other departments to provide treatment that that impose minimal burden on the patient's overall well-being. The outpatient clinic treats all gastrointestinal diseases and also offers Helicobacter pylori outpatient clinic.

2. Endoscopic examination

Endoscopic examinations are conducted for screening, investigating symptomatic cases, and precisely diagnosing and treating lesions. Additionally, procedures such as gastric fistula construction, ileus tube insertion, and stent placement are also performed.

When a lesion is detected, a magnifying endoscope is used to closely examine the surface microvasculature and pit patterns. Endoscopic treatment options encompass cold polypectomy, hot snare polypectomy using bipolar snare, EMR (Endoscopic Mucosal Resection), and ESD (Endoscopic Submucosal Dissection), selected based on the size, morphology, and depth diagnosis of the detected lesion.

The number of endoscopies and treatments during the year were as follows; upper endoscopy: 753 cases, colonoscopy: 521 cases, gastric ESD: 6 cases, other gastric treatments: 3 cases, colorectal ESD: 9 cases, and other colorectal treatments: 239 cases. We plan to significantly increase both the number of endoscopic examinations and treatments in the next year and beyond. We also plan to participate in single-center and multi-center clinical studies.

3. Treatment of drug-resistant Helicobacter pylori infection

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, we make correct diagnosis of infection by multiple modalities, give eradication therapy for these refractory patients, and achieve high rates of successful eradication.

The number of patients who visited the Helicobac-

ter pylori outpatient clinic in the past year was 94 cases, (including 18 cases with allergies, 19 cases with difficulty in achieving third-stage sterilization, and 15 cases with fourth-stage sterilization and beyond.)

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Department of Surgery
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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

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, Dr. Sakuyama, Dr. Onoyama, Dr. Kojima, and Dr. Monma), 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. We also started robotic surgery for colon cancer in September, 2022.

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Our clinical practice and clinical studies have been focused on (1) risk management of medical electronic devices in Research Hospital. (2) anesthetic management in patients undergoing major surgery including joint arthroplastic surgery for hemophilia patients, variable surgical procedures for translational researches (3) assessment of functional failure of the internal valve of anesthesia machine (4) assessment of reliability of cardiac output measurements .

1. 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.

2. Anesthetic management for carrier hemophilia.

Hemophilia is X-linked gene disease with the activity abnormality of the coagulation factor. The hemophilia A is caused by factor VIII abnormality, and the hemophilia B is caused by factor Ⅸ abnormality. Careful hemostatic management is required in perioperative care of the hemophilic patients. It is usually recommended that we perform coagulation factor replacement therapy and hemostatic monitoring.

We experienced anesthesia management of the orthopedic surgery of patients with hemophilia B that underwent living-donor liver transplantation for cirrhosis due to the hepatitis C virus this time. We carried out hemostatic monitoring and perioperative management, but did not require coagulation factor replacement therapy. There were no complications such as postoperative bleeding and infection.

Female hemophilia patients are often not informed as carriers themselves, and there is a possibility that medical practice may be performed without recognizing them as hemophilia patients. We experienced anesthesia of female hemophilia patients and safety managed anesthesia with appropriate hemostatic management.

3. Assessment of 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 anes-

thesia.

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.

4. 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.

IMSUT Hospital

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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 hemophilia by concentrates, however there are few hospitals focus on surgical treatments except us. Many hemophilia 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 2023, more than 270 surgical treatments for hemophilia induced other coagulation diseases such as deficiency factor VII, Von Willebrand diseases of afibrinogenemia. Some of them have the deficiency factor antibody as well.

Publication 2023

Kumiko Ono, Jun Hirose, Megumi Noguchi, Kayoko Asano, Masaaki Yasuda, Hideyuki Takedani Extension contracture stiff knee in haemophilia: Surgical timing and procedure for total knee arthroplasty. Mod Rheumatol. 2023 Jul 4;33(4):851-855. doi: 10.1093/mr/roac067.

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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

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 melano-

ma 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 112 operations were carried out in 2023 including 107 gliomas and 3 olfactory neuroblastomas. More than 100 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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IMSUT Hospital

Department of Urology
泌尿器科

Professor	Haruki Kume, M.D., Ph.D.		教　授	博士(医学)	久　米　春　喜
Project Senior Assistant Professor	Sayuri Takahashi, M.D., Ph.D.		特任講師	博士(医学)	高　橋　さゆり
Project Assistant Professor	Mariko Tabata, M.D.		特任助教		田　畑　真梨子

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 842 cases of urological surgery including 140 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

The WNT signaling pathways (canonical and non-canonical) regulate prostate cancer progression, but the precise mechanism by which this regulation occurs has yet to be established. We investigated expression levels of WNTs in prostate cancer tissues and found that WNT5A is expressed at higher levels in cancer cells and in the stroma of non-cancerous regions than in the epithelium in prostate of cancer in patient samples. Prostate stroma-derived WPMY1 cells also highly express WNT5A. WNT5A knockdown in WPMY1 cells (WPMY1-shW5A) results in a reduction of cancer related genes, EMT-related genes, and several cytokines by microarray analysis. WNT5A expression is directly regulated by ligand inducible androgen receptor (AR) bound to the WNT5A promoter. Receptor tyrosine kinase-like orphan receptor 1 (ROR1), one of WNT5A receptors is highly expressed in samples from prostate cancer patients and in prostate cancer cell lines. ROR1 knockdown in PC3 cells (PC3-shRO1) abrogated WNT5A-induced cell proliferation. These results show that WNT5A in stroma cells regulates prostate cancer proliferation and invasion, at least in part, through the ROR1 signaling pathway. Our results suggest that cell-to-cell communication between stromal cells and prostate cancer cells enhances prostate cancer progression and ROR1 may be a novel therapeutic target for the disease.

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 1,680 patients visited our department in 2023 for the purpose of thorough examinations for diagnosis or surgical treatments.

We totally performed 292 surgical operations in 2023: 43 cases of Robotic-assisted prostatectomy, 10 Robotic-assisted partial nephrectomy, one Laparoscopic nephroureterectomy, three Radical cystectomy with ileal conduit urinary diversion, 16 open surgery, 28 Trans-urethral resection of bladder tumor, eight Trans-urethral resection of prostate, 14 Trans-urethral lithotripsy, 23 Ureteroscopy, 41 Ureteral stenting, and five Botox injection for overactive bladder

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IMSUT Hospital

Department of Medical Informatics
医療情報部

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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, and in late 2023, we updated the system. 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

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 been supporting the cell processing for translational studies preformed in 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 for treatment-resistant severe acute graft-versus host disease, acute cerebral injury, and radiation injury.

1. Transfusion medicine and related tests

Abe Y, Ogami K, Iwasawa N, Yokoyama K, 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.

2. Cell Processing and quality tests for Hematopoietic stem cell transplantation and clinical trials.

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

For autologous peripheral blood stem cell transplantation (PBSCT), 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 test CD34-positive cells in the graft of PBSC, bone marrow, and cord blood as the quality tests for hematopoietic transplantations. We process the cells for clinical trials including collection (apheresis), cryopreservation, and thawing with or without washing upon the requests.

3. Exploring the therapeutic application of UC-MSCs for severe acute graft-versus-host disease (aGVHD) and non-infectious pulmonary complications (NIPC) after hematopoietic stem cell transplantation

Nagamura-Inoue T, Takahashi A, Hori A, Miharu Y, Mori Y, Nagamura F, Yokoyama K

We investigated the immunosuppressive mechanisms of UC-MSCs on inflammatory cells. A phase I dose-escalation trial, IMSUT-CORD for steroid-resist-

ant aGVHD using allogeneic umbilical cord-derived mesenchymal stromal cells (IMSUT-CORD) have been safely completed (Int J Hematol. 2022 Nov; 116(5):754-769.). From 2022 to 2023, clinical trial of NIPC treated with UC-MSCs have been implemented. We continued to prepare the next clinical trials by obtaining the pre-clinical POC for aGVHD and NIPC in vitro and in vivo.

4. Study of therapeutic application of UC-MSCs to acute brain injury and importance of microglia

Sei K, Mori Y, Mukai T, Nagamura-Inoue T

Based on the efficacy of proof of concept using UC-MSCs for cerebral palsy by Mukai T et al, a clinical trial (Phase I/II) for cerebral palsy treated with UC-MSCs was implemented from 2021 to 2023, and completed safely. Furthermore, we investigated the efficiency of UC-MSCs for the treatment of acute encephalitis (AE) mimicking the viral encephalitis. We found the improvement of the neuron degeneration and part of behavior abnormalities in AE by intravenous injection of 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

Mori Y, Nagamura-Inoue T, 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, cleft palate, and hemorrhagic arthropathy using mice models in collaboration with companies.

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

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

The Research Cord Blood bank / resource was established in 2004 and supported by the Ministry of Education, Culture, Sports, Science and Technology for the development of regenerative medicine, immunological cell therapy, infection research, modified gene cell therapy, and drug discovery. Since July 2012, this project has been incorporated into the National BioResource Project (NBRP). The research umbilical cord blood (CB) bank provides processed and cryopreserved CB units (nucleated cells, mononuclear cells, and CD34 + cells) to researchers worldwide via the RIKEN Bioresource Center. The website is at http://www.nbrp.jp/.

7. Institute of Medical Science, University of Tokyo, Cell Resource Center (IMSUT-CRC)

Takahashi A, Miharu Y, Hori A, Mori 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–), 6) aAVC-WT1 cell therapy (2017–), and (7) dendritic cell (DC) therapy using DCs pulsed with neoantigen (2020–).

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

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IMSUT Hospital

Surgical Center
手術部

Project Professor Minoru Tanaka, M.D., Ph.D.

特任教授 博士(医学) 田 中 実

IMSUT hospital provides seamless support for translational research. Our mission is the management and operation of the surgical center to achieve a safe and organized environment where surgical procedures can be performed in high quality. A da Vinci surgical system (da Vinci Xi), a robotic technology that allows surgeons to perform minimally invasive procedures, was introduced in November 2020. Robot-assisted Radical Prostatectomies (RARP) for prostate cancer and robotic rectal surgery for tumors including rectal cancer and GIST are performed. The Medtronic Stealth Autoguide Platform was introduced in April 2023.

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. The Medtronic Stealth Autoguide Platform was introduced in April 2023. It is a robotic guidance system intended for instrument holders' spatial positioning and orientation. It is based on a pre-operative plan and feedback from an image-guided navigation system with 3D imaging software. Oncolytic virus therapy using the Stealth Autoguide Robotic system was started. The number of surgeries has increased over the past few years due to the introduction of surgical-assist robots, with a marked increase in da Vinci surgeries and oncolytic virus therapy.

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 2023

Total number of operations	671
Planned operations	655
Emergency operations	16
General anesthesia	323
Spinal	89
Epidural	210
Local	207
Others	49

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.

* Only achievements related to clinical laboratory medicine are shown here, and others overlapping across affiliations have been omitted. Please refer to each respective affiliation.

1. Umbilical cord blood and cord tissue banking as somatic stem cell resources to support medical cell modalities.

Tokiko NAGAMURA-INOUE.

Human umbilical cord blood (CB) and umbilical cord tissue (UC) are attractive sources of somatic stem cells for gene and cell therapies. UC-derived mesenchymal stromal cells (UC-MSCs) have a higher proliferation potency than those derived from adult tissues and can be used anon-HLA restrictively. We established the off-the-shelf cord blood/cord bank IMSUT CORD to support novel cell therapy modalities, including the CB-derived immune cells, MSCs, MSCs-derived extracellular vesicles, biological carriers loaded with chemotherapy drugs, prodrug, oncolytic viruses, nanoparticles, human artificial chromosome, combinational products with a scaffold, bio3D printing, and so on. [Inflamm Regen. 2023.]

2. Research for quantification of immune function of chimeric antigen receptor T (CAR-T) cells by use of new molecular imaging flow cytometry (MI-FCM).

Tomohiro ISHIGAKI.

Assays to evaluate CAR T-cell function more sensitively are required in clinical and basic research. However, conventional methods, such as cytotoxicity assays and in-vitro cytokine production assays, require a long cell-culture processing time and are less

quantitative. We focused on the aggregation of CAR molecules, which is observed after the antigen recognition and the immune response of CAR-T cells. We established the quantitative assay to analyze the change in the localization of CAR molecules using novel molecular imaging flow cytometry (MI-FCM). We found the parameter that is highly correlated with killing activity in various CAR T-cells. Quantifying the CAR localization in CAR T-cells by MI-FCM could be useful for evaluating their immune function. [The 70th annual meeting of JSLM. 2023.]

3. Morphological changes in abnormal lymphocytes in a case of adult T-cell leukemia/lymphoma (ATL) that transformed from indolent to acute type.

Clinical hematology team, Tomohiro ISHIGAKI, and Tokiko NAGAMURA-INOUE.

Recently, accurate identification of adult T-cell leukemia/lymphoma (ATL) cells has been possible through methods such as flow cytometry, but cell morphology diagnosis using microscopy remains very important in routine clinical practice. Abnormal lymphocytes in ATL differ from leukemic blast cells, and their differentiation is often very challenging and requires expertise. The changes in morphology and staining (color mixing of CMYK) of abnormal lymphocytes were examined in a case of ATL that transformed from an indolent to an acute type. The results showed that abnormal lymphocytes with larger cell size and nucleus size emerged at the time of acute transformation. There was also an increase in nuclear indentations and lobulations. While there are limitations in assessing color tone changes visually, it was possible to significantly detect slight differences in color tones between normal and abnormal lymphocytes by using CMYK values for evaluation on the same specimen. [Motoko Mizukami reported at the 70th annual meeting of JSLM. 2023.]

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 6 clinical investigations and trials conducted in this hospital, including treatments using new drugs and new cell therapy. 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

Head, Professor	Yasuhito Nannya, M.D., D.M.Sc.	教　授　博士(医学)	南　谷　泰　仁

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, 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
Nurse Manager	Mika 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　佳
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 StewardshipTeam) to promote the practice of hospital infection control and prevent the spread of antimicrobial resistant organisms. The ICT consists of control , an infection control nurse, a pharmacist, a clinical laboratory technician and an administrative staff.

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 patient 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 2023, 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, phase II clinical trial with novel gene-induced adjuvant cells for acute myelogenous leukemia, administration of vaccine against the COVID-19 infection, and dendritic cell therapy for ATLL.

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 2023, we supported 27 basic researches (24: other than IMSUT), 17 preclinical studies (12: other than IMSUT), and 11 clinical studies (4: other than IMSUT) under this program. The number of studies we assist has been increasing year by year. Efficient operation of the organization is required.

5. Statistical consulting for basic research

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. Motoki Amai, Masanori Nojima, Yoshikazu Yuki, Hiroshi Kiyono, Fumitaka Nagamura. A review of criteria strictness in "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Vaccine. 2023 Aug 31;41(38):5622-5629. doi: 10.1016/j.vaccine.2023.07.072. Epub 2023 Aug 1.
2. Tokiko Nagamura-Inoue , Fumitaka Nagamura . Umbilical cord blood and cord tissue banking as somatic stem cell resources to support medical cell modalities. Inflamm Regen 2023 Dec 5;43(1):59. doi: 10.1186/s41232-023-00311-4.
3. Tokiko Nagamura-Inoue, Seiko Kato, Yuho Najima, Masamichi Isobe, Noriko Doki, Hisashi Yamamoto, Naoyuki Uchida, Atsuko Takahashi, Akiko Hori, Masanori Nojima, Kazuteru Ohashi, Fumitaka Nagamura, Arinobu Tojo. Immunological influence of serum-free manufactured umbilical cord-derived mesenchymal stromal cells for steroid-resistant acute graft-versus-host disease. Int J Hematol. 116(5):754-769, 2023.
4. Nogami K, Fujii T, Sawada A, Nagao A, Nagae C, Nojima M, Suzuki N, Nosaka D, Shimura T, Sugao Y, Amano K. Association of physical activity with bleeding events and safety in patients with haemophilia A starting emicizumab prophylaxis: an interim analysis of the TSUBASA study. Int J Hematol. 2023 Dec 15. doi: 10.1007/s12185-023-03679-8. Epub ahead of print. PMID: 38100026.
5. Nagao A, Chikasawa Y, Sawada A, Kanematsu T, Yamasaki N, Takedani H, Nojima M, Fujii T, Suzuki N, Matsushita T, Higasa S, Amano K; ADVANCE Japan Working Group. Haemophilia and cardiovascular disease in Japan: Low incidence rates from ADVANCE Japan baseline data. Haemophilia. 2023 Oct 8. doi: 10.1111/hae.14876. Epub ahead of print. PMID: 37806778.
6. Watanabe K, Nojima M, Nakase H, Sato T, Matsuura M, Aoyama N, Kobayashi T, Sakuraba H, Nishishita M, Yokoyama K, Esaki M, Hirai F, Nagahori M, Nanjo S, Omori T, Tanida S, Yokoyama Y, Moriya K, Maemoto A, Handa O, Ohmiya N, Tsuchiya K, Shinzaki S, Kato S, Uraoka T, Tanaka H, Takatsu N, Nishida A, Umeno J, Nakamura M, Mishima Y, Fujiya M, Tsuchida K, Hiraoka S, Okabe M, Toyonaga T, Matsuoka K, Andoh A, Hirota Y, Hisamatsu T; J-COMBAT study group. Trajectory analyses to identify persistently low responders to COVID-19 vaccination in patients with inflammatory bowel disease: a prospective multicentre controlled study, J-COMBAT. J Gastroenterol. 2023 Aug 10. doi: 10.1007/s00535-023-02029-z. Epub ahead of print. PMID: 37561155.

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 Professor	Minoru Tanaka, M.D., Ph.D.		特任教授	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 2023, 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), including G47Δ and various armed third-generation oncolytic 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 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; 2021–2023), and noninfectious pulmonary complication after allogeneic hematopoietic stem cell transplantation NIPC; 2022–2023). Our 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, Sakai T, Shibuya Y, Nagaya N, Ogami K, Mukai T, Nagamura F

Human umbilical cord blood (CB) and umbilical cord tissue (UC) are attractive sources of somatic stem cells for gene and cell therapies. CB and UC can be obtained noninvasively from donors. CB, a known source of hematopoietic stem cells for transplantation, has attracted attention as a new source of immune cells, including universal chimeric antigen receptor-T cell therapy (CAR-T) and, more recently, universal CAR-natural killer cells. UC-derived mesenchymal stromal cells (UC-MSCs) have a higher proliferation potency than those derived from adult tissues and can be used anon-HLA restrictively. We have established a CB/UC bank at the IMSUT hospital (IMSUT CORD) to collect CB and UC tissue after informed consent from the mothers in collaboration with the obstetricians. After receiving them, we stock the UC-tissue, and to manufacture master cells and product cells for research and clinical use.

To maintain quality control, we have introduced the ISO 9001:2015 quality management standards in IMSUT CORD since 2018. We have transferred the manufacturing and testing technologies to the client companies, where they apply our techniques and standards in their clinical trials including therapies for acute GVHD, cerebral palsy, and COVID-19 related acute respiratory distress syndrome (ARDS). The IMSUT CORD mission is to supply domestic UC-

MSCs and CB as a source of allogeneic somatic stem cells in research and clinical use. We have supplied clinical-grade UC-MSC products for clinical trials including severe acute graft-versus-host disease (GVHD; 2018-2020), COVID-19-related ARDS (2020-2022), cerebral palsy (PVL)(2021-2023), and non-infectious pulmonary disease after allogeneic hematopoietic stem cell transplantation NIPS; 2022-2023), after approval by the review board of IMSUT CORD and 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), where the manufacturing license was obtained in 2023.

Visit our website:
https://plaza.umin.ac.jp/imsutcord/

Publications

1. Nagamura-Inoue T, Nagamura F., Umbilical cord blood and cord tissue banking as somatic stem cell resources to support medical cell modalities, Inflamm Regen. 2023 Dec 5;43(1):59.

IMSUT Hospital

Department of Nursing
看護部

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

看護部長	看護管理者	吉　井　栄　子
副看護部長		久　原　みな代
副看護部長		小　澤　昌　子
看護師長		成　田　初　子
看護師長	修士(看護学)	小　粥　美　香
看護師長	修士(看護学)	佐　藤　朋　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
看護師長	修士(心理学)	砂　田　純　子
看護師長		杉　山　栄美子

The Department of Nursing aims to contribute to the medical team by providing high-quality patient-centered nursing care in order to respond to diversifying needs in line with changes in social conditions and advances in medicine. In 2023, the epidemic of the new coronavirus infection has subsided, and the nursing department structure has been changed in order to contribute to the maintenance of the medical care system.

In January 2023, the eighth wave of the new coronavirus infection (COVID-19) caused nurses to be infected, and clusters occurred, but they were contained within a week. Since then, the number of hospitalized patients due to COVID-19 and the number of staff infections have gradually decreased, and by February, no patients were hospitalized. Since May 8, the classification of COVID-19 infectious diseases has been shifted from Category 2 to Category 5, and measures against nosocomial infections have been relaxed.

However, due to the impact of the medical system on COVID-19 over the past three years, the number of nurses leaving their jobs and taking leave has increased, and this fiscal year has been decided to start with 20 fewer nurses than usual. Thirteen nurses had retired by September, including nurses who had extended their retirement dates and temporary nurses. In order to secure human resources, the Nursing Department Management Office engaged in various recruitment activities, such as the use of temporary staffing and recruitment agencies and nurse recruitment sites. As a result, applicants began to gather around July, and seven nurses were secured as of January 2024. During this time, we have been able to maintain medical and nursing services by dispatching support from other departments to wards that are understaffed.

In addition, due to a shortage of human resources, it became impossible to maintain the facility standard of 7:1 for the allocation of nurses for the basic hospitalization fee, and it was decided to make the 4th floor ward and the 5th floor ward one nursing unit, making it a three-ward system. From June, we tried to operate the department as a ward on the 4th and 5th floors. At first, the nurses complained, but the nurse heads of the two wards took time to talk with the nurses and listen to their thoughts. Arrangements were made so that nurses could experience working in both wards,

and by October it was back on track as a ward on the 4th and 5th floors. This achievement is thanks to the cooperation of the head nurses and nurses of the 4th and 5th floor wards.

With regard to medical and nursing services, due in part to the influence of the super-aging society, the number of hospitalized patients with deterioration in activities of daily living and cognitive ability has increased, and the number of patients who meet the end of their lives at our hospital has increased. As a result, psychological support for patients, daily life support, and monitoring to prevent falls and falls have become the main focus of nursing, and the mental burden is also large. There were also more opportunities to hold conferences with doctors, pharmacists, and other professionals on support for life after discharge. In recuperation, nurses are involved in nursing care while cherishing the time that they feel is good for the patient and their family. Sometimes, I would say, "I'm glad I was hospitalized here."

The number of surgeries has also increased overall, with an increase in the number of total cystectomy and ileal duct rearrangement in urology, as well as an increase in opportunities to be involved in postoperative stoma care. Sponsored by related departments, we held a study session on stoma care by a nurse who is Certified Nurse in Wound, Ostomy and Continence Nursing, and we were able to give back to the nursing of patients. It was also useful for the learning of nurses in other departments. Overall, a system has been established in which nurses are prepared for practical skills by holding study sessions in each department before engaging in new medical care and nursing.

This year, as part of our social contribution and nurse training, we accepted clinical nursing training for nursing students and training to support nurses returning to work after leaving their jobs. The clinical nursing training was highly evaluated by nursing students and faculty members, and the return-to-work support training was well received, so it was decided to continue it in the next academic year. Through this experience, the nurses were given the opportunity to improve their training skills and think about career development.

Publication

・嶋津千陽、山口恭子："臓器のきゃら"と一緒に学ぶ！消化器解剖と病態生理　消化器ナーシング vol.28 NO.4　P.16-28、メディカ出版　2023

・村松言子、真田ひかる："臓器のきゃら"と一緒に学ぶ！消化器解剖と病態生理　消化器ナーシング vol.28 NO.4　P.64-75、メディカ出版　2023

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・砂田純子、入院時から市中感染型MRSAを想定した治療を行い奏功したHIV感染者における蜂窩織炎の一例、第32回日本創傷・オストミー・失禁管理学会学術集会、仙台国際センター、2023. 7. 8・9

・野口麻衣子、山花玲子、砂田純子、「専門性の高い看護師による訪問看護師への遠隔相談の試行と実用可能性の検討」、第13回日本在宅看護学会学術集会、クロス・ウェーブ船橋、2030. 11. 18・19

・上山美香、中澤光子、中川沙織、織田ひとみ、千葉陽子、中村智子、砂田純子、当院においてHIV薬を特攻性注射薬に変更した患者の反応について、第37回日本エイズ学会学術集会　2023. 12. 3-5

IMSUT Hospital

Department of Pharmacy
薬剤部

Director	Seiichiro Kuroda
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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) Iimura Y, Furukawa N, Kuroda S. Delayed Oxaliplatin-Related Severe Neurotoxicity in Metastatic Colorectal Cancer: A Case Report. Cureus. 2023 Jan 10;15(1):e33578. doi: 10.7759/cureus.33578.
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3) Iimura Y, Iihara H, Aoyama T, Ishibashi M, Sasuga C, Furukawa N, Anzai E, Ijichi Y, Takahashi S, Tabata M, Niimi F, Kaneko J, Izukuri K, Baba K, Boku N, Kuroda S. Study Protocol for a Prospective Observational Study to Evaluate the Efficacy of Fosnetupitant for Long-Delayed Chemotherapy-Induced Nausea and Vomiting in Patients Receiving Platinum-Based Chemotherapy (LODEC-N). J Clin Trials Vol. 13 Iss. 4 No: 1000532.
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<Conference presentation>

1) 古川直樹、飯村洋平、石橋正祥、流石智恵子、馬場啓介、阿彦友佳、小野山温那、柵山尚紀、愛甲丞、志田大、野島正寛、黒田誠一郎、朴成和　カペシタビン誘発性手足症候群に対するヒドロコルチゾン外用剤の予防効果に関する第II相試験（T-CRACC study）　第一報　医療薬学会2023仙台
2) 流石智恵子、飯村洋平、古川直樹、石橋正祥、馬場啓介、中澤光子、佐藤朋子、都留由香里、吉井栄子、冨樫仁美、本田友絵、松原康朗、黒田誠一

郎、朴成和　がん化学療法施行患者における多職種連携チーム活動の臨床効果に関する前向き観察研究（M-CAST　study）　第一報　医療薬学会2023仙台

3) 石橋正祥、飯村洋平、黒田誠一郎　薬学生のアンケート結果からみたCOVID-19の流行に伴うオンライン・実地ハイブリッド実習の評価　医療薬学会2023仙台

IMSUT Hospital

Department of AIDS Vaccine Development
エイズワクチン開発担当

Invited 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. Impaired protective role of HLA-B*57:01/58:01 in HIV-1 CRF01_AE infection: a cohort study in Vietnam.

Tam Tran Thi Minh[1], Yuta Hikichi[2], Shoji Miki[2], Yuriko Imanari[2], Shigeru Kusagawa[2], Midori Okazaki[2], Thao Dang Thi Thu[3], Teiichiro Shiino[2], Saori Matsuoka[2], Hiroyuki Yamamoto[2], Jun Ohashi[4], William W. Hall[5], Tetsuro Matano, Lan Anh Nguyen Thi[1], Ai Kawana-Tachikawa: [1]Centre for Bio-Medical Research, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; [2]AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; [3]Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan; [4]Department of Biological Sciences, Graduate School of Sciences, University of Tokyo, Tokyo, Japan; [5]Center for Research in Infectious Diseases, School of Medicine & Medical Science, University College Dublin, Dublin, Ireland

Human Leukocyte Antigen *HLA-B*57:01* and *B*58:01* are considered anti-HIV-1 protective alleles. HLA-B*57:01/58:01-restricted HIV-1 Gag TW10 (TSTLQEQIGW, Gag residues 240-249) epitope-specific $CD8^+$ T cell responses that frequently select for a Gag escape mutation, T242N, with viral fitness cost are crucial for HIV-1 control. Although this finding has been observed in cohorts where HIV-1 subtype B or C predominates, the protective impact of *HLA-B*57:01/58:01* has not been reported in Southeast Asian countries where HIV-1 CRF01_AE is the major circulating strain. In this study, the effect of *HLA-B*57:01/58:01* on CRF01_AE infection was investigated in a CRF01_AE-infected Vietnamese cohort (N = 280). *HLA-B*57:01/58:01*-positive individuals mostly had HIV-1 with T242N (62/63) but showed neither a significant reduction in viral load nor increased CD4 counts relative to *B*57:01/58:01*-negative participants. *In vitro* and *in vivo* analyses revealed a significant reduction in viral fitness of CRF01_AE with T242N. *In silico* analysis indicated reduced presentation of epitopes in the context of CRF01_AE compared to subtype B or C in 10/16 HLA-B*57:01/58:01-restricted HIV-1 epitopes. These results indicate that the protective impact of *HLA-B*57:01/58:01* on CRF01_AE infection is impaired despite strong suppressive pressure by TW10-specific $CD8^+$ T cells.

2. Plasmacytoid dendritic cells stimulated with Lactococcus lactis strain Plasma produce soluble factors to suppress SARS-CoV-2 replication.

Hiroshi Ishii[2], Kenta Jounai[6], Ryohei Tsuji[6], Konomi Ohshio[6], Daiki Kaneda, Midori Okazaki[2], Shigeyoshi

Harada[2], Daisuke Fujiwara[6], Tetsuro Matano: [6]Kirin Central Research Institute, Kirin Holdings, Co., Ltd., Japan

Innate immune responses are important in the control of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication. We have previously found a lactic acid bacteria species, *Lactococcus lactis* strain Plasma (LC-Plasma), which possesses specific feature to activate plasmacytoid dendritic cells (pDCs) and thus may affect innate immune responses. In this study, we investigated the impact of pDC activation by LC-Plasma on SARS-CoV-2 replication *in vitro*. Addition of the culture supernatant of pDCs stimulated with LC-Plasma resulted in suppression of SARS-CoV-2 replication in Vero and Calu-3 cells. We confirmed interferon-α (IFN-α) secretion in the supernatant of pDCs stimulated with LC-Plasma and induction of IFN-stimulated genes in cells treated with the pDC supernatant. Anti-IFN-α antibody impaired the suppression of SARS-CoV-2 replication by the supernatant of LC-Plasma-stimulated pDCs, suggesting that IFN-α plays an important role in the SARS-CoV-2 suppression. These results indicate the potential of LC-Plasma to induce inhibitory responses against SARS-CoV-2 replication through pDC stimulation with IFN-α secretion.

3. HTLV-1 proliferation after CD8$^+$ cell depletion by monoclonal anti-CD8 antibody administration in latently HTLV-1-infected cynomolgus macaques.

Midori Nakamura-Hoshi[2], Takushi Nomura[3], Masako Nishizawa[2], Trang Thi Thu Hau[2], Hiroyuki Yamamoto[2], Midori Okazaki[2], Hiroshi Ishii[2], Kenzo Yonemitsu[7], Yuriko Suzaki[7], Yasushi Ami[7], Tetsuro Matano: [7]Management Department of Biosafety, Laboratory Animal, and Pathogen Bank, National Institute of Infectious Diseases, Tokyo, Japan

HTLV-1 induces chronic asymptomatic latent infection with substantial proviral load but without significant viral replication *in vivo*. Cumulative studies have indicated involvement of CD8$^+$ cells including virus-specific CD8$^+$ T cells in the control of HTLV-1 replication. However, whether HTLV-1 expression from latently infected cells *in vivo* occurs in the absence of CD8$^+$ cells remains unclear. In this study, we examined the impact of CD8$^+$ cell depletion by monoclonal anti-CD8 antibody administration on proviral load in HTLV-1-infected cynomolgus macaques. Five cynomolgus macaques were infected with HTLV-1 by inoculation with HTLV-1-producing cells. Administration of monoclonal anti-CD8 antibody in the chronic phase resulted in complete depletion of peripheral CD8$^+$ T cells for approximately two months. All five macaques showed an increase in proviral load following CD8$^+$ cell depletion, which peaked just before the reappearance of peripheral CD8$^+$ T cells. Tax-specific CD8$^+$ T cell responses were detected in these recovered CD8$^+$ T cells. Importantly, anti-HTLV-1 antibodies also increased after CD8$^+$ cell depletion, indicating HTLV-1 antigen expression. These results provide evidence indicating that HTLV-1 can proliferate from the latent phase in the absence of CD8$^+$ cells and suggest that CD8$^+$ cells are responsible for the control of HTLV-1 replication.

4. Breadth and durability of SARS-CoV-2 specific T cell responses following long-term recovery from COVID-19.

Dang Thi Thu Thao[3], Alitzel Anzurez[2], Kaori Nakayama-Hosoya[2], Shoji Miki[2], Kazuo Yamashita[8], Mark de Souza[2], Tetsuro Matano, Ai Kawana-Tachikawa: [8]KOTAI Biotechnologies, Inc., Japan

T cell immunity is crucial for long-term immunological memory, but the profile of SARS-CoV-2-specific memory T cells in individuals who recovered from COVID-19 (COVID-19-convalescent individuals) is not sufficiently assessed. In this study, the breadth and magnitude of SARS-CoV-2-specific T cell responses were determined in COVID-19-convalescent individuals in Japan. Memory T cells against SARS-CoV-2 were detected in all convalescent individuals, and those with more severe disease exhibited a broader T cell response relative to cases with mild symptoms. Comprehensive screening of T cell responses at the peptide level was conducted for spike (S) and nucleocapsid (N) proteins, and regions frequently targeted by T cells were identified. Multiple regions in S and N proteins were targeted by memory T cells, with median numbers of target regions of 13 and 4, respectively. A maximum of 47 regions were recognized by memory T cells for an individual. These data indicate that SARS-CoV-2-convalescent individuals maintain a substantial breadth of memory T cells for at least several months following infection. Broader SARS-CoV-2-specific CD4$^+$ T cell responses, relative to CD8$^+$ T cell responses, were observed for the S but not the N protein, suggesting that antigen presentation is different between viral proteins. The binding affinity of predicted CD8$^+$ T cell epitopes to HLA class I molecules in these regions was preserved for the Delta variant and at 94 to 96% for SARS-CoV-2 Omicron subvariants, suggesting that the amino acid changes in these variants do not have a major impact on antigen presentation to SARS-CoV-2-specific CD8$^+$ T cells.

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IMSUT Distinguished Professor Unit

Division of Virology
ウイルス感染部門

Project Professor　Yoshihiro Kawaoka, D.V.M., Ph.D.　特任教授　獣医学博士　河　岡　義　裕

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. Characterization of SARS-CoV-2 Omicron BA.2.75 clinical isolates.

Uraki R, Iida S[1], Halfmann PJ[2], Yamayoshi S, Hirata Y[1], Iwatsuki-Horimoto K, Kiso M, Ito M, Furusawa Y, Ueki H, Sakai-Tagawa Y, Kuroda M[2], Maemura T[2], Kim T[2], Mine S[1], Iwamoto N[3], Li R[4], Liu Y[4], Larson D[4], Fukushi S[5], Watanabe S[6], Maeda K[6], Wang Z[4], Ohmagari N[3], Theiler J[7], Fischer W[8,9], Korber B[8,9], Imai M, Suzuki T[1], Kawaoka Y. [1]Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan. [2]Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA. [3]Disease Control and Prevention Center, National Center for Global Health and Medicine Hospital, Tokyo, Japan. [4]Department of Animal, Dairy, and Veterinary Sciences, College of Agriculture and Applied Sciences, Utah State University, Logan, UT, USA. [5]Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan. [6]Center for Influenza and Respiratory Virus Research, National Institute of Infectious Diseases, Tokyo, Japan. [7]Space Data Science and Systems, Los Alamos National Laboratory, Los Alamos, USA. [8]Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, USA. [9]New Mexico Consortium, Los Alamos, USA.

The prevalence of the Omicron subvariant BA.2.75 rapidly increased in India and Nepal during the summer of 2022, and spread globally. However, the virological features of BA.2.75 are largely unknown. Here, we evaluated the replicative ability and pathogenicity of BA.2.75 clinical isolates in Syrian hamsters. Although we found no substantial differences in weight change among hamsters infected with BA.2, BA.5, or BA.2.75, the replicative ability of BA.2.75 in the lungs is higher than that of BA.2 and BA.5. Of note, BA.2.75 causes focal viral pneumonia in hamsters, characterized by patchy inflammation interspersed in alveolar regions, which is not observed in BA.5-infected hamsters. Moreover, in competition assays, BA.2.75 replicates better than BA.5 in the lungs of hamsters. These results suggest that BA.2.75 can cause more severe respiratory disease than BA.5 and BA.2 in a hamster model and should be closely monitored.

2. In vitro and in vivo characterization of SARS-CoV-2 strains resistant to nirmatrelvir

Kiso M, Furusawa Y, Uraki R, Imai M, Yamayoshi S, Kawaoka Y.

Nirmatrelvir, an oral antiviral agent that targets a SARS-CoV-2 main protease (3CLpro), is clinically useful against infection with SARS-CoV-2 including its omicron variants. Since most omicron subvariants

have reduced sensitivity to many monoclonal antibody therapies, potential SARS-CoV-2 resistance to nirmatrelvir is a major public health concern. Several amino acid substitutions have been identified as being responsible for reduced susceptibility to nirmatrelvir. Among them, we selected L50F/E166V and L50F/E166A/L167F in the 3CLpro because these combinations of substitutions are unlikely to affect virus fitness. We prepared and characterized delta variants possessing Nsp5-L50F/E166V and Nsp5-L50F/E166A/L167F. Both mutant viruses showed decreased susceptibility to nirmatrelvir and their growth in VeroE6/TMPRSS2 cells was delayed. Both mutant viruses showed attenuated phenotypes in a male hamster infection model, maintained airborne transmissibility, and were outcompeted by wild-type virus in co-infection experiments in the absence of nirmatrelvir, but less so in the presence of the drug. These results suggest that viruses possessing Nsp5-L50F/E166V and Nsp5-L50F/E166A/L167F do not become dominant in nature. However, it is important to closely monitor the emergence of nirmatrelvir-resistant SARS-CoV-2 variants because resistant viruses with additional compensatory mutations could emerge, outcompete the wild-type virus, and become dominant.

3. In vitro and in vivo characterization of SARS-CoV-2 resistance to ensitrelvir.

Kiso M, Yamayoshi S, Iida S[1], Furusawa Y, Hirata Y, Uraki R, Imai M, Suzuki T[1], Kawaoka Y.

Ensitrelvir, an oral antiviral agent that targets a SARS-CoV-2 main protease (3CLpro or Nsp5), is clinically useful against SARS-CoV-2 including its omicron variants. Since most omicron subvariants have reduced sensitivity to most monoclonal antibody therapies, SARS-CoV-2 resistance to other antivirals including main protease inhibitors such as ensitrelvir is a major public health concern. Here, repeating passages of SARS-CoV-2 in the presence of ensitrelvir revealed that the M49L and E166A substitutions in Nsp5 are responsible for reduced sensitivity to ensitrelvir. Both substitutions reduced in vitro virus growth in the absence of ensitrelvir. The combination of the M49L and E166A substitutions allowed the virus to largely evade the suppressive effect of ensitrelvir in vitro. The virus possessing Nsp5-M49L showed similar pathogenicity to wild-type virus, whereas the virus possessing Nsp5-E166A or Nsp5-M49L/E166A slightly attenuated. Ensitrelvir treatment of hamsters infected with the virus possessing Nsp5-M49L/E166A was ineffective; however, nirmatrelvir or molnupiravir treatment was effective. Therefore, it is important to closely monitor the emergence of ensitrelvir-resistant SARS-CoV-2 variants to guide antiviral treatment selection.

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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. SELEX targeting virus-like particle generates RNA aptamers inhibiting the chikungunya virus

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Nucleic acid aptamers are a promising drug modality, but it remains difficult to generate virus-neutralizing aptamers because of the lack of a robust system for the discovery of aptamers targeting virus particles of interest. We report that a platform technology to develop virus neutralizing RNA aptamers targeting chikungunya virus (CHIKV) as a model target by adopting our SELEX method with virus-like particles (VLPs), VLP-SELEX. An identified aptamer raised against CHIKV-VLPs, Apt#1, and its truncated derivatives showed neutralizing activity with IC50

values in the nanomolar range in a cell-based assay system using a pseudoviral particle of CHIKV (CHIKVpp). An antiviral-based chemical genetic approach for probing the interaction area of Apt#1 showed additive effects of Apt#1 with most assessed antivirals, whereas potent competitiveness with suramin, a reported interactant with domain A of E2 envelope protein (E2DA), was revealed in both cell-based and surface plasmon resonance analyses, predicting E2DA to be the Apt#1 interface. Further, Apt#1 interfered with the attachment process of CHIKVpp, suggesting it acts as an attachment inhibitor targeting E2DA in CHIKV in concert with certain antivirals. These results suggest that VLP-SELEX may be a novel feasible option for exploring virus-neutralizing agents targeting virus-particles of interest.

Publications

1. Daniel S Pereira, Akita K, Robert B Bhisitkul, Nishihata T, Yusuf Ali, Nakamura E, Nakamura Y: Safety and tolerability of intravitreal umedaptanib pegol (anti-FGF2) for neovascular age-related macular degeneration (nAMD): a phase 1, open-label study." Eye (Lond), 2023 Dec 1, doi: 10.1038/s41433-023-02849-6. Online ahead of print.
2. Daniel S Pereira, Raj K Maturi, Akita K, Vinaya Mahesh, Robert B Bhisitkul, Nishihata T, Sakota E, Yusuf Ali, Nakamura E, Padma Bezwada, Nakamura Y.: Clinical proof of concept for anti-FGF2 therapy in exudative age-related macular degeneration (nAMD): phase 2 trials in treatment-naïve and anti-VEGF pretreated patients. Eye (Lond), 2023 Nov 30, doi: 10.1038/s41433-023-02848-7. Online ahead of print.

Social Cooperation Research Program

Project Division of International Advanced Medical Research

国際先端医療社会連携研究部門

Project Associate Professor　Koichiro Yuji, M.D., Ph.D.　特任准教授　博士（医学）　湯　地　晃一郎

The mission of the Project Division is to apply changes in advanced medical research at the Institute of Medical Science at the University of Tokyo (IMSUT). Our activities include field research in which innovative medicine will be implemented; cross-disciplinary education of physicians, researchers, and professionals; collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development; and establishing projections of the future healthcare system of Japan, which will be the first fully fledged aged society.

Implementing advanced medical research at IMSUT

Yuji, K.

The Project Division was established in November 2014. Our mission is to contribute to the progress of advanced medical research at IMSUT; to perform field research in which innovative medicine will be implemented; and to further the cross-disciplinary education of physicians, researchers, and professionals. Our future plans include collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development.

Projections on the future healthcare system in Japan, the first fully fledged aged society

Yuji, K.

Japan is rapidly becoming a fully fledged aged society, and the increasing dependence of the elderly population is a significant concern. We have simulated both the supply and demand features of Japan's future healthcare system.

Publications

1. 湯地 晃一郎. ビッグデータとしての臨床検査情報HL7FHIRとその展開　臨床検査の利活用に向けてPHR/EHRの共通基盤.　日本臨床検査医学会誌. 71(8);547-551, 2023.
2. 湯地 晃一郎. 第７回 日本臨床薬理学会 関東・甲信越地方会を終えて. 臨床薬理. 54(6);249-253, 2023.

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. Analytical Method for Experimental Validation of Computer-Designed Antibody

Tanabe A, Tsumoto K.

In the computational design of antibodies, the interaction analysis between target antigen and antibody is an essential process to obtain feedback for validation and optimization of the design. Kinetic and thermodynamic parameters as well as binding affinity (KD) allow for a more detailed evaluation and understanding of the molecular recognition. In this chapter, we summarize the conventional experimental methods which can calculate KD value (ELISA, FP), analyze a binding activity to actual cells (FCM), and evaluate the kinetic and thermodynamic parameters (ITC, SPR, BLI), including high-throughput analysis and a recently developed experimental technique.

2. Structural Classification of CDR-H3 in Single-Domain VHH Antibodies

Kuroda D, Tsumoto K.

The immune systems protect vertebrates from foreign molecules or antigens, and antibodies are important mediators of this system. The sequences and structural features of antibodies vary depending on species. Many of antibodies from vertebrates, including camelids, have both heavy and light chain variable domains, but camelids also have antibodies that lack the light chains. In antibodies that lack light chains, the C-terminal variable region is called the VHH domain. Antibodies recognize antigens through six complementarity-determining regions (CDRs). The third CDR of the heavy chain (CDR-H3) is at the center of the antigen-binding site and is diverse in terms of sequence and structure. Due to the impor-

tance of antibodies in basic science as well as in medical applications, there have been many studies of CDR-H3s of antibodies that possess both light and heavy chains. However, nature of CDR-H3s of single-domain VHH antibodies is less well studied. In this chapter, we describe current knowledge of sequence-structure-function correlations of single-domain VHH antibodies with emphasis on CDR-H3. Based on the 370 crystal structures in the Protein Data Bank, we also attempt structural classification of CDR-H3 in single-domain VHH antibodies and discuss lessons learned from the ever-increasing number of the structures.

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. Molecular mechanism underlying the increased risk of colorectal cancer metastasis caused by single nucleotide polymorphisms in LI-cadherin gene

Yui A, Kuroda D, Maruno T, Nakakido M, Nagatoishi S, Uchiyama S, Tsumoto K.

LI-cadherin is a member of the cadherin superfamily. LI-cadherin mediates Ca2 + -dependent cell-cell adhesion through homodimerization. A previous study reported two single nucleotide polymorphisms (SNPs) in the LI-cadherin-coding gene (CDH17). These SNPs correspond to the amino acid changes of Lys115 to Glu and Glu739 to Ala. Patients with colorectal cancer carrying these SNPs are reported to have a higher risk of lymph node metastasis than patients without the SNPs. Although proteins associated with metastasis have been identified, the molecular mechanisms underlying the functions of these proteins remain unclear, making it difficult to develop effective strategies to prevent metastasis. In this study, we employed biochemical assays and molecular dynamics (MD) simulations to elucidate the molecular mechanisms by which the amino acid changes caused by the SNPs in the LI-cadherin-coding gene increase the risk of metastasis. Cell aggregation assays showed that the amino acid changes weakened the LI-cadherin-dependent cell-cell adhesion. In vitro assays demonstrated a decrease in homodimerization tendency and MD simulations suggested an alteration in the intramolecular hydrogen bond network by the mutation of Lys115. Taken together, our results indicate that the increased risk of lymph node metastasis is due to weakened cell-cell adhesion caused by the decrease in homodimerization tendency.

5. Design of single-domain VHH antibodies to increase the binding activity in SPR amine coupling

Hirao A, Nagatoishi S, Ikeuchi E, Yamawaki T, Mori C, Nakakido M, Tsumoto K.

Single-domain antibodies, or VHH, nanobodies, are attractive tools in biotechnology and pharmaceuticals due to their favorable biophysical properties. Single-domain antibodies have potential for use in sensing materials to detect antigens, and in this paper, we propose a generic design strategy of single-domain antibodies for the highly efficient use of immobilized antibodies on a sensing substrate. Amine coupling was used to immobilize the single-domain antibodies on the substrate through a robust covalent bond. First, for two model single-domain antibodies with lysines at four highly conserved positions (K48, K72, K84, and K95), we mutated the lysines to alanine and measured the binding activity of the mutants (the percentage of immobilized antibodies that can bind antigen) using surface plasmon resonance. The two model single-domain antibodies tended to have higher binding activities when K72, which is close to the antigen binding site, was mutated. Adding a Lys-tag to the C-terminus of single-domain antibodies also increased the binding activity. We also mutated the lysine for another model single-domain antibodies with the lysine in a different position than the four residues mentioned above and measured the binding activity. Thus, single-domain antibodies immobilized

in an orientation accessible to the antigen tended to have a high binding activity, provided that the physical properties of the single-domain antibodies themselves (affinity and structural stability) were not significantly reduced. Specifically, the design strategy of single-domain antibodies with high binding activity included mutating the lysine at or near the antigen binding site, adding a Lys-tag to the C-terminus, and mutating a residue away from the antigen binding site to lysine. It is noteworthy that mutating K72 close to the antigen binding site was more effective in increasing the binding activity than Lys-tag addition, and immobilization at the N-terminus close to the antigen binding site did not have such a negative effect on the binding activity compared to immobilization at the K72.

6. Targeting hemoglobin receptors IsdH and IsdB of Staphylococcus aureus with a single VHH antibody inhibits bacterial growth

Valenciano-Bellido S, Caaveiro JMM, Nakakido M, Kuroda D, Aikawa C, Nakagawa I, Tsumoto K.

Methicillin-resistant Staphylococcus aureus, or MRSA, is one of the major causative agents of hospital-acquired infections worldwide. Novel antimicrobial strategies efficient against antibiotic-resistant strains are necessary and not only against S. aureus. Among those, strategies that aim at blocking or dismantling proteins involved in the acquisition of essential nutrients, helping the bacteria to colonize the host, are intensively studied. A major route for S. aureus to acquire iron from the host organism is the Isd (iron surface determinant) system. In particular, the hemoglobin receptors IsdH and IsdB located on the surface of the bacterium are necessary to acquire the heme moiety containing iron, making them a plausible antibacterial target. Herein, we obtained an antibody of camelid origin that blocked heme acquisition. We determined that the antibody recognized the heme-binding pocket of both IsdH and IsdB with nanomolar order affinity through its second and third complementary-determining regions. The mechanism explaining the inhibition of acquisition of heme in vitro could be described as a competitive process in which the complementary-determining region 3 from the antibody blocked the acquisition of heme by the bacterial receptor. Moreover, this antibody markedly reduced the growth of three different pathogenic strains of MRSA. Collectively, our results highlight a mechanism for inhibiting nutrient uptake as an antibacterial strategy against MRSA.

7. Raman Spectroscopic Analysis of Highly-Concentrated Antibodies under the Acid-Treated Conditions

Sato Y, Nagatoishi S, Noguchi S, Tsumoto K.

Antibody drugs are usually formulated as highly-concentrated solutions, which would easily generate aggregates, resulting in loss of efficacy. Although low pH increases the colloidal dispersion of antibodies, acid denaturation can be an issue. Therefore, knowing the physical properties at low pH under high concentration conditions is important. Raman spectroscopy was used to investigate pH-induced conformational changes of antibodies at 50 mg/ml. Experiments in pH 3 to 7 were performed for human serum IgG and recombinant rituximab. We detected the evident changes at pH 3 in Tyr and Trp bands, which are the sensitive markers of intermolecular interactions. Thermal transition analysis over the pH range demonstrated that the thermal transition temperature (Tm) was highest at pH 3. Acid-treated and neutralized one showed higher Tm than that of pH 7, indicating that their extent of intermolecular interactions correlated with the Tm values. Onset temperature was clearly different between concentrated and diluted samples. Colloidal analyses confirmed the findings of the Raman analysis. Our studies demonstrated the positive correlation between Raman analysis and colloidal information, validating as a method for evaluating antibody conformation associated with aggregation propensities.

8. Development of a 1:1-binding biparatopic anti-TNFR2 antagonist by reducing signaling activity through epitope selection

Akiba H, Fujita J, Ise T, Nishiyama K, Miyata T, Kato T, Namba K, Ohno H, Kamada H, Nagata S, Tsumoto K

Conventional bivalent antibodies against cell surface receptors often initiate unwanted signal transduction by crosslinking two antigen molecules. Biparatopic antibodies (BpAbs) bind to two different epitopes on the same antigen, thus altering crosslinking ability. In this study, we develop BpAbs against tumor necrosis factor receptor 2 (TNFR2), which is an attractive immune checkpoint target. Using different pairs of antibody variable regions specific to topographically distinct TNFR2 epitopes, we successfully regulate the size of BpAb-TNFR2 immunocomplexes to result in controlled agonistic activities. Our series of results indicate that the relative positions of the two epitopes recognized by the BpAb are critical for controlling its signaling activity. One particular antagonist, Bp109-92, binds TNFR2 in a 1:1 manner without unwanted signal transduction, and its structural basis is determined using cryo-electron microscopy. This antagonist suppresses the proliferation of regulatory T cells expressing TNFR2. Therefore, the BpAb format would be useful in designing specific and distinct antibody functions.

9. Modulation of a conformational ensemble by a small molecule that inhibits key protein-protein interactions involved in cell adhesion

Senoo A, Nagatoishi S, Kuroda D, Ito S, Ueno G, Caaveiro JMM, Tsumoto K.

Small molecules that regulate protein-protein interactions can be valuable drugs; however, the development of such small molecules is challenging as the molecule must interfere with an interaction that often involves a large surface area. Herein, we propose that modulating the conformational ensemble of the proteins participating in a given interaction, rather than blocking the interaction by directly binding to the interface, is a relevant strategy for interfering with a protein-protein interaction. In this study, we applied this concept to P-cadherin, a cell surface protein forming homodimers that are essential for cell-cell adhesion in various biological contexts. We first determined the crystal structure of P-cadherin with a small molecule inhibitor whose inhibitory mechanism was unknown. Molecular dynamics simulations suggest that the inhibition of cell adhesion by this small molecule results from modulation of the conformational ensemble of P-cadherin. Our study demonstrates the potential of small molecules altering the conformation ensemble of a protein as inhibitors of biological relevant protein-protein interactions.

10. Arginine cluster introduction on framework region in anti-lysozyme antibody improved association rate constant by changing conformational diversity of CDR loops

Maeta S, Nakakido M, Matsuura H, Sakai N, Hirata K, Kuroda D, Fukunaga A, Tsumoto K.

Antibodies are used for many therapeutic and biotechnological purposes. Because the affinity of an antibody to the antigen is critical for clinical efficacy of pharmaceuticals, many affinity maturation strategies have been developed. Although we previously reported an affinity maturation strategy in which the association rate of the antibody toward its antigen is improved by introducing a cluster of arginine residues into the framework region of the antibody, the detailed molecular mechanism responsible for this improvement has been unknown. In this study, we introduced five arginine residues into an anti-hen egg white lysozyme antibody (HyHEL10) Fab fragment to create the R5-mutant and comprehensively characterized the interaction between antibody and antigen using thermodynamic analysis, X-ray crystallography, and molecular dynamics (MD) simulations. Our results indicate that introduction of charged residues strongly enhanced the association rate, as previously reported, and the antibody-antigen complex structure was almost the same for the R5-mutant and wild-type Fabs. The MD simulations indicate that the mutation increased conformational diversity in complementarity-determining region loops and thereby enhanced the association rate. These observations provide the molecular basis of affinity maturation by R5 mutation.

11. Anti-InlA single-domain antibodies that inhibit the cell invasion of Listeria monocytogenes

Yamazaki T, Nagatoishi S, Yamawaki T, Nozawa T, Matsunaga R, Nakakido M, Caaveiro JMM, Nakagawa I, Tsumoto K.

Listeriosis, caused by infection with Listeria monocytogenes, is a severe disease with a high mortality rate. The L. monocytogenes virulence factor, internalin family protein InlA, which binds to the host receptor E-cadherin, is necessary to invade host cells. Here, we isolated two single-domain antibodies (VHHs) that bind to InlA with picomolar affinities from an alpaca immune library using the phage display method. These InlA-specific VHHs inhibited the binding of InlA to the extracellular domains of E-cadherin in vitro as shown by biophysical interaction analysis. Furthermore, we determined that the VHHs inhibited the invasion of L. monocytogenes into host cells in culture. High-resolution X-ray structure analyses of the complexes of VHHs with InlA revealed that the VHHs bind to the same binding site as E-cadherin against InlA. We conclude that these VHHs have the potential for use as drugs to treat listeriosis.

12. Biophysical insight into protein-protein interactions in the Interleukin-11/Interleukin-11Rα/glycoprotein 130 signaling complex

Mori C, Nagatoishi S, Matsunaga R, Kuroda D, Nakakido M, Tsumoto K.

Interleukin-11 (IL-11) is a member of the interleukin-6 (IL-6) family of cytokines. IL-11 is a regulator of multiple events in hematopoiesis, and IL-11-mediated signaling is implicated in inflammatory disease, cancer, and fibrosis. All IL-6 family cytokines signal through the signal-transducing receptor, glycoprotein 130 (gp130), but these cytokines have distinct as well as overlapping biological functions. To understand IL-11 signaling at the molecular level, we performed a comprehensive interaction analysis of the IL-11 signaling complex, comparing it with the IL-6 complex, one of the best-characterized cytokine complexes. Our thermodynamic analysis revealed a clear difference between IL-11 and IL-6. Surface plasmon resonance analysis showed that the interaction between IL-11 and IL-11 receptor α (IL-11Rα) is entropy driven, whereas that between IL-6 and IL-6 receptor α (IL-6Rα) is enthalpy driven. Our analysis using isothermal titration calorimetry revealed that the bind-

ing of gp130 to the IL-11/IL-11Rα complex results in entropy loss, but that the interaction of gp130 with the IL-6/IL-6Rα complex results in entropy gain. Our hydrogen-deuterium exchange mass spectrometry experiments suggested that the D2 domain of gp130 was not involved in IL-6-like interactions in the IL-11/IL-11Rα complex. It has been reported that IL-6 interaction with gp130 in the signaling complex was characterized through the hydrophobic interface located in its D2 domain of gp130. Our findings suggest that unique interactions of the IL-11 signaling complex with gp130 are responsible for the distinct biological activities of IL-11 compared to IL-6.

13. High-throughput analysis system of interaction kinetics for data-driven antibody design

Matsunaga R, Ujiie K, Inagaki M, Fernández Pérez J, Yasuda Y, Mimasu S, Soga S, Tsumoto K.

Surface plasmon resonance (SPR) is widely used for antigen-antibody interaction kinetics analysis. However, it has not been used in the screening phase because of the low throughput of measurement and analysis. Herein, we proposed a high-throughput SPR analysis system named "BreviA" using the Brevibacillus expression system. Brevibacillus was transformed using a plasmid library containing various antibody sequences, and single colonies were cultured in 96-well plates. Sequence analysis was performed using bacterial cells, and recombinant antibodies secreted in the supernatant were immobilized on a sensor chip to analyze their interactions with antigens using high-throughput SPR. Using this system, the process from the transformation to 384 interaction analyses can be performed within a week. This system utility was tested using an interspecies specificity design of an anti-human programmed cell death protein 1 (PD-1) antibody. A plasmid library containing alanine and tyrosine mutants of all complementarity-determining region residues was generated. A high-throughput SPR analysis was performed against human and mouse PD-1, showing that the mutation in the specific region enhanced the affinity for mouse PD-1. Furthermore, deep mutational scanning of the region revealed two mutants with > 100-fold increased affinity for mouse PD-1, demonstrating the potential efficacy of antibody design using data-driven approach.

14. Experimental modification in thermal stability of oligomers by alanine substitution and site saturation mutagenesis of interfacial residues

Hoya M, Matsunaga R, Nagatoishi S, Tsumoto K.

For certain industrial applications, the stability of protein oligomers is important. In this study, we demonstrated an efficient method to improve the thermal stability of oligomers using the trimeric protein chloramphenicol acetyltransferase (CAT) as the model. We substituted all interfacial residues of CAT with alanine to detect residues critical for oligomer stability. Mutation of six of the forty-nine interfacial residues enhanced oligomer thermal stability. Site saturation mutagenesis was performed on these six residues to optimize the side chains. About 15% of mutations enhanced thermal stability by more than 0.5 °C and most did not disrupt activity of CAT. Certain combinations of mutations further improved thermal stability and resistance against heat treatment. The quadruple mutant, H17V/N34S/F134A/D157C, retained the same activity as the wild-type after heat treatment at 9 °C higher temperature than the wild-type CAT. Furthermore, combinations with only alanine substitutions also improved thermal stability, suggesting the method we developed can be used for rapid modification of industrially important proteins.

15. Quantitative analysis of antibody aggregates by combination of pinched-flow fractionation and coulter method

Nagatoishi S, Toyoshima T, Furukawa K, Tsumoto K.

For the pharmaceutical development of proteins, multiple methods of analysis are recommended for evaluating aggregation, and the development of more quantitative and simpler analytical techniques for subvisible particles is expected. This study introduces the Pinched-Flow Fractionation (PFF)-Coulter method, which combines the Pinched-flow fractionation (PFF) and Coulter methods to analyze the concentration of submicron-sized particles. The PFF method separates the particles by size. Separated particles were individually detected using the Coulter method. We have utilized the PFF-Coulter method to quantitatively analyze particle concentrations using standard particles, evaluate detection limits, variability, and correlation between theoretical and measured values, and analyze mixtures of different particle sizes. The PFF-Coulter method allows for quantitatively analyzing of particle sizes from 0.2 to 2.0 μm. The quantifiable weight concentration range was 2.5 × 10-2 - 50 μg/mL, and the number concentration range was 104-1010 particles/mL. The sample volume was small (<10 μL). The PFF-Coulter method is capable of quantitative analysis that complements data from conventional measurement techniques, and when used in conjunction with existing submicron-size particle analysis techniques, will enable more accurate particle analysis.

16. Conformational features and interaction mechanisms of VH H antibodies with β-hairpin CDR3: A case of Nb8-HigB2 interaction

Yamamoto K, Nagatoishi S, Matsunaga R, Nakakido M, Kuroda D, Tsumoto K.

The β-hairpin conformation is regarded as an important basic motif to form and regulate protein-protein interactions. Single-domain VH H antibodies are potential therapeutic and diagnostic tools, and the third complementarity-determining regions of the heavy chains (CDR3s) of these antibodies are critical for antigen recognition. Although the sequences and conformations of the CDR3s are diverse, CDR3s sometimes adopt β-hairpin conformations. However, characteristic features and interaction mechanisms of β-hairpin CDR3s remain to be fully elucidated. In this study, we investigated the molecular recognition of the anti-HigB2 VH H antibody Nb8, which has a CDR3 that forms a β-hairpin conformation. The interaction was analyzed by evaluation of alanine-scanning mutants, molecular dynamics simulations, and hydrogen/deuterium exchange mass spectrometry. These experiments demonstrated that positions 93 and 94 (Chothia numbering) in framework region 3, which is right outside CDR3 by definition, play pivotal roles in maintaining structural stability and binding properties of Nb8. These findings will facilitate the design and optimization of single-domain antibodies.

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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

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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], Yoichiro Kamatani[2], Takayuki Morisaki[1],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.

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. Consenting employees of NTT group companies who undertake regular/annual physical examinations were recruited to a comprehensive survey program of genetic testing using microarray analysis and healthcare data collection. 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 study aims to enable inclusion of multi-layered biomedical information into current company-based health care systems.

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.

In 2023, a grant from the Japanese Cabinet Office's Cross-ministerial Strategic Innovation Promotion (SIP) Program on Development of an Integrated Healthcare System was awarded to develop and implement a life-record-type digital twin. This project utilizes the serial healthcare/clinical data including biomedical information of the NTT working generation cohort to analyse and visualize disease onest and progression including risks.

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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

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Since the opening of our new laboratory under a joint research agreement with Daiichi Sankyo RD Novare Co., Ltd, we have been struggling to establish our experimental flow for drug-sensitivity screening using primary tumor specimens derived from patients with hematological malignancies. In order to finally establish a novel platform for precision medicine projects combining DSS and comprehensive multi-omics analyses, we aimed to optimize our tissue culture methods for PTS from acute myeloid leukemia (AML) patients. Using clinical specimens kindly provided by the Department of Hematology and Oncology, IMSUT Hospital, we were able to evaluate optimal tissue culture conditions for 3 to 9 days of ex vivo incubation with drugs. From these experiments, we were able to establish our in-house tissue culture medium that can maintain hematopoietic stem/ progenitor cell-like components of AML cells for the period of ex vivo drug treatments. While we handle these cell processing procedures, we could also make progress in high-throughput assay system with automation technologies. Our efforts are highly expected to provide us with tools to understand the pathogenesis of hematological malignancies and develop further therapeutics.

1. Clinical precision research for hematological malignancies

Kimihito Cojin Kawabata[1], Hironobu Komori[1,2], Hayato Tsuji[1,2], Yoshiharu Takama[1,2], Seiko Kato[1], Maiko Morita[1], Sanae Suzuki[1], Tetsushi Oka[2], Yoshimasa Ono[2], Kenji Wakabayashi[2], Gen Kudo[2], Yasuhito Nannya[1], Satoshi Takahashi[1].

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The aim of this study is to perform a comprehensive multi-omics analysis covering genetic and epigenetic alterations as well as gene expressions, which can be compared side-by-side with functional analyses such as ex vivo drug responses. All these experiments use primary tumor specimens (PTS) derived from patients with hematopoietic malignancies attending either the IMSUT or collaborating hospitals, together with the Department of Hematology and Oncology. Currently, our special focus is on acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).

Currently, in the aforementioned network, we have performed whole genome or whole exome analyses on the PTS of patients with hematopoietic malignancies, along with RNA expression, transcriptome analyses. We further aim to organize these omics approaches with higher resolution at the single cell level. These omics profiles can be immediately compared with the clinical course of the actual patients. The NGS data compared to the clinical profile are dis-

cussed in detail in regular Tumor Board meetings (biweekly).

Drug sensitivity screening (DSS) using PTS is another axis of the group's efforts. In this topic, several other groups have already published their data on similar aspects (Nature. 2018;562(7728):526-31, Cancer Discovery. 2022;12(2):388-401, etc.). However, the more samples and compounds we use at multiple doses, and the more intensive work and time we need in these high-throughput procedures. In this sense, this field is still in its infancy. Therefore, we are trying to install more updated and modernized technologies in these wet experiments and subsequent analyses. One of them is a fully automated ex vivo drug screening system, which uses a robotic arm to integrate several machines that can automatically handle the entire process from cell seeding, drug injection, cell incubation, liquid handling to flow cytometry. After data acquisition, drug sensitivity profile data is also collected through an automated data processing pipeline. After installation, we have completed the optimization of the entire system so that each PTS we have stored in the laboratory is analyzed in a 384-well plate format. We are currently evaluating the significance of these results.

To date, there is a great deal of variability in the methods used for ex vivo culture of PTS in the field of DSS. These methods need to be compared side-by-side and ultimately further optimized to obtain drug sensitivity profiles using cells in a better state. To reiterate, the limited number of cells collected from patients and the use of all available frozen vials in initial screening experiments are two major problems in experiments using PTS. In order to expand the possibilities of this AML/MDS PTS for more advanced applications, we have conducted experiments to compare different ex vivo culture methods side by side. At the Clinical Precision Research Platform, we have already collected 159 different PTS aliquoted in 1495 cryovials. They are continuously used either in the automated DSS experiments, OMICS analyses or in these ex vivo culture assays. We have found that 1. Short term culture protocols (up to 7 days) optimized for the current DSS assays make some differences regardless of changing concentrations of serum or other supplements for culture media. 2. The use of stromal feeder cells is superior to other conventional stroma-free culture methods currently used for normal hematopoietic stem cells. Moreover, 3. Recently, we have installed the most updated culture methods to challenge for stroma-free, serum-free ex vivo culture system optimized for PTS. The idea for the updated culture media is derived from ongoing collaborative research projects on normal hematopoietic stem cell culture. Initial data on this topic continue to fuel our research motivation on a daily basis. Details of these findings will be updated as they become available.

2. Generation of antigen-specific T cells derived from cord blood

Maiko Morita[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

The aim of this project is to establish a non-gene transfer method for the generation and expansion of viral antigen-specific T cells from naive human cord blood (CB)-derived T cells.

Previous studies have shown that cGAMP, a type of STING ligand, induces an IFN-type 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 CB-derived naive T cells were cultured with cGAMP and viral antigen peptides twice for 14 days, significant production of inflammatory cytokines such as IFN-γ and TNF-α was observed. As a result of optimization of culture conditions, supplementation with ascorbic acid, ITS-G supplement, and an amino acid was shown to effectively produce inflammatory cytokines from CB-derived CTLs. In some samples, strong inflammatory cytokine production was observed even after 5th stimulation with viral antigen peptides, and its amount correlated with the number of stimulations. However, repeated experiments showed that cell proliferation and cytokine production were largely dependent on individual differences. As a next step, we plan to perform multi-omics analysis or single-cell RNA-seq to investigate the difference between PB-derived CTLs and CB-derived CTLs and to identify the factors for efficient induction of CTLs from naive 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.		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

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 study the optimization of diagnosis and treatment, providing innovative approaches to intractable diseases and conditions.

1. Research and development of novel diagnostics to evaluate immune response

Hiroshi Yasui, Hiroshi Yotsuyanagi, Kiyosumi Ochi, Asako Kobayashi, Reika Li, Mikiko Suzuki, Keiji Hirano[1], Yuma Oka[1], Yoshinori Murakami[2], ArinobuTojo[3], Masatoshi Yanagida[1], Noriko Doki[4]

[1]Central Research Laboratories, Sysmex Corporation

[2]Division of Molecular Pathology, IMSUT

[3]Tokyo Medical and Dental University

[4]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital

Novel immunodiagnostics to analyze immune function is important for the evaluate the activity of autoimmune diseases as well as development of cancer immunotherapy. We study to develop novel immunodiagnostics to evaluate activities of immune cells in patients with allogenic hematopoietic stem cell transplantation to diagnose severity of graft-versus-host disease. It will be also expected to contribute the development of the novel cancer immunotherapy in hematologic malignancies.

2. Investigator-initiated clinical trials under an Investigational New Drug application for the development of novel cancer therapeutics and biomarkers

Hiroshi Yasui, Mikiko Suzuki, Yataro Daigo[1], Fumitaka Nagamura[2]

[1]Dean's Office, IMSUT

[2]Center for Translational Research, IMSUT Hospital, IMSUT

Genome medicine and genome research, including pharmacogenomics and pharmacogenetics, are important for developing novel therapeutic agents for cancer and incurable diseases and identifying biomarkers. Our research aims to develop efficient approaches for conducting investigator-initiated clinical trials under Investigational New Drug (IND) applications to promote translational research and discover biomarkers for prediction of the safety and efficacy of novel therapeutics through omics analyses, including genomics. We were conducting, supporting, summarizing or preparing three investigator-initiated clinical trials under INDs applications for the development of academic-oriented innovative anticancer

drug especially novel cancer immunotherapy.

3. Program for supporting biospecimen analysis for the diagnosis and treatment of hematological malignancies

Hiroshi Yasui, Arinobu Tojo[1], Kaoru Uchimaru[2], Yoshihisa Yamano[3], Toshiki Watanabe[3]
[1]Tokyo Medical and Dental University
[2]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo
[3]St. Marianna University School of Medicine

To support cancer scientists in promoting translational research and genome medicine, we have established a platform for supporting cohort studies and biospecimen analysis. Under this program, we are collecting and managing clinical materials, including tumor cells, serum, and peripheral blood mononuclear cells from patients at high risk of hematologic malignancies as well as patients with blood cancer. We provide support for obtaining and/or analyzing biomaterials, as requested by researchers, and contribute to their clinical studies and publications.

4. Support and management of translational research

Hiroshi Yasui

To promote translational research and genome medicine, we participate in the "Translational Research Network Program, Japanese Translational Research and Clinical Trials Core Centers" supported by the Japan Agency for Medical Research and Development, as members of the Translational Research Advancement Center of the University of Tokyo. The aim of the program is to promote translational research and investigator-led clinical trials aiming for practical applications of basic studies in academia, managing the assessment of scientific seeds and intellectual property rights, and therefore promoting the development of advanced medical arts, including genome medicine.

Publications

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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

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. The phase 2 part of this trial is ongoing.

A recombinant herpes simplex type 1 with human bevacizumab expression, T-BV

Phase II trials of G47Δ in glioblastoma showed efficacy and safety, but cases of temporary brain edema were observed during the induction of anti-tumor immunity by G47Δ. To further improve the safety of viral therapy for brain tumors, we have developed T-BV, a G47Δ-based recombinant HSV-1 expressing bevacizumab that can reduce brain edema without systemic administration. We have produced clinical-grade T-BV and will soon initiate clinical trials.

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
Professor	Fumitaka Nagamura, M.D., Ph.D.	教　授	博士(医学)	長　村　文　孝
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 Mutsuhiro Takekawa, M.D., Ph.D.

教 授 博士(医学) 武 川 睦 寛

Our major missions are to coordinate institutional projects and to enhance the mutual cooperation and 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.
In May 2023, the Project Coordination Office merged with the former International Affairs Office, which was responsible for public relations and language support, to strengthen its functions. On the public relations front, the Office continues to focus on an effective PR strategy including publishing new information about a variety of scientific research conducted by IMSUT on its official website and social media. The Office is also working to increase IMSUT's international presence through press releases in both Japanese and English, press conferences, and IMSUT's PR magazines. On the language support side, the Office provides translation support from Japanese to English to create a favourable working environment for the international members of IMSUT.

1. Support for the management of institutional projects

Kiyomi Nakagawa, Yoko Udagawa, Ayako Miyake

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)
- On-campus job project for student financial support FY 2023

2. International Joint Usage/Research Center Program of MEXT

Kaori Inoue, Junko Tsuzuku

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 per-

taining 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. 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!".

5. 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 the PR magazine "PLATINUM STREET TIMES" in June and December 2023, featuring patient first medicine at IMSUT Hospital and IMSUT's research achievements in the latest gene therapy, regenerative medicine, and cell therapy.

6. Language support

Kazuyo Ohara

In 2023, with the reclassification of the COVID-19 category to Class 5 and the gradual resumption of research activities by IMSUT members, the Office worked closely with the administrative staff to provide the international members of IMSUT with important notices and updates in English. These included the Dean's messages and notices encouraging the members to actively participate in awareness-raising seminars and training sessions led by the Administration Bureau. The Office also vigorously undertook various translation and proofreading work, aiming to create a favourable working environment for the international members of IMSUT by offering tailored language services.

7. 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
Project Professor	Yataro Daigo, M.D., Ph.D.	特任教授	博士(医学)	醍 醐 弥太郎

"Platforms for Advanced Technologies and Research Resources" (platform.umin.jp/) was launched in fiscal year (FY) 2022 under the new framework of the Grant-inAid 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 52 universities and 22 research institutions nationwide which provide more than 70 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 22 members participated: four platform representatives and 18 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, Atsuko Ishizaki, Miyuki Kawakami, Yataro Daigo, Jun-ichiro Inoue, and Mutsuhiro Takekawa

The following activities have been performed under the management of this office in 2023.

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 holding the luncheon seminar, joining the exhibitions of various scientific meetings and posting the article on The Science News.
7. Facilitating cooperative networks between our platforms and other domestic or international groups that support life science researches.

Dean's Office

BioBank Japan
バイオバンク・ジャパン

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	医学博士	中　西　　真
Professor	Yuji Yamanashi, Ph.D.	教　授	理学博士	山　梨　裕　司
Project Professor	Koichi Matsuda, M.D., Ph.D.	特任教授	博士(医学)	松　田　浩　一
Cooperative Professor	Yoichiro Kamatani, M.D., Ph.D.	連携教授	博士(医科学)	鎌　谷　洋一郎
Visiting Professor	Takayuki Morisaki, M.D., Ph.D.	客員教授	医学博士	森　崎　隆　幸

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

1. Masato Akiyama, Saori Sakaue, Atsushi Takahashi, Kazuyoshi Ishigaki, Makoto Hirata, Koichi Matsuda, Yukihide Momozawa, Yukinori Okada, Toshiharu Ninomiya, Chikashi Terao, Yoshinori Murakami, Michiaki Kubo and Yoichiro Kamatani. Genome-Wide Association Study Reveals BET1L Associated with Survival Time in the 137,693 Japanese Individuals. Commun Biol. 6 (1):143, 2023.
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SCIENTIFIC MEETINGS & SEMINARS

50th IMSUT Founding Commemorative Symposium The Battle Against COVID-19

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「COVID-19 との戦い」というテーマで講演を行った。

日　時：令和 5 年 5 月 26 日（金）　13:00 ～ 17:00
会　場：医科学研究所 1 号館講堂

Hiroshi Yotsuyanagi（Division of Infectious Diseases Advanced Clinical Research Center, IMSUT）
A Review of COVID-19 in Japan for three years

Hitoshi Oshitani（Department of Virology, Tohoku University Graduate School of Medicine）
Pandemic prevention, preparedness, and response over the past 30 years

Koichi Fukunaga（Division of Pulmonary Medicine, Department of Medicine, Keio University, School of Medicine）
Strength in Solidarity

Kei Sato（Division of Systems Virology, Department of Microbiology and Immunology, IMSUT）
Evolution of SARS-CoV-2

Hisashi Arase（Department of Immunochemistry, Research Institute for Microbial Diseases, Osaka University / Laboratory of Immunochemistry, WPI Immunology Frontier Research Center, Osaka University）
Host factors involved in SARS-CoV-2 infection

学友会セミナー

（令和５年１月～令和５年 12 月）

１月４日　演題：　HSV プロテインキナーゼ UL13 を介した病原性発現制御機構の解明
　　演者：　小柳　直人
１月４日　演題：　Toll Like Receptor におけるリガンド認識機構の解明
　　演者：　柴田　琢磨
１月６日　演題：　臍帯血移植における肝中心静脈閉塞症 / 類洞閉塞症候群
　　演者：　加藤　せい子
１月16日　演題：　がんの分子病態に基づいたトランスレーショナルリサーチとヒト生体試料による研究支援活動
　　演者：　醍醐　弥太郎
１月16日　演題：　Why can 3rd mRNA vaccination induce Omicron-neutralizing antibodies? -Contribution of antibody feedback to memory B cell selection-
　　演者：　井上　毅
１月18日　演題：　機能欠損リボソームに起因する翻訳異常の認識・解消機構の解析
　　演者：　李　思涵
１月26日　演題：　ワクチン開発の為の中和抗体エピトープ・パラトープ解析
　　演者：　郭　悠
１月27日　演題：　老化細胞に対する免疫監視機構
　　演者：　王　徳瑋　Teh Wei Wang
１月30日　演題：　RNA ウイルスの複製を抑制する宿主因子とその仕組みを模倣した創薬の試み
　　演者：　山根　大典
１月30日　演題：　ヒト iPS 細胞を駆使した三次元軟骨組織の再構成
　　演者：　大場　敬義
２月３日　演題：　社会ウイルス学が解き明かす麻疹ウイルス神経病原性獲得メカニズム
　　演者：　白銀　勇太
２月３日　演題：　ウイルス感染症を細胞レベルから世界レベルまで考える
　　演者：　古瀬　祐気
２月７日　演題：　胎児発育不全に起因する神経発達障害に対する間葉系幹細胞を用いた予防法開発
　　演者：　辻　雅弘
２月10日　演題：　次世代医療技術開発のための診断技術創出基盤
　　演者：　安井　寛
２月14日　演題：　非腫瘍細胞の異常を起点とする良性腫瘍の悪性化
　　演者：　山内（井上）　茜

２月15日　演題：　Development of New Therapies Targeting the Tumor Microenvironment to Treat Glioblastoma
　　演者：　Susana de Vega Paredes
３月17日　演題：　自然免疫分子 STING の細胞内物質輸送経路による厳密な制御機構
　　演者：　田口　友彦
３月17日　演題：　プロテオミクス解析を用いた肥大型心筋症の予後予測と病態生理の解明
　　演者：　島田　悠一
３月23日　演題：　SAPKK の時空間制御による生体ストレス応答機構の解明
　　演者：　森泉　寿士
３月28日　演題：　クロマチン特性解析による骨髄異形成症候群の病態解明
　　演者：　大島　基彦
４月６日　演題：　ブロモドメインタンパク質を標的とした大腸がんの新たな治療戦略
　　演者：　山口　貴世志
４月14日　演題：　Host-Pathogen interactions in the context of Leishmaniasis
　　演者：　Jalal ALSHAWEESH
４月28日　演題：　基質の構造的特徴に応じたタンパク質品質管理機構
　　演者：　冨田　拓哉
５月９日　演題：　神経幹細胞の休眠におけるプロテオスタシス制御
　　演者：　小林　妙子
５月22日　演題：　Inherited Breast and Ovarian Cancer and the Resolution of a Paradox
　　演者：　Mary-Claire King
６月６日　演題：　シクロデキストリンを基盤としたワクチンアジュバントの開発と細胞外微粒子の一粒子評価法の確立
　　演者：　林　智哉
６月８日　演題：　Genome sequence representation from the segment to the whole in the era of large language models
　　演者：　張　耀中
６月12日　演題：　CRISPR 随伴トランスポゾンによるヒト細胞における RNA 誘導性 DNA 挿入技術の開発
　　演者：　齋藤 諒
６月13日　演題：　エンベロープウイルス感染症の治療法開発に関する基礎研究
　　演者：　合田　仁
６月23日　演題：　Targeting selenoprotein dependency of AML
　　演者：　中田　大介
７月18日　演題：　Learning cytometers and beyond
　　演者：　太田 禎生
７月21日　演題：　Interactions between malaria parasite species: Do they matter?
　　演者：　Richard Culleton

７月21日　演題：　Immune responses to malaria infections
　　演者：　Julius Hafalla
７月24日　演題：　The powerful biology of senescence in cancer and aging
　　演者：　Scott W. Lowe
７月27日　演題：　Towards automated and efficient methods of analyzing and modifying biologically relevant strings and graphs
　　演者：　Robert Daniel Barish
８月９日　演題：　SARS-CoV-2 の流行・進化の理解と予測
　　演者：　伊東 潤平
８月30日　演題：　筋ジストロフィーに対する間葉系細胞を用いた炎症制御治療
　　演者：　笠原　優子
９月12日　演題：　ファージゲノム解析を基盤とした新規治療法の開発
　　演者：　植松　智
10月11日　演題：　医療分野への活用に向けた新規ゲノム編集技術の開発研究
　　演者：　吉見　一人
10月27日　演題：　肝虚血再灌流障害におけるインフラマソーム非依存性の IL-1 β産生機序の解明
　　演者：　佐田友　藍
10月30日　演題：　Metabolic changes in aging and disease
　　演者：　Peter J Mullen
10月30日　演題：　セレノエピジェネティクス
　　演者：　廣澤　瑞子
11月８日　演題：　Spatial Transcriptomics breast cancer expression signatures: basic machine learning and new opportunities
　　演者：　Carsten Oliver Daub
11月９日　演題：　内視鏡による消化管癌への挑戦
　　演者：　南出　竜典
12月１日　演題：　抗ウイルス因子 APOBEC3 とヘルペスウイルスとの攻防
　　演者：　有井　潤
12月11日　演題：　スーパーコンピュータ「富岳」を用いた大規模全ゲノム解析とその高速化
　　演者：　伊東　聰
12月12日　演題：　高深度ニュートリオミクスから迫るがん悪性化機構の解明
　　演者：　大澤　毅
12月13日　演題：　研究的思考に基づく持続可能な麻酔科診療チームの確立
　　演者：　坊垣　昌彦
12月25日　演題：　造血幹細胞～発生・休眠制御・不均一性～
　　演者：　田中　洋介
12月25日　演題：　Genetic Heterogeneity in Gene-Edited Hematopoietic Stem Cells
　　演者：　ベッカー　ハンス　次郎

12月25日　演題：　新たな造血幹細胞移植法：経胎盤移植法の開発と応用
　　演者：　全　孝静
12月27日　演題：　健康医療データの利活用と患者・市民の権利保護の研究―日本と欧州の動向
　　演者：　李　怡然

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。2023 年度は「老化研究の新展開と応用」というテーマの下で次のようなセミナーが行われた。

老化研究の新展開と応用

	月　日	講師名		演　　題
1	4月10日	小林　武彦	東京大学定量生命科学研究所 教授	ゲノムの不安定性と細胞老化
2	4月17日	茶本　健司	京都大学医学研究科 特定准教授	老化によるがん免疫治療抵抗性とその克服
3	4月24日	北島　智也	理化学研究所 生命機能科学研究センター チームリーダー	老化による卵子の染色体数異常のメカニズム
4	5月1日	高橋　暁子	公益財団法人がん研究会 部長	老化細胞における SASP 制御機構
5	5月8日	南野　　徹	順天堂大学大学院医学研究科 教授	Seno therapeutics の展望
6	5月15日	伊藤　貴浩	京都大学医生物学研究所 教授	Metabolic regulation of stem cell fates in cancer
7	5月22日	原　　英二	大阪大学微生物病研究所 教授	細胞老化の制御と役割：微生物との関与について
8	5月29日	秋下　雅弘	東京大学大学院医学系研究科 教授	ヒトの老化と老年疾患
9	10月2日	三浦　恭子	熊本大学大学院 生命科学研究部 准教授	最長寿齧歯類ハダカデバネズミにおける抗老化・がん耐性のメカニズム
10	10月16日	西村　栄美	東京大学医科学研究所 老化再生生物学分野 教授	幹細胞から解く皮膚の老化メカニズム
11	10月23日	馬渕　　洋	順天堂大学大学院医学研究科 特任准教授	組織幹細胞老化と幹細胞治療
12	10月30日	城村　由和	金沢大学がん進展制御研究所 教授	細胞老化の制御機構とそれを標的とした先進的な健康寿命延伸法の創出
13	11月6日	中西　　真	東京大学医科学研究所 癌防御シグナル分野 教授	老化を制御する

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５年12月９日（土）９：15 ～ 16：40
　　令和５年12月10日（日）９：30 ～ 16：40
場　所：医科学研究所１号館講堂

教員および題目

12 月 9 日（土）

講　師　名		題　　目
武川　睦寛	基礎医科学部門 分子シグナル制御分野	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	ゲノム解析がつくる健康社会
柴田　琢磨	感染・免疫部門 感染遺伝学分野	核酸代謝異常を認識する免疫機構の役割
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
山梨　裕司	癌・細胞増殖部門 腫瘍抑制分野	神経筋シナプス（NMJ）形成・維持シグナルと NMJ 標的治療

12 月 10 日（日）

講　師　名		題　　目
野島　正寛	先端医療研究センター 先端医療開発推進分野	新医薬品、ワクチン開発と臨床試験
佐伯　　泰	基礎医科学部門 タンパク質代謝制御分野	ユビキチン研究の最前線とタンパク質分解誘導剤
伊東　潤平	感染・免疫部門 システムウイルス学分野	データ駆動アプローチで紐解くウイルスの流行と進化
志田　　大	先端医療研究センター フロンティア外科学分野	どんどん進化する大腸がん手術 ～開腹手術、腹腔鏡手術、そしてロボット手術～

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