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Annual Report

2020

Preface

It is our pleasure to present Annual Report 2020 of the Institute of Medical Science, The University of Tokyo (IMSUT). The predecessor organization of IMSUT was founded in 1892 as the Institute of Infectious Diseases (IID) by Dr. Shibasaburo Kitasato and incorporated into the University of Tokyo in 1916. In that era, infectious diseases were the greatest threat to public health and the IID was the center for research into infectious diseases in Asia and a top center for the world, as well. As its successor, IMSUT has been contributing to basic, translational, and clinical research efforts fighting against SARS-CoV-2 and COVID-19, as you will see in the report.

In 1967, this institute received its present name when we reorganized it to encompass a wider range of medical sciences to meet the demands of modern medical care after World War II. Now, based on a history and tradition of over 129 years since the IID's foundation, IMSUT's mission has grown to contribute to the development and welfare of human society through research in cutting-edge medical science and the implementation of state-of-the-art medical care. To achieve this mission, IMSUT promotes interdisciplinary research and develops it into a wide range of practical applications, from the establishment of artificial intelligence, AI, supporting the most advanced AI medical care, to the development of drugs, including gene, virus, and vaccine therapies, cell and organ transplantation using stem cells and iPS cells, and new dimensions of genomic medicine.

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

Importantly, IMSUT was authorized in 2018 as the only International Joint Usage/Research Center serving the life science field by the Minister of Education, Culture, Sports, Science and Technology, Japan. By utilizing this platform, we are supporting 34 international joint research projects in fiscal year 2020. As a world-leading medical science institute, we fervently desire to further contribute to the development of global communities of basic, translational, and clinical research.

This annual report summarizes our scientific achievements in 2020. I sincerely hope that these achievements will inspire yet further advances, promote worldwide collaborations with our scientists, and ultimately contribute to improve health care around the world.

January 2021

Yuji Yamanashi, 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, 2020〉

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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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RESEARCH ACTIVITIES

Department of Microbiology and Immunology

Division of Virology
ウイルス感染分野

Professor	Yoshihiro Kawaoka, D.V.M., Ph.D.	教　授	獣医学博士	河　岡　義　裕
Associate Professor	Masaki Imai, D.V.M., Ph.D.	准教授	博士(獣医学)	今　井　正　樹
Project Associate Professor	Seiya Yamayoshi, D.V.M., Ph.D.	特任准教授	博士(医学)	山　吉　誠　也
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Assistant Professor	Shinya Yamada, Ph.D.	助　教	博士(医学)	山　田　晋　弥
Project Assistant Professor	Maki Kiso, D.V.M., Ph.D.	特任助教	博士(医学)	木　曽　真　紀
Project Assistant Professor	Hiroshi Ueki, D.V.M., Ph.D.	特任助教	博士(医学)	植　木　紘　史
Research Associate	Yuko Sakai-Tagawa, Ph.D.	助　手	博士(医学)	坂井(田川)優子

Viruses can cause devastating diseases. The long-term goal of our research is to understand the molecular pathogenesis of viral diseases by using influenza virus, Ebola virus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections as models. Interactions between viral and host gene products during viral replication determine the consequences of infection (i.e., the characteristics of disease manifestation, whether limited or widespread); hence, our research has centered on such interactions during these viral infections.

1. Protective Immunity and Persistent Lung Sequalae in Domestic Cats after SARS-CoV-2 Infection.

Chiba S[1], Halfmann PJ[1], Hatta M[1], Maemura T[1], Fan S[1], Armbrust T[1], Swartley OM[1], Crawford LK[1], Kawaoka Y: [1]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, USA.

SARS-CoV-2 readily transmits between domestic cats (see also Project 6 in this report). We found that domestic cats that recover from an initial infection might be protected from reinfection. However, we also found long-term persistence of inflammation and other lung lesions after infection in cats, despite a lack of clinical symptoms and limited viral replication in the lungs.

2. Comparison of Rapid Antigen Tests for COVID-19.

Yamayoshi S, Sakai-Tagawa Y, Koga M[2], Akasaka O[3], Nakachi I[4], Koh H[5], Maeda K[6], Adachi E[7], Saito M[2], Nagai H[7], Ikeuchi K[2], Ogura T[8], Baba R[4], Fujita K[8], Fukui T[5], Ito F[5], Hattori SI[6], Yamamoto K[9], Nakamoto T[9], Furusawa Y, Yasuhara A, Ujie M, Yamada S, Ito M, Mitsuya H[6], Omagari N[9], Yotsuyanagi H[2], Iwatsuki-Horimoto K, Imai M, Kawaoka Y: [2]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Japan. [3]Emergency Medical Center, Fujisawa City Hospital, Japan. [4]Pulmonary Division, Department of Internal Medicine, Saiseikai Utsunomiya Hospital, Japan. [5]Division of Pulmonary Medicine, Department of Internal Medicine, Tachikawa Hospital, Japan. [6]Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Japan. [7]Department of Infectious Diseases and Applied Immunology, IMSUT Hospital of Institute of Medical

Science, the University of Tokyo, Japan. [8]Department of Emergency Medicine and Critical Care Medicine, Saiseikai Utsunomiya Hospital, Japan. [9]Disease Control and Prevention Center, National Center for Global Health and Medicine Hospital, Japan.

Reverse transcription-quantitative PCR (RT-qPCR)-based tests are widely used to diagnose coronavirus disease 2019 (COVID-19). Because local clinics lack the capability to do RT-qPCR testing, rapid antigen tests (RATs) for COVID-19 based on lateral flow immunoassays are used for rapid diagnosis. However, their sensitivity compared with each other and with RT-qPCR and infectious virus isolation has not been examined. Here, we compared the sensitivity among four RATs by using SARS-CoV-2 isolates and several types of COVID-19 patient specimens and compared their sensitivity with that of RT-qPCR and infectious virus isolation. Although the RATs read the samples containing large amounts of virus as positive, even the most sensitive RAT read samples containing small amounts of virus as negative. Moreover, all RATs tested failed to detect viral antigens in several specimens from which virus was isolated. The current RATs will likely miss some COVID-19 patients who are shedding infectious SARS-CoV-2.

3. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo.

Hou YJ[10], Chiba S[1], Halfmann P[1], Ehre C[11], Kuroda M[1], Dinnon KH 3rd[12], Leist SR[10], Schäfer A[10], Nakajima N[13], Takahashi K[13], Lee RE[11], Mascenik TM[11], Graham R[10], Edwards CE[10], Tse LV[10], Okuda K[11], Markmann AJ[14], Bartelt L[14], de Silva A[12], Margolis DM[10], Boucher RC[11], Randell SH[11], Suzuki T[13], Gralinski LE[10], Kawaoka Y, Baric RS[10]: [10]Department of Epidemiology, University of North Carolina at Chapel Hill, USA. [11]Marsico Lung Institute, University of North Carolina at Chapel Hill, USA. [12]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, USA. [13]Department of Pathology, National Institute of Infectious Diseases, Japan. [14]Department of Medicine, University of North Carolina at Chapel Hill, USA.

The spike aspartic acid–614 to glycine (D614G) substitution is prevalent in global SARS-CoV-2 strains, but its effects on viral pathogenesis and transmissibility remain unclear. We, therefore, engineered a SARS-CoV-2 variant containing this substitution. The variant exhibits more efficient infection, replication, and competitive fitness in primary human airway epithelial cells but maintains similar morphology and in vitro neutralization properties, compared with the ancestral wild-type virus. Infection of human angiotensin-converting enzyme 2 (ACE2) transgenic mice and Syrian hamsters with the variant or wild-type virus resulted in similar viral titers in respiratory tissues and pulmonary disease. However, the D614G variant transmitted much faster and displayed increased competitive fitness compared with the wild-type virus in hamsters. These data show that the D614G substitution enhances SARS-CoV-2 infectivity, competitive fitness, and transmissibility in primary human cells and animal models.

4. Effectiveness of Face Masks in Preventing Airborne Transmission of SARS-CoV-2.

Ueki H, Furusawa Y, Iwatsuki-Horimoto K, Imai M, Kabata H[14], Nishimura H[15], Kawaoka Y: [14]Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, Japan. [15]Clinical Research Division, Virus Research Center, National Hospital Organization Sendai Medical Center, Japan

Guidelines from the CDC and the WHO recommend the wearing of face masks to prevent the spread of COVID-19; however, the protective efficiency of such masks against airborne transmission of infectious SARS-CoV-2 droplets/aerosols is unknown. Here, we developed an airborne transmission simulator of infectious SARS-CoV-2-containing droplets/aerosols produced by human respiration and coughs and assessed the transmissibility of the infectious droplets/aerosols and the ability of various types of face masks to block the transmission. We found that cotton masks, surgical masks, and N95 masks all have a protective effect with respect to the transmission of infective droplets/aerosols of SARS-CoV-2 and that the protective efficiency was higher when masks were worn by a virus spreader. Importantly, medical masks (surgical masks and even N95 masks) were not able to completely block the transmission of virus droplets/aerosols even when completely sealed. Our data will help medical workers understand the proper use and performance of masks and determine whether they need additional equipment to protect themselves from infected patients.

5. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development.

Imai M, Iwatsuki-Horimoto K, Hatta M[1], Loeber S[16], Halfmann PJ[1], Nakajima N[13], Watanabe T, Ujie M, Takahashi K[13], Ito M, Yamada S, Fan S[1], Chiba S[1], Kuroda M[1], Guan L[1], Takada K, Armbrust T[1], Balogh A[1], Furusawa Y, Okuda M, Ueki H, Yasuhara A, Sakai-Tagawa Y, Lopes TJS, Kiso M, Yamayoshi S, Kinoshita N[9], Ohmagari N[9], Hattori SI[6], Takeda M[17], Mitsuya H[6], Krammer F[18], Suzuki T[13], Kawaoka Y: [16]Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, USA. [17]Department of Virology 3,

National Institute of Infectious Diseases, Japan. [18]Department of Microbiology, Icahn School of Medicine at Mount Sinai, USA.

Here, we assessed the replicative ability and pathogenesis of SARS-CoV-2 isolates in Syrian hamsters. SARS-CoV-2 isolates replicated efficiently in the lungs of hamsters, causing severe pathological lung lesions following intranasal infection. In addition, microcomputed tomographic imaging revealed severe lung injury that shared characteristics with SARS-CoV-2–infected human lung, including severe, bilateral, peripherally distributed, multilobular ground glass opacity, and regions of lung consolidation. SARS-CoV-2–infected hamsters mounted neutralizing antibody responses and were protected against subsequent rechallenge with SARS-CoV-2. Moreover, passive transfer of convalescent serum to naïve hamsters efficiently suppressed the replication of the virus in the lungs even when the serum was administrated two days post-infection of the serum-treated hamsters. Collectively, these findings demonstrate that this Syrian hamster model will be useful for understanding SARS-CoV-2 pathogenesis and testing vaccines and antiviral drugs.

6. Transmission of SARS-CoV-2 in Domestic Cats.

Halfmann PJ[1], Hatta M[1], Chiba S[1], Maemura T[1], Fan S[1], Takeda M[17], Kinoshita N[9], Hattori SI[6], Sakai-Tagawa Y, Iwatsuki-Horimoto K, Imai M, Kawaoka Y

Reports of human-to-feline transmission of SARS-CoV-2 prompted us to evaluate nasal shedding of this virus from inoculated cats and its subsequent transmission by direct contact between virus-inoculated cats and cats with no previous infection with the virus. SARS-CoV-2 replicated efficiently in cats and transmitted well between cats without any symptoms. These results suggest that cats may be a silent intermediate host of SARS-CoV-2.

Publications

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Division of Infectious Genetics
感染遺伝学分野

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

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

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

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

2. Nucleosides drive histiocytosis in SLC29A3 disorders by activating TLR7

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

3. Toll-like receptor 7 is a factor of type 1 diabetes in NOD mice

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Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of beta cells by infiltrating $CD8^+$ T cells in the islet. Autoantibody production (e.g. anti-GAD65) also contributes to the development of T1D, thus acquired immune system is thought as main player of progression for T1D. Although pathogenic roles of the acquired immune system are well established, a role of the innate immune system in T1D remains unclear.

Toll-like receptor 7 (TLR7), an RNA-sensing TLR, is known to drive a variety of autoimmune diseases. We here show that TLR7 drives disease progression in T1D. In WT Non-Obese Diabetes (NOD) mice, the onset rate of T1D reached about 70% by 7 mo of age, whereas the onset rate of *Tlr7*$^{-/-}$ NOD mice was as low as 30 % at 7 mo. The infiltration of immune cells into islets was attenuated in *Tlr7*$^{-/-}$ NOD mice. The number of $CD11b^+/Ly6C^-/Fc\gamma R4^+/TLR7^+$ monocytes (patrolling monocyte, patMC) in spleen and the expression of FcγR4 on the subset were decreased by TLR7 deficiency. Autoantibody production was reduced by TLR7 deficiency, however, the number of plasma cells was not changed. These results suggest that TLR7 drives disease progression through the activation of patrolling monocyte/macrophages in NOD mice.

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

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

1. Identification of a Herpes Simplex Virus 1 Gene Encoding Neurovirulence Factor by Chemical Proteomics

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Identification of the complete set of translated genes of viruses is important to understand viral replication and pathogenesis as well as for therapeutic approaches to control viral infection. Here, we use chemical proteomics, integrating bio-orthogonal non-canonical amino acid tagging and high-resolution mass spectrometry, to characterize the newly synthesized herpes simplex virus 1 (HSV-1) proteome in infected cells. In these infected cells, host cellular protein synthesis is shut-off, increasing the chance to preferentially detect viral proteomes. We identify nine previously cryptic orphan protein coding sequences whose translated products are expressed in HSV-1-infected cells. Functional characterization of one identified protein, designated piUL49, shows that it is critical for HSV-1 neurovirulence in vivo by regulating the activity of virally encoded dUTPase, a key enzyme that maintains accurate DNA replication. Our results demonstrate that cryptic orphan protein coding genes of HSV-1, and probably other large DNA viruses, remain to be identified.

2. Phosphoregulation of a conserved herpesvirus tegument protein by a virally encoded protein kinase in viral pathogenicity and potential linkage between its evolution and viral phylogeny

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Us3 proteins of herpes simplex virus 1 (HSV-1) and HSV-2 are multifunctional serine-threonine protein kinases. Here, we identified an HSV-2 tegument protein, UL7, as a novel physiological substrate of HSV-2 Us3. Mutations in HSV-2 UL7, which precluded Us3 phosphorylation of the viral protein, significantly reduced mortality, viral replication in the vagina, and development of vaginal disease in mice following vaginal infection. These results indicated that Us3 phosphorylation of UL7 in HSV-2 was required for efficient viral replication and pathogenicity in vivo. Of note, this phosphorylation was conserved in UL7 of chimpanzee herpesvirus (ChHV), which phylogenetically forms a monophyletic group with HSV-2 and the resurrected last common ancestral UL7 for HSV-2 and ChHV. In contrast, the phosphorylation was not conserved in UL7s of HSV-1, which belongs to a sister clade of the monophyletic group, the resurrected last common ancestor for HSV-1, HSV-2, and ChHV, and other members of the genus Simplexvirus that are phylogenetically close to these viruses. Thus, evolution of Us3 phosphorylation of UL7 coincided with the phylogeny of simplex viruses. Furthermore, artificially induced Us3 phosphorylation of UL7 in HSV-1, in contrast to phosphorylation in HSV-2, had no effect on viral replication and pathogenicity in mice. Our results suggest that HSV-2 and ChHV have acquired and maintained Us3 phosphoregulation of UL7 during their evolution because the phosphoregulation had an impact on viral fitness in vivo, whereas most other simplex viruses have not because the phosphorylation was not necessary for efficient fitness of the viruses in vivo.

3. Role of phosphatidylethanolamine biosynthesis in herpes simplex virus 1-infected cells on progeny virus morphogenesis in the cytoplasm and on viral pathogenicity in vivo

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Glycerophospholipids are major components of cell membranes. Phosphatidylethanolamine (PE) is a glycerophospholipid that is involved in multiple cellular processes, such as membrane fusion, the cell cycle, autophagy, and apoptosis. In this study, we investigated the role of PE biosynthesis in herpes simplex virus 1 (HSV-1) infection by knocking out the host cell gene encoding phosphate cytidylyltransferase 2, ethanolamine (Pcyt2), which is a key rate-limiting enzyme in one of the two major pathways for PE biosynthesis. Pcyt2 knockout reduced HSV-1 replication and caused an accumulation of unenveloped and partially enveloped nucleocapsids in the cytoplasm of an HSV-1-infected cell culture. A similar phenotype was observed when infected cells were treated with meclizine, which is an inhibitor of Pcyt2. In addition, treatment of HSV-1-infected mice with meclizine significantly reduced HSV-1 replication in the mouse brains and improved their survival rates. These results indicated that PE biosynthesis mediated by Pcyt2 was required for efficient HSV-1 envelopment in the cytoplasm of infected cells and for viral replication and pathogenicity in vivo. The results also identified the PE biosynthetic pathway as a possible novel target for antiviral therapy of HSV-associated diseases and raised an interesting possibility for meclizine repositioning for treatment of these diseases, since it is an over-the-counter drug that has been used for decades against nausea and vertigo in motion sickness.

4. ESCRT-III controls nuclear envelope deformation induced by progerin

Jun Arii, Fumio Maeda, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasuko Mori & Yasushi Kawaguchi

Hutchinson-Gilford progeria syndrome (HGPS) is a premature aging disorder, caused by mutation in the gene encoding lamin A/C, which produces a truncated protein called progerin. In cells from HGPS patients, progerin accumulates at the nuclear membrane (NM), where it causes NM deformations. In this study, we investigated whether progerin-induced NM deformation involved ESCRT-III, a protein complex that remodels nuclear and cytoplasmic membranes. The ESCRT-III protein CHMP4B was recruited to sites of aberrant NM proliferation in human cells ectopically expressing progerin and in patient-derived HGPS fibroblasts. Derepression of NM deformation in these cells was observed following depletion of CHMP4B or an ESCRT-III adaptor, ALIX. Treatment with rapamycin (which induce autophagic clearance of progerin and reverse progerin-induced cellular phenotypes) down-regulated progerin-induced NM deformation, whereas treatment with bafilomycin A1 (an inhibitor of autophagy and lysosome-based degradation) or CHMP4B depletion antagonized the effects of rapamycin. These results indicate that the ALIX-mediated ESCRT-III pathway plays a suppressive role in progerin-induced NM deformation and suggest that autophagy down-regulates progerin-induced NM deformation in a manner dependent on ESCRT-III machinery.

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Division of Vaccine Science
ワクチン科学分野

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

1. Researches and development of a mRNA vaccine and monoclonal antibodies against SARS-CoV-2

In 2020, the coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to the successful development of two mRNA-based vaccines, encoding the full length of the viral surface spike protein, with high efficacy and reasonable safety. However, reactogenicity, such as fever, caused by innate immune responses to the vaccine formulation remains to be improved. To overcome this potential issue, we developed a lipid nanoparticle (LNP)-based mRNA vaccine, encoding the SARS-CoV-2 spike protein receptor-binding domain (LNP-mRNA-RBD), that improved immunogenicity by removing reactogenic materials from the vaccine formulation in mice and conferred protection against SARS-CoV-2 infection. The vaccine is filed for patent, finished for pre-clinical development, and is underway to first in human clinical trial by the end of March 2021.

In addition to vaccine development, we started to generate monoclonal antibody against SARS-CoV-2 obtained from the volunteer recovered from COVID-19. We have obtained several clones with very high affinity, filed for patent, and now is underway for its efficacy in the animal model.

2. Discovery of Self-Assembling Small Molecules as Vaccine Adjuvants

Immune potentiators, termed adjuvants, trigger early innate immune responses to ensure the generation of robust and long-lasting adaptive immune responses of vaccines. Presented here is a study that takes advantage of a self-assembling small-molecule library for the development of a novel vaccine adjuvant. Cell-based screening of the library and subsequent structural optimization led to the discovery of a simple, chemically tractable deoxycholate derivative (molecule 6, also named cholicamide) whose well-defined nanoassembly potently elicits innate immune responses in macrophages and dendritic cells. Functional and mechanistic analyses indicate that the virus-like assembly enters the cells and stimulates the innate immune response through Toll-like receptor 7 (TLR7), an endosomal TLR that detects single-stranded viral RNA. As an influenza vaccine adjuvant in mice, molecule 6 was as potent as Alum, a clinically used adjuvant. The studies described here pave the way for a new approach to discovering and designing

self-assembling small-molecule adjuvants against pathogens, including emerging viruses.

3. IL-33 Is Essential for Adjuvant Effect of Hydroxypropyl-β-Cyclodexrin on the Protective Intranasal Influenza Vaccination

Vaccine adjuvants are traditionally used to augment and modulate the immunogenicity of vaccines, although in many cases it is unclear which specific molecules contribute to their stimulatory activity. We previously reported that both subcutaneous and intranasal administration of hydroxypropyl-β-cyclodextrin (HP-β-CD), a pharmaceutical excipient widely used to improve solubility, can act as an effective adjuvant for an influenza vaccine. However, the mechanisms by which mucosal immune pathway is critical for the intranasal adjuvant activity of HP-β-CD have not been fully delineated. Here, we show that intranasally administered HP-β-CD elicits a temporary release of IL-33 from alveolar epithelial type 2 cells in the lung; notably, IL-33 expression in these cells is not stimulated following the use of other vaccine adjuvants. The experiments using gene deficient mice suggested that IL-33/ST2 signaling is solely responsible for the adjuvant effect of HP-β-CD when it is administered intranasally. In contrast, the subcutaneous injection of HP-β-CD and the intranasal administration of alum, as a damage-associated molecular patterns (DAMPs)-inducing adjuvant, or cholera toxin, as a mucosal adjuvant, enhanced humoral immunity in an IL-33-independent manner, suggesting that the IL-33/ST2 pathway is unique to the adjuvanticity of intranasally administered HP-β-CD. Furthermore, the release of IL-33 was involved in the protective immunity against influenza virus infection which is induced by the intranasal administration of HP-β-CD-adjuvanted influenza split vaccine. In conclusion, our results suggest that an understanding of administration route- and tissue-specific immune responses is crucial for the design of unique vaccine adjuvants.

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マラリア免疫学分野

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Malaria, caused by Plasmodium parasites, often leads to severe complications such as cerebral malaria and death. The immune pathology causing these complications is poorly defined. In our lab, we elucidate the mechanisms involved in the host immune system and Plasmodium parasites interactions. We develop new techniques to understand these interactions. Our final goal is to develop vaccines and vaccine modalities against malaria and other infectious diseases.

1. The host targeting effect of chloroquine in malaria

Cevayir Coban[1,2]: [1]Division of Malaria Immunology, Department of Microbiology and Immunology, The Institute of Medical Science (IMSUT), The University of Tokyo; [2]Laboratory of Malaria Immunology, Immunology Frontier Research Center (IFReC), Osaka University.

Due to the rapid onset and spread of the COVID-19 pandemic, the treatment of COVID-19 patients by hydroxychloroquine alone or in combination with other drugs has captured a great deal of attention and triggered considerable debate. Historically, the worldwide use of quinoline based-drugs has led to a spectacular reduction in death from malaria. Unfortunately, scientists have been forced to seek alternative drugs to treat malaria due to the emergence of chloroquine-resistant parasites in the 1960s. The repurposing of hydroxychloroquine against viral infections, various types of cancer and autoimmune diseases has been ongoing for more than 70 years, with no clear understanding of its mechanism of action (MOA). Here, in the current review article, we closely examine the MOA of this old but influential drug in and beyond malaria. Better insights into how chloroquine targets the host's cellular and immune responses may help to develop applications against to new pathogens and diseases, and perhaps even restore the clinical utility of chloroquine against malaria.

2. Heparin induces neutrophil elastase-dependent vital and lytic NET formation

Patrick M. Lelliott[1], Masatoshi Momota[2,3], Takayuki Shibahara[2,3], Michelle S. J. Lee[1], Nicholas I Smith[4], Ken J. Ishii[2,3,5], and Cevayir Coban[1,6]: [1]Laboratory of Malaria Immunology, and [2]Laboratory of Vaccine Science, Immunology Frontier Research Center (IFReC), Osaka University, [3]Laboratory of Adjuvant Innovation, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka, [4]Biophotonics Laboratory, Immunology Frontier Research Center (IFReC), Osaka University, [5]Division of Vaccine Science, and [6]Division of Malaria Immunology, The Institute of Medical Science (IMSUT), The University of Tokyo

We recently developed an automated analysis method by using imaging flow cytometry that allows quantifying neutrophil extracellular traps (NETs) in vitro and in vivo (*Lelliott et al., Cytometry A, 2019*). We next demonstrated a new finding, an ability of heparin to induce NETs. Heparin is used extensively as an anticoagulant in a broad range of diseases and proce-

dures; however, its biological effects are not limited to coagulation and remain incompletely understood. For instance, heparin usage can lead to the life-threatening complication known as heparin-induced thrombocytopenia (HIT), caused by the development of antibodies against heparin/PF4 complexes. We found that after heparin stimulation, NETs occurred with cell lysis and death, but live neutrophils releasing extracellular DNA strands, known as vital NETs, also occurred abundantly. Formation of NETs was time and dose dependent, and required reactive oxygen species and neutrophil elastase. Other compounds related to heparin such as low molecular weight heparin, fondaparinux and heparan sulfate either failed to induce NETs, or did so to a much lesser extent. Our findings suggest the ability of heparin to directly induce NET formation and it should be considered in the context of heparin treatment and HIT pathogenesis.

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人癌病因遺伝子分野

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Human cancers develop and progress toward malignancy through accumulation of multiple genetic and epigenetic alterations. Elucidation of these alterations is essential to provide molecular targets for prevention, diagnosis, and treatment of cancer. Our current interest is to understand the roles of cell-cell interaction in invasion, metastasis, drug resistance and immunological responses of cancer. Genomic and epigenomic abnormalities involved in human tumors, including small cell lung cancer, adult T-cell leukemia, cholangiocarcinoma, lung, breast, head and neck and urological cancers, as well as genetic susceptibility to various common diseases are also being investigated.

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

Takeshi Ito, Yumi Tsuboi, Yutaka Kasai, Toko Funaki, Yuki Azuma, Yoshiaki Kanamura, Etsu Kaku, Ryuki Shibata, Yuto Tanaka, Mizuki Tominaga, Miko Komiya, Yuya Sohinohara, Tomoko Masuda, Hiromi Ichihara, Kaoru Kiguchi, Motoi Oba, Zenichi Tanei[1], Akiteru Goto[2], Daisuke Matsubara[3] and Yoshinori Murakami; [1]Department of Cancer Pathology, Faculty of Medicine, Hokkaido University, Sapporo, [2]Department of Cellular and Organ Pathology, Graduate School of Medicine, Akita University, Akita, [3]Department of Integrative Pathology, Jichi Medical University, Shimotsuke, Japan

Disruption of cell adhesion is a critical step to invasion and metastasis of human cancer and their acquired resistance to several anti-cancer and molecular targeting drugs. CADM1/TSLC is an immunoglobulin superfamily cell adhesion molecule (IgCAMs) and acts as a tumor suppressor in various epithelial cancers. By contrast, CADM1 promotes cell invasion and metastasis in adult T-cell leukemia (ATL) or small cell lung cancer (SCLC). We are currently investigating molecular pathways of CADM1 underlying its dual roles in oncogenesis. For this purpose, we investigated CADM1-associated proteins comprehensively using mass spectrometry of immune-precipitates of CADM1 and identified a transmembrane adaptor protein, Csk-binding protein (Cbp), known to suppress Src-mediated transformation, as a binding partner of CADM1. CADM1 co-immunoprecipitated with Cbp and c-Src and suppresses c-Src activation, wound healing and tumorigenicity of a human colon cancer cell. CADM1 would act as a colon tumor suppressor by intervening oncogenic c-Src signaling through binding with Cbp besides its authentic cell adhesion activity (1).

On the other hand, CADM1 is highly expressed in SCLC and promotes colony formation of SCLC cells in soft agar. We found that one of the 4.1-binding proteins, 4.1R, was responsible for suppression of colony formation by CADM1 in SCLC cells. In primary SCLC, CADM1 expression was correlated with membranous localization of 4.1R and was associated with more advanced tumor stage. These results suggest that the formation of CADM1–4.1R complex would

promote malignant features of SCLC (2).

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

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

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

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

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

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

To unveil additional molecular mechanisms underlying multistage carcinogenesis, genomic, epigenomic, and transcriptional alterations in key molecules in human tumorigenesis were examined in various cancers. We examined somatic alterations at sites of CNV in DNA from 92 breast cancers using a CNV-specific Comparative Genomic Hybridization array with 412,000 sites or quantitative PCR analysis. Somatic copy number alterations (CNAs) were detected in 39.9% of the CNV probes examined. When CNA fragments were categorized according to their size, CNAs smaller than 10kb correlated significantly with ER-positivity and lower NG, providing a novel mechanism in ER-positive breast carcinogenesis (3).

Furthermore, germline mutations of cancer-related genes were examined in a large number of patients from prostate, pancreatic and colorectal cancer using the samples in BioBank Japan (BBJ) in collaboration with scientist in Riken (4-6). Molecular mechanisms of cancer progression in response to hypoxia and transcriptional regulation of lung cancer were also analyzed in collaboration with former colleagues (7-10).

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

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

To understand the molecular mechanisms of diseases and their genetic risks of individuals, genome-wide association studies (GWAS) were carried out or polygenic risk scores (PRS) were evaluated (11,12) using genomic and clinical information of patients accumulated in BBJ in collaboration with others. The targets include pancreatic, endometrial and cervical cancers (13-15), coronary artery diseases and other common diseases (16-19), hematopoietic clonality (20-22) and various phenotypes (23-29). These results provide not only direct information for preventing and treating human diseases but also a novel concept in understanding various diseases and genomic heterogeneity.

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Department of Cancer Biology

Division of Cellular and Molecular Biology
分子発癌分野

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Gene expression is largely regulated by signal transduction triggered by various stimulations. Several lines of evidence indicate that genetic defects of molecules involved in the signal transduction or the gene expression lead to abnormal cell differentiation or tumor formation. Our goal is to understand the molecular mechanisms of disease pathogenesis and oncogenesis by elucidating normal regulation of intracellular signal transduction and gene expression involved in cell proliferation and differentiation. We have identified and been interested in Tumor necrosis factor receptor-associated factor 6 (TRAF6), which acts as an E3 ubiquitin ligase to generate Lys63-linked polyubiquitin chains that are crucial for transducing signals emanating from the TNFR superfamily or the TLR/IL-1R family leading to activation of transcription factor NF-κB and AP-1. By generating TRAF6-deficient mice, we found that TRAF6 is essential for osteoclastogenesis, immune self-tolerance, lymph node organogenesis and formation of skin appendices. We are currently focusing on molecular mechanisms underlying TRAF6-mediated activation of signal transduction pathways and how TRAF6 is involved in osteoclastogenesis and self-tolerance. In addition, NF-κB is constitutively activated in various cancer cells and this activation is likely involved in the malignancy of tumors. Thus, we are also investigating the molecular mechanisms of the constitutive activation of NF-κB and how this activation leads to the malignancy of breast cancers and adult T cell leukemia (ATL). In addition, we are investigating novel molecular mechanisms how tumor microenvironments and inflammation are regulated.

1. Molecular mechanism of the regulation of NF-κB transcription factor

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Transcription factor NF-κB binds specifically to a decameric motif of nucleotide, κB site, and activates transcription. The activation of NF-κB has been demonstrated to be carried out post-translationally upon extracellular stimuli through membrane receptors such as members of the TLR/IL-1R family and of TNFR superfamily. In canonical NF-κB pathway, NF-κB forms a complex with regulatory protein, IκB, and is sequestered in the cytoplasm prior to stimulation. Upon stimulation, IκB is rapidly phosphorylated on two specific serine residues by IκB kinase (IKK) complex followed by lysine 48 (K48)-linked ubiquitination and proteasome-dependent degradation of IκB. NF-κB subsequently translocates to the nucleus to activate transcription of target genes. This project is to identify molecules that regulate signal from membrane receptors to NF-κB/IκB complex. We have previously identified upstream activators of NF-κB, tu-

mor necrosis factor receptor-associated factor (TRAF) 6. TRAF6 contains RING domain in the N-terminus and acts as an E3 ubiquitin-ligase to catalyze the lysine 63 (K63)-linked polyubiquitination of several signaling molecules and TRAF6 itself. To understand the molecular mechanisms of TRAF6-mediated NF-κB activation, we try to identify proteins that are ubiquitinated by TRAF6 upon stimulation. We took advantage of using the peptide that specifically binds K63-linked polyubiquitin chain to purify such proteins. We have confirmed that the peptide-based affinity column is useful for specific concentration of recombinant K63-linked polyubiquitin chain, suggesting that it also works for purification of the proteins of our interest. We are also interested in noncanonical NF-κB pathway, which is crucial for immunity by establishing lymphoid organogenesis and B-cell and dendritic cell (DC) maturation. RelB is a major NF-κB subunit in the pathway. To elucidate the mechanism of the RelB-mediated immune cell maturation, a precise understanding of the relationship between cell maturation and RelB expression and activation at the single-cell level is required. Therefore, we generated knock-in mice expressing a fusion protein between RelB and fluorescent protein (RelB-Venus) from the *Relb* locus. The *Relb*$^{Venus/Venus}$ mice developed without any abnormalities observed in the *Relb*$^{-/-}$ mice, allowing us to monitor RelB-Venus expression and nuclear localization as RelB expression and activation. *Relb*$^{Venus/Venus}$ DC analyses revealed that DCs consist of RelB^{-}, RelB^{low} and RelB^{high} populations. The RelB^{high} population, which included mature DCs with projections, displayed RelB nuclear localization, whereas RelB in the RelB^{low} population was in the cytoplasm. Although both the RelB^{low} and RelB^{-} populations barely showed projections, MHC II and co-stimulatory molecule expression were higher in the RelB^{low} than in the RelB^{-} splenic conventional DCs. Taken together, our results identify the RelB^{low} population as a possible novel intermediate maturation stage of cDCs and the *Relb*$^{Venus/Venus}$ mice as a useful tool to analyze the dynamic regulation of the non-canonical NF-κB pathway.

2. Molecular mechanism of RANK signaling in osteoclastogenesis

Saya Bando, Mizuki Yamamoto and Jun-ichiro Inoue

Bone is an important organ, which supports body structure and hematopoiesis. Osteoclasts are large multinucleated cells, which have ability to degrade bone matrixes, and play a crucial role in bone homeostasis in concert with osteoblast, which generates bone matrix. As a result of excess formation or activation of osteoclasts, pathological bone resorption is observed in postmenopausal osteoporosis, rheumatoid arthritis and bone metastasis. Therefore, elucidating the molecular mechanism of osteoclastogenesis is important for understanding bone diseases and developing novel strategies to treat such diseases. Osteoclasts are differentiated from hematopoietic stem cells upon stimulation with macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL). It is known that the activation of signal transduction pathway emanating from receptor RANK is essential for osteoclastogenesis. The RANK signal activates transcriptional factors, NF-κB and AP-1, through the E3 ubiquitin ligase TRAF6, and induces activation of PLCγ2-mediated Ca^{2+} signaling pathway. These signals lead to the induction of NFATc1, a master transcriptional factor in osteoclastogenesis. We have previously demonstrated that RANK has a functional amino acid sequences, named Highly Conserved domain in RANK (HCR), which does not have any homology of amino-acid sequence with other proteins. The HCR acts as a platform for formation of signal complex including TRAF6, PLCγ2 and adaptor protein Gab2. This formation of signal complex is involved in sustaining activation of RANK signaling, and is essential for the NFATc1 induction and osteoclastogenesis. To elucidate the physiological function of HCR, we have generated mice lacking HCR domain of RANK.

3. Intratumoral bidirectional transitions between epithelial and mesenchymal cells in triple-negative breast cancer

Mizuki Yamamoto, Aya Watanabe, Yoko Hirayama and Jun-ichiro Inoue

Epithelial-mesenchymal transition (EMT) and its reverse process, MET, are crucial in several stages of cancer metastasis. EMT allows cancer cells to move to proximal blood vessels for intravasation. However, because EMT and MET processes are dynamic, mesenchymal cancer cells are likely to undergo MET transiently and subsequently re-undergo EMT to restart the metastatic process. Therefore, spatiotemporally-coordinated mutual regulation between EMT and MET could occur during metastasis.

To elucidate such regulation, we chose HCC38, a human triple-negative breast cancer cell line, because HCC38 is composed of epithelial and mesenchymal populations at a fixed ratio. We established E-cadherin- and Vimentin-reporter expressing HCC38 cells to analyze EMT status using FACS analysis. Using this cell, we performed CRISPR/Cas9-mediated screening for intratumoral EMT/MET-regulating genes and found 21 EMT-inducers and 3 MET-inducers. Now, we are analyzing molecular mechanisms of EMT and MET by candidate genes to develop novel therapeutic strategies for triple-negative breast cancer.

4. Molecular mechanism of the Flavi virus E-protein-mediated membrane fusion.

Mizuki Yamamoto, Yusuke Fujinami, Aya Watanabe and Jun-ichiro Inoue

We have developed a cell-based fusion assay for prME protein of Flavi virus in a low pH-dependent manner, using Aedes albopictus cell line C6/36 cells expressing Renilla luciferase (RL)-based split reporter proteins. Using this assay, we are performing chemical screening to investigate molecular mechanisms for the E-protein-mediated membrane fusion.

5. Mint3 depletion attenuates proliferation in pancreatic cancer cells

Akane Kanamori, Jun-ichiro Inoue and Takeharu Sakamoto

Pancreatic cancer is one of the deadliest cancers. Although severe hypoxia is characteristic for pancreatic cancer, most cancer cells grow in normoxic and modest hypoxic areas. Hypoxia inducible factor-1 (HIF-1) is a master transcriptional factor for hypoxic response and thought to promote the malignancy of pancreatic cancer not only in severe hypoxic area but also in normoxic and modest hypoxic areas where cancer cells grow. However, the importance of HIF-1 in pancreatic cancer proliferation under the normoxic condition remains unclear. To address this, we focused on Mint3 which activates HIF-1 even in normoxia in cancer cells. Mint3 depletion attenuated proliferation in human pancreatic cancer AsPC1, BxPC3, Panc-1, and MIA-PaCa2 cells. Further analyses revealed that Mint3 depletion caused cell cycle arrest with increased p21 and p27 expression in pancreatic cancer cells in a HIF-1-dependent manner. In addition, Mint3 depletion attenuated epithelial mesenchymal transition, stemness features, and chemoresistance in pancreatic cancer cells. Even in vivo, Mint3 depletion attenuated orthotopic tumor growth of AsPC1 cells in immunodeficient mice. Database analyses showed that high Mint3 expression correlates with poor prognosis of pancreatic cancer patients. Thus, Mint3 is a possible target for pancreas cancer treatment.

6. Mint3 controls polarization of tumor-associated macrophages

Yuya Fukui, Jun-ichiro Inoue and Takeharu Sakamoto

Tumor-associated macrophages (TAMs) are thought to promote tumor malignancy. TAMs can be classified into two subtypes; an inflammatory M1-like subtype and an immune-suppressive M2-like subtype. Most TAMs polarize to the M2-like subtype, resulting in decreased anti-tumor immunity and enhanced tumor progression. It has been reported that HIF-1 depletion attenuates expression of M2-related genes in TAMs. This prompted us to evaluate whether a HIF-1 activator, Mint3, can also control TAM polarization in vivo. Myeloid-cell specific Mint3 knockout (cKO) mice showed decreased tumor growth of subcutaneously inoculated LLC and MC38 cells compared with control mice. TAMs from tumors of Mint3 cKO mice less polarized to the M2-like subtype. Further analyses of tumor infiltrated leucocytes showed that $CD8^+$ T cells increased while Tregs decreased in tumors of Mint3 cKO mice, indicating improved anti-tumor immunity in tumors of Mint3 cKO mice.

Publications

Niederkorn, M., Hueneman, K, Choi, K, Varney, M.E., Romano, L., Pujato, M.A., Greis, K.D., Inoue, J., Meetei, R. and Starczynowski. D.T. TIFAB regulates USP15-mediated p53 signaling during stressed and malignant hematopoiesis. *Cell Rep.* 30, 2776-2790. e6. doi:10.1016/j.celrep.2020.01.093

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

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

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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 of the neuromuscular junction (NMJ), providing a new insight into RTK-mediated signaling. It seems possible that local levels of cytoplasmic activators, like Dok-7, control the activity of RTKs in concert with their extracellular ligands.

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

chestrated 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 have recently found that forced expression of Dok-7 lacking the C-terminal region rescued Dok-7 knockout mice from neonatal lethality caused by the lack of NMJs, indicating restored MuSK activation and NMJ formation. However, these mice showed only marginal activation of MuSK and died by 3 weeks of age apparently due to an abnormally small number and size of NMJs. Therefore, Dok-7's C-terminal region plays a key, but not fully essential, role in MuSK activation and NMJ formation.

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

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

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

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

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

paired 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.

3. Pathophysiological mechanisms underlying *DOK7* myasthenia.

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

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

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

4. *DOK7* gene therapy that enlarges NMJs.

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

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

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

Inoue-Yamauchi, A., Wu, W., Sato, T., Jozawa, H.,

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

6. Roles of C/EBPδ.

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

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

7. Omic analyses.

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

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

8. Screening of chemical compound and siRNA libraries.

Eguchi, T., Ueta, R., Nagatoishi, S., Tsumoto, K., and Yamanashi, Y.

In addition to the omic analyses described above, we performed high throughput screenings of chemical compound and siRNA libraries, aiming to intervene in pathogenic signals or to gain insights into signaling mechanisms underlying a variety of biological events. We are investigating in vivo effects, including therapeutic ones in mouse models of human diseases, of hit compounds or down-regulation of candidate genes, and continue the ongoing screenings to further collect appropriate hit compounds and candidate genes that may regulate important signalings. We are also investigating target proteins for the hit compounds to understand their modes of actions.

Publications

Eguchi T., Tezuka T., Fukudome T., Watanabe Y., Sagara H., Yamanashi Y. Overexpression of Dok-7 in skeletal muscle enhances neuromuscular transmission with structural alterations of neuromuscular junctions: Implications in robustness of neuromuscular transmission. Biochem Biophys Res Commun. 523(1):214-219, 2020.

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Department of Cancer Biology

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

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Several lines of evidence have underpinned the prominent role of senescent cells in aging and healthspan although a general understanding of molecular basis underlying senescence-associated pathogenesis is still in its early stages. Our research interests are to understand the common pathological basis underlying various age-associated disorders. We are currently focusing on identification and characterization of senescent cells in vivo. To do so, we have generated a mouse model in which p16-positive senescent cells are visualized by fluorescent labelling. In addition, we are interested in developing innovative anti-aging technologies through regulating and/or eliminating senescent cells in vivo (senotherapy). A molecular link between DNA methylation and genomic integrity is also under investigation.

1. Generation of p16 reporter mouse models and characterization of p16^{high} cells *in vivo*

Yoshikazu Johmura, Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Dan Li, Wang Tehwei, Takehiro Yamanaka, Harris Alexander Stephen, Narumi Suzuki, Satotaka Ohmori, Liu Xianuyang, Yue Zhang, Sakura Yumoto, Lindo Nakamura, Yao Jiayi, Kim Jiwoo, Toshiro Migita, Yasuhiro Yamada[1], Masashi Yanagisawa[2], Seiya Imoto[3], Yoichi Furukawa[4], Kouji Matsushima[5], Hiroki R Ueda[6], Atsushi Miyajima7, and Makoto Nakanishi: [1]Division of Stem Cell Pathology, [3]Division of Health Medical Intelligence, Human Genome Center, [4]Division of Clinical Genome Research, IMSUT, [2]International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, [5]Division of Molecular Regulation of Inflammatory and Immune Diseases, Research Institute of Biomedical Sciences, Tokyo University of Science, [6]Department of Systems Pharmacology, Graduate School of Medicine, The University of Tokyo, [7]Laboratory of Stem Cell Therapy, Institute for Quantitative Biosciences, The University of Tokyo,

Cell senescence plays a key role in age-associated organ dysfunction, but the *in vivo* pathogenesis is largely unclear. To address this question, we generated a p16-Cre^{ERT2}-tdTomato mouse model to analyze the *in vivo* characteristics of p16^{high} cells at a single-cell level. We found tdTomato-positive p16^{high} cells detectable in all organs, which were enriched with age. We also found that these cells failed to proliferate, and had half-lives ranging from 2.6 to 4.2 months, depending on the tissue examined. Single-cell transcriptomics in the liver and kidneys revealed that p16^{high} cells were present in various cell types, though most dominant in hepatic endothelium and in renal proximal and distal tubule epithelia, and that these cells exhibited heterogeneous senescence-associated phenotypes. Further, elimination of p16^{high} cells ameliorated nonalcoholic steatohepatitis-related hepatic lipidosis and immune cell infiltration. Our new mouse model and single-cell analysis provide a powerful resource to enable the discovery of previously unidentified senescence functions *in vivo*.

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

Yoshikazu Johmura, Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Dan Li, Wang Tehwei, Takehiro Yamanaka, Harris Alexander Stephen, Narumi Suzuki, Satotaka Ohmori, Liu Xianuyang, Yue Zhang, Sakura Yumoto, Lindo Nakamura, Yao Jiayi, Kim Jiwoo, Toshiro Migita, Makoto Suematsu[1], Masaki Matsumoto[2], Makoto Arita[3], Masataka Sugimoto[4], Keiichi I Nakayama[5], Yoichi Furukawa[6], Seiya Imoto[7], and Makoto Nakanishi: [1]Department of Biochemistry, Keio University School of Medicine, [2]Department of Omics and Systems Biology, Niigata University Graduate School of Medical and Dental Sciences, [3]RIKEN Center for Integrative Medical Sciences, [4]Research Institute, National Center for Geriatrics and Gerontology, [5]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, [6]Division of Health Medical Intelligence, Human Genome Center, [7]Division of Clinical Genome Research, IMSUT

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

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

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

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

4. HPF1-dependent PARP activation promotes LIG3-XRCC1-mediated backup pathway of Okazaki fragment ligation.

Atsuya Nishiyama, Chieko Konishi, Yoshie Chiba, Soichiro Kumamoto, Ryota Miyashita, Tomomi Nagatani, and Makoto Nakanishi:

DNA Ligase 1 (LIG1) is known as the major DNA ligase responsible for Okazaki fragment joining. Recent studies have implicated LIG3 complexed with XRCC1 as an alternative player in Okazaki fragment joining in cases where LIG1 is not functional, although the underlying mechanisms are largely unknown. Using a cell-free system derived from *Xenopus* egg extracts, we demonstrated the essential role of PARP1-HPF1 in LIG3-dependent Okazaki fragment joining. We found that Okazaki fragments were eventually ligated even in the absence of LIG1, employing in its place LIG3-XRCC1 which was recruited onto chromatin. Concomitantly, LIG1 deficiency induces ADP-ribosylation of histone H3 in a PARP1-HPF1-dependent manner. The depletion of PARP1 or HPF1 resulted in a failure to recruit LIG3 onto chromatin and a subsequent failure in Okazaki fragment joining in LIG1-depleted extracts. Importantly, Okazaki fragments were not ligated at all when LIG1 and XRCC1 were co-depleted. Our results suggest that a unique form of ADP-ribosylation signaling promotes the recruitment of LIG3 on chromatin and its mediation of Okazaki fragment joining as a backup system for

LIG1 perturbation.

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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. Impairment of spatial memory accuracy improved by *Cbr1* copy number resumption and $GABA_B$ receptor-dependent enhancement of synaptic inhibition in Down syndrome model mice

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Down syndrome is a complex genetic disorder caused by the presence of three copies of the chromosome 21 in humans. The most common models, carrying extra-copies of overlapping fragments of mouse chromosome 16 that is syntenic to human chromosome 21, are Ts2Cje, Ts1Cje and Ts1Rhr mice. In electrophysiological analyses using hippocampal slices, we found that the later phase of the depolarization during tetanic stimulation, which was regulated by $GABA_B$ receptors, was significantly smaller in Ts1Cje and Ts2Cje mice than that in WT controls but not in Ts1Rhr mice. Furthermore, isolated $GABA_B$ receptor-mediated inhibitory synaptic responses were larger in Ts1Cje mice. To our knowledge, this is the first report that directly shows the enhancement of $GABA_B$ receptor-mediated synaptic currents in Ts1Cje mice. These results suggest that $GABA_B$ receptor-mediated synaptic inhibition was enhanced in Ts1Cje and Ts2Cje mice but not in Ts1Rhr mice. The *Cbr1* gene, which is present in three copies in Ts1Cje and Ts2Cje but not in Ts1Rhr, encodes carbonyl reductase that may facilitate $GABA_B$-receptor activity through a reduction of prostaglandin E2 (PGE2). Interestingly, we found that a reduction of PGE2 and a memory impairment in Ts1Cje mice were alleviated when only *Cbr1* was set back to two copies (Ts1Cje;$Cbr1^{+/+/-}$). However, the $GABA_B$ receptor-dependent enhancement of synaptic inhibition in Ts1Cje mice was unaltered in Ts1Cje;$Cbr1^{+/+/-}$ mice. These results indicate that *Cbr1* is one of the genes responsible for DS cogni-

tive impairments and the gene(s) other than *Cbr1*, which is included in Ts1Cje but not in Ts1Rhr, is responsible for the $GABA_B$ receptor-dependent over-inhibition.

2. Hyperactivity, impulsiveness and abnormal motor coordination observed in lemur kinase 1 knockout mice

Kobayashi, S., Takahashi, M.[5], Sugiyama, A.[5], Wei, R.[5], Fukuda, K.[6], Nishino, H.[5], Takahashi, R.[5], Tsutsumi, K.[5], Kita, I.[7], Ando, K.[5], Kamiguchi, H.[8], Tomomura, M.[9], Hisanaga, S.-i.[5] and Manabe, T.: [5]Laboratory of Molecular Neuroscience, Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan, [6]Developmental Biology, Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan, [7]Laboratory of Behavioral Neuroscience, Department of Human Health Sciences, Tokyo Metropolitan University, Tokyo, Japan, [8]Laboratory for Neural Cell Dynamics, RIKEN Center for Brain Science, Saitama, Japan, [9]Department of Oral Health Sciences, Meikai University School of Health Sciences, Chiba, Japan.

Lemur kinase 1 (LMTK1), previously called apoptosis-associated tyrosine kinase (AATYK), remains an uncharacterized Ser/Thr protein kinase that is predominantly expressed in the brain. It is recently reported that LMTK1A, an isoform of LMTK1, binds to recycling endosomes through its palmitoylation and regulates endosomal trafficking by suppressing the activity of Rab11 small GTPase. In neurons, knockdown or knockout of LMTK1 results in longer axons and greater branching of dendrites, suggesting that LMTK1 plays a role in neuronal circuit formation. However, its *in vivo* function remained to be investigated. Here, we examined the brain structures and behaviors of LMTK1 knockout (KO) mice. LMTK1 was expressed in most neurons throughout the brain. The overall brain structure appeared to be normal in LMTK1 KO mice, but the numbers of presynaptic puncta and area of synaptic vesicles in the presynaptic region were increased. LMTK1 KO mice exhibited psychiatric behaviors such as hyperactivity, impulsiveness and high motor coordination without impairment of social interaction. Some of these abnormal behaviors represent core features of attention-deficient hyperactive disorder (ADHD), suggesting the possible involvement of LMTK1 in the pathogenesis of ADHD.

3. Protrudin-deficient mice manifest depression-like behavior with abnormalities in activity, attention, and cued fear-conditioning

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Protrudin is a protein that resides in the membrane of the endoplasmic reticulum and is highly expressed in the nervous system. Although mutations in the human protrudin gene (*ZFYVE27*, also known as *SPG33*) give rise to hereditary spastic paraplegia (HSP), the physiological role of the encoded protein has been largely unclear. We therefore generated mice deficient in protrudin and subjected them to a battery of behavioral tests designed to examine their intermediate phenotypes. The protrudin-deficient mice were found to have a reduced body size and to manifest pleiotropic behavioral abnormalities, including hyperactivity, depression-like behavior, and deficits in attention and fear-conditioning memory. They exhibited no signs of HSP, however, consistent with the notion that HSP-associated mutations of protrudin may elicit neural degeneration, not as a result of a loss of function, but rather as a result of a gain of toxic function. Overall, our results suggest that protrudin might play an indispensable role in normal neuronal development and behavior.

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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. Stress-responsive MTK1 SAPKKK serves as a redox sensor that mediates delayed and sustained activation of SAPKs by oxidative stress.

Moe Matsushita, Takanori Nakamura, Hisashi Moriizumi, Hiroaki Miki[1], and Mutsuhiro Takekawa: [1]Department of Cellular Regulation, Research Institute for Microbial Diseases, Osaka University.

Living organisms are frequently exposed to a variety of cellular stresses such as ultraviolet (UV)-light, γ irradiation, DNA-damaging reagents, osmotic stress, heat shock, accumulation of misfolded proteins, and oxidative stress. Of these stressors, oxidative stress is an inevitable consequence of aerobic life and arises because of an imbalance between reactive oxygen species (ROS) generation and the extent of antioxidant defenses. When the excessive ROS production overwhelms the defense system in vivo, various biological macromolecules, including proteins and nucleic acids, undergo oxidative damage, which eventually leads to the perturbation of cellular homeostasis and functions. Indeed, oxidative damage is a major cause of cell injury and death under various pathological conditions including aging, cancer, neurodegenerative disorders, diabetes, atherosclerosis, and inflammatory diseases.

Besides these etiological aspects, ROS also serve as second messengers in physiological signaling events that regulate key biological processes such as cell proliferation, differentiation, apoptosis, and innate immunity. For instance, upon contact with microorganisms, phagocytic cells [e.g., macrophages (MFs)] generate large amounts of ROS by activating NADPH oxidases. This process, termed the respiratory burst, initiates the killing of infected pathogens and promotes the production of proinflammatory cytokines including TNFα and IL-6, and is vital for the host defense against pathogens. Therefore, elucidation of the effects of ROS on cellular homeostasis is of paramount importance to understand these critical biological processes and to develop therapeutic interventions for many chronic and degenerative diseases. Nevertheless, the molecular mechanisms underlying the cellular responses to ROS remain elusive.

This year, we discovered that the MAP three ki-

nase 1 (MTK1) MAPKKK functions as an oxidative-stress sensor that perceives the cellular redox state and transduces it into stress-activated p38 and JNK signaling. Following oxidative stress, MTK1 is rapidly oxidized and subsequently gradually reduced at evolutionarily conserved cysteine residues in its N-terminal regulatory domain. These coupled oxidation-reduction modifications of MTK1 elicit its catalytic activity. Therefore, this novel stress sensor is unique in that it does not simply detect intracellular oxidation events but it responds to coupled oxidation and reduction reactions, and it also acts as an effector to induce delayed and sustained activation of SAPK signaling through its C-terminal kinase domain. Gene knockout experiments showed that oxidative stress–induced p38/JNK signaling is mediated by coordinated activation of the two MAPKKKs, MTK1 and apoptosis signal–regulating kinase 1 (ASK1), which have different time and dose-response characteristics. We found that the MTK1-mediated redox sensing system is crucial for delayed and sustained p38/JNK activities and dictates cell fate decisions including cell death and IL-6 production during respiratory burst in macrophages. Our results delineate a molecular mechanism by which cells generate optimal biological responses under fluctuating redox environments. Given that oxidative stress–induced cell death and IL-6 production are both involved in various pathological conditions including chronic inflammatory diseases, metabolic disorders, cancer, and neurodegenerative diseases and that anti-IL-6 therapies are highly effective against certain diseases, MTK1 could be a potential target for the development of novel therapeutic interventions for human diseases in which oxidative insults are involved.

2. Identification of novel substrates of human mitogen-activated protein kinases.

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

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

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

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

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

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

formed by other mechanisms. For instance, drugs or lipid mediators that target eIF4F, such as pateamine A, hippuristanol and 15d-PGJ2, inhibit translation initiation and thereby initiate SG assembly independently of eIF2α phosphorylation.

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

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

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

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

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

Ayaka Sakurai, Shiho Sameshima, Shiho Hirose, Mariko Saito, Yusuke Takagi, Noriko Nishizumi-Tokai, Yuji Kubota, and Mutsuhiro Takekawa

The initial cellular response to various environmental cues, such as growth factors, environmental stresses, and cytokines, is the transcriptional regulation of a set of genes that control a wide variety of biological functions. MAPK signaling pathways are known to play crucial roles in this process. Previous studies have shown that MAPKs directly phosphorylate and activate a bunch of transcription factors and regulators. For instance, the transcription factor ELK-1, which is a member of the ternary complex factor (TCF) subfamily, is a substrate of ERK. TCFs interact with a second transcription factor, serum response factor (SRF), and these two transcription factors jointly bind and activate serum response elements (SREs) in the promoters of immediately early genes (IEGs). Moreover, upon stress stimulation, p38 and JNK MAPKs directly phosphorylate activating transcription factor 2 (ATF2). ATF2 binds either to CRE response elements as a homodimer, or to both AP-1 and CRE sequences as a heterodimer, in which ATF2 forms a complex with other members of the ATF family or with Jun/Fos family members, thereby inducing target gene expression.

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

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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. Profiling the principles of the transcriptional control encoded in the genomic regulatory sequences of co-expressed genes from scRNA-seq

Luis Augusto Eijy Nagai, Alexis Vandenbon[1] and Kenta Nakai: [1]Institute for Frontier Life and Medical Sciences, Kyoto University

Previous works of this lab have demonstrated that a common structure exists in the upstream region of co-expressed genes, exhibiting tissue-specific or developmental time-specific gene expression programs of various organisms. However, a tissue is composed of multiple cell-types with different expression patterns. With the advance of NGS, transcriptomics in a single-cell resolution can trace cell differentiation at all developmental stages in nematodes. Using this kind of data we aim to attempt to extract a group of co-expressed genes in high accuracy and establish a method that automatically extracts the common structures existing in the upstream sequence. Using a published atlas of *C. elegans* developmental stages, we were able to re-analyze and extract the promoter sequences from scRNA-seq data. We then obtained sets of co-expressed genes based on the distribution of the principal components. These sets of genes had more statistically meaningful gene ontology terms that could describe each cluster than the shuffled groups used as control. Preliminary results have shown that distinct enriched transcription factor motifs could be identified in different clusters suggesting a cluster-specific profile. Further results are still necessary to confirm the validity of our analysis.

2. Searching for a novel epigenetic code that affects the nature of human embryonic stem cell

Yasuhisa Ishikawa and Kenta Nakai

It is well known that epigenetic factors such as DNA methylation and histone modifications influence gene expression and cell-specific natures. For example, DNA demethylation modifications producing 5-hydroxymethylcytosine (5hmC) are highly accumulated in embryonic stem cells (ESCs) and thought to interact with histone modifications to influence the acquisition of ESC potency. Furthermore, in recent years, the concept of "epigenetic code" has been proposed. This is a concept corresponding to "genetic code", in which a plurality of epigenetic factors form a kind of code, which results in a change in gene expression and influences cell-specific natures. Therefore, we conducted research aiming to discover a novel epigenetic code containing 5hmC, which has the possibility to influence the natures of human ESCs. As a result, a novel epigenetic code candidate consisting of 5hmC and two histone modifications (H3K4me1 and H4K8ac) was discovered. It was confirmed that these epigenetic factors caused ESC-specific expression by co-occurring. Furthermore, this co-occurrence was found to be present in pluripotent stem cell marker genes (Nanog, Oct3/4). Theses finding has the potential to help better understand the nature of ESCs and its relationship to epigenetic factors.

3. A semi-supervised deep learning approach for predicting the functional effects of genomic non-coding variations

Hao Jia, Sung-Joon Park and Kenta Nakai

Understanding the functional effects of non-coding variants is important as they are often associated with gene-expression alteration and disease development. In this work, we propose a novel method, employing a semi-supervised deep-learning model with pseudo labels, which takes advantage of learning from both experimentally annotated and unannotated data. We prepared known functional non-coding variants with histone marks, DNA accessibility, and sequence context in GM12878, HepG2, and K562 cell lines. Applying our method to the dataset demonstrated its outstanding performance, compared with that of existing tools. Our results also indicated that the semi-supervised model with pseudo labels achieves higher predictive performance than the supervised model without pseudo labels. The semi-supervised deep learning model coupled with pseudo labeling has advantages in studying with limited datasets, which is not unusual in biology. Our study provides an effective approach in finding non-coding mutations potentially associated with various biological phenomena, including human diseases.

4. Characterizing promoter and enhancer sequences by a deep learning method

Xin Zeng, Sung-Joon Park and Kenta Nakai

Promoters and enhancers are well known regulatory elements modulating gene expression. As confirmed by high-throughput sequencing technologies, these regulatory elements are bidirectionally transcribed. That is, promoters produce stable mRNA in the sense direction and unstable RNA in the antisense direction, while enhancers transcribe unstable RNA in both directions. Although it is thought that enhancers and promoters share a similar architecture of transcription start sites (TSSs), how the transcriptional machinery distinctly uses these genomic regions as promoters or enhancers remains unclear. To address this issue, we developed a deep learning method by utilizing a Convolutional Neural Network (CNN) and the saliency algorithm. In comparison with other classifiers, our CNN presented higher predictive performance, suggesting the overarching importance of the high-order sequence features, captured by the CNN. Moreover, our method revealed that there are substantial sequence differences between the enhancers and promoters. Our CNN-based method can capture the complex TSS architectures. We found that the genomic regions around TSSs for promoters and enhancers contribute to RNA stability and show some GC-biased characteristics as a critical determinant for promoter TSSs.

5. Developing a database and web application for detecting microbial contaminants from next generation sequencing data

Sung-Joon Park, Satoru Onizuka[2], Masahide Seki[3], Yutaka Suzuki[3], Takanori Iwata[4] and Kenta Nakai: [2]Division of Periodontology, Department of Oral Function, Kyushu Dental University: [3]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo: [4]Department of Periodontology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

Microorganisms infect and contaminate eukaryotic cells during the course of biological experiments. Because microbes influence host cell biology and may therefore lead to erroneous conclusions, a computational platform that facilitates decontamination is indispensable. Recent studies show that next-generation sequencing (NGS) data can be used to identify the presence of exogenous microbial species. However, as one of the difficulties, since the host cells are often contaminated by multiple microorganisms that possess higher sequence homology, it is required careful attention to intra- and interspecies sequence

similarities among potential contaminants. We are developing a computational approach to improve detection rates. We are building it as a web application along with a large-scale database. Currently, the database includes over 700 statistically significant microbial genera that have been detected from the publicly-available 5,000 WGS and RNA-seq dataset. Since contaminants have complex characteristics, including variability across laboratories and sequencing protocols, we believe that our computational platform is useful to improve our understanding of how and why microbial species infect and contaminate host cells and the impact on the interpretation of experimental results.

6. Transcriptome analysis of multipotent mesenchymal stem cells derived from oral and maxillofacial tissues

Sung-Joon Park, Satoru Onizuka[2], Takanori Iwata[4] and Kenta Nakai

Multipotent mesenchymal stem cells (MSCs) are a well-known candidate for therapeutic applications and they are isolated from various tissues. Despite their importance, the molecular characteristics of MSCs are strongly relying on the origin of tissue. Here, we analyzed RNA-seq data from human MSCs from three different bones and publicly-available various MSCs and embryonic stem cells. Although the MSCs may be divided into two distinct groups that are originated from over the neck or not, the expression patterns of all CD antigen genes were similar among different types of MSCs, except for ESCs. Remarkably, we found that the MSCs derived from tissues of the maxillofacial regions are HOX-negative, those derived from other tissues are HOX-positive. The genes including MSX1, LHX8, and BARX1, an essential regulator of craniofacial development, were highly expressed in maxillofacial tissue-derived MSCs. These results revealed that MSCs from different anatomical locations have remarkable differences in gene expression and positional memory, implying the importance of choosing an appropriate cell source for regenerative therapy.

7. Analyzing ploidy-dependent gene expression in mouse early embryos by bulk and single-cell RNA-seq data

Sung-Joon Park, Miho Ohsugi[5] and Kenta Nakai: [5]Department of Biological Sciences, Graduate School of Science, The University of Tokyo

Polyploidy is a biological phenomenon that dominates the genome evolution in various organisms. In particular, mammalian diploid embryos exhibits higher rates in development compared to haploid embryos that arrest development before implantation. However, the molecular mechanisms underlying the ploidy dependency remain unclear. Here, we analyzed gene expression patterns at bulk and single-cell resolutions during mouse early embryonic stages; 1-cell, 2-cell, and 4-cell stages. The embryos are parthenogenetic diploid (D/C=1.0), conventional haploid (D/C=0.5), and another type of haploid embryos (D/C=1.0) whose total amount of genomic DNA and the DNA/cytoplasm ratio (D/C ratio) have been halved. This study revealed that although the onset of zygotic gene activation occurs for all types, the ploidy-dependent marker genes are different from each other. Particularly, the up-regulated genes in D/C=0.5 haploid 2-cell embryos exhibit abnormality in the regulation of apoptosis and translation elongation. By investigating such transcriptomic features in the three types of embryos, we seek to understand how the reduced D/C ratio causes early developmental arrest and why.

8. Analyzing the impact of inter-chromosomal interactions on B cell development by linear regression modeling

Sung-Joon Park and Kenta Nakai

Recent advances in characterizing 3D genome organization have highlighted the functional involvement of the higher-order chromatin structures during cell differentiation and disease development. In particular, intra-chromosomal interactions and TADs have been intensively studied. On the other hand, non-homologous chromosomal contacts (NHCCs) that are interactions among different chromosomes are also thought to play a crucial role in the coordination of chromatin structure with transcription but remain poorly understood. Here, with the public NGS datasets of human B cells, we designed a regression-based computational approach and applied it to analyze the impact of NHCCs on gene regulation. As a result, the modeling captured significant regulators attributed to the 3D chromatin structure, which suggests dynamic and complex NHCC regulatory activities during B cell development. As previous studies have reported that NHCCs form poised chromatin hubs, our results implied the involvement of NHCCs for rapid cytokine response in activated B cells.

9. Extreme value theory as a general framework for understanding mutation frequency distribution in cancer genomes

Natsuki Tokutomi, Kenta Naka and Sumio Sugano[6]: [6]Medical Research Institute, Tokyo Medical and Dental University

Currently, there is no recognized population genetics framework describing the population dynamics of cancer cells that is applicable to real cancer ge-

nome data. By focusing on cancer as a Darwinian evolutionary system, we formulated and analyzed the observed mutation frequency among tumors (MFaT) as a proxy for the hypothesized sequence read frequency and beneficial fitness effect of a cancer driver mutation. Analogous to intestinal crypts, we assumed that sample donor patients are separate culture tanks where proliferating cells follow certain population dynamics described by extreme value theory (EVT). To validate this, we analyzed three large-scale cancer genome datasets, each harboring > 10 000 tumor samples and in total involving > 177 898 observed mutation sites. We clarified the necessary premises for the application of EVT in the strong selection and weak mutation (SSWM) regime in relation to cancer genome sequences at scale. We also confirmed that the stochastic distribution of MFaT is likely of the Fr_echet type, which challenges the well-known Gumbel hypothesis of beneficial fitness effects. Based on statistical data analysis, we demonstrated the potential of EVT as a population genetics framework to understand and explain the stochastic behavior of driver-mutation frequency in cancer genomes as well as its applicability in real cancer genome sequence data.

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

Phit Ling Tan, Ken Ishii[7], Florent Ginhoux[8] and Kenta Nakai: [7]Division of Vaccine Science, The Institute of Medical Science, The University of Tokyo, [8]Singapore Immunology Network (SIgN), A*STAR

Previously, we found a potentially interesting new group of cells while studying the aggregated single-cell transcriptome data of human blood/bone marrow cells: a subtype of putative dendritic cell precursors (pre-DCs) that expresses certain neutrophil progenitor markers. Other than expressing some neutrophil progenitor markers, this subtype of putative pre-DCs exhibits similar expression profiles to conventional dendritic cells (cDCs) and $Axl^{+}Siglec6^{+}$pre-cDCs. Despite having similar expression profiles to cDCs and pre-cDCs, trajectory inference analyses suggest that this group of cells is a precursor of DCs. In order to validate these *in silico* results that suggest the existence of such putative pre-DCs, flow cytometry and *in vitro* DC differentiation experiments are being prepared to identify this group of cells and to study its potential differentiation behaviors. We will also perform index sort single-cell RNA-seq experiments based on the previous validation analysis to definitively validate our analysis.

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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. The repertoire of mutational signatures in human cancer

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Somatic mutations in cancer genomes are caused by multiple mutational processes, each of which generates a characteristic mutational signature. Here, as part of the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA), we characterized mutational signatures using 84,729,690 somatic mutations from 4,645 whole-genome and 19,184 exome sequences that encompass most types of cancer. We identified 49 single-base-substitution, 11 doublet-base-substitution, 4 clustered-base-substitution and 17 small insertion-and-deletion signatures. The substantial size of our dataset, compared with previous analyses, enabled the discovery of new signatures, the separation of overlapping signatures and the decomposition of signatures into components that may represent associated-but distinct-DNA damage, repair and/or replication mechanisms. By estimating the contribution of each signature to the mutational catalogues of individual cancer genomes, we revealed associations of signatures to exogenous or endogenous exposures, as well as to defective DNA-maintenance processes. However, many signatures are of unknown cause. This analysis provides a systematic perspective on the repertoire of mutational processes that contribute to the development of human cancer.

2. Genetic landscape of external auditory canal squamous cell carcinoma

Sato K[1,2], Komune N[2], Hongo T[2,3], Koike K[2,4], Niida A[5], Uchi R[2], Noda T[2], Kogo R[2], Matsumoto N[3], Yamamoto H[3], Masuda M[1], Oda Y[3], Mimori K[4], Nakagawa T[2]; [1]Department of Head and Neck Surgery, National Hospital Organization KyushuCancer Center, Fukuoka, Japan. [2]Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. [3]Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. [4]Department of Surgery, Kyushu University Beppu Hospital, Beppu, Oita, Japan [5]Laboratory of Molecular Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

External auditory canal squamous cell carcinoma (EACSCC) is an extremely rare and aggressive malignancy. Due to its rarity, the molecular and genetic characteristics of EACSCC have not yet been elucidated. To reveal the genetic alterations of EACSCC, we performed whole exome sequencing (WES) on 11 primary tumors, 1 relapsed tumor and 10 noncancerous tissues from 10 patients with EACSCC, including 1 with a rare case of synchronous bilateral EACSCC of both ears. WES of the primary tumor samples showed that the most frequently mutated gene is TP53 (63.6%). In addition, recurrent mutations in CDKN2A, NOTCH1, NOTCH2, FAT1 and FAT3 were detected in multiple samples. The mutational signature analysis of primary tumors indicated that the mutational processes associated with the activation of apolipoprotein B mRNA- editing enzyme catalytic polypeptide-like (APOBEC) deaminases are the most common in EACSCC, suggesting its similarity to SCC from other primary sites. Analysis of arm-level copy number alterations detected notable amplification of chromosomes 3q, 5p and 8q as well as deletion of 3p across multiple samples. Focal chromosomal aberrations included amplifications of 5p15.33 (ZDHHC11B) and 7p14.1 (TARP) as well as deletion of 9p21.3 (CDKN2A/B). The protein expression levels of ZDHHC11B and TARP in EACSCC tissues were validated by immunohistochemistry. Moreover, WES of the primary and relapsed tumors from a case of synchronous bilateral EACSCC showed the intrapatient genetic heterogeneity of EACSCC. In summary, this study provides the first evidence for genetic alterations of EACSCC. Our findings suggest that EACSCC mostly resembles other SCC.

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

1. Genome-wide association study of various diseases and phenotypes

Identification of a novel uterine leiomyoma GWAS locus in a Japanese population.

Uterine leiomyoma is one of the most common gynaecologic benign tumours, but its genetic basis remains largely unknown. Six previous GWAS identified 33 genetic factors in total. Here, we performed a two-staged GWAS using 13,746 cases and 70,316 controls from the Japanese population, followed by a replication analysis using 3,483 cases and 4,795 controls. The analysis identified 9 significant loci, including a novel locus on 12q23.2 (rs17033114, P = 6.12 × 10-25 with an OR of 1.177 (1.141-1.213), LINC00485). Subgroup analysis indicated that 5 loci (3q26.2, 5p15.33, 10q24.33, 11p15.5, 13q14.11) exhibited a statistically significant effect among multiple leiomyomas, and 2 loci (3q26.2, 10q24.33) exhibited a significant effect among submucous leiomyomas. Pleiotropic analysis indicated that all 9 loci were associated with at least one proliferative disease, suggesting the role of these loci in the common neoplastic pathway. Furthermore, the risk T allele of rs2251795 (3q26.2) was associated with longer telomere length in both normal and tumour tissues. Our findings elucidated the significance of genetic factors in the pathogenesis of leiomyoma.

Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases

The overwhelming majority of participants in current genetic studies are of European ancestry. To elucidate disease biology in the East Asian population, we conducted a genome-wide association study (GWAS) with 212,453 Japanese individuals across 42 diseases. We detected 320 independent signals in 276 loci for 27 diseases, with 25 novel loci (P < 9.58 × 10-9). East Asian-specific missense variants were identified as candidate causal variants for three novel loci, and we successfully replicated two of them by analyzing independent Japanese cohorts; p.R220W of ATG16L2 (associated with coronary artery disease) and p. V326A of POT1 (associated with lung cancer). We further investigated enrichment of heritability within 2,868 annotations of genome-wide transcription factor occupancy, and identified 378 significant enrichments across nine diseases (false discovery rate < 0.05) (for example, NKX3-1 for prostate cancer). This large-scale GWAS in a Japanese population provides in-

sights into the etiology of complex diseases and highlights the importance of performing GWAS in non-European populations.

Claudin-2 deficiency associates with hypercalciuria in mice and human kidney stone disease

The major risk factor for kidney stone disease is idiopathic hypercalciuria. Recent evidence implicates a role for defective calcium reabsorption in the renal proximal tubule. We hypothesized that claudin-2, a paracellular cation channel protein, mediates proximal tubule calcium reabsorption. We found that claudin-2-null mice have hypercalciuria due to a primary defect in renal tubule calcium transport and papillary nephrocalcinosis that resembles the intratubular plugs in kidney stone formers. Our findings suggest that a proximal tubule defect in calcium reabsorption predisposes to papillary calcification, providing support for the vas washdown hypothesis. Claudin-2-null mice were also found to have increased net intestinal calcium absorption, but reduced paracellular calcium permeability in the colon, suggesting that this was due to reduced intestinal calcium secretion. Common genetic variants in the claudin-2 gene were associated with decreased tissue expression of claudin-2 and increased risk of kidney stones in 2 large population-based studies. Finally, we describe a family in which males with a rare missense variant in claudin-2 have marked hypercalciuria and kidney stone disease. Our findings indicate that claudin-2 is a key regulator of calcium excretion and a potential target for therapies to prevent kidney stones.

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Human Genome Center

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

Shimizu E, Kasajima R, Katayama K, Yamaguchi K, Yokoyama K, Fujimoto K, Yadome M, Hyugaji T, Komura M, Yamamoto M, Saito A, Kobayashi M, Ogawa M, Takei T, Yuji K, Takane K, Ikenoue T, Robert B, Shibuya T, Hiroshima Y, Miyagi Y, Muto K, Uematsu S, Tojo A, Furukawa Y, Miyano S, Yamaguchi R, 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.

2. Metagenome Analysis of Intestinal Microbiota

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

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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. Genome-wide association studies and heritability analysis reveal the involvement of host genetics in the Japanese gut microbiota

Ishida S[4], Kato K[5], Tanaka M[4], Odamaki T[5], Kubo R[4], Mitsuyama E[5], Xiao J[5], Yamaguchi R, Uematsu S, Miyano S, Imoto S: [4]DeNA Life Science, [5]Morinaga Milk Industry Co., Ltd.

Numerous host extrinsic and intrinsic factors affect the gut microbiota composition, but their cumulative effects do not sufficiently explain the variation in the microbiota, suggesting contributions of missing factors. The Japanese population possesses homogeneous genetic features suitable for genome-wide association study (GWAS). Here, we performed GWASs for human gut microbiota using 1068 healthy Japanese adults. To precisely evaluate genetic effects, we corrected for the impacts of numerous host extrinsic and demographic factors by introducing them as covariates, enabling us to discover five loci significantly associated with microbiome diversity measures: HS3ST4, C2CD2, 2p16.1, 10p15.1, and 18q12.2. Nevertheless, these five variants explain only a small fraction of the variation in the gut microbiota. We subsequently investigated the heritability of each of the 21 core genera and found that the abundances of six genera are heritable. We propose that the gut microbiota composition is affected by a highly polygenic architecture rather than several strongly associated variants in the Japanese population.

3. Development of Computational Methods for Genomic Data

a. Neoantimon: a multifunctional R package for identification of tumor-specific neoantigens

Hasegawa T, Hayashi S, Shimizu E, Mizuno S[6], Niida A, Yamaguchi R, Miyano S, Nakagawa H[7], Imoto S: [6]Kyushu University, [7]Riken

It is known that some mutant peptides, such as those resulting from missense mutations and frameshift insertions, can bind to the major histocompatibility complex and be presented to antitumor T cells on the surface of a tumor cell. These peptides are termed neoantigen, and it is important to understand this process for cancer immunotherapy. Here, we introduce an R package termed Neoantimon that can predict a list of potential neoantigens from a variety of mutations, which include not only somatic point mutations but insertions, deletions and structural variants. Beyond the existing applications, Neoantimon is capable of attaching and reflecting several additional information, e.g. wild-type binding capability, allele specific RNA expression levels, single nucleotide polymorphism information and combinations of mutations to filter out infeasible peptides as neoantigen.

b. Theoretical foundation of the performance of phylogeny-based somatic variant detection

Moriyama T, Imoto S, Miyano S, Yamaguchi R

We study the performance of a variant detection method that is based on a property of tumor phylogenetic tree. Our major contributions are two folds. First, we show the property of tumor phylogenetic

tree: the total patterns of mutations are restricted if a multi-regional mutation profile follows a corresponding tumor phylogenetic tree, where a multi-regional mutation profile is a matrix in which predictions of somatic mutations at the corresponding tumor regions are listed. Second, we evaluate the lower and upper bounds of specificity and sensitivity of a phylogeny-based somatic variant detection method under several situations. In the evaluation, we conduct patient-wise variant detection from a noisy multi-regional mutation profile matrix for some genomic positions by utilizing the phylogenetic property; we assume that the phylogenetic information can be extracted from another mutation profile matrix that contains accurate candidates at different genomic positions from the noisy ones. From the evaluation, we find that higher sensitivity is not guaranteed in the phylogeny-based variant detection, but higher specificity is guaranteed for several cases. These findings indicate the tumor phylogeny gives more merit for variant detection based on erroneous long-read sequencers (e.g. Oxford nanopore sequencers) than that based on accurate short-read sequencers (e.g., Illumina sequencer).

Publications (Jan 2020 – March 2020)

c. Nanopore basecalling from a perspective of instance segmentation

Zhang Y-Z, Akdemir A, Tremmel G, Imoto S, Miyano S, Shibuya T, Yamaguchi R

Nanopore sequencing is a rapidly developing third-generation sequencing technology, which can generate long nucleotide reads of molecules within a portable device in real-time. Through detecting the change of ion currency signals during a DNA/RNA fragment's pass through a nanopore, genotypes are determined. Currently, the accuracy of nanopore basecalling has a higher error rate than the basecalling of short-read sequencing. Through utilizing deep neural networks, the-state-of-the art nanopore basecallers achieve basecalling accuracy in a range from 85% to 95%.

In this work, we proposed a novel basecalling approach from a perspective of instance segmentation. Different from previous approaches of doing typical sequence labeling, we formulated the basecalling problem as a multi-label segmentation task. Meanwhile, we proposed a refined U-net model which we call UR-net that can model sequential dependencies for a one-dimensional segmentation task. The experiment results show that the proposed basecaller UR-nano achieves competitive results on the in-species data, compared to the recently proposed CTC-featured basecallers.

d. 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.

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Human Genome Center

Department of Public Policy
公共政策研究分野

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

1. Japanese insurers' attitudes toward adverse selection and genetic discrimination

Since the 1990s, insurance has been the primary field focused on the social disadvantages of using genetic test results because of the concerns related to adverse selection. Although life insurance is popular in Japan, Japan does not currently have any regulations on the use of genetic information and insurers have largely kept silent for decades. To reveal insurers' attitudes on the topic, we conducted an anonymous questionnaire survey with 100 insurance company employees and recruited nine interviewees from the survey respondents. We found that genetic discrimination is not generally considered as a topic of human rights. We also found that insurers have uncertain fears and concerns about adverse selection in terms of actuarial fairness but not regarding profits. When it comes to preparing guidelines on the use of genetic information by Japanese insurers, we believe that public dialog and consultation are necessary to gain understanding of the people.

2. Japanese citizens' behavioral changes and preparedness against COVID-19

The Japanese government instituted countermeasures against COVID-19, a pneumonia caused by the new coronavirus, in January 2020. Seeking "people's behavioral changes," in which the government called on the public to take precautionary measures or exercise self-restraint, was one of the important strategies. The purpose of this study is to investigate how and from when Japanese citizens have changed their precautionary behavior under circumstances in which the government has only requested their cooperation. This study uses micro data from a cross-sectional survey conducted on an online platform of an online research company, based on quota sampling that is representative of the Japanese population. By the end of March 2020, a total of 11,342 respondents, aged from 20 to 64 years, were recruited. About 85 percent reported practising the social distancing measures recommended by the government including more females than males and more older than younger participants. Frequent handwashing is conducted by 86 percent of all participants, 92 percent of female, and 87.9 percent of over-40 participants. The most important event influencing these precautionary actions was the infection aboard the Diamond Princess cruise ship, which occurred in early February 2020 (23 percent). Information from the central and local govern-

ments, received by 60 percent of the participants, was deemed trustworthy by 50 percent. However, the results also showed that about 20 percent of the participants were reluctant to implement proper prevention measures. The statistical analysis indicated that the typical characteristics of those people were male, younger (under 30 years old), unmarried, from lower-income households, a drinking or smoking habit, and a higher extraversion score. To prevent the spread of infection in Japan, it is imperative to address these individuals and encourage their behavioural changes using various means to reach and influence them.

3. Professional Commitment to Ethical Discussions Needed From Epidemiologists in the COVID-19 Pandemic

The global threat due to the novel coronavirus 2019 infection (COVID-19) pandemic has given rise to various ethical challenges in public health, and increasingly more articles are being published in medical journals on ethical issues concerning COVID-19. One major theme concerns the moral distress of balancing the importance of epidemiological surveillance and digital patient tracing with privacy concerns. Given the extraordinary current circumstances, other pressing ethical questions have also been posed, including the following: Which studies should be continued or cancelled? What constitutes an ethical study design and rationale? How should the participant burden/risk be balanced against the benefits of research? Should protocol procedures be limited or modified to ensure participant safety, at the risk of decreasing research integrity? Should we justify a shortcut through a fast-track research review and an expedited submission-review process, with the potential downside of lower research quality and more faulty publications? How should the scientific community avoid jeopardizing the pandemic public health management by the rapid and uncontrolled flood of clinical/epidemiological data dissemination in the media? Should we justify conducting urgently required research in developing countries with more treatment-naïve potential research populations, even though the peak of infection expansion in developed countries has already waned? Conventionally, academic journals have been one of the most powerful venues for scholarly discussion. However, since the COVID-19 expansion, the extent to which scholarly journals of epidemiology have served as discussion forums for ethical issues on COVID-19 has become unclear, even though many of the ethical questions noted above would likely be important for epidemiologists as well. Against that backdrop, we conducted a small bibliometric survey on June 11, 2020. Specifically, we performed a database search of PubMed using the constraints of "COVID-19," and "ethics." This identified 424 articles, of which approximately two-thirds (285) were published in scholarly journals; the remaining 139 were in commercial journals. Of the former 424, 13 were published in Nature/Science, 33 in five major medical journals (*NEJM, BMJ, JAMA, Lancet, Annals of Internal Medicine*), 192 in other medical journals, 39 in public health/epidemiological journals, 64 in bioethics journals, and 36 in others. Of the 39 published in public health/epidemiological journals, only one was published in an epidemiological journal. Accordingly, our findings suggest that unfortunately, epidemiologist engagement in ethical issues on COVID-19 is minimal at best.

We speculate that this may be caused by the following factors:(1) Not all—but many—epidemiologists might be indifferent to ethical issues on epidemiology, or have left these arguments in the hands of others such as bioethicists; (2) While they may be concerned with ethical issues, epidemiologists may be less familiar with developing ethical arguments; (3) Appropriate forums for mediation of ethical discussions in epidemiological journals may be few in number. Notably, this third factor should be rejected because nearly all of the top 10 epidemiological journals with "epidmiol-" or the equivalent in their names, being indexed in the 2018 Journal Citation Reports® (except *Journal of Epidemiology*, ranked 10th), provide sufficient space for unsolicited and unstructured longer articles (1,500–3,000 words) for development of debates or perspectives, in addition to the *Letter to the Editor* section. Therefore, we suspect that most epidemiologists are simply not using the available forums well. We believe that ethical issues directly concerning epidemiology should be shared and discussed, first and foremost, among epidemiologists. Because epidemiologists are health professionals, they shoulder the responsibility to maintain ethical integrity in their epidemiological endeavors. Utilizing such forums effectively and actively to exchange opinions on the ethics of epidemiology will promote the healthy development of epidemiology as a science and will contribute greatly to public health policy making as well.

4. An educational workshop designed for research ethics consultants to educate investigators on ethical considerations

The role of research ethics consultants in biomedical research has increased to the point that they have an advisory capacity at all research institutes. For such professionals, we have established an educational system, which includes teaching materials, training methods, and nationwide educational workshops. These workshops have served to examine the developed system's usefulness and to provide realistic training for consultant candidates. In addition, we have used the current workshop to encourage clinical research investigators (and related personnel) to participate. Subsequently, we examined its usefulness as an opportunity to provide exposure to research eth-

ics. In October 2019, we held a 1-day pilot workshop in Tokushima, Japan, which included a basic lecture in research ethics. During the lecture, two sets of materials were used for case discussion: case 1, covering issues related to a clinical trial, and case 2, covering issues related to human biological specimens. At the end of the workshop, a 30-item self-reporting anonymous questionnaire was provided. Of the 13 total participants, 9 (70%) were clinical research investigators and related personnel, while 6 (46%) had no direct intention to act as consultants. Respondents indicated that case 2 was more difficult than case 1. However, both cases were generally accepted as educational materials; thus, satisfaction was expressed in relation to both. As the evaluations of the cases were generally positive, we will further examine the usefulness of participation in the workshop in the cultivation of research ethics in the investigator community.

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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, such as algorithm theory, big data technologies, artificial intelligence, data mining, and privacy preserving technologies. Medical data, especially genome data are increasing exponentially in medical science from basics to clinical research. Our aim is to innovate the entire medical science with novel data informatics solutions.

1. Development of Artificial Intelligence Technologies for Biomedical Data

a. Natural Language Processing Methods for International Medical Text Databases

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Morphological information is important for many sequence labeling tasks in Natural Language Processing (NLP). Yet, existing approaches rely heavily on manual annotations or external software to capture this information. We propose using subword contextual embeddings for languages with rich morphology. Evaluated on Dependency Parsing (DEP) and Named Entity Recognition (NER) tasks, which are shown to benefit highly from morphological information, subword contextual embeddings consistently outperformed other approaches on all languages tested (Hungarian, Finnish, Czech and Turkish). Besides, the novel network architecture we propose, coupled with a Bayesian hyperparameter optimization suite, achieved state-of-the-art results for both tasks for the Turkish language. Finally, we experimented with different multi-task learning architectures to analyze the effect of jointly learning the two tasks.

Deep Neural Network (DNN) based Machine Learning models achieved remarkable success in many fields of research. Yet, many recent studies show the limitations of these approaches to generalize to unseen examples and to new domains such as the biomedical domain. Besides, supervised-learning based DNN models require a substantial amount of labeled data which is not readily available for many tasks such as the biomedical question answering task. Transfer Learning is shown to mitigate these challenges by transferring information from auxiliary tasks to improve the performance on a source task, and shown to be especially useful for low-resource tasks. These observations and findings motivated us to investigate the effect of transfer learning and multi-task learning on the biomedical question answering task. We proposed a novel multi-task learning model to learn biomedical entities and questions simultaneously. In this work, we explain the three different neural models we used to participate for the BioASQ 8B challenge. Our results showed that transferring information from the biomedical entity recognition task brings improvement for the biomedical question answering task.

b. Artificial Intelligence Techniques for Molecular Data Analysis

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We are developing artificial intelligence techniques for various molecular data analysis, including protein 3-D structures, metagenome NGS data. As for the metagenome data, we developed deep learning models that couple CNN (Convolutional Neural Network) with RNN (Recurrent Neural Network) for virus classification from metagenome next generation sequencer data. Our model called BiRNN_CNN achieves 0.891 AUC, which outperforms previous state-of-the-art methods.

2. Development of Privacy Preserving Technologies for Medical Data

a. Differential Privacy Methods for Medical Data

Tatsuki Koga[1], Akito Yamamoto[1], Robert Daniel Barish[1], Tetsuo Shibuya[1]

Privacy-preserving machine learning is being increasingly important for a variety of applications in medical science because we often need to handle sensitive data including personal information. Using differentially private empirical risk minimization (DP-ERM) algorithms is one of the most common approaches to obtain privatized predictors in supervised learning. However, minimizing the empirical risk with current algorithms may negatively affect the classification performance under class imbalance in terms of metrics suited for imbalanced datasets. In this case, ERM with class-dependent weights is a procedure typically used for non-private ERM. We extend two fundamental DP-ERM algorithms to minimize the empirical risk with class-dependent weights so that they perform better on imbalanced datasets. We also propose an algorithm to tune hyperparameters in terms of the area under the receiver operating characteristic curve (AUC) with the privacy guarantee. We show through experiments that when datasets have class imbalance and are large enough, the proposed algorithms outperform the existing algorithms.

Analyses of datasets which contain personal genomic information are very important for revealing associations between diseases and genomes. Genome-wide association studies (GWAS), which are large-scale genetic statistical analyses, often involve tests with contingency tables. However, if the statistics obtained by these tests are made public as they are, sensitive information of individuals might be leaked. Existing studies have proposed privacy protection methods for statistics in the chi-squared test with 3 × 2 contingency tables, but they do not cover all the tests used in GWAS. In addition, existing methods for releasing p-values are not practical. In this work, we propose methods to release p-values in the chi-squared test with a 3 × 2 contingency table, chi-squared statistics and p-values in the chi-squared test with a 2 × 2 contingency table, p-values in the Fisher's exact test, and chi-squared statistics and p-values in the Cochran-Armitage 's trend test while preserving both personal privacy and utility. The above statistical tests are used for comparative evaluation of allele frequencies and genotype frequencies, and the Fisher's exact test is often applied when the entries of contingency tables are small. We make theoretical guarantees by showing the sensitivity of the above statistics based on the concept of differential privacy. From our experimental results, we evaluate the utility of the proposed methods and show the appropriate thresholds for using the private statistics in statistical tests.

b. RAM Simulator Data Structures for Privacy Preserving Computation

Taku Onodera[4], Tetsuo Shibuya[1]: [4]Department of Computer Science, University of Helsinki

Wear leveling — a technology designed to balance the write counts among memory cells regardless of the requested accesses — is important for security-critical applications. We completely determine the problem parameter regime for which Security Refresh — one of the most well-known existing wear leveling schemes for PCM — is optimal by providing a positive result and a matching negative result. In particular, Security Refresh does not achieve optimality for the practically relevant regime of large-scale memory. We also propose a novel scheme that achieves an almost optimal lifetime, time/space overhead, and wear-free space for the relevant regime not covered by Security Refresh. Unlike existing studies, we give rigorous theoretical lifetime analyses, which is necessary to assess and control the security risk.

3. Development of Biomedical Database Technologies

a. Development of Algorithms for Next Generation Sequencer Data

Kazushi Kitaya[1], Tetsuo Shibuya[1]

Many bioinformatics tasks are achieved using a set of k-mers and the de Bruijn Graph represented by it for fast and space-saving processing. While much work has been done on how to efficiently compress and store a single set of k-mers or a de Bruijn Graph, methods for compressing multiple k-mer sets have been less studied. We propose a data structure that

can efficiently represent multiple k-mer sets constructed from genomic data and from which it is possible to efficiently reconstruct the original data. In addition, the proposed data structure does not require reference data, i.e., no additional information other than the data to be compressed is needed. Given 3292 k-mer sets constructed from whole genome sequence data for E. coli, we successfully reduced the amount of data by more than 50% compared to compressing them individually. This data structure is useful for researchers who want to use multiple k-mer sets or multiple de Bruijn Graphs and for administrators of genome-related databases who want to store their data on disk efficiently.

b. Integrating Viruses and Cellular Organisms for Pathway Maps

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

Publications

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Human Genome Center

Division of Metagenome Medicine
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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.

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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 are now developing the isolation method of intestinal viruses and are generating analysis pipeline of metagenome analysis of them. We also generating the method to analyze host-parasite association identified based on the shotgun sequencing data of the bacterial flora and viral plexus.

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

In recent years, not only in intestinal diseases such as inflammatory bowel disease, but also in autoimmune diseases, diabetes, cardiovascular diseases, and autism, dysbiosis have been detected. It is now clear that dysbiosis is closely related to the pathogenesis of the diseases. In our laboratory, we perform metagenomic analysis of intestinal bacteria and virrus in dysbiosis-related diseases, search for microorganisms related to the pathological condition (pathobiont), and develop phage therapy to specifically control the pathobiont.

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Center for Experimental Medicine and Systems Biology

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

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

1. Identification of distinct loci for de novo DNA methylation by DNMT3A and DNMT3B during mammalian development

Masaki Yagi, Mio Kabata[1,2], Akito Tanaka[1], Tomoyo Ukai , Sho Ohta , Kazuhiko Nakabayashi[3], Masahito Shimizu[2], Kenichiro Hata[3], Alexander Meissner [4,5,6], Takuya Yamamoto [1,7,8,9], Yasuhiro Yamada[7] : [1]Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University, [2]Department of Gastroenterology/Internal Medicine, Gifu University Graduate School of Medicine, [3]Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development, [4]Department of Genome Regulation, Max Planck Institute for Molecular Genetics, [5]Department of Stem Cell and Regenerative Biology, Harvard University, [6] Broad Institute of MIT and Harvard, [7]AMED-CREST, AMED, [8] Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University, [9] Medical-risk Avoidance based on iPS Cells Team, RIKEN Center for Advanced Intelligence Project (AIP)

De novo establishment of DNA methylation is accomplished by DNMT3A and DNMT3B. Here, we analyze de novo DNA methylation in mouse embryonic fibroblasts (2i-MEFs) derived from DNA-hypomethylated 2i/L ES cells with genetic ablation of *Dnmt3a* or *Dnmt3b*. We identify 355 and 333 uniquely unmethylated genes in *Dnmt3a* and *Dnmt3b* knockout (KO) 2i-MEFs, respectively. We find that *Dnmt3a* is exclusively required for de novo methylation at both TSS regions and gene bodies of Polycomb group (PcG) target developmental genes, while *Dnmt3b* has a dominant role on the X chromosome. Consistent with this, tissue-specific DNA methylation at PcG target genes is substantially reduced in *Dnmt3a* KO embryos. Finally, we find that human patients with *DNMT3* mutations exhibit reduced DNA methylation at regions that are hypomethylated in *Dnmt3* KO 2i-MEFs. In conclusion, here we report a set of unique de novo DNA methylation target sites for both DNMT3 enzymes during mammalian development that overlap with hypomethylated sites in human patients.

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

Jumpei Taguchi, Hirofumi Shibata[1], Mio Kabata[1], Masaki Kato[10], Kei Fukuda[3], Akito Tanaka[1], Sho Ohta, Tomoyo Ukai, Kanae Mitsunaga[1], Yosuke Yamada[1], So Nagaoka[11], Sho Yamazawa[12], Kotaro Ohnishi[1], Knut Woltjen[1], Tetsuo Ushiku[12], Manabu Ozawa[13], Mitinori Saitou[1,11,8], Yoichi Shinkai[10], Takuya Yamamoto[1,7,8,9] and Yasuhiro Yamada[7] : [10] Cellular Memory Laboratory, RIKEN Cluster for Pioneering Research, [11] Department of Anatomy and Cell Biology, Graduate School of Medicine, [12] Department of Pathology, Graduate School of Medicine, The University of Tokyo, [13]Laboratory of Reproductive Systems Biology, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, The University of Tokyo

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

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Center for Experimental Medicine and Systems Biology

Laboratory of Innate Immunity
自然免疫研究分野

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

教 授 医学博士 三 宅 健 介

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

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

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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. TLR3-mTORC2 axis is required for response against HSV-1 infection

Ryota Sato[1*], Akihisa Kato[2, 3], Takahiko Chimura[4], Shin-Ichiroh Saitoh[1], Takuma Shibata[1], Ryutaro Fukui[1], Jun Arii[2, 3], Tsuneo Ikenoue[5], Toshiya Manabe[4], Yasushi Kawaguchi[2, 3], Kensuke Miyake[1,6]
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Toll-like receptor 3 (TLR3) is a double-stranded RNA (dsRNA) sensor indispensable for defense against Herpes Simplex Virus (HSV-1) infection in the brain. We show here that TLR3 was required for innate immune responses to HSV-1 in neurons and astrocytes. In HSV-1 infection, TLR3 recruited the mammalian target of rapamycin complex 2 (mTORC2) that lead to induction of chemokines and trafficking of TLR3 to the cell periphery. TLR3 trafficking enabled activation of molecules required for type I interferon (type I IFN) induction including mTORC1. Intracranial HSV-1 infection in mice was exacerbated by impairing TLR3 responses with an mTOR inhibitor, and significantly rescued by potentiating TLR3 responses with an agonistic anti-TLR3 antibody. These results suggest that the TLR3-mTORC2 axis might be a therapeutic target to combat herpes simplex encephalitis.

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

Ryota Sato [1,] Tatjana Reuter [1 2], Ryosuke Hiranuma [1], Takuma Shibata [1], Ryutaro Fukui [1], Yuji Motoi [1], Yusuke Murakami [1], Hiroki Tsukamoto [3 4], Satoshi Yamazaki [5], Kaiwen Liu [1], Shin-Ichiroh Saitoh [1], Eicke Latz [2 6 7], Kensuke Miyake [1,8] Division of Innate Immunity, Department of Microbiology and Immunology, [2] Institute of Innate Immunity, Biomedical Center, Venusberg-Campus, University of Bonn, Bonn, Germany. [3]Laboratory of Oncology, Pharmacy Practice and Sciences, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan. [4]Department of Pharmaceutical Sciences, School of Pharmacy at Fukuoka, International University of Health and Welfare, Fukuoka, Japan. [5]Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan. [6]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. [7]Department of Infectious Diseases and Immunology, UMass Medical School, Worcester, MA, USA. [8]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

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

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Center for Experimental Medicine and Systems Biology

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

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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. NELL2-mediated lumicrine signaling through OVCH2 is required for male fertility

Daiji Kiyozumi[1,2], Taichi Noda[1,2], Ryo Yamaguchi[2,3], Tomohiro Tobita[2,4], Takafumi Matsumura[2,3], Kentaro Shimada[2,3], Mayo Kodani[2,3], Takashi Kohda[5] , Yoshitaka Fujihara[1,2], Manabu Ozawa , Zhifeng Yu[6] , Gabriella Miklossy[6], Kurt M. Bohren[6], Masato Horie[7] , Masaru Okabe[1,2,3], Martin M. Matzuk[6,8,9,10], Masahito Ikawa[1,2,3,4] : [1]Immunology Frontier Research Center, Osaka University, [2]Research Institute for Microbial Diseases, Osaka University, [3]Graduate School of Pharmaceutical Sciences, Osaka University, [4]Graduate School of Medicine, Osaka University, [5]Faculty of Life and Environmental Sciences, [6]Center for Drug Discovery and Department of Pathology and Immunology, Baylor College of Medicine, [7]Department of CNS Research, Otsuka Pharmaceutical, [8] Department of Molecular and Cellular Biology, Baylor College of Medicine, [9] Department of Molecular and Human Genetics, Baylor College of Medicine, [10] Department of Pharmacology and Chemical Biology, Baylor College of Medicine.

The lumicrine system is a postulated signaling system in which testis-derived (upstream) secreted factors enter the male reproductive tract to regulate epididymal (downstream) pathways required for sperm maturation. Until now, no lumicrine factors have been identified. We demonstrate that a testicular germ-cell–secreted epidermal growth factor–like protein, neural epidermal growth factor–like–like 2 (NELL2), specifically binds to an orphan receptor tyrosine kinase, c-ros oncogene 1 (ROS1), and mediates the differentiation of the initial segment (IS) of the caput epididymis. Male mice in which *Nell2* had been knocked out were infertile. The IS-specific secreted proteases, ovochymase 2 (OVCH2) and A disintegrin and metallopeptidase 28 (ADAM28), were expressed upon IS maturation, and OVCH2 was required for processing of the sperm surface protein ADAM3, which is required for sperm fertilizing ability. This work identifies a lumicrine system essential for tes-

tis-epididymis-spermatozoa (NELL2-ROS1-OVCH2-ADAM3) signaling and male fertility.

2. RNA-binding protein Ptbp1 regulates alternative splicing and transcriptome in spermatogonia and maintains spermatogenesis in concert with Nanos3

Manami Senoo[11], Hiroshi Hozoji[11], Yu Ishikawa-Yamauchi, Takashi Takijiri,[11] Sho Ohta[12], Tomoyo Ukai[12], Mio Kabata[13], Takuya Yamamoto[13,14,15,16], Yasuhiro Yamada[12], Masahito Ikawa[2], and Manabu Ozawa : [11]The Graduate School of Frontier Sciences, The University of Tokyo, [12]Division of Stem Cell Pathology, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, The University of Tokyo, [13]Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University, [14] Institute for the Advanced Study of Human Biology (WPI-ASHBi), [15]AMED-CREST, [16] Medical-risk Avoidance based on iPS Cells Team, RIKEN Center for Advanced Intelligence Project (AIP)

PTBP1, a well-conserved RNA-binding protein, regulates cellular development by tuning posttranscriptional mRNA modification such as alternative splicing (AS) or mRNA stabilization. We previously revealed that the loss of Ptbp1 in spermatogonia causes the dysregulation of spermatogenesis, but the molecular mechanisms by which PTBP1 regulates spermatogonium homeostasis are unclear. In this study, changes of AS or transcriptome in Ptbp1-knockout (KO) germline stem cells (GSC), an in vitro model of proliferating spermatogonia, was determined by next generation sequencing. We identified more than 200 differentially expressed genes, as well as 85 genes with altered AS due to the loss of PTBP1. Surprisingly, no differentially expressed genes overlapped with different AS genes in Ptbp1-KO GSC. In addition, we observed that the mRNA expression of Nanos3, an essential gene for normal spermatogenesis, was significantly decreased in Ptbp1-KO spermatogonia. We also revealed that PTBP1 protein binds to Nanos3 mRNA in spermatogonia. Furthermore, Nanos3+/−;Ptbp1+/− mice exhibited abnormal spermatogenesis, which resembled the effects of germ cell-specific Ptbp1 KO, whereas no significant abnormality was observed in mice heterozygous for either gene alone. These data implied that PTBP1 regulates alternative splicing and transcriptome in spermatogonia under different molecular pathways, and contributes spermatogenesis, at least in part, in concert with NANOS3. Key words: Alternative splicing, PTBP1, RNA-binding protein, Spermatogonia, Spermatogenesis

3. Polypyrimidine tract binding protein (Ptbp1) expression by Sertoli cells is essential for sperm production

Alternative splicing (AS), by which multiple forms of protein from a single gene are translated, is an important mechanisms for regulating proper development or homeostasis of specific type of cells in mammals. Testis shows more AS than any other tissue except bran. Although AS regulation in testicular germ cells during spermatogenesis is becoming to be uncovered, its role in Sertoli cells, testicular somatic cells and play an essential role to support spermatogenesis through the life period, is almost unknown. In the present study, we showed that expression of polypyrimidine tract binding protein (PTBP1), known as one of key factors to regulate AS, in Sertoli cells is essential for spermatogenesis. We show that PTPB1 is abundantly expressed by Sertoli cells and spermatogonia in the testis, and less evident or none in spermatocyte or spermatid. Eighty-three percent (five out of six) of Sertoli cell specific Ptbp1 KO male (Ptbp1 cKO) were infertile, and sperm counts from epididymis at 8 to 9-week-old was almost 50-fold lower compared to the Control (0.5×10^6 vs 24.2×10^6, $P<0.01$). Immunohistochemical analysis revealed that Ptbp1 cKO shows an increase of apoptosis of germ cells but not of Sertoli cells, and the presence of giant multinucleated cells in the lumen of seminiferous tubules. Furthermore, aberrant expression of Connexin43, a key protein of gap junction contributing blood-testis-barrier, and abnormal detachment of Sertoli cells from basal membrane were frequently observed in the Ptbp1 cKO. The data suggests that Ptbp1 plays an important role to regulate germ cell differentiation into spermatid. NGS analysis for determining transcriptome and splicome of Ptbp1 cKO Sertoli cells are currently ongoing.

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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 Zinc finger nucleases (ZFNs), TAL effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) nucleases (CRISPR/Cas), have widely used in life science and medical science. We are developing novel genome editing tools to overcome technical and patent limitation of CRISPR-Cas9 system. We are also developing the efficient genome editing strategies with these tools in rodents. These technologies facilitate easy and flexible gene editing in living organisms.

Development of CRISPR-Cas3 system for human genome editing

Kazuto Yoshimi, Yoko Yamauchi, Hiromi Taniguchi, Tomoaki Fujii and Tomoji Mashimo

Although single-component Class 2 CRISPR systems, such as type II Cas9 or type V Cas12a (Cpf1), are widely used for genome editing in eukaryotic cells, the application of multi-component Class 1 CRISPR has yet to be developed. We demonstrate that type I-E CRISPR, which is composed of Escherichia coli Cascade, Cas3, and programmable pre-crRNA, mediates distinct DNA cleavage activity in human cells. Notably, Cas3, which possesses helicase and nuclease activity, predominantly triggered several thousand base pair deletions upstream of the 5′-ARG protospacer adjacent motif (PAM), without prominent off-target activity. This Cas3-mediated directional and broad DNA degradation can be used to introduce functional gene knockouts and knock-ins. As an example of potential therapeutic applications, we show Cas3-mediated exon-skipping of the Duchenne muscular dystrophy (DMD) gene in patient-induced pluripotent stem cells (iPSCs). These findings broaden our understanding of the Class 1 CRISPR system, which may serve as a novel and unique genome editing tool in eukaryotic cells distinct from the Class 2 CRISPR system.

Rapid and accurate detection of novel coronavirus SARS-CoV-2 using CRISPR-Cas3

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Novel coronavirus SARS-CoV-2 outbreaks have rapidly spread to multiple countries, highlighting the urgent necessity for fast, sensitive, and specific diagnostic tools for virus surveillance. Here, the previously unknown collateral single-stranded DNA cleavage we observed with type I CRISPR-Cas3 highlights its potential for development as a Cas3-mediated rapid (within 40 min), low-cost, instrument-free detection

method for SARS-CoV-2. This Cas3-based assay is comparable with Cas12- and real-time reverse-transcriptase PCR-based assays in its speed and sensitivity, but offers greater specificity for single-base-pair discrimination while negating the need for highly trained operators. These findings support the use of CRISPR diagnostics for point-of-care testing in patients with suspected SARS-CoV-2 infections.

Combination of NHEJ and HDR for efficient and precise plasmid knock-ins in mice and rats

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

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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. In this study, we 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. G0 KI mice carried NHEJ-dependent indel mutations at one targeting site that was designed at the intron region, and HDR-dependent precise KIs of the various donor cassettes spanning from 1 to 5 kbp, such as EGFP, mCherry, Cre, and genes of interest, at the other exon site. These findings indicate that 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.

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Yoshimi K, Takeshita K, Yamayoshi S, Shibumura S,Yamauchi Y, Yamamoto M, Yotsuyanagi H, Kawaoka Y and Mashimo T. *medRivx*:2020-0602 doi: https://doi.org/10.1101/2020.06.02.20119875

Center for Experimental Medicine and Systems Biology

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

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

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

Laboratories that consist the Core

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

Cutting edge genome editing technologies

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

Supporting generation of gene manipulated mouse or rat models through the core lab and AdAMS platform

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

to this platform.

Number of mice or rat strains we have developed in 2020

In 2020, our core has provided 20 and 11 strains of gene manipulated mice through the core lab and AdAMS, respectively. In case of rats, 1 and 8 strains of gene manipulated rats have also been provided through the core lab and AdAMS, respectively.

Advanced Clinical Research Center

Division of Molecular Therapy
分子療法分野

Professor	Arinobu Tojo, M.D., D.M.Sc.	教　授	医学博士	東　條　有　伸
Associate Professor	Satoshi Takahashi, M.D., D.M.Sc.	准教授	博士(医学)	高　橋　　聡
Assistant Professor	Muneyoshi Futami, M.D., D.M.Sc.	助　教	博士(医学)	二　見　宗　孔
Assistant Professor	Masamichi Isobe M.D., D.M.Sc.	助　教	博士(医学)	磯　部　優　理

The main theme of our research is toward the development of novel therapeutic options against intractable malignant disorders including leukemia, lymphoma and various cancers. For this purpose, we are making every effort to master the mechanisms of normal and neoplastic stem cells on the basis of molecular and cellular biology as well as medical informatics. We also try to develop novel therapies in the field of regenerative medicine using bone marrow-derived mesenchymal stromal cells.

(1) Molecular and cellular analysis of hematological malignancies toward clinical practice: Tumor-specific genetic alterations often result in transcriptional dysregulation and activation of signal transduction pathways as well as defective tumor suppressors, which appear to be the primary cause of those tumors. We are studying the molecular and cellular aspects of hematological malignancies as a model system. Furthermore, we performed clinical sequencing in tight collaboration with Human Genome Center to establish a platform for precision medicine.

(2) Development of a novel cell therapy using the genome editing with CRISPR/Cas9: Cell therapy using mesenchymal stem cells and chimeric antigen receptor expressing-T cells (CAR-T cells) are promising therapeutic options for refractory diseases. While cell therapies are remarkably effective, very expensive cost hampers them to be applied for regular clinical use. We used CRISPR/Cas9 for the gene editing to generate a universal cell therapy.

(3) Clinical study of clonal evolution of HTLV-1-infected T cells into leukemia: Adult T-cell leukemia is a T cell malignancy which develops in HTLV-1 infected individuals after long latency period. HTLV-1 infected cells are regarded to transform through multi-step oncogenesis process. We are analyzing HTLV-1 infected cells in different stages of transformation whose phenotypes such as CD7 and CADM1 expression vary in each stage by sorting them using flow cytometer. These analyses will provide useful information regarding molecular mechanism to develop ATL.

1. Development of a novel cell therapy using the genome editing with CRISPR/Cas9

Meshitsuka S, Ikeda M, Futami M, Tojo A

Division of Molecular Therapy

Cell therapies using mesenchymal stem cells (MSCs) are effective for the treatment of graft versus

host disease (GvHD) following allogeneic stem cell transplantation. However, human leukocyte antigen (HLA) alleles differ between patients and donors, and transplanted MSCs are eventually rejected by the host T cells. The knockout (KO) of HLA gene would unlock the HLA restriction and facilitate the development of universal cell therapy. For better retention of transplanted MSCs in recipients, a genome modification to knockout HLA molecule was performed. Because HLA class I molecules are expressed on the cell surface together with β-2 microglobulin (B2M), knockout (KO) of B2M leads to loss of expression of HLA. Using the electroporation, MSCs were transfected with Cas9 protein and a short guide RNA (sgRNA) targeting B2M. Successful KO of B2M and HLA class I was confirmed with a high efficiency. We confirmed that B2M-/- MSCs retains the immunosuppressive effect as strong as parental MSCs using the mixed lymphocyte reaction (MLR) in the presence of MSCs. Although loss of HLA would protect MSCs from cytotoxic T lymphocytes (CTLs), loss of HLA deprives a protective effect of HLA through the binding to inhibitory receptors on the natural killer (NK) cells. To avoid from both CTLs and NK cells, HLA-G, an almost invariant non-classical HLA, was fused with B2M, and the B2M/HLA-G fusion was introduced. Fer the better and safer gene transduction into MSCs, we compared with different transduction methods including lentiviral vector, AAV vector, and electroporation, and we found that AAV vector is the most safe and efficient method to insert the B2M/HLA-G fusion into MSCs. Using the method that we have confirmed, two different MSCs ($B2M^{-/-}$, $B2M^{-/-}$ substituted with B2M/HLA-G) were co-cultured with peripheral blood nuclear cells. The substitution of B2M/HLA-G into B2M clearly protected MSCs from cytotoxicity by NK cells, and the MSCs showed a longer retention and prolonged anti-T cell effects (eg. inhibition of IFN-g and TNF-α). These gene modified MSCs retained the pluripotency to differentiate into osteoblasts, chondrocytes, adipocytes, as compared with parent MSCs. In summary, B2M-/- breaks the HLA barrier, substitution of B2M with B2M/HLA-G prolongs MSC retention and immunomodulatory effects, and the gene-modified cells retained an equivalent pluripotency to parent MSCs. These results encourage us to utilize the B2M/HLA-G modified MSCs for a novel cellular therapy.

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

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Immunoglobulin superfamily member 8 (IGSF8 or CD316), a cell surface protein containing 4 IG domains, interacts with several tetraspanins including CD9 and CD81, and modulates cell migration and motility. We examined the role of Igsf8 in normal and leukemic hematopoiesis using conditional knockout mice (*Igsf8* f/f; Vav-Cre or *Igsf8* f/f; Rosa26-CreERT). *Igsf8* was ubiquitously expressed in normal blood cells and leukemia cells. $Igsf8^{-/-}$ did not significantly affect adult hematopoiesis in peripheral blood and bone marrow. $Igsf8^{-/-}$ LT-HSC ($CD34^-$ $Flk2^-$ KSL cells) reduced colony forming ability *in vitro*, and showed comparable donor chimerism by 3 months, but led to reduced donor chimerism at 4 months and those after second transplantation *in vivo*, suggesting that Igsf8 does not affect adult hematopoiesis, but that may affect repopulating ability of HSCs. In *MLL-AF9* and $NRAS^{G12V}$-driven AML, or *BCR-ABL* and *NUP98-HOXA9*-driven CML-BC mice models, $Igsf8^{-/-}$ led to a dramatic decrease in the number of leukemic colonies formed *in vitro*. $Igsf8^{-/-}$ leukemia mice showed significantly longer survival and depleted leukemia cells in bone marrow and spleen *in vivo*. $Igsf8^{-/-}$ leukemia cell triggered an increment of apoptotic cell death, which contribute to significantly lower proportion of LSCs in spleen of $Igsf8^{-/-}$ leukemic mice. Given that $Igsf8^{-/-}$ did not affect homing ability of leukemia cells, these results indicate that Igsf8 is required for propagation of myeloid leukemia and maintenance of LSC.

Gene set enrichment analysis exhibited increase apoptosis related genes and decrease Wnt/β-catenin related genes in $Igsf8^{-/-}$ leukemic cells, but not in LT-HSCs. Increase pro-apoptosis genes and decrease anti-apoptosis genes and Wnt/β-catenin target genes in $Igsf8^{-/-}$ AML stem cells were validated in quantitative polymerase chain reaction analysis. Further, expression levels of β-catenin protein in $Igsf8^{-/-}$ leukemic cells were significantly lower compared to $Igsf8^{+/+}$ leukemic cells, but not in normal hematopoietic stem and progenitor cells. These results suggest that Igsf8 might be critical for myeloid leukemia maintenance via Wnt/β-catenin signaling pathway. Knockdown of IGSF8 by small hairpin RNA in myeloid leukemia cell lines (THP-1, MV4-11, SKM-1, and K562) and primary patient-derived AML cells exhibited reduced numbers of colony forming cells *in vitro*. Knockdown of *IGSF8* also caused decrease expression of β-CATENIN in AML cell lines. Taken together, our present study reveals that Igsf8 is indispensable for myeloid leukemia, but not adult hematopoiesis, suggesting that IGSF8 inhibition should be considered for targeting myeloid leukemia.

3. A gain-of-function mutant of WASp perturbs hematopoiesis and causes impaired neutrophil development.

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Division of Molecular Therapy

X-linked neutropenia (XLN) is a very rare type of severe congenital neutropenia (SCN) caused by a gain-of-function mutation in the Wiscott-Aldrich syndrome gene (*WAS*), the product of which (WASp) is expressed only in blood cells and upregulated especially during neutrophil maturation. Four types of WASp mutations in its GBD domain (L270P, S272P, I290T, and I294T) have been described so far, and we reported the first case with WAS p.I290T. Thereafter, 2 mouse models harboring WASp.L272P or I296T (XLN WASp homologs) were developed. Both mutations render neutrophils hyperactive, but neither could recapitulate neutropenic phenotype. Herein, we established a novel knock-in mouse carrying WASp.I292T and extensively analyzed its peripheral blood and bone marrow. Confocal microscopy after phalloidin-staining demonstrated constitutively enhanced actin polymerization in WASp.I292T neutrophils in contrast with wild type neutrophils. While the number of PB neutrophils and the relative proportion of BM progenitor compartments were comparable between WASp.I292T mutant and wild type mice, mutant mice showed significantly increased number of BM neutrophils and prominent extramedullary hematopoiesis in spleen, suggesting ineffective granulopoiesis. Intriguingly, *in vitro* colony forming ability of KSL cells from mutant mice was up- or down-regulated depending on the combination of stimulating factors. Furthermore, BM neutrophils from mutant mice had reduced transmigration ability in response to various chemokines and chemoattractants. Gene set enrichment analysis of granulocyte-macrophage progenitors from wild type and mutant mice revealed attenuation of both NF-κB and TP53 signaling pathways in mutant progenitors. These data suggests that WASp.I292T-triggered enhancement of actin polymerization perturbs hematopoiesis and causes impaired neutrophil development possibly through suppression of both NF-κB and TP53 signaling pathways.

4. Small-molecule HDAC and Akt inhibitors suppress tumor growth and enhance immunotherapy in multiple myeloma

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Multiple myeloma (MM) patients may undergo relapse and experience resistance to existing therapies. Cereblon (CRBN) is key mediator of the bioactivities of immunomodulatory drugs (IMiDs), including lenalidomide. Moreover, genetic alteration of CRBN is frequently detected in IMiD-resistant patients and considered to contribute to IMiD resistance. Thus, overcoming resistance to IMiDs, is expected to improve clinical outcomes. Binding of IMiDs to CRBN mediates the recruitment of IKZF1 or IKZF3 to E3 ubiquitin ligase for subsequent degradation, resulting in c-Myc downregulation. We revealed that histone deacetylase (HDAC) inhibitors suppressed tumor growth in CRBN-disrupted MM cells through downregulation of IKZF1 and IKZF3. It was previously reported that IKZF1 and IKZF3 are negative regulators for NK cell-activating ligands including MICA. We found that HDAC inhibitors augmented the antibody-dependent cellular cytotoxicity (ADCC) of therapeutic antibodies via enhancing expression of MICA. Analysis of an integrated gene expression profile and disease prognosis database comprising gene expression profiles from 414 newly diagnosed MM patients (GenomicScape, http://www.genomicscape.com) demonstrated that higher MICA expression is significantly associated with better overall survival (OS) of MM patients. Lenalidomide resistance is also attributed to increased stabilization of c-Myc by phosphorylation and inhibition of glycogen synthase kinase-3 (p-GSK-3), a down-stream target of PI3K/Akt. GSK-3 α/β actively reduces c-Myc, while p-GSK-3 α/β is an inactive form leading to increase in c-Myc. We found that CRBN knockdown in MM cells enhanced p-GSK-3 and c-Myc expression by lenalidomide. Akt inhibitor downregulated c-Myc by blocking GSK-3 phosphorylation, which was enhanced by HDAC inhibitors. HDAC and Akt inhibitors showed a synergistic effect on cell cytotoxicity and c-Myc suppression when combined. This anti-myeloma effect of HDAC and Akt inhibitors was partially restored by GSK-3 inhibitors. According to clinical trial data, patients with high GSK-3 α/β expression had better OS than those with low GSK-3 α/β expression. Further, patients with high Myc expression had poorer OS than those with low expression. These results are suggestive of the negative effect of GSK-3 α/β inactivation-related c-Myc stabilization on MM patient prognosis. CUDC-907, a dual HDAC and PI3K inhibitor, showed a cytotoxic effect and enhanced immunotherapy in myeloma cell lines including multi drug-resistant and primary cells from lenalidomide-resistant patients. CUDC-907 was also effective in myeloma xenograft model of using CRBN-knockout cells. In conclusion, the use of a dual HDAC and Akt inhibitor with or without therapeutic antibodies, is a promising therapeutic approach for relapsed/refractory MM patients.

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

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Mucosal-associated invariant T (MAIT) cells are a type of innate lymphocyte and recognize riboflavin (vitamin B2) synthesis products presented by MHC-related protein 1. We investigated long-term reconstitution of MAIT cells and its association with chronic graft-versus-host disease (cGVHD) in a cross-sectional cohort of 173 adult patients after allogeneic hematopoietic cell transplantation. According to donor source, the number of MAIT cells significantly correlated with time after cord blood transplantation (CBT) but not with time after bone marrow transplantation or peripheral blood stem cell transplantation. The number of MAIT cells was significantly lower in patients with cGVHD compared with patients without cGVHD. We also examined the association between MAIT cell reconstitution and gut microbiota as evaluated by 16S ribosomal sequencing of stool samples 1 mo post-CBT in 27 adult patients undergoing CBT. The diversity of gut microbiota was positively correlated with better MAIT cell reconstitution after CBT. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States analysis indicated that amounts of *ribB* and *ribA* genes were significantly higher in the microbiomes of patients with subsequent MAIT cell reconstitution after CBT. In conclusion, long-term MAIT cell reconstitution is dependent on the type of donor source. Our data also unveiled an important role for the interaction of circulating MAIT cells with gut microbiota in humans.

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

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Acute myeloid leukemia (AML) relapse is considered to occur due to escape of tumor cells from anti-tumor immunity and contribution of immune checkpoints CD155/CD112 to AML progression is assumed. However, both the activation receptor DNAM-1 and inhibitory receptor TIGIT present on natural killer (NK) and T cells bind to CD155/CD112. It is unclear how changes in the expression of CD155/CD112 affect tumor immunity. The Raf-MEK-ERK pathway, related to regulation of CD155/CD112 expression, is one of the targets of FLT3 inhibitors. We investigated the effect of FLT3 inhibitors on the expression of CD155/CD112 and its effects on NK and T cell cytotoxicity. CD155/CD112 expression in AML cell lines with or without treatment of the FLT3 inhibitor quizartinib was analyzed. The direct cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) of NK cells under FLT3 inhibition were determined by luciferase reporter assay. The cytotoxicity of γδ T cells was also analyzed. CD155/CD112 expression was specifically downregulated by the FLT3 inhibitor in FLT3 mutated cell lines. The direct cytotoxicity and ADCC of NK cells were enhanced. However, the cytotoxicity of γδ T cells with decreased TIGIT expression as compared to that of NK cells was not enhanced. Analysis of clinical trials from the database revealed that high CD155/CD112 expression is associated with poor overall survival. The enhanced cytotoxicity of NK cells against the cells that were treated with FLT3 inhibitors suggests that CD155/CD112 are possible target of FLT3 inhibitors in AML.

In addition, we tried to generate genetic engineered NK cells with enhanced anti-tumor cytotoxicity. First, we introduced human IL-15 using lentivirus vector into NK-92 cells, NK cell lines derived from human NK cell lymphoma. Although NK-92 cells are cytokine dependent cell lines, the introduction of IL-15 made NK-92 cells proliferative independent of cytokines. This genetic engineered NK cells were supposed to be useful for our future analyses especially in in vivo analyses using patient-derived xenografts (PDX) mouse model. Furthermore, we performed genome editing in NK-92 cells using CRISPR-Cas9 systems. The cytotoxicity of DNAM-1$^+$/TIGIT$^-$ NK-92 cells against AML cells with or without FLT3 mutations was enhanced as compared with that of DNAM-1$^-$/TIGIT$^+$ NK-92 cells. These results indicate the usefulness of genome editing of immune check point-related genes in NK cells to enhance their anti-tumor cytotoxicity. We are in the process of genome editing of immune check point-related genes in *induced pluripotent stem* (iPS) cells and differentiation-induction of genome edited iPS cells into NK cells.

7. The impact of circulating tumor DNA status on acute myeloid leukemia and myelodysplastic syndromes with alloSCT: Interim results of a prospective study.

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We previously reported the utility of residual circulating tumor DNA (ctDNA) status for identifying patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) at high risk for relapse post myeloablative allogeneic hematopoietic stem cell transplantation (alloSCT) in the retrospective setting. However, it remains to be elucidated whether this approach could be useful in the prospective setting as well. Here, we report the interim results from a Japanese multicenter prospective observational study, examining the clinical utility of this approach (KSGCT1702, conducted on behalf of Kanto Study Group for Cell Therapy). Since June 2018, we've enrolled patients with AML and MDS planning to undergo myeloablative alloSCT. We collected tumor and matched serum samples at diagnosis and serum samples every month until the fourth month post alloSCT. We subjected tumor DNA (bone marrow or peripheral blood), and buccal swab DNA, to next generation sequencing, identifying candidate driver mutations. We designed allele specific droplet digital PCR (ddPCR) assays for each to monitor ctDNA. The median detection limit of these assays was 0.04% (Nakamura et al, Blood 2019). The primary endpoint was to compare the cumulative incidence of relapse rate (CIR) within 1 year post alloSCT according to residual ctDNA status. The log-rank test was used for group comparison. As of November 2019, a total of 38 patients were enrolled and 28 patients (26 AML and 2 MDS) of whom received alloSCT. The median follow up period post alloSCT was 119.5 (1-365) days. The median age was 54 years (25-66 years) including 15 males (53.6%). The conventional risk category was adverse or high risk in 42.9% of patients. To date, we've finished sequencing in 24 (85.7%) patients. A total of 33 somatic driver mutations/fusion genes were detected in all 24 patients, with one, two and three mutations found in 17, 5, and 2 individuals, respectively. These mutations included: single nucleotide variants, DNMT3A, CEBPA, NRAS, CEBPA, TET2, PTPN11, NPM1, IDH2, TP53, SMC3, RUNX1, JAK2, and FLT3; structural variants, CBFB/MYH11, KMT2A/MLLT3, and KMT2A/AFDN. We could construct ddPCR assays for 20 of 24 (83.3%) patients. Notably, there was a clear correlation of VAF between diagnostic ctDNA and matched tumor DNA from BM in available patients (r^2=0.86; p=0.0009). Of these 24 patients, one patient died without relapse, with six in clinical relapse with a median of 4 months (range, 1 to 10 months), and with the remaining 17 in remission. Of 20 patients available for personalized assays, fourteen patients were available for ctDNA status 3 months post alloSCT, and 4 of whom were positive ctDNA status with the average allele frequency of 1.52 (0.12 to 5.0) %. Most importantly, when we compared CIR rate according to residual ctDNA status, positive ctDNA status at 3 months post alloSCT was associated with higher CIR at 10 months: 100% in positive patients vs. 0% in negative patients (p=0.0259, Figure1). Our ctDNA monitoring could identify patients who were more likely to relapse. Additional enrollment of patients and further follow-up are needed to confirm this promising result.

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Advanced Clinical Research Center

Division of Cellular Therapy
細胞療法分野

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

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

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

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

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

2. Molecular targeting therapies using small molecule compounds

Naru Sato, Yasutaka Hayashi, Yousuke Tanaka, Akiho Tsuchiya, Toshiyuki Kawashima, Yukinori Minoshima, Tomofusa Fukuyama, Susumu Goyama, and Toshio Kitamura:

STAT3 is frequently activated in many cancers and leukemias, and is required for transformation of NIH3T3 cells. We previously isolated a STAT3 inhibitor in collaboration with a biotech venture company in USA.

In addition to the STAT3 inhibitor, we have recently started a new project to develop STAT5 inhibitors in collaboration with a pharmaceutical company. To this end, we have developed a screening method to search for STAT5 inhibitors. In addition to STAT3/5 inhibitors, we have started several collaborations with several domestic and global pharmaceutical companies to evaluate the efficacies of a variety of molecular targeted therapies in our established mouse MDS/AML/MPN models.

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

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

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

We have recently established Rosa26-knock-in mice for ASXL1-MT. These KI mice did not develop MDS in their lives, but presented disturbed differentiation of erythroid cells and mild macrocytic anemia. Combination with other mutations (eg.Runx1mutation) and insertional mutagenesis experiments have demonstrated that these mice are in the pre-leukemic states. Now we use the ASXL1-MT-KI mouse as a model for "clonal hematopoiesis". Clonal hematopoiesis is a state in which one or in rare case 2 leukemia-associated mutations are found in more than 1~2 %blood cells in old (>65 years) healthy people. People with clonal hematopoiesis have 10-times higher risk for developing hematological malignancies. In addition, they have 2-times higher risks for developing stroke and acute myocardial infarction. In addition, in cancer patients after chemotherapy, clonal hematopoiesis is identified 20~30%. Importantly, cancer patients with clonal hematopoiesis have higher ratios of recurrence and poorer prognosis. No we are characterizing ASXL1-MT-KI mice to clarify how people with clonal hematopoiesis develop a variety of diseases.

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

Tomofusa Fukuyama, Susumu Goyama, Toshio Kitamura

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

rently under investigation.

5. Development of RUNX1-targeted therapy for AML

Taishi Yonezawa, Tomofusa Fukuyama, Yosuke Tanaka, Hirotaka Takahashi[5], Tatsuya Sawasaki[5], Toshio Kitamura, Susumu Goyama
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RUNX1 is a transcription factor and plays important roles in hematopoiesis. Recent reports have shown that RUNX1 promotes the development of leukemias as well as several solid cancers. CBFB is a partner protein of RUNX1 composing heterodimer, and it increases the stability and DNA-binding ability of RUNX1. Therefore, inhibition of RUNX1-CBFB interaction has been considered a promising therapeutic strategy for RUNX1-dependent tumors. However, the effects of RUNX1-CBFB inhibition have not been proven in clinical trials.

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

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

6. Targeting TP53 and antitumor immunity for leukemia therapy

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

The negative regulator of p53, MDM2, is frequently overexpressed in acute myeloid leukemia (AML) that retains wild-type *TP53* alleles. Targeting of p53-MDM2 interaction to reactivate p53 function is therefore an attractive therapeutic approach for AML. We showed that an orally active inhibitor of p53-MDM2 interaction, DS-5272, causes drastic tumor regressions of MLL-AF9-driven AML *in vivo* with a tolerable toxicity. However, the antileukemia effect of DS-5272 was markedly attenuated in immunodeficient mice, indicating the critical impact of systemic immune responses that drive p53-mediated leukemia suppression. In relation to this, DS-5272 triggered immune-inflammatory responses in MLL-AF9 cells including upregulation of Hif1α and PD-L1, and inhibition of the Hif1α-PD-L1 axis sensitized AML cells to p53 activation. Interestingly, we found that NK cells, but not cytotoxic T cells, are important mediators of antileukemia immunity. Our findings suggests that dual activation p53 and NK cells could be a promising therapeutic strategy for AML.

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

Yosuke Tanaka, Tsuyoshi Fukushima, Susumu Goyama, Toshio Kitamura

CML LSCs (Chronic myeloid leukemia leukemic stem cells) were thought to be quiescent (in G_0 phase). Their quiescent state is thought to be a major reason why CML LSCs are resistant to several BCR-ABL tyrosine kinase inhibitors (TKIs). We planned to examine relationships between quiescent states of CML LSCs, their leukemia-initiating potential and their resistance to TKIs. To this end, we have established mouse CML model with G_0M. Briefly, we retrovirally overexpressed BCR-ABL fusion gene in bone marrow (BM) cells from 5FU-treated G_0M mice and then injected them into lethally irradiated wild type mice to develop CML mouse model. These mice developed CML within 3 weeks. We found that G_0M and CD27, a marker for CML stem and progenitor cells, could split the conventional CML LSC fraction (BCR-ABL+, cKit+, Sca1+, lineage marker negative; CML KSL), into 4 fractions and identified that CML LSCs and CML progenitor cells were enriched in G_0M-postive CD27-postive CML KSL fraction and G_0M-negative CD27-postive CML KSL fraction, respectively. We confirmed that the CML LSCs were resistant to imatinib. RNA-Seq analysis exhibited that NFkB signaling pathways were enriched in imatinib-treated CML LSCs as compared with vehicle-treated CML LSCs, indicating that NFkB signal pathways are important for their resistance to imatinib. To inhibit NFkB signal pathways, we used IRAK1/4 inhibitor, which can block IL1R/TLR4 signals to activate NFkB. Combination therapy of imatinib with IRAK1/4 inhibitor was effective at eliminating CML LSCs as compared with mono therapies. We extended the examination of efficacy of IRAK1/4 inhibitor on CML LSCs to CML-CP patient samples. IRAK1/4 inhibitor in combination

with imatinib was effective at eliminating CD34+CD38-CD90+ CML LSCs in vitro and xenograft model. These results exhibited that IRAK1/4-NFkB signal axis was crucial for maintenance of imatinib resistant CML LSCs.

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

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

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Division of Infectious Diseases
感染症分野

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

1. Clinical research of COVID-19

Makoto Saito, Eisuke Adachi[1], Hiroyuki Nagai[1], Shinya Yamamoto, Lim Lay Ahyoung, Kazuhiko Ikeuchi, Michiko Koga, Takeya Tsutsumi, Hiroshi Yotsuyanagi

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Since the emergence and spread of SARS-CoV2 in Japan in the beginning of 2020, many patients with COVID-19 were admitted to the IMSUT Hospital. There are more than 250 COVID-19 patients in 2020. By the observation of these patients, we noticed a lot of clinical questions and investigated various factors such as patients' backgrounds, clinical findings, and laboratory data. We have obtained many novel and interesting findings and published them in international journals. The medical care for COVID-19 patients is ongoing at the IMSUT Hospital and the number of patients is accumulating, therefore we continue the investigation which will contribute to the improvement of patient care and understanding of COVID-19.

2. Basic research for the control of COVID-19

Michiko Koga, Makoto Saito, Eisuke Adachi[1], Hiroyuki Nagai[1], Shinya Yamamoto, Kazuhiko Ikeuchi, Takeya Tsutsumi, Aya Ishizaka[2], 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 started COVID-19-related research at the beginning of 2020, and we have been working in collaboration with some of these laboratories. Our main mission is to obtain and share clinical samples from COVID-19 patients, but we are also conducting basic research by ourselves such as microbiomes in those patients. Additionally, we are attempting to perform high-resolution transcriptomic analysis of blood immune cells from disease progression to recovery in COVID-19 in order to enhance a better understanding

of the protective and pathogenic immune responses of the disease. Specifically, we are performing single-cell RNA 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.

3. Analysis of genetic sequence of hepatitis viruses.

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

We sometimes see patients with acute hepatitis at IMSUT Hospital. Most of the causes are viral hepatitis, induced by hepatitis A, B, C, or E virus. Actually, in 2018, there was the outbreak of hepatitis A in HIV-infected patients, and still in 2020, we see some patients with hepatitis A. Concerning hepatitis B and C, due to the similar route of infection, every year we see some patients who are also infected with HIV. Using sera and/or stools obtained from these patients, we cloned a total or part of viral genome and determined the genetic sequence of the viruses, to identify the transmission route of the viruses and also the drug-resistant mutations or vaccine escape mutations. Concerning hepatitis B, we have also been examining the HBV-positive samples derived from blood donors who were accidentally found to be HBsAg-positive. As for hepatitis E, we are collaborating with outside researchers in Tokyo as well as Hokkaido where the hepatitis E virus infection is sometimes observed. By cloning the virus from samples derived from not only the patients but also suspectable foods or wild animals, we try to investigate the transmission routes of the virus.

4. Evaluation of the efficacy of HA vaccine for HIV-MSM

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

Due to the outbreak of hepatitis A in Japan around 2018, we started to vaccinate HIV-MSM with HA vaccine "Aimmugen®" in IMSUT Hospital. Aimmugen® is shown to be highly effective and induce IgG-HA antibodies for almost all of healthy people by twice vaccination. However, there have been the efficacy for HIV-infected people, especially in case of twice vaccination. Therefore, we evaluated the efficacy of Aimmugen® among HIV-MSM, particularly focused on twice vaccination. By October 2019, 147 HIV-MSM were vaccinated at least once with Aimmugen®. Among them, 134 finished the second vaccination and 114 were tested for IgG-HA antibodies. Ninety-five HIV-MSM were seropositive for IgG-HA, indicating the seropositive rate after second vaccination is 71.1%, which is lower than healthy adults. Now we are investigating the factors associated with the efficacy of Aimmugen® among HIV-MSM.

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

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

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

Drug-resistance test had been performed on national-wide HIV-1-infected cases newly diagnosed. The overall prevalence of TDR was about 6.4% in 2019.

6. Study of HIV and the host genome database construction

Michiko Koga, Ayako Kamisato[6], Eisuke Adachi[1], Tadashi Kikuchi[1,3], Takeya Tsutsumi, Hiroshi Yotsuyanagi, Ai Tachikawa-Kawana[3], Teiichiro Shiino[3], Tetsuro Matano[3]
[6]Division of Bioethics, IMSUT

We have made the database of HIV genome, the enterobacterial flora gene of feces and the host genome with Japan Agency for Medical Research and Development grant. Database of the viral genome, the host genome, the clinical information of HIV cases examined in HIV researches will contribute to HIV prevention by promoting elucidation of HIV transmission trend, prevention of disease progression, research on the disease pathology. It is another important thing that the public use of the genome information related to infectious disease has many ethical, legal and social issues (ELSI), so we have the study to solve the ELSI problem also.

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

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

The eradication of HIV provirus from HIV-infected patients is a critical point in 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 a latency-reversing agent, allowing eradication by the host immune system. This approach is known as the "kick-and-kill" strategy. It has been reported that histone acetyltransferase inhibitor SAHA strongly reactivates latent HIV reservoir. To identify the novel chemical which act as a latency-reversing agent, we investigated the reactivation of latently infected cells *ex vivo* by treatment with Inhibitor A, a specific inhibitor of histone modifying enzyme, using CD4+ T-cells derived from HIV-infected patients. Consequently, Inhibitor A treatment triggered the expression of cellular HIV-1 mRNA derived from provirus at the same levels as observed with the HDAC inhibitor SAHA treatment, indicating that Inhibitor A acts as a latency-reversing agent on latent CD4+ T-cells.

8. Analysis of the HIV-associated gut microbiome

Aya Ishizaka[2], Michiko Koga, Prince Kofi Parbie[3], Tadashi Kikuchi[1,3], Eisuke Adachi[1], Tomohiko Koibuchi[1], Taketoshi Mizutani[2], Tetsuro Matano[3,7], Hiroshi Yotsuyanagi

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

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

Takeya Tsutsumi, Kazuhiko Ikeuchi, Diki Prawisuda, Kazuya Okushin[8], Kyoji Moriya[8], Kazuhiko Koike[9], Hiroshi Yotsuyanagi

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

10. Clinical epidemiology of malaria in pregnancy

Makoto Saito

Malaria is the leading cause of mortality in the tropics. Pregnant women are particularly vulnerable and malaria in pregnancy causes an adverse impact on the mother and fetus. In the global collaboration with the colleagues in sub-Saharan Africa and Asia, we have conducted a pooled meta-analysis of the clinical data on falciparum malaria in pregnancy worldwide. Currently, further analyses of a clinical trial conducted in Thailand are being conducted, focusing on the impact of antimalarial resistance on the treatment outcomes in pregnant women.

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Our research is aimed to apply results of basic cancer research in clinics. We have been working on the following five projects, 1) elucidation of the role of Wnt/β-catenin signaling pathway in gastrointestinal carcinogenesis, 2) discovery of Wnt inhibitor through a screening of large-scale chemical libraries, 3) establishment and investigation of mouse models of human cancer, 4) understanding the genetic features of rare cancers and mechanisms of their development, and 5) clinical sequencing for the implementation of genomic medicine.

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

Kiyoshi Yamaguchi, Yoichi Furukawa

Aberrant activation of the Wnt/β-catenin signaling pathway has been found in various types of cancers. This activation leads to the accumulation of β-catenin in the nucleus, followed by the transactivation of the TCF/LEF family. Therefore, comprehensive understanding of genes directly transactivated by the heterodimeric β-catenin/TCF transcriptional complex results in the better understanding of the role of Wnt/β-catenin pathway in human carcinogenesis. To date, we have identified the Wnt target genes such as *RNF43*, *SP5*, *CLDN1*, *ENC1*, *APCDD1*, and *FRMD5* in colorectal cancer cells. Recently, we also identified another set of Wnt target genes through the transcriptome analysis of hepatocellular carcinoma (HCC) cells carrying activated Wnt signaling. One of the target genes, odontogenic ameloblast associated (ODAM), has been reported to be associated with the formation of the bone, dentin, and enamel, whereas association of ODAM with the pathway and its biological role in hepatoma cells remains largely unknown. Promoter analysis and subsequent ChIP assay determined the direct transcriptional regulation of *ODAM* by the β-catenin/TCF. In addition, we performed RNA-seq analysis of ODAM-depleted HCC cells. Interpretation of the differentially expressed genes will help understanding of the biological role of ODAM and uncover a new role of the pathway involved in carcinogenesis.

2. Cancer drug discovery through a large chemical library screening

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Establishment of well-designed high-throughput screening system is an essential for the identification of small molecules that inhibit a signaling pathway or a molecule of interest. Various types of cell-based assays have contributed to the discovery of small molecules that modulate Wnt signaling. We developed a sensitive and specific cell-based reporter assay for the

detection of the Wnt/β-catenin signaling activity. By leveraging this assay, we established a high-throughput screening system, and performed a screening of small molecule and natural compound libraries. As a result, we have identified several candidate chemicals for the Wnt inhibitors. With the support of the Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS) program, we have investigated the structure-activity relationship (SAR) of these chemical probes. In addition, we have started target identification of the probes.

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

Tsuneo Ikenoue, Yoichi Furukawa

Genetically engineered mice are useful tools for studying human diseases, including cancer. In this project, we have demonstrated that liver-specific expression of oncogenic *Kras* cooperates homozygous *Pten* deletion induced intrahepatic cholangiocarcinoma (ICC) but not hepatocellular carcinoma (HCC) in mice. We have also established another ICC mouse model carrying a cancer-associated mutant allele of *Fbxw7* in combination with an oncogenic *Kras* allele.

In addition, we have investigated roles of cancer-associated hotspot mutations of *IDH1* and *IDH2* in hepatocarcinogenesis using liver-specific knockin mice of these mutations. We have shown that *IDH1* and *IDH2* mutations promote the development ICCs in the background oncogenic *Kras* mutation. Intensive analysis of these mice should provide better understanding of mechanisms of carcinogenesis associated with *IDH1/2* mutations and facilitate the development of new therapies to tumors carrying these mutations.

4. Elucidation of genetic characteristics of human tumors and mechanisms of their development

Kiyoko Takane, Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Kotoe Katayama[1], Seiya Imoto[1], Rui Yamaguchi[2], Satoru Miyano[3]: [1]Division of Health Medical Intelligence, Human Genome Center, IMSUT, [2]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute, [3]Systems Biology for Intractable Diseases, Medical Research Institute, Tokyo Medical and Dental University

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

Recently, we performed DNA methylome analysis of 15 clinical PMPs and 10 non-cancerous samples using Infinium 850K BeadChip. After merging two cases from the GEO database into the methylome data, we stratified PMPs by hierarchical clustering analysis. PMP was stratified into two epigenotypes: high-methylation and normal like-methylation. High-methylation PMP neoplasms were significantly correlated with *KRAS* mutations, but not *GNAS* mutation. To comprehensively understand genetic alterations in PMP, we extensively analyze PMP tumors and matched normal colonic mucosa by the whole genome sequencing and RNA sequencing. Analysis of these data will facilitate the discovery of biomarkers of PMP, selection of effective anti-cancer drugs, and personalized medical care.

5. Clinical sequencing for the implementation of genomic medicine

Kiyoko Takane, Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Eigo Shimizu[1], Rika Kasajima[1], Seiya Imoto[1], Tetsuo Shibuya[2], Kazuaki Yokoyama[3], Arinobu Tojo[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]Division of Molecular Therapy, [4]Project Division of International Advanced Medical Research, Advanced Clinical Research Center, IMSUT, [5]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute, [6]Systems Biology for Intractable Diseases, Medical Research Institute, Tokyo Medical and Dental University

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

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

tary nonpolyposis colorectal cancer syndrome (HNPCC), is the most common cause of hereditary colon cancer. Germline variants in the mismatch repair (MMR) genes are responsible for the disease. In the collaborative study with the Japanese Society for Colorectal Cancer (JSCCR), we have previously identified substantial number of structural variations (SVs) in the MMR genes. Since detection of SVs using short-read NGS is a challenging work, we took advantage of Oxford Nanopore MinION, a long read-sequencer, and tested the efficacy of long read-sequencing coupled with target enrichment for the detection of SVs. Application of this system successfully identified all SVs tested including large deletions and duplication. These data suggest that long read-sequencing will be help the identification of pathogenic SVs in patients with hereditary diseases.

In addition, we have been working on the implementation of genomic data in clinics. We opened an outpatient clinic in IMSUT hospital for the consultation of patients with rare or intractable cancer. Patients who visited this clinic and gave 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 IBM Watson for Genomics (WfG). The results of WfG including predicted driver mutations and suggested actionable drugs were discussed in the Tumor Board meeting of this project, which is held every two weeks.

Publications

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Advanced Clinical Research Center

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

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任準教授	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

Our Laboratory is focused on developing oncolytic virus therapies for various malignant tumors. Oncolytic viruses are engineered to kill tumor cells without affecting normal cells. G47Δ, a recombinant, triple-mutated oncolytic herpes simplex virus type 1 (HSV-1), exhibits potent anti-tumor efficacy while maintaining safety. Three clinical trials using G47Δ and one using IL-12 expressing virus (T-hIL12) have been conducted at IMSUT Hospital.

Development of novel recombinant oncolytic HSV-1

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

Translational research of G47Δ was performed totally by this laboratory, including invention, preclinical tests, clinical lot manufacturing and clinical trials since 2003. It is expected that G47Δ be approved as the world's first oncolytic virus product for malignant brain tumors in 2021. Besides malignant brain tumors, we have meticulously accumulated pre-clinical data with the intention to expand the application of G47Δ for other cancers, including renal cancer, prostate cancer, bladder cancer, malignant mesothelioma, tongue cancer, esophageal cancer, gastric cancer, colon cancer, lung cancer, breast cancer, nasopharyngeal cancer, cholangiocarcinoma, hepatic cancer, pancreatic cancer, malignant melanoma, and malignant lymphoma. Preclinical research has revealed that G47Δ is universally effective for all types of solid tumors, and is expected as an innovative treatment for cancers in the near future. Human IL-12-expressing G47Δ (T-hIL12) has been developed and a joint clinical trial with Shinshu University has started in January 2020 in patients with melanoma.

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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

Division of Advanced Medicine Promotion was established in 2011. Our mission is to assist the clinical development and the conduct of clinical trials, especially for translational research. For this purpose, it is critical to discover new "seeds" and to eradicate blockades until the clinical utilization. In this sense, our role is the translation from the results of basic research to the conduct of clinical trials at IMSUT Hospital. At IMSUT Hospital, we work together with staffs of Center for Translational Research. Concurrently, to concur blockades on translational research, we have been engage in research on regulatory science and biostatistics.

1. Assistance of Clinical Trials/TRs at Research Hospital

Minako Kouno, Riyo Owada,, Masanori Nojima, Fumitaka Nagamura

At IMSUT Hospital, we work together with staffs of Center for Translational Research. The assistance of Translational (Clinical) Research Coordinators is indispensable for the conduct of clinical trials, especially for TR. The activities of Coordinators are results of the collaboration between Division of Advanced Medicine Promotion and Center for Translational Research. In 2020, we supported 4 sponsor-investigator clinical trials and 3 non-IND clinical studies.

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

Miwako Okada, Fumitaka Nagamura

Ministry of Education, Culture, Sports, Science and Technology launched "Translational Research Network Program" to promote translational research based on the results of basic science at academia. This grogram was transferred to Japan Agency for Medical Research and Development in 2015 and has been expected to support TRs from basic science to seek obtaining intellectual property to early stage of clinical trial. In 2020, we supported 23 basic researches (11: other than IMSUT), 14 preclinical studies (5: other than IMSUT), and 12 clinical studies (3: other than IMSUT). The number of studies we assist has been increasing year by year. Organization reinforcement is the urgent problem.

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

Masanori Nojima

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

4. Statistical consulting for basic research

Masanori Nojima

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

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Advanced Clinical Research Center

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

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

The goals of our researches are to identify the mechanisms and to establish novel therapies especially for cancers and inflammatory diseases of the digestive system. One of the research fields is the inflammatory diseases, in which we investigated the molecular pathogenesis of gastritis, cholangitis and inflammatory bowel disease. Another research field is the malignancies. Using genetically engineered mice, we have elucidated the carcinogenic mechanisms driven by gene mutations.

1. Role of IL-33 in the gastrointestinal homeostasis

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

Our previous study unveiled the important role of IL-33 in the carcinogenesis of bile duct. To investigate the role of IL-33 in intestinal tract, we have established conditional IL-33 expression mouse (LSL-IL-33 mouse) and crossed with GI-tract specific cre expression mice line. Stomach specific IL-33 expression (TFF1-LSL-IL33, Mist1creERT-LSL-IL33) induced gastritis characterized by inflammatory cell infiltration not only in the lamina propria, but also in the muscular layer and serosa. Gastric epithelial cells also showed loss of parietal cells and chief cells indicating gastric atrophy with metaplasia. In clear contrast, intestine specific IL-33 expression (villin-LSL-IL33) did not exhibit inflammatory phenotype. We are currently investigating the characteristics and the mechanism of IL-33 mediated gastrointestinal diseases.

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

Yoshihiro Hirata

Squamo-columnar junction (SCJ) is one of the transitional zones in body where two different cell types merge. The origin of SCJ tumors and the process of tumorigenesis are largely unknown. We have established a mouse model in which invasive tumors developed specifically at gastric SCJ. We try to identify cancer initiating cells as well as stem cells specific to gastric SCJ.

3. Role of acetylcholine signaling in the inflammatory bowel diseases

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

Intestinal homeostasis is governed by multiple factors of host and luminal contents like microbes and food antigens. However, little is known about the role of hormonal and neural responses in the development of IBD. We examined the role of acetylcholine signaling using murine colitis model. Administration of nicotine, a ligand of nicotinic acetylcholine receptor, to IL-10 knockout mice showed reduced inflammatory cell infiltration in the colonic mucosa and retained goblet cells. Intestinal organoids underwent

cystic ballooning morphological changes after co-culture with pathogenic dendritic cells, which are attenuated by the treatment of nicotine, but this nicotine induced phenotype was absent in organoids co-cultured with dendritic cells (DCs) from alpha7nicotinic acetylcholine receptor KO mice. IL-10 KO mice with DC-specific alpha7nicotinic acetylcholine receptor deletion exhibited more severe colitis than IL-10 KO mice indicating the importance of acetylcholine signaling on DCs.

4. Mechanism of gastric metaplasia and cancer development

Masahiro Hata[1], Mayo Tsuboi[1], Hiroto Kinoshita, Yoku Hayakawa, Yuki Hayata[1], Hayato Nakagawa, Yoshihiro Hirata, Kazuhiko Koike.

Gastric metaplasia is recognized as a precursor of intestinal type gastric cancer. Its origin and pathogenesis is not defined so far. To examine the molecules involved in gastric metaplasia, we developed stomach epithelial cell specific genetic modification mouse (TFF1-cre mouse), which enabled gene manipulation predominantly in gastric pit cell lineage. Kras activation or Pten inactivation in gastric pit cell lineage leads to foveolar hyperplasia and metaplastic change of gastric gland. When cdh1 gene encoding E-cadherin is inactivated in gastric pit cells, transient signet ring cell formation and spontaneous pit cell shedding in gastric gland lead to metaplastic squamous cell expansion from squamo-columnar junction.

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

Yoshihiro Hirata, Hisayoshi Natomi, Hayato Nakagawa, Kazuhiko Koike

Primary sclerosing cholangitis is a rare form of biliary inflammation which can progress to cirrhosis and cancer. The cause of the disease is not clarified so far. Especially, the life style factors which affect the severity and the progression of biliary diseases are not well understood, and the mechanisms of disease modification are not clarified. We are currently investigating the effects of life style factors, such as smoking and obesity, as well as the role of intestinal microflora, on cholangitis and biliary cancer using originally developed mouse biliary disease models.

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

Ryosuke Muroyama, Naoya Kato, Yoshihiro Hirata

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

Publications

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Advanced Clinical Research Center

Division of Bioethics
生命倫理研究分野

Associate Professor Ayako Kamisato, Ph.D.

准教授　博士(法学)　神　里　彩　子

Division of Bioethics is a laboratory established in 2017.
New ethical, legal and social issues (ELSI) may occur when conducting advanced clinical research or clinical practice. In our laboratory, we study about how and what decisions should be made by a nation, society, or individual when such issues arise.

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

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo

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

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

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

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

Currently, we have 1635 members and 629 institutions registered with our program as of 15 January,

2021. We constantly assess our programs through questionnaires to improve each program. We have consistently received high scores from our users.

2. The REC Education program for Researchers

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo

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

- Module 1. Clinical Research Act-What kind of research is subject to Clinical Research Act?
- Module 2. Clinical Research Act-Procedures for conducting specific clinical research.

3. Large scale survey of medical doctors on public understanding of medical research terms

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo

In 2019, we conducted an Internet survey to understand the public's "recognition rate" and "understanding rate" of basic 12 medical research terms, such as "informed consent" "clinical trial" "cohort study" "intervention study" "double blind randomized trial". The results revealed that most of these medical research terms had a low recognition rate and also that the understanding rate was very low, in general.

Based on this result, we conducted an Internet survey of medical doctors in 2020 to investigate how doctors predict the general public understanding on medical research terms. This survey found that doctors predict that the general public have a low level of understanding.

4. Survey on ordinances of fetal appendage "Ena"

Ayako Kamisato, Hong Hyunsoo

Recently, fetal appendages are attracting attention as raw materials for regenerative medicine products. However, some areas have special ordinances called "Ena ordinance" that regulate the handling of fetal appendages. We identified where these special ordinances were in place. Then, in order to understand whether the fetal appendages can be used in regenerative medicine products, we conducted interview survey with the persons in charge of each local government.

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

Ayako Kamisato

As the infection with the new coronavirus spreads, it is expected that patients who need to use a ventilator or ECMO will be triaged. Dr.Kamisato conducted an Internet survey to see the public's understanding and attitude on triage.

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

Ayako Kamisato

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

In addition, she is a member of the Council on clinical use of using genomic editing technology for human fertilized embryos set by the Ministry of Health, Labour and Welfare Ministry (MHLW) and she recommended that a law be enacted.

7. Production of Common IC Form for "Center of Healthy Aging Innovation project"

Ayako Kamisato, Kazuyo Arisawa

"Center of Healthy Aging Innovation project" promoted by Hirosaki University is one of the projects of JST Center of Innovation (COI) Program. One goal of this project is to build a platform of big data on medical and health. In order to achieve this goal, it is necessary to integrate data obtained from multiple cohort studies. To accelerate data integration, we support researchers on making ethical review documents.

Publication

・神里彩子「第6章　臨床研究のルールと手続き」有田悦子・足立智孝編集『薬学人のための事例で学ぶ倫理学』（南江堂，2020年）
・神里彩子，吉田幸恵.「医学研究用語に対する一般市民の認知度・理解度調査 ― インターネット調査結果からの考察　―」臨床薬理第51巻第4号pp.187-198（2020年7月）
・神里彩子・長村文孝・洪賢秀・長村登紀子「再生医療等製品への周産期付属物の利用と胞衣条例」再生医療vol.19,　No.4（メディカルレビュー社，2020年11月）
・神里彩子「胞衣取扱条例からみる周産期付属物（胞衣）の価値」医学のあゆみ274巻7号（医歯薬出版株式会社，2020年8月）
・神里彩子「「ヒト受精胚に遺伝情報改変技術等を用いる研究に関する倫理指針」の概要とヒト受精胚研究に関する制度設計」医事法研究第2号（甲斐克則責任編集，信山社，2020年6月）
・神里彩子「臨床研究法」周産期医学（特集　知っておきたい周産期にかかわる法律・制度）Vol.50, No.1（東京医学社）2020年1月）

Advanced Clinical Research Center

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

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

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

1. Introduction

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

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

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

See NO17-9 (Department of Surgery, IMSUT Hospital).

3. Making Evidence for gastrointestinal malignancy

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

4. Publications (as a member of IMSUT)

Kudose Y, Ahiko Y, Shida D.

Response to: Comment on "Evaluation of Recurrence Risk After Curative Resection for Patients With Stage I to III Colorectal Cancer Using the Hazard Function: Retrospective Analysis of a Single-institution Large Cohort".

Ann Surg. 2020 Dec 18;Publish Ahead of Print. doi: 10.1097/SLA.0000000000004686. Online ahead of print.

Takamizawa Y, Shida D, Boku N, Nakamura Y, Ahiko Y, Yoshida T, Tanabe T, Takashima A, Kanemitsu Y.

Nutritional and inflammatory measures predict survival of patients with stage IV colorectal cancer.

BMC Cancer. 2020 Nov 11;20(1):1092. doi: 10.1186/s12885-020-07560-3.

Yasui K, Shida D, Nakamura Y, Ahiko Y, Tsukamoto S, Kanemitsu Y.

Postoperative, but not preoperative, inflammation-based prognostic markers are prognostic factors in stage III colorectal cancer patients.

Br J Cancer. 2020 Dec 1. doi: 10.1038/s41416-020-01189-6. Online ahead of print.

Center for Stem Cell Biology and Regenerative Medicine

Division of Regenerative Medicine
再生医学分野

Professor	Hideki Taniguchi, MD, PhD		教　授	博士(医学)	谷　口　英　樹
Project Associate Professor	Tomoyuki Yamaguchi, PhD		特任准教授	博士(医学)	山　口　智　之
Assistant Professor	Yun-Zhong Nie, PhD		助　教	博士(医学)	聶　　運　中
Project Assistant Professor	Yasuharu Ueno, PhD		特任助教	博士(医学)	上　野　康　晴

For patients with end-stage organ failure, organ transplantation is the only effective treatment; however, the scarcity of transplantable organs hinders this treatment for most patients. Recently, regenerative medicine on the generation of transplantable organs has attracted much attention. Regenerative medicine is a challenging scientific field that converts the pioneering knowledge of developmental biology and stem cell biology to clinical application. Our laboratory is developing a novel therapeutic strategy to substitute organ transplantation. We have established novel organoid culture technologies to reconstruct human organs from stem cells, including human induced pluripotent stem cells (iPSCs). Currently, we try to realize transplantation of human liver primordia (liver buds [LBs]) generated from iPSCs to treat liver diseases, such as metabolic disease and liver failure. Moreover, we also expand the established technologies to reconstruct artificial refractory cancer tissue (cancer organoid) with a tumor microenvironment. Based on this unique cancer organoid, we develop a new drug-screening platform to discover potential compounds that prevent cancer relapse and metastasis.

1. Development of markers for detecting undifferentiated cells during human iPSC-LB production

Keisuke Sekine[1], Yasuharu Ueno[1], Yoshiki Kuse[1], Syusaku Tsuzuki[1], Eriko Kanai[1], Takashi Okumura[1], Yumi Horie[1], Toshiharu Kasai[1], Shinya Matsumoto[1] and Hideki Taniguchi[1,2]: [1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, [2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Organ transplantation is the only curative method for treating end-stage organ failure. Over the past decade, the demand for organ transplantation has increased worldwide because of the increased incidence of organ failure. However, a critical shortage of donor organs has highlighted the urgent need to generate organs from human induced pluripotent stem cells (iPSCs). Human iPSCs derived liver bud (iPSC-LB) is a potential solution to the ongoing donor-organ shortage in liver transplantation. Notably, the production of iPSC-LBs for clinical application has to follow the national guidelines strictly. Here, we developed chemically defined, animal origin-free (CD-AOF) media under BSE/TSE guideline (EMEA/410/01 Rev.3) for iPSC-LBs production with the collaboration of Ajinomoto Co., Inc. (Sekine K, Taniguchi H, et al., Sci Rep. 21; 10 (1): 17937, 2020.). Meanwhile, to ensure therapeutic safety, it is essential to establish a method of evaluating the frequency of undifferentiated iPSCs in iPSC-LBs. *LIN28A* has been reported as a potential marker for evaluating the residual undifferentiated iPSCs during cellular differentiation. However, he-

patic endoderm cells differentiated from iPSCs express *LIN28A* at a high level, making it impossible to detect the residual undifferentiated iPSCs in hepatic endoderm cells. Therefore, we searched for markers for detecting undifferentiated cells during the differentiation of hepatic endoderm cells from hiPSCs and extracted three promising markers (Sekine K, Taniguchi H, et al., Sci Rep. 10(1):10293. 2020. Patent application 2018-115025). In collaboration with EIKEN CHEMICAL CO., LTD. (Manuscript in preparation. Patent application 2019-207004, 2019-207002), we also established the high-precision detection method (Loop-mediated isothermal amplification: LAMP method) for those markers under the serious nucleic acid contamination (world's highest detection sensitivity 0.0001%). This cell evaluation technique with the extracted markers is considered useful for the safe clinical application of iPSC-LBs.

2. Therapeutic effects of human iPSC-LB transplantation for urea cycle disorders

Yasuharu Ueno[1], Tomoyuki Yamaguchi[1], Yoshiki Kuse[1], Takashi Okumura[1], Yumi Horie[1], Toshiharu Kasai[1], Eriko Kanai[1], Syusaku Tsuzuki[1], Shinya Matsumoto[1], Satoshi Okamoto[2], Tomomi Tadokoro[2], Soichiro Murata[2] and Hideki Taniguchi[1,2]: [1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, [2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Urea cycle disorders are inherited metabolic deficiencies with hyperammonemia caused by a single gene defect of the urea cycle enzymes or transporters. Ornithine transcarbamylase (OTC) is a rate-limiting enzyme in the urea cycle, and OTC deficiency (OTCD) is the most common urea cycle disorder in humans. Unlike diffuse liver diseases, most urea cycle disorder patients show normal liver histology. Therefore, the transplantation of iPSC-LB with OTC activity might be an expected regenerative medicine technique for the treatment of OTCD. To establish a new therapy with human iPSC-LB transplantation for patients with urea cycle disorders, we are proceeding with verification of the effectiveness of iPSC-LB transplantation and preparation for clinical research. For the proof of principle (POC) experiment, we performed genome editing technology to establish NOG-OTC^{spf} mice, a severe OTCD immunodeficiency model with abnormally high blood ammonia and urinary orotic acid. The renal capsule transplantation of iPSC-LBs could exhibit hepatic functions in NOG-OTC^{spf} mice with an improvement of hyperammonemia, indicating the effective iPSC-LBs transplantation for OTCD. Currently, we are preparing to carry out clinical trials of OTCD.

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

Tomoyuki Yamaguchi[1], Yasuharu Ueno[1], Yoshiki Kuse[1], Kenta Takeuchi[1], Eriko Kanai[1], Takashi Okumura[1], Yumi Horie[1], Syusaku Tsuzuki[1], and Hideki Taniguchi[1,2]: [1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, [2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Early detection of pancreatic cancer is difficult because of poor characteristic clinical symptoms. Pancreatic cancer is highly recurrent and has a high metastasis rate, resulting in a poor prognosis. Based on the organ bud technology developed in our regenerative medicine studies, we used primary pancreatic cancer cells isolated from Japanese pancreatic cancer patients to create a human primary pancreatic cancer organoid that reproduces the pancreatic cancer microenvironment. In this year, we developed an improved method in generating pancreatic cancer organoids, which closely recapitulated the structure of clinical tissue compared to conventional organoids. These new pancreatic cancer organoids possess cancer cells with cyst structures, vascular networks, and organoids are rich in the stroma. Moreover, cancer cells in new organoids retain their proliferation ability as primary cancer cells and have cancer stem cell characteristics and epithelial-mesenchymal transition (EMT). The formation of tumor blood vessels is also observed in this organoid. To conclude, this novel cancer organoid has successfully improved in reproducing the characteristic of pancreatic cancer in vitro. Therefore, applying this new cancer organoid in therapeutic drug screening and biological analysis may play a vital role in contributing to the new findings.

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

Tomomi Tadokoro[2], Yoshiki Kuse[1], Yasuharu Ueno[1], Takashi Okumura[1], Tomoyuki Yamaguchi[1] and Hideki Taniguchi[1,2]: [1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, [2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Microgravity in orbit does not cause subsidence or convection and is considered advantageous in expanding cells in three-dimension. By utilizing this microgravity environment, we aim to develop a novel method for generating human iPSCs-derived liver tissue in collaboration with Japan Aerospace Explora-

tion Agency (JAXA). In detail, we attempt to establish the new technique of generating three-dimensional organs containing large blood vessels. We prepared human iPSCs derived liver buds (hiPSC-LBs) and artificial vessels on the earth. These were placed into the culture container and launched to the International Space Station "KIBO". We confirmed that hiPSC-LBs were successfully gathered onto the artificial vessel under microgravity, as *in silico* simulation suggested. After culturing hiPSC-LBs for a predetermined period in the incubator installed in "KIBO", samples are planning to transfer to the earth. We will compare the difference in their growth and differentiation with the ground control group. This finding will uncover the effects of gravity on cell growth and differentiation. We expect the research results obtained in space experiment contribute to the subsequent development and understanding: (1) Development of a new technique in human three-dimensional tissue preservation and transportation, which is crucial to the product under regenerative medicine before practical use. (2) Establishment of a novel technique in generating human organs joined with large blood vessels. (3) Development of a new three-dimensional culture device simulating the microgravity environment on the earth.

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

Yun-Zhong Nie[1], Xia Yang[1], Yang Li[1], Richie Plummer[1], Eriko Kanai[1], Hideki Taniguchi[1,2]: [1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, [2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

The development of new transplantable human livers from human induced pluripotent stem cells (iPSCs) is urgently needed due to the persistent profound shortage of transplantable donors to treat end-stage liver diseases. By mimicking the process of liver organogenesis, our lab has developed three-dimensional vascularized liver organoids (LOs) from iPSCs that can help to rescue the drug-induced lethal liver failure model in vivo. We are currently investigating the clinical application of the LOs for liver diseases, including inherited metabolic liver disease and liver cirrhosis; however, the established method for LO generation is not suitable for human-scale production because of the time-consuming, expensive, and high-risk of iPSC contamination. Here, we aim to develop an efficiently massive production system of LOs based on iPSC derived proliferative progenitors, including hepatoblasts, fetal hepatic stellate cells, and endothelial progenitors, and hope to find a promising, safe, and effective therapeutic strategy for liver diseases.

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Center for Stem Cell Biology and Regenerative Medicine

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

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

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

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

HSC functions, highlighting a key role for age-associated cell-intrinsic defects in HSC aging.

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

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

EZH1 and EZH2 are enzymatic components of polycomb repressive complex (PRC) 2, which catalyzes histone H3K27 tri- methylation (H3K27me3) to repress the transcription of PRC2 target genes. We previously reported that the hematopoietic cell-specific *Ezh2* deletion ($Ezh2_{\Delta/\Delta}$) induced a myelodysplastic syndrome (MDS)-like disease in mice. We herein demonstrated that severe PRC2 insufficiency induced by the deletion of one allele Ezh1 in Ezh2-deficient mice ($Ezh1_{+/-}Ezh2_{\Delta/\Delta}$) caused advanced dyserythropoiesis accompanied by a differentiation block and enhanced apoptosis in erythroblasts. p53, which is activated by impaired ribosome biogenesis in del(5q) MDS, was specifically activated in erythroblasts, but not in hematopoietic stem or progenitor cells in $Ezh1_{+/-}Ezh2_{\Delta/\Delta}$ mice. *Cdkn2a*, a major PRC2 target encoding $p19_{Arf,}$ which activates p53 by inhibiting MDM2 E3 ubiquitin ligase, was de-repressed in $Ezh1_{+/-}Ezh2_{\Delta/\Delta}$ erythroblasts. The deletion of *Cdkn2a* as well as p53 rescued dyserythropoiesis in $Ezh1_{+/-}Ezh2_{\Delta/\Delta}$ mice, indicating that PRC2 insufficiency caused p53-dependent dyserythropoiesis via the de-repression of Cdkn2a. Since PRC2 insufficiency is often involved in the pathogenesis of MDS, the present results suggest that p53-dependent dyserythropoiesis manifests in MDS in the setting of PRC2 insufficiency.

3. Efficacy of the novel tubulin polymerization inhibitor PTC-028 for myelodysplastic syndrome

Cheng Zhong[1], Kensuke Kayamori[1,2], Shuhei Koide[1], Daisuke Shinoda[1,3], Motohiko Oshima[1], Yaeko Nakajima-Takagi[1], Yurie Nagai[2], Naoya Mimura[4], Emiko Sakaida[2], Satoshi Yamazaki[5], Satoshi Iwano[6], Atsushi Miyawaki[6], Ryoji Ito[7], Kaoru Tohyama[8], Kiyoshi Yamaguchi[9], Yoichi Furukawa[9], William Lennox[10], Josephine Sheedy[10], Marla Weetall[10], Atsushi Iwama[1] : [1]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [2]Department of Endocrinology, Hematology and Gerontology, Chiba University Graduate School of Medicine, Chiba, Japan; [3]Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan; [4]Department of Transfusion Medicine and Cell Therapy, Chiba University Hospital, Chiba, Japan; [5]Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan; [6]Laboratory for Cell Function Dynamics, Center for Brain Science, RIKEN, Saitama, Japan; [7]Humanized Model Laboratory, Central Institute for Experimental Animals, Kanagawa, Japan; [8]Department of Laboratory Medicine, Kawasaki Medical School, Okayama, Japan; [9]Division of Clinical Genome Research, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [10]PTC Therapeutics, South Plainfield, NJ, USA

Monomer tubulin polymerize into microtubules, which are highly dynamic and play a critical role in mitosis. Therefore, microtubule dynamics are an important target for anti-cancer drugs. The inhibition of tubulin polymerization or de-polymerization was previously targeted and exhibited efficacy against solid tumors. The novel small molecule PTC596 directly binds tubulin, inhibits microtubule polymerization, down-regulates MCL-1, and induces p53-independent apoptosis in acute myeloid leukemia cells. We herein investigated the efficacy of PTC-028, a structural analog of PTC596, for myelodysplastic syndrome (MDS). PTC-028 suppressed growth and induced apoptosis in MDS cell lines. The efficacy of PTC028 in primary MDS samples was confirmed using cell proliferation assays. PTC-028 synergized with hypomethylating agents, such as decitabine and azacitidine, to inhibit growth and induce apoptosis in MDS cells. Mechanistically, a treatment with PTC-028 induced G2/M arrest followed by apoptotic cell death. We also assessed the efficacy of PTC-028 in a xenograft mouse model of MDS using the MDS cell line, MDS-L and the AkaBLI bioluminescence imaging system, which is composed of AkaLumine-HCl and Akaluc. PTC-028 prolonged the survival of mice in xenograft models. The present results suggest a chemotherapeutic strategy for MDS through the disruption of microtubule dynamics in combination with DNA hypomethylating agents.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Transplantation
幹細胞移植分野

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We are conducting clinical stem cell transplantation, especially using cord blood as a promising alternative donor for clinical use and investigating optimal strategies to obtain the best results in this area. We are also generating pre-clinical study to utilize virus-specific CTL for immune competent patients such as post-transplantation. Our goal is as allogeneic transplantation to be safer therapeutic option and to extend for older patients.

1. Impact of intestinal microbiota on reconstitution of circulating monocyte, dendritic cell,and natural killer cell subsets in adults undergoing single cord blood transplantation.

Konuma T[1, 2], Oiwa-Monna M[1], Mizusawa M[1], Isobe M[1, 2], Kato S[1, 2], Takahashi S[1, 2, 3], Tojo A[1, 2, 3]. : [1] Department of Hematology/Oncology, IMSUT Hospital, [2] Division of Molecular Therapy, [3] Division of Stem Cell Transplantation

The intestinal microbiota plays a fundamental role in the development of host innate immune cells, such as monocytes, dendritic cells (DCs), and natural killer (NK) cells. We examined the association between intestinal microbiota and subsequent immune reconstitution of circulating monocyte, DC, and NK cell subsets in 38 adult patients undergoing single-unit cord blood transplantation (CBT). A higher diversity of intestinal microbiota at 1 month was significantly associated with higher counts of plasmacytoid DCs at 7 months after CBT, as measured by the Chao1 index. Principal coordinate analysis of unweighted UniFrac distances showed significant differences between higher and lower classical monocyte reconstitution at 7 months post-CBT. The families Neisseriaceae, Burkholderiaceae, Propionibacteriaceae, and Coriobacteriaceae were increased in higher classical monocyte reconstitution at 7 months post-CBT, whereas the family Bacteroidaceae was increased in lower classical monocyte reconstitution at 7 months post-CBT. These data show that intestinal microbiota composition affects immune reconstitution of classical monocyte and plasmacytoid DCs following single-unit CBT.

2. Clinical outcomes of persistent colonization with multidrug-resistant Gram-negative rods is associated with non-relapse mortality in adult patients undergoing single-unit cord blood transplantation.

Mizusawa M[1], Konuma T[1, 2], Kato S[1, 2], Isobe M[1, 2], Shibata H[3], Suzuki M[3], Takahashi O[3], Oiwa-Monna M[2], Takahashi S[1,2, 4], Tojo A[1,2, 4].: [1] Department of Hematology/Oncology, IMSUT Hospital, [2] Division of Molecular Therapy, [3] Department of Laboratory Medicine, IMSUT Hospital, [4] Division of Stem Cell Transplantation

Severe bacterial infections are a serious problem after cord blood transplantation (CBT). Colonization with multidrug-resistant Gram-negative rods (MRGNR) is associated with increased morbidity and mortality after allogeneic hematopoietic cell transplantation. However, its impact on outcomes after

CBT is unclear. We aim to explore the impact of colonization with MRGNRs in adult patients undergoing CBT. We retrospectively analyzed 145 adult patients who received single-unit CBT in our institute. As a standard practice in our institute, all patients were screened for colonization with MRGNR by oral cavity swabs, urine, and stool specimens between the day of admission for CBT and the day of discharge or day 100 after CBT. There were 62 incidents of colonization with MRGNR in 52 patients, of which 25 involved Stenotrophomonas maltophilia, 19 multidrug-resistant Pseudomonas spp., and 18 extended-spectrum beta-lactamase-producing Enterobacteriaceae. On multivariate analysis, MRGNR persistence significantly affected increase in non-relapse mortality (NRM) (hazard ratio [HR], 8.96; 95% CI 1.85-43.46; P = 0.006) and the subsequent development of bloodstream infection due to MRGNR (HR 11.82; 95% CI 2.15-64.87; P = 0.004), but not MRGNR clearance, compared with non-colonized patients. These data suggest that persistent colonization with MRGNR is significantly associated with higher NRM in CBT for adults.

3. Effects of Acute and Chronic Graft-versus-myelodysplastic Syndrome on Long-term Outcomes Following Allogeneic Hematopoietic Cell Transplantation

Konuma T[1,2], and Adult Myelodysplastic Syndrome Working Group of the Japan Society for Hematopoietic Cell Transplantation. : [1] Department of Hematology/Oncology, IMSUT Hospital, [2] Division of Molecular Therapy

The major limitation of cord blood transplantation (CBT) for adults remains the delayed hematopoietic recovery and higher incidence of graft failure, which result in a higher risk of early mortality in CBT. We evaluated early overall survival (OS), non-relapse mortality (NRM), neutrophil engraftment, acute graft-vs-host disease, and cause of early death among 9678 adult patients who received single-unit CBT in Japan between 1998 and 2017. The probability of OS at 100 days was 64.4%, 71.7%, and 78.9% for the periods 1998 to 2007, 2008 to 2012, and 2013 to 2017, respectively (P < .001). The cumulative incidences of NRM at 100 days during the same period were 28.3%, 20.8%, and 14.6%, respectively (P < .001). The cumulative incidences of neutrophil engraftment were also improved during the same period (P < .001). The most common cause of death within 100 days after CBT was bacterial infection in 1998 to 2007 and primary disease in the latter two time periods. Across the three time periods, the proportions of deaths from bacterial and fungal infection, graft failure, hemorrhage, sinusoidal obstructive syndrome, and organ failure decreased in a stepwise fashion. Landmark analysis of OS and NRM after 100 days showed that OS did not change over time in the multivariate analysis. Our registry-based data demonstrated a significant improvement of early OS after CBT for adults over the past 20 years. The landmark analysis suggested that improvement of early mortality could lead to an improvement of long-term OS after CBT.

4. Impact of a prior history of cancer on prognosis after myeloablative single-unit cord blood transplantation

Okabe M[1], Konuma T[1, 2], Oiwa-Monna M[2], Isobe M[1, 2], Kato S[1, 2], Takahashi S[1, 2, 3], Tojo A[1, 2, 3]. : [1] Department of Hematology/Oncology, IMSUT Hospital, [2] Division of Molecular Therapy, [3] Division of Stem Cell Transplantation

A prior history of cancer was associated with higher non-relapse mortality or overall mortality in patients undergoing allogeneic haematopoietic cell transplantation. Because it is unclear whether the outcomes after cord blood transplantation are influenced by a prior history of cancer, we retrospectively assessed the prevalence and prognostic impact of a prior history of cancer in adult patients undergoing myeloablative single-unit cord blood transplantation in our institute between 2004 and 2020. The univariate analysis showed that a prior history of cancer did not affect the probability of overall survival; the cumulative incidence of relapse; or non-relapse mortality. In the multivariate analysis, prior history of cancer was not associated with overall mortality, relapse or non-relapse mortality. No patients with a prior history of cancer had experienced prior cancer relapse. A prior history of cancer was not associated with non-relapse mortality or overall mortality following single-unit cord blood transplantation.

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Center for Stem Cell Biology and Regenerative Medicine

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

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

教 授 医学博士 北 村 俊 雄

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

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

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

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

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

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

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

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

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

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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

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Stem cells represent a valuable cell source in the field of regenerative medicine. Hematopoietic stem cells represent a valuable cell source for transplantation medicine, whereas pluripotent stem cells are newly emerging types of stem cells that have been utilized either for the basic research or to develop curative treatment for various diseases. We have been focusing especially on the utilization of induced pluripotent stem cells as a research platform to elucidate pathophysiology of intractable diseases based on their proper modeling. Our goal is to establish safe and efficacious treatment for the patients suffering from various types of devastating diseases with no curative treatment currently available

Elucidation of pathophysiology and treatment of RAS-associated autoimmune lymphoproliferative syndrome-like disease (RALD)

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RAS-associated autoimmune lymphoproliferative syndrome-like disease (RALD) is a rare genetic disorder of the immune system. RALD is characterized by lymphadenopathy, splenomegaly, autoimmunity, and occasional abnormalities in myeloid cell compartments such as monocytes. RALD shares many features with autoimmune lymphoproliferative syndrome, for which a somatic oncogenic mutation in NRAS or KRAS is found to play a causal role. *RAS* is the most commonly mutated oncogene found in various types of cancers. How the mutant RAS contributes to oncogenesis remains unclear amongst the complex genetic landscape of cancer. This issue has been commonly discussed in solid tumors, but many studies show that oncogenic RAS also has an important role in blood cancers. In this project, we have succeeded in generation of induced pluripotent stem (iPS) cells from bone marrow of RALD patients. Reflecting a nature of somatic mutations, we obtained not only a group of iPS cells harboring the genomic change compatible with G13C-KRAS, but also those having intact *KRAS* gene. Having these isogenic sets of patient-iPS cells, we sought to elucidate the effects of this mutation, and to develop a new treatment modality for RALD. Recently, some progress has been made for direct targeting of the mutant RAS protein, although it has only been successful for the G12C isoform. Considering the difficulty and certain disadvantages in direct targeting of G13C-KRAS, we are aiming at development of the way to specifically target mutant cells that have developed unique biological characteristics as a result of the mutation. Identification of therapeutic targets uniquely equipped to the mutant cells would lead to discovery of drugs effective for RALD patients. Since the mutant clones in patients may easily develop drug resistance over time when only a single inhibitor is used, we are taking an approach toward establishment of combination therapy. The results from this project may be applied to improve the treatment of not only RALD, but also

other types of cancers, of which RAS mutations are found causative. We are now in the process of expanding a series of RALD-related iPS cells, using genome-editing technologies. To accelerate the overall drug screening processes, modification of a protocol is also underway to facilitate large-scale differentiation from iPS cells to hematopoietic cells. Having identified several inhibitors possibly usable in combination therapy, the effects of each candidate will be tested in our iPS cell-based study platform, of which results can hopefully culminate in discovery of ideal treatment strategies for RAS-associated intractable disorders.

Center for Stem Cell Biology and Regenerative Medicine

Division of Experimental Pathology
幹細胞病理学分野

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

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

1. Identification of distinct loci for de novo DNA methylation by DNMT3A and DNMT3B during mammalian development

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De novo establishment of DNA methylation is accomplished by DNMT3A and DNMT3B. Here, we analyze de novo DNA methylation in mouse embryonic fibroblasts (2i-MEFs) derived from DNA-hypomethylated 2i/L ES cells with genetic ablation of *Dnmt3a* or *Dnmt3b*. We identify 355 and 333 uniquely unmethylated genes in *Dnmt3a* and *Dnmt3b* knockout (KO) 2i-MEFs, respectively. We find that *Dnmt3a* is exclusively required for de novo methylation at both TSS regions and gene bodies of Polycomb group (PcG) target developmental genes, while *Dnmt3b* has a dominant role on the X chromosome. Consistent with this, tissue-specific DNA methylation at PcG target genes is substantially reduced in *Dnmt3a* KO embryos. Finally, we find that human patients with *DNMT3* mutations exhibit reduced DNA methylation at regions that are hypomethylated in *Dnmt3* KO 2i-MEFs. In conclusion, here we report a set of unique de novo DNA methylation target sites for both DNMT3 enzymes during mammalian development that overlap with hypomethylated sites in human patients.

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

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

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Center for Stem Cell Biology and Regenerative Medicine

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

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

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

1. Long-term ex vivo expansion of mouse hematopoietic stem cells.

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Utilizing multipotent and self-renewing capabilities, hematopoietic stem cells (HSCs) can maintain hematopoiesis throughout life. However, the mechanism behind such remarkable abilities remains undiscovered, at least in part because of the paucity of HSCs and the modest ex vivo expansion of HSCs in media that contain poorly defined albumin supplements such as bovine serum albumin. Here, we describe a simple platform for the expansion of functional mouse HSCs ex vivo for >1 month under fully defined albumin-free conditions. The culture system affords 236- to 899-fold expansion over the course of a month and is also amenable to clonal analysis of HSC heterogeneity. The large numbers of expanded HSCs enable HSC transplantation into nonconditioned recipients, which is otherwise not routinely feasible because of the large numbers of HSCs required. This protocol therefore provides a powerful approach with which to interrogate HSC self-renewal and lineage commitment and, more broadly, to study and characterize the hematopoietic and immune systems.

2. Activated HoxB4-induced hematopoietic stem cells from murine pluripotent stem cells via long-term programming

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Hematopoietic stem cells (HSCs) are multipotent cells that form the entire blood system and have the potential to cure several pathogenic conditions directly or indirectly arising from defects within the HSC compartment. Pluripotent stem cells (PSCs) or induced pluripotent stem cells (iPSCs) can give rise to all embryonic cell types; however, efficient in vitro differentiation of HSCs from PSCs remains challenging. HoxB4 is a key regulator orchestrating the differentiation of PSCs into all cells types across the mesodermal lineage, including HSCs. Moreover, the ectopic expression of HoxB4 enhances the in vitro generation and expansion of HSCs. However, several aspects of HoxB4 biology including its regulatory functions are not fully understood. Here, we describe the role of HoxB4 in indirectly inhibiting the emergence of mature $CD45^+$ HSCs from iPSCs in vitro. Forced activation of HoxB4 permitted long-term maintenance of functional hematopoietic stem and progenitor cells (HSPCs), which efficiently reconstituted hematopoiesis upon transplantation. Our method enables an easy and scalable in vitro platform for the generation of HSCs from iPSCs, which will ultimately lead to a better understanding of HSC biology and facilitate preparation of the roadma for producing an unrestricted supply of HSCs for several curative therapies.

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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 state-of-art flow cytometry (FCM) services for internal and external researcher. We offer assistance in the following areas, (1) initial project planning (2) antibody panel design and optimization (3) instrument operation and maintenance (4) data analysis.

Instruments at the FACS Core Laboratory

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

FCM usage performance in 2020

FCM analysis and sorting is performed either by the FACS Core staff or by trained users. There were about 2,500 cases of FCM use in 2020.

Seminar and Training

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

International Research Center for Infectious Diseases

Department of Special Pathogens
高病原性感染症系

Professor Yoshihiro Kawaoka, D.V.M., Ph.D.

教 授 獣医学博士 河 岡 義 裕

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

Multicolor two-photon imaging of in vivo cellular pathophysiology upon influenza virus infection using the two-photon IMPRESS.

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In vivo two-photon imaging is a valuable technique for studies of viral pathogenesis and host responses to infection in vivo. In this protocol, we describe a methodology for analyzing influenza virus–infected lung in vivo by two-photon imaging microscopy. We describe the surgical procedure, how to stabilize the lung, and an approach to analyzing the data. Further, we provide a database of fluorescent dyes, antibodies, and reporter mouse lines that can be used in combination with a reporter influenza virus (Color-flu) for multicolor analysis. Setup of this model typically takes ~30 min and enables the observation of influenza virus–infected lungs for >4 h during the acute phase of the inflammation and at least 1 h in the lethal phase. This imaging system, which we termed two-photon IMPRESS (imaging pathophysiology research system), is broadly applicable to analyses of other respiratory pathogens and reveals disease progression at the cellular level in vivo.

G Protein Pathway Suppressor 1 Promotes Influenza Virus Polymerase Activity by Activating the NF-κB Signaling Pathway

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Influenza virus relies heavily on cellular machinery to replicate in host cells. Therefore, to better understand the influenza virus life cycle, it is important to identify which host proteins are involved and how they function in virus replication. Previously, we identified G protein pathway suppressor 1 (GPS1) to be a matrix protein 2 (M2)-interacting host protein. GPS1 is a component of the COP9 signalosome, which regulates the NF-κB signaling pathway. Here, we found that the downregulation of GPS1 expression reduced influenza virus replication by more than 2 log units. Although GPS1 was not involved in the early and late stages of virus replication, such as viral entry, uncoating, assembly, or budding, we found that viral polymerase activity was impaired in GPS1-downregulated cells. Moreover, our results suggest that M2 activates the NF-κB signaling pathway in a GPS1-dependent manner and that activation

of NF-κB signaling leads to the upregulation of influenza virus polymerase activity. Our findings indicate that GPS1 is involved in the transcription and replication of influenza virus genomic RNA through the activation of the NF-κB signaling pathway.

Influenza A variants with reduced susceptibility to baloxavir isolated from Japanese patients are fit and transmit through respiratory droplets.

Imai M, Yamashita M, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Kiso M, Murakami J, Yasuhara A, Takada K, Ito M, Nakajima N[1], Takahashi K[1], Lopes TJS, Dutta J, Khan Z[2], Kriti D[2], van Bakel H[2], Tokita A[3], Hagiwara H[4], Izumida N[5], Kuroki H[6], Nishino T[7], Wada N[8], Koga M[9], Adachi E[10], Jubishi D, Hasegawa H[1,11], Kawaoka Y. : [1]Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan; [2]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [3]Clinic Bambini, Tokyo, Japan; [4]Hagiwara Clinic, Tokyo, Japan; [5]Akebonocho Clinic, Tokyo, Japan; [6]Sotobo Children's Clinic, Chiba, Japan; [7]Alpaca Kids Ent Clinic, Tokyo, Japan; [8]Wada Pediatric Clinic, Tokyo, Japan; [9]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [10]Department of Infectious Diseases and Applied Immunology, IMSUT Hospital of the Institute of Medical Science, University of Tokyo, Tokyo, Japan; [11]Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo, Japan

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International Research Center for Infectious Diseases

Department of Infectious Disease Control
感染制御系

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

1. Role of phosphatidylethanolamine biosynthesis in herpes simplex virus 1-infected cells on progeny virus morphogenesis in the cytoplasm and on viral pathogenicity in vivo

Jun Arii[1], Ayano Fukui, Yuta Shimanaka[2], Nozomu Kono[2], Hiroyuki Arai[2], Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasuko Mori[1], Yasushi Kawaguchi: [1] Division of Clinical Virology, Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, Hyogo [2]Laboratory of Health Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo

Glycerophospholipids are major components of cell membranes. Phosphatidylethanolamine (PE) is a glycerophospholipid that is involved in multiple cellular processes, such as membrane fusion, the cell cycle, autophagy, and apoptosis. In this study, we investigated the role of PE biosynthesis in herpes simplex virus 1 (HSV-1) infection by knocking out the host cell gene encoding phosphate cytidylyltransferase 2, ethanolamine (Pcyt2), which is a key rate-limiting enzyme in one of the two major pathways for PE biosynthesis. Pcyt2 knockout reduced HSV-1 replication and caused an accumulation of unenveloped and partially enveloped nucleocapsids in the cytoplasm of an HSV-1-infected cell culture. A similar phenotype was observed when infected cells were treated with meclizine, which is an inhibitor of Pcyt2. In addition, treatment of HSV-1-infected mice with meclizine significantly reduced HSV-1 replication in the mouse brains and improved their survival rates. These results indicated that PE biosynthesis mediated by Pcyt2 was required for efficient HSV-1 envelopment in the cytoplasm of infected cells and for viral replication and pathogenicity in vivo. The results also identified the PE biosynthetic pathway as a possible novel target for antiviral therapy of HSV-associated diseases and raised an interesting possibility for meclizine repositioning for treatment of these diseases, since it is an over-the-counter drug that has been used for decades against nausea and vertigo in motion sickness.

2. Phosphoregulation of a conserved herpesvirus tegument protein by a virally encoded protein kinase in viral pathogenicity and potential linkage between its evolution and viral phylogeny

Misato Shibazaki, Akihisa Kato, Kosuke Takeshi-

ma, Jumpei Ito[1], Mai Suganami[1], Naoto Koyanagi, Yuhei Maruzuru, Kei Sato[1], Yasushi Kawaguchi: [1]Division of Systems Virology, Department of Infectious Disease Control, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo

Us3 proteins of herpes simplex virus 1 (HSV-1) and HSV-2 are multifunctional serine-threonine protein kinases. Here, we identified an HSV-2 tegument protein, UL7, as a novel physiological substrate of HSV-2 Us3. Mutations in HSV-2 UL7, which precluded Us3 phosphorylation of the viral protein, significantly reduced mortality, viral replication in the vagina, and development of vaginal disease in mice following vaginal infection. These results indicated that Us3 phosphorylation of UL7 in HSV-2 was required for efficient viral replication and pathogenicity in vivo. Of note, this phosphorylation was conserved in UL7 of chimpanzee herpesvirus (ChHV), which phylogenetically forms a monophyletic group with HSV-2 and the resurrected last common ancestral UL7 for HSV-2 and ChHV. In contrast, the phosphorylation was not conserved in UL7s of HSV-1, which belongs to a sister clade of the monophyletic group, the resurrected last common ancestor for HSV-1, HSV-2, and ChHV, and other members of the genus Simplexvirus that are phylogenetically close to these viruses. Thus, evolution of Us3 phosphorylation of UL7 coincided with the phylogeny of simplex viruses. Furthermore, artificially induced Us3 phosphorylation of UL7 in HSV-1, in contrast to phosphorylation in HSV-2, had no effect on viral replication and pathogenicity in mice. Our results suggest that HSV-2 and ChHV have acquired and maintained Us3 phosphoregulation of UL7 during their evolution because the phosphoregulation had an impact on viral fitness in vivo, whereas most other simplex viruses have not because the phosphorylation was not necessary for efficient fitness of the viruses in vivo.

3. Identification of a Herpes Simplex Virus 1 Gene Encoding Neurovirulence Factor by Chemical Proteomics

Akihisa Kato, Shungo Adachi[1], Shuichi Kawano[2], Kousuke Takeshima, Mizuki Watanabe, Shinobu Kitazume[3], Ryota Sato[4], Hideo Kusano[1], Naoto Koyanagi, Yuhei Maruzuru, Jun Arii, Tomohisa Hatta[1], Tohru Natsume[1] & Yasushi Kawaguchi: [1]Molecular Profiling Research Center for Drug Discovery (molprof), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo [2]Department of Computer and Network Engineering, Graduate School of Informatics and Engineering, The University of Electro-Communications, Tokyo [3]Preparing Section for New Faculty of Medical Science, Fukushima Medical University, Fukushima City, Fukushima [4]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo

Identification of the complete set of translated genes of viruses is important to understand viral replication and pathogenesis as well as for therapeutic approaches to control viral infection. Here, we use chemical proteomics, integrating bio-orthogonal non-canonical amino acid tagging and high-resolution mass spectrometry, to characterize the newly synthesized herpes simplex virus 1 (HSV-1) proteome in infected cells. In these infected cells, host cellular protein synthesis is shut-off, increasing the chance to preferentially detect viral proteomes. We identify nine previously cryptic orphan protein coding sequences whose translated products are expressed in HSV-1-infected cells. Functional characterization of one identified protein, designated piUL49, shows that it is critical for HSV-1 neurovirulence in vivo by regulating the activity of virally encoded dUTPase, a key enzyme that maintains accurate DNA replication. Our results demonstrate that cryptic orphan protein coding genes of HSV-1, and probably other large DNA viruses, remain to be identified.

4. ESCRT-III controls nuclear envelope deformation induced by progerin

Jun Arii, Fumio Maeda, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasuko Mori & Yasushi Kawaguchi

Hutchinson-Gilford progeria syndrome (HGPS) is a premature aging disorder, caused by mutation in the gene encoding lamin A/C, which produces a truncated protein called progerin. In cells from HGPS patients, progerin accumulates at the nuclear membrane (NM), where it causes NM deformations. In this study, we investigated whether progerin-induced NM deformation involved ESCRT-III, a protein complex that remodels nuclear and cytoplasmic membranes. The ESCRT-III protein CHMP4B was recruited to sites of aberrant NM proliferation in human cells ectopically expressing progerin and in patient-derived HGPS fibroblasts. Derepression of NM deformation in these cells was observed following depletion of CHMP4B or an ESCRT-III adaptor, ALIX. Treatment with rapamycin (which induce autophagic clearance of progerin and reverse progerin-induced cellular phenotypes) down-regulated progerin-induced NM deformation, whereas treatment with bafilomycin A1 (an inhibitor of autophagy and lysosome-based degradation) or CHMP4B depletion antagonized the effects of rapamycin. These results indicate that the ALIX-mediated ESCRT-III pathway plays a suppressive role in progerin-induced NM deformation and suggest that autophagy down-regulates progerin-induced NM deformation in a manner dependent on ESCRT-III machinery.

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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

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

1. Influenza virus-induced oxidized DNA activates inflammasomes.

Moriyama M, Nagai M, Maruzuru Y, Koshiba T, Kawaguchi Y, and Ichinohe T.

Influenza virus M2 and PB1-F2 proteins have been proposed to activate the Nod-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome in macrophages by altering intracellular ionic balance or mitochondrial reactive oxygen species (ROS) production. However, the precise mechanism by which these viral proteins trigger the NLRP3 inflammasome activation remains unclear. Here we show that influenza virus stimulates oxidized DNA release from macrophages. Ion channel activity of the M2 protein or mitochondrial localization of the PB1-F2 protein was required for oxidized DNA release. The oxidized DNA enhanced influenza virus-induced IL-1β secretion, whereas inhibition of mitochondrial ROS production by antioxidant Mito-TEMPO decreased the virus-induced IL-1β secretion. In addition, we show that influenza virus stimulates IL-1β secretion from macrophages in an AIM2-dependent manner. These results provide a missing link between influenza viral proteins and the NLRP3 inflammasome activation and reveal the importance of influenza virus-induced oxidized DNA in inflammasomes activation.

Publications

Moriyama M, Nagai M, Maruzuru Y, Koshiba T, Kawaguchi Y, Ichinohe T. Influenza virus-induced oxidized DNA activates inflammasomes. *iScience*. 23:101270, 2020.

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感染制御系・システムウイルス学分野

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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. 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. A tissue level atlas of the healthy human virome

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Human-resident microbes can influence both health and disease. Investigating the microbiome using next-generation sequencing technology has revealed examples of mutualism and conflict between microbes and humans. Comparing to bacteria, the viral component of the microbiome (i.e. the "virome") is understudied. Somatic tissues of healthy individuals are usually inaccessible for the virome sampling, therefore, thus there is limited understanding of the presence and distribution of viruses in tissues in healthy individuals and how virus infection associates with human gene expression and perturbs immunological homeostasis. To characterize the human virome in a tissue-specific manner, here we performed meta-transcriptomic analysis using the RNA-sequencing dataset from the Genotype-Tissue Expression (GTEx) Project. We analyzed the 8,991 RNA-sequencing data obtained from 51 somatic tissues from 547 individuals and successfully detected 39 viral species in at least one tissue. We then investigated associations between virus infection and human gene expression and human disease onset. We detected some expected relationships; for instance, hepatitis C virus infection in the liver was strongly associated with interferon-stimulated gene upregulation and pathological findings of chronic hepatitis. Presence of herpes simplex virus type 1 in one subject's brain strongly associated with immune gene expression. While torque teno virus was detected in a broad range of human tissues, it was not associated with interferon responses. Being notable in light of its association with lymphoproliferative disorders, Epstein-Barr virus infection in the spleen and blood was associated with an increase in plasma cells in healthy subjects. Human herpesvirus 7 was often detected in the stomach; intriguingly, it associated with the proportion of human leukocytes in the stomach as well as digestive gene expression. Moreover, virus infections in the local tissues associated with systemic immune responses in circulating blood. To our knowledge, this study is the first comprehensive investigation of the human virome in a variety of tissues in healthy individuals through meta-transcriptomic analysis. Further investigation of the associations described here, and appli-

cation of this analytical pipeline to additional datasets, will be useful to reveal the impact of viral infections on human health.

2. Multiomics investigation revealing the characteristics of HIV-1-infected cells *in vivo*

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For eradication of HIV-1 infection, it is important to elucidate the detailed features and heterogeneity of HIV-1-infected cells in vivo. To reveal multiple characteristics of HIV-1-producing cells in vivo, we used a hematopoietic stem cell-transplanted humanized mouse model infected with GFP-encoding replication-competent HIV-1. We performed multiomics experiments using recently developed technology to identify the features of HIV-1-infected cells. Genome-wide HIV-1 integration site analysis revealed that productive HIV-1 infection tends to occur in cells with viral integration into transcriptionally active genomic regions. Bulk transcriptome analysis revealed that a high level of viral mRNA is transcribed in HIV-1-infected cells. Moreover, single-cell transcriptome analysis showed the heterogeneity of HIV-1-infected cells, including CXCL13high cells and a subpopulation with low expression of interferon-stimulated genes, which can contribute to efficient viral spread in vivo. Our findings describe multiple characteristics of HIV-1-producing cells in vivo, which could provide clues for the development of an HIV-1 cure.

3. SARS-CoV-2 ORF3b is a potent interferon antagonist whose activity is further increased by a naturally occurring elongation variant

Yoriyuki Konno‡, Izumi Kimura‡, Keiya Uriu, Masaya Fukushi[7], Takashi Irie[7], Yoshio Koyanagi[6], Daniel Sauter[8], Robert J. Gifford[9], USFQ-COVID19 consortium, So Nakagawa[10], Kei Sato (‡Equal contribution): [6]Kyoto University, Japan; [7]Hiroshima University, Japan; [8]Ulm University, Germany; [9]University of Glasgow, UK; [10]Tokai University, Japan

One of the features distinguishing SARS-CoV-2 from its more pathogenic counterpart SARS-CoV is the presence of premature stop codons in its ORF3b gene. Here, we show that SARS-CoV-2 ORF3b is a potent interferon antagonist, suppressing the induction of type I interferon more efficiently than its SARS-CoV ortholog. Phylogenetic analyses and functional assays revealed that SARS-CoV-2-related viruses from bats and pangolins also encode truncated ORF3b gene products with strong anti-interferon activity. Furthermore, analyses of approximately 17,000 SARS-CoV-2 sequences identified a natural variant, in which a longer ORF3b reading frame was reconstituted. This variant was isolated from two patients with severe disease and further increased the ability of ORF3b to suppress interferon induction. Thus, our findings not only help to explain the poor interferon response in COVID-19 patients, but also describe the emergence of natural SARS-CoV-2 quasispecies with an extended ORF3b gene that may potentially affect COVID-19 pathogenesis.

4. A role for gorilla APOBEC3G in shaping lentivirus evolution including transmission to humans

Yusuke Nakano[6], Keisuke Yamamoto[6], Mahoko Takahashi Ueda[10], Andrew Soper[6], Yoriyuki Konno, Izumi Kimura, Keiya Uriu, Ryuichi Kumata, Hirofumi Aso, Naoko Misawa[6], Shumpei Nagaoka, Soma Shimizu[6], Keito Mitsumune[6], Yusuke Kosugi[6], Guillermo Juarez-Fernandez[6], Jumpei Ito, So Nakagawa[10], Terumasa Ikeda[11], Yoshio Koyanagi[6], Reuben S Harris[11] and Kei Sato: [6]Kyoto University, Japan; [10]Tokai University, Japan; [11]University of Minnesota, USA

The APOBEC3 deaminases are potent inhibitors of virus replication and barriers to cross-species transmission. For simian immunodeficiency virus (SIV) to transmit to a new primate host, as happened multiple times to seed the ongoing HIV-1 epidemic, the viral infectivity factor (Vif) must be capable of neutralizing the APOBEC3 enzymes of the new host. Although much is known about current interactions of HIV-1 Vif and human APOBEC3s, the evolutionary changes in SIV Vif required for transmission from chimpanzees to gorillas and ultimately to humans are poorly understood. Here, we demonstrate that gorilla APOBEC3G is a factor with the potential to hamper SIV transmission from chimpanzees to gorillas. Gain-of-function experiments using SIVcpzPtt Vif revealed that this barrier could be overcome by a single Vif acidic amino acid substitution (M16E). Moreover, degradation of gorilla APOBEC3F is induced by Vif through a mechanism that is distinct from that of human APOBEC3F. Thus, our findings identify virus adaptations in gorillas that preceded and may have facilitated transmission to humans.

5. Endogenous retroviruses drive KRAB zinc-finger protein family expression for tumor suppression

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Gene expression aberration is a hallmark of cancers, but the mechanisms underlying such aberrations remain unclear. Human endogenous retroviruses (HERVs) are genomic repetitive elements that potentially function as enhancers. Since numerous HERVs are epigenetically activated in tumors, their activation could cause global gene expression aberrations in tumors. Here, we show that HERV activation in tumors leads to the upregulation of hundreds of transcriptional suppressors, namely Krüppel-associated box domain-containing zinc-finger family proteins (KZFPs). KZFP genes are preferentially encoded nearby the activated HERVs in tumors and transcriptionally regulated by these adjacent HERVs. Increased HERV and KZFP expression in tumors was associated with better disease conditions. Increased KZFP expression in cancer cells altered the expression of genes related to the cell cycle and cell-matrix adhesion and suppressed cellular growth, migration, and invasion abilities. Our data suggest that HERV activation in tumors drives the synchronized elevation of KZFP expression, presumably leading to tumor suppression.

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International Research and Development Center for Mucosal Vaccines

Division of Mucosal Barriology
粘膜バリア学分野

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

1. Osteoprotegerin-dependent M cell self-regulation balances gut infection and immunity.

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Microfold cells (M cells) are responsible for antigen uptake to initiate immune responses in the gut-associated lymphoid tissue (GALT). Receptor activator of nuclear factor-κB ligand (RANKL) is essential for M cell differentiation. Follicle-associated epithelium (FAE) covers the GALT and is continuously exposed to RANKL from stromal cells underneath the FAE, yet only a subset of FAE cells undergoes differentiation into M cells. We showed that M cells express os-

teoprotegerin (OPG), a soluble inhibitor of RANKL, which suppresses the differentiation of adjacent FAE cells into M cells. Notably, OPG deficiency increases M cell number in the GALT and enhances commensal bacterium-specific immunoglobulin production, resulting in the amelioration of disease symptoms in mice with experimental colitis. By contrast, OPG-deficient mice are highly susceptible to Salmonella infection. Thus, OPG-dependent self-regulation of M cell differentiation is essential for the balance between the infectious risk and the ability to perform immunosurveillance at the mucosal surface.

2. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice.

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Antibiotics and dietary habits can affect the gut microbial community, thus influencing disease susceptibility. Although the effect of microbiota on the postnatal environment has been well documented, much less is known regarding the impact of gut microbiota at the embryonic stage. We showed that maternal microbiota shapes the metabolic system of offspring in mice. During pregnancy, short-chain fatty acids produced by the maternal microbiota dictate the differentiation of neural, intestinal, and pancreatic cells through embryonic GPR41 and GPR43. This developmental process helps maintain postnatal energy homeostasis, as evidenced by the fact that offspring from germ-free mothers are highly susceptible to metabolic syndrome, even when reared under conventional conditions. Thus, our findings elaborate on a link between the maternal gut environment and the developmental origin of metabolic syndrome.

3. Microfold cell-dependent antigen transport alleviates infectious colitis by inducing antigen-specific cellular immunity.

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Infectious colitis is one of the most common health issues worldwide. Microfold (M) cells actively transport luminal antigens to gut-associated lymphoid tissue to induce IgA responses; however, it remains unknown whether M cells contribute to the induction of cellular immune responses. We reported that M cell-dependent antigen transport plays a critical role in the induction of Th1, Th17, and Th22 responses against gut commensals in the steady state. The establishment of commensal-specific cellular immunity was a prerequisite for preventing bacterial dissemination during enteropathogenic Citrobacter rodentium infection. Therefore, M cell-null mice developed severe colitis with increased bacterial dissemination. This abnormality was associated with mucosal barrier dysfunction. These observations suggest that antigen transport by M cells may help maintain gut immune homeostasis by eliciting antigen-specific cellular immune responses.

4. Microbiota-derived butyrate limits the autoimmune response by promoting the differentiation of follicular regulatory T cells.

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Dysbiosis of the intestinal microbiota has been observed in rheumatoid arthritis patients, although the pathological relevance has remained obscure. Follicular regulatory T (TFR) cells play critical regulatory roles in the pathogenesis of autoimmune diseases, including RA. Reduced number of circulating TFR cells has been associated with the elevation of autoantibodies and disease severity in RA. However, the contribution of commensal microbe-derived butyrate in controlling TFR cell differentiation remains unknown. We examined the contribution of microbe-derived butyrate in controlling autoimmune arthritis using collagen-induced arthritis (CIA) and SKG arthritis models. Microbe-derived butyrate suppressed the development of autoimmune arthritis. The immunization of type II collagen (CII) caused hypertrophy of the GALT in the colon by amplifying the GC reaction prior to the onset of the CIA. Butyrate mitigated these pathological events by promoting TFR cell differentiation. Butyrate directly induced the differentiation of functional TFR cells in vitro by enhancing histone acetylation in TFR cell marker genes. This effect was attributed to histone deacetylase (HDAC) inhibition by butyrate, leading to histone hyperacetylation in the promoter region of the TFR-cell marker genes. The adoptive transfer of the butyrate-treated iTFR cells reduced CII-specific autoantibody production and thus ameliorated the symptoms of arthritis. Accordingly, microbiota-derived butyrate serves as an environmental cue to enhance TFR cells, which suppress autoantibody production in the systemic lymphoid tissue, eventually ameliorating RA. Our findings provide mechanistic insights into the link between the gut environment and RA risk.

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International Research and Development Center for Mucosal Vaccines

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

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

1. Development of next-generation vaccine inducing both antigen-specific systemic and mucosal immunity

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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 were acquired by antigen boosting of the target organs. This immunization effectively regulated *Streptococcus pneumoniae* infection. Moreover, vaccination for *Clostridium ramosum* (*C. ramosum*), a representative causative commensal microbiota for obesity and diabetes, alleviated high-fat diet-induced obesity in mice by controlling the number of *C. ramosum* in the mucosa. Collectively, this vaccine strategy induces strong antigen-specific mucosal and systemic immunity and has the potential to prevent infections and commensal microbiota-associated diseases. The patent of this new vaccine strategy was granted in 2019 in Japan and in 2020 in US. We are currently conducting monkey experiments for formulation in human on the basis of collaboration with Mitsubishi Tanabe Pharmaceutical company. We started collaboration with Medicago in Canada and are developing an IgA-inducing mucosal vaccine with this system by using SARS-CoV-2 Viral-like particle as an antigen which have been developed by Medicago.

2. Analysis of resident macrophages in small intestinal LP

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$CD11c^{int}CD11b^{int}$ cells in small intestinal LP are resident macrophages. They specifically express chemokine receptor CX3CR1 in intestinal LP. Their phagocytotic activity is very strong. Although they express MHC class II, they cannot move from LP to draining lymph nodes effectively, suggesting that

they may be involved in local immune responses in intestine. They express TLR4, TLR7 and TLR9 and produce TNF-α and IL-10 by TLR stimulation. We performed microarray analysis in the CD11c^{int}CD11b^{int} cells, CD11c^{hi}CD11b^{hi} cells, splenic CD11c^{+} DCs and peritoneal macrophages with or without stimulation of TLR ligand and compared signaling pathways among them. We found several candidate genes which specifically express in CD11c^{int}CD11b^{int} cells. We generated gene-targeting mice and are examining the *in vivo* function of them in CD11c^{int}CD11b^{int} cells.

3. Development a new therapy for radiation injury in mucosa.

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High-dose ionizing radiation induces severe DNA damage in the epithelial stem cells in small intestinal crypts and causes gastrointestinal syndrome (GIS). Although the tumor suppressor p53 is a primary factor inducing death of crypt cells with DNA damage, its essential role in maintaining genome stability means inhibiting p53 to prevent GIS is not a viable strategy. Here, we show that the innate immune receptor Toll-like receptor 3 (TLR3) is critical for the pathogenesis of GIS. *Tlr3*$^{-/-}$ mice show substantial resistance to GIS owing to significantly reduced radiation-induced crypt cell death. Despite showing reduced crypt cell death, p53-dependent crypt cell death is not impaired in *Tlr3*$^{-/-}$ mice. p53-dependent crypt cell death causes leakage of cellular RNA, which induces extensive cell death via TLR3. An inhibitor of TLR3–RNA binding ameliorates GIS by reducing crypt cell death. Thus, we propose blocking TLR3 activation as a novel and preferable approach to treat GIS. We are analyzing the role of TLR3 in radiation-induced oral mucositis.

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International Research and Development Center for Mucosal Vaccines

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

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To explore new avenues for mucosal vaccine development and immune-regulation, investigators have begun to employ novel adjuvants and targeting mucosal tissues and immune cells for vaccine delivery and elucidate the mechanisms of immune-regulation in the mucosal tissues. Despite recently 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 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 the 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. We are currently testing the roles of these molecules for the induction of mucosal tissue-specific immune responses by using gain and lost function approaches.

2. Roles of Secretory IgA antibodies for the maintenance of the homeostasis in the oral cavity

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Secretory IgA (SIgA) plays a central role in preventing bacterial and viral infections on mucosal surfaces by neutralizing toxins and viruses and inhibiting bacterial attachment to epithelial cells. However, the role of salivary SIgA antibodies (Abs) in regulating oral flora is still unknown. This study aimed to evaluate the association among oral bacteria, their metabolites and periodontitis in IgA-deficient (IgA KO) and wild-type (WT) control mice. Microcomputed tomography (micro-CT) analysis was used to assess alveolar bone resorption as development of periodontitis. The bacterial profiles of saliva were determined using the next-generation sequencing assays. Furthermore, the metabolites in saliva were measured and compared using CE-TOFMS. Salivary microbiota of IgA KO mice revealed a remarkably decreased frequency of Streptococcus, and increased percentages of *Aggregatibacer*, *Actinobacillus*, and *Prevotella* at the genus level when compared with those of WT. Compared to WT control mice of the same age, the level of alveolar bone loss was significantly increased in IgA KO mice, and infiltration of osteoclasts was found on the surface of the alveolar bone. The metabolome profile indicated that the metabolites of IgA KO mice had greater variability in carbon metabolic, urea cycle, and lipid pathways than WT mice. These results suggest that salivary SIgA plays an important role in regulating and maintaining normal oral microflora to prevent the development of the periodontal disease.

3. Stratified layer analysis revealsintrinsic hormone stimulates cryptal mesenchymal cells forcontrolling mucosal homeostasis

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Mesenchymal cells in the crypt play indispensable roles in the maintenance of intestinal epithelial homeostasis through their contribution to the preservation of stem cells. However, the acquisition properties of the production of stem cell niche factors by the mesenchymal cells have not been well elucidated, due to technical limitations regarding the isolation and subsequent molecular and cellular analyses of cryptal mesenchymal cells. To evaluate the function of mesenchymal cells located at the large intestinal crypt, we established a novel method through which cells are harvested according to the histologic layers of the mouse colon, and we compared cellular properties between microenvironmental niches, the luminal mucosa and crypts. The gene expression pattern in the cryptal mesenchymal cells showed that receptors of the hormone/cytokine leptin were highly expressed, and we found a decrease in Wnt2b expression under conditions of leptin receptor deficiency, which also induced a delay in cryptal epithelial proliferation. Our novel stratified layer isolation strategies thus revealed new microenvironmental characteristics of colonic mesenchymal cells, including the intrinsic involvement of leptin in the control of mucosal homeostasis.

4. Orally desensitized mast cells form a regulatory network with Treg cells for the control of food allergy

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Oral immunotherapy (OIT) is an effective approach to controlling food allergies. Although the detailed molecular and cellular mechanisms of OIT are unknown currently, they must be understood to advance the treatment of allergic diseases in general. To elucidate the mechanisms of OIT, especially during the immunological transition from desensitization to allergy regulation, we generated a clinical OIT murine model and used it to examine immunological features after OIT. We found that in mice that completed OIT successfully, desensitized mast cells (MCs) showed functionally beneficial alterations, such as increased expression of regulatory cytokines and enhanced expansion of regulatory T cells. Importantly, regulatory-T-cell-mediated inhibition of allergic responses was decreased in mice in which desensitized MCs were depleted during OIT. Collectively, these findings show that the desensitization process modulates the activation of MCs, leading directly to enhanced induction of regulatory-T-cell expansion and promotion of clinical allergic unresponsiveness. Our results suggest that efficiently inducing regulatory MCs is a novel strategy for the treatment of allergic diseases.

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International Research and Development Center for Mucosal Vaccines

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Mucosal vaccine is a prospective strategy for the vaccine development against pathogens invading through mucosal tissues. We have examined the immunological functions of commensal and pathogenic microorganisms as well as diets and applied them to the development of adjuvants and antigen delivery for the efficient immune responses against mucosal vaccines. These findings also could be extended to the development of mucosal immunotherapy against allergic, inflammatory, and infectious diseases.

1. Emerging roles of dietary lipids and vitamin B1 for the regulation of allergic inflammatory diseases and immune system development

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Immune system is regulated by dietary materials and their metabolites. We previously found that dietary intake of linseed oil, rich in $\omega 3$ α-linolenic acid, led to the amelioration of allergic responses in the gut and nasal mucosa through the metabolic conversion of α-linolenic acid into anti-allergy and anti-inflammatory lipid mediators of 17,18-epoxyeicosatetraenoic acid and 15-hydroxyeicosapentaenoic acid. Here, we extended our view by showing the evidence that

maternal intake of linseed oil led to the alleviation of allergic symptoms in the offspring skin. Lipidomic analysis revealed that breast milk contained much amount of 14-hydroxydocosapentaenoic acid (14-HDPA) when mouse dams were fed with linseed oil in comparison to conventional soybean oil. We found that 14-HDPA exhibited potent bioactivity in the induction of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on plasmacytoid dendritic cells in offspring, which led to the inhibition of inflammatory cytokines production from T cells in the skin. Our another course of study revealed that vitamin B1-deficiency resulted in thymic involution with remarkable reduction of the number of CD4 and CD8 α double positive thymocytes. We further found that vitamin B1 deficient mice showed enhanced maturation phenotype of $\gamma\delta$ thymocytes by increased expression of TGFβ superfamily cytokines due to the vitamin B1-mediated metabolic impairment and accumulation of branched-chain α-keto acids in thymic stromal cells. These results collectively demonstrate that dietary lipid and vitamin B1 plays key roles in the control of immune systems, which can be applied to the development and optimization of vaccine against infectious and allergic diseases.

2. The mechanism for a commensal bacterium *Alcaligenes* to cohabit inside intestinal lymphoid tissue and application as a vaccine adjuvant

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Lymphoid-tissue–resident commensal bacteria (LRCs), including *Alcaligenes faecalis*, colonize within dendritic cells (DCs) in intestinal lymphoid tissue including the Peyer's patches (PPs) of mammals. LRCs modulate the host immune system to promote intestinal IgA antibody responses. Indeed, we previously demonstrated that *A. faecalis* activates DCs to produce IL-6, an IgA production-enhancing cytokine, through the weak agonistic activity of its lipopolysaccharide (LPS) against toll-like receptor (TLR) 4 together with low inflammatory activity, indicating that *Alcaligenes* spp. maintain their homeostatic environment in PPs without inducing an excessive inflammatory response. Here, we show an intracellular symbiotic system in which the LRC *Alcaligenes* creates a unique energy shift in DCs. Whereas DCs showed low mitochondrial respiration when they were co-cultured with non-symbiotic *Escherichia coli*, DCs carrying *A. faecalis* maintained increased mitochondrial respiration. Furthermore, *E. coli* induced apoptosis of DCs but *A. faecalis* did not. Regarding an underlying mechanism, *A. faecalis*—unlike *E. coli*—did not induce intracellular nitric oxide (NO) production in DCs due to the low activity of its LPS. Therefore, *A. faecalis*, an example of LRCs, may persist within intestinal lymphoid tissue because they elicit little NO production in DCs. In addition, the symbiotic DCs exhibit characteristic physiologic changes, including a low rate of apoptosis and increased mitochondrial respiration.

Unique characteristics of *Alcaligenes* LPS, which moderately activate host immune responses, promoted us to be examined for application as a vaccine adjuvant. Indeed, we previously reported that *Alcaligenes* LPS promoted antigen-specific immune responses including IgG antibody and Th17 responses without excessive inflammation. Here, we chemically synthesized *Alcaligenes* lipid A, an active part of LPS, and examined its efficacies as a vaccine adjuvant. In both systemic and nasal vaccination, *Alcaligenes* lipid A enhanced antigen-specific IgG/IgA antibody and Th17 responses. Further, in nasal vaccination with pneumococcal surface protein A (PspA) as a vaccine candidate antigen, *Alcaligenes* lipid A induced the protective immunity against *Streptococcus pneumoniae* infection. These findings suggest that *Alcaligenes* lipid A is a useful and applicable synthetic adjuvant for both systemic and nasal vaccine development including *S. pneumoniae* vaccine.

3. A Nanogel-based trivalent PspA nasal vaccine protects macaques from intratracheal challenge with pneumococci.

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Current polysaccharide-based pneumococcal vaccines are effective but not compatible with all serotypes of *Streptococcus pneumoniae*. We previously developed an adjuvant-free cationic nanogel nasal vaccine containing pneumococcal surface protein A (PspA) which is expressed on the surfaces of all pneumococcal serotypes. Because PspA proteins have sequence diversity, we formulated and tested a nanogel-based trivalent pneumococcal nasal vaccine to demonstrate the vaccine's immunogenicity and protective efficacy in macaques. Nasal vaccination of macaques with cationic cholesteryl pullulan nanogel (cCHP)-trivalent PspA vaccine effectively induced PspA-specific IgGs that bound to pneumococcal surfaces and triggered complement C3 deposition. The immunized macaques were protected from pneumococcal intratracheal challenge through both inhibition of lung inflammation and elimination of the bacteria from the lungs. These results demonstrated that the cCHP-trivalent PspA vaccine is an effective candidate vaccine for broad protection against pneumococcal infections.

4. Comparative whole-genome and proteomics analyses of the next seed bank and the original master seed bank of MucoRice-CTB 51A line, a rice-based oral cholera vaccine

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We have previously developed a rice-based oral vaccine against cholera diarrhea, MucoRice-CTB. Using Agrobacterium-mediated co-transformation, we produced the selection marker–free MucoRice-CTB line 51A, which has three copies of the cholera toxin B subunit (CTB) gene and two copies of an RNAi cassette inserted into the rice genome. We determined the sequence and location of the transgenes on rice chromosomes 3 and 12. The expression of alpha-amylase/trypsin inhibitor, a major allergen protein in rice, is lower in this line than in wild-type rice. Line 51A was self-pollinated for five generations to fix the transgenes, and the seeds of the sixth generation produced by T5 plants were defined as the master seed bank (MSB). T6 plants were grown from part of the MSB seeds and were self-pollinated to produce T7 seeds (next seed bank; NSB). NSB was examined and its whole genome and proteome were compared with those of MSB. First, we re-sequenced the transgenes of NSB and MSB and confirmed the positions of the three CTB genes inserted into chromosomes 3 and 12. The DNA sequences of the transgenes were identical between NSB and MSB. Next, using whole-genome sequencing, we compared the genome sequences of three NSB with three MSB samples, and evaluated the effects of SNPs and genomic structural variants by clustering. No functionally important mutations (SNPs, translocations, deletions, or inversions of genic regions on chromosomes) between NSB and MSB samples were detected. Analysis of salt-soluble proteins from NSB and MSB samples by shot-gun MS/MS detected no considerable differences in protein abundance. No difference in the expression pattern of storage proteins and CTB in mature seeds of NSB and MSB was detected by immuno-fluorescence microscopy.

In Conclusions, all analyses revealed no considerable differences between NSB and MSB samples. Therefore, NSB can be used to replace MSB in the near future.

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International Research and Development Center for Mucosal Vaccines

Division of Mucosal Symbiosis
粘膜共生学分野

Project Associate Professor Yoshiyuki Goto, Ph.D.
Invited Professor Tetsuro Matano, M.D., D.M.Sc.

特任准教授 博士(医学) 後藤義幸
委嘱教授 博士(医学) 俣野哲朗

The gastrointestinal tract is a unique organ that is constitutively exposed by various antigens, including commensal microbiota. In order to create a symbiotic environment for non-pathogenic luminal microorganisms, epithelial cells (ECs) and immune cells cooperatively establish homeostasis of the intestinal microenvironment. We aim to identify the mechanisms of epithelial α1, 2-fucosylation, one of the symbiotic factors between host and microbiota, and uncover the role of ECs-immune cell network in the establishment of intestinal homeostasis. We also aim to understand host-microbe as well as microbe-microbe interaction in the gut.

1. Innate lymphoid cells govern intestinal epithelial α1, 2-fucosylation

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α1, 2-fucosyl linkages located to terminal carbohydrate moiety expressed on intestinal epithelial cells is catalyzed by fucosyltransferase 2 (Fut2). Epithelial α1, 2-fucose is one of the symbiotic factors that mediate host–microbiota interaction. For example, epithelial α1, 2-fucose is utilized as a dietary carbohydrate by various symbiotic bacteria such as *Bacteroides*. Therefore, disruption of Fut2 leads to dysbiosis both in mice and humans and predisposed to the development of inflammatory diseases such as Crohn's disease. Despite the importance of intestinal and systemic homeostasis, the molecular and cellular mechanisms of the induction of epithelial Fut2, and subsequent α1, 2-fucosylation remains unknown. We found that group 3 innate lymphoid cells (ILC3) are critical inducers of intestinal epithelial Fut2 expression and fucosylation that is mediated by the production of interleukin 22 and lymphotoxin from ILC3 in a commensal bacteria–dependent and -independent manner, respectively. Fut2-deficient mice are susceptible to the infection by pathogenic microorganisms. These data unveil a novel function of ILC3 in creating the appropriate symbiotic environment and protective platform against pathogenic microorganisms through regulating the epithelial α1, 2-fucosylation.

2. Commensal microbiota prevent fungi from colonizing the gastrointestinal tract.

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Intestinal epithelial cells are the first line of defense against infection by pathogenic microorganisms. *Candida albicans* are one of the commensal fungi reside in the mucosal surface including the gastrointestinal tract. However, *C. albicans* also have been reported to exert pathogenic effects in the immunocompromised host and expand to the systemic compartments, which is called invasive candidiasis. Invasive candidiasis triggered by *C. albicans* colonization in the gut is one of the serious infectious diseases in the world. So far, it is unclear what kind of factors which regulate *C. albicans* colonization in the gut. To investigate this, we focused on the role of commensal bacteria against colonization by *C. albicans*. We found that germfree and several antibiotic-treated mice allow colonization of *C. albicans* in the gut. Furthermore, oral administration of feces isolated from normal mice excluded *C. albicans* from the gut. This data suggests that commensal bacteria prevent the colonization of *C. albicans* in the gut, and commensal bacteria may be a useful therapeutic target for protection against *C. albicans* infection.

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Health Intelligence Center

Division of Health Medical Data Science
健康医療データサイエンス分野

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Our mission is to utilize genomic big data and time series health medical data to realize methods for prediction and prevention of diseases and keeping/improving our health. For this purpose, we develop novel computational data analysis technologies by integrating Bayesian statistical theory and high-performance computing on supercomputer system.

1. Development of Computational Platform for Clinical Sequence and Interpretation

Shimizu E, Kasajima R, Katayama K, Yamaguchi K, Yokoyama K, Komura M, Yamamoto M, Saito A, Kobayashi M, Ogawa M, Takei T, Yuji K, Takane K, Ikenoue T, Shibuya T, Hasegawa T, Miyagi Y, Muto K, Tojo A, Furukawa Y, Miyano S, Yamaguchi R, Imoto S

For implementing genomic medicine enhanced with advanced sequencing technologies in the Institute of Medical Science, we formed a research team composed of researchers and technicians who have various academic backgrounds including medicine, biology, pharmacology, genetics, statistics, computer science, ethics, etc. A highly secure infrastructure for analyzing personal genome was constructed; in the system, next generation sequencers are directly connected to a part (disconnect to internet) of supercomputer system in Human Genome Center and, for keeping traceability, laboratory information management system (LIMS) is implemented to record all logs of wet experiments and computational analyses. Together with sequencing analyses, we now intensively focus on a method for translating personal genome information into valuable clinical information. In July 2015, we started to use IBM Watson for cancer research to interpret the results of genomic analyses. The results of sequencing analysis and the clinical translation by IBM Watson are evaluated and discussed in biweekly expert panel meeting. Also, multi-omics data including whole genome, transcriptome and epigenome were obtained for integrative analysis that has the potential to achieve highly precise interpretation. This research is also performed as a part of the University of Tokyo's Center of Innovation (COI) program "Self-Managing Healthy Society".

2. Health Medical Big Data Analysis

a. Integration of the records of health examination, microbiome and genomic data for predicting disease risks

Hasegawa T, Kakuta M, Yamaguchi R, Imoto S

Owing to increasing medical expenses, researchers have attempted to grasp clinical signs and preventive measures of diseases using electronic health record (EHR). In particular, time-series EHRs collected by periodic medical check-up enable us to clarify the relevance among check-up results and individual environmental factors such as lifestyle. However, usually such time-series data have many missing observations, and some results are strongly correlated to each

other. These problems make the analysis difficult and there exists strong demand to detect clinical findings beyond them.

We focus on blood test values in medical check-up results and apply a time-series analysis methodology using a state space model. It can infer the internal medical states emerged in blood test values and handle missing observations. The estimated models enable us to predict one's blood test values under specified condition and predict the effect of intervention, such as changes of body composition and lifestyle.

We use time-series data of EHRs periodically collected in the Hirosaki cohort study in Japan and elucidate the effect of 17 environmental factors to 38 blood test values. Using the estimated model, we then simulate and compare time-transitions of participant's blood test values under several lifestyle scenarios. It visualizes the impact of lifestyle changes for the prevention of diseases. Finally, we exemplify that prediction errors under participant's actual lifestyle can be partially explained by genetic variations, and some of their effects have not been investigated by traditional association studies.

b. Pan-cancer analysis of whole genomes

Imoto S, Yamaguchi R, Hasegawa T, Shuto H, Masashi Fujita[1], Fan Zhang[2], Shimizu E, Komura M, Miyano S, Zhang Z[2], Nakagawa H[1]: [1]Riken, [2]Peking University

We were involved in the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA). We reported the integrative analysis of 2,658 whole-cancer genomes and their matching normal tissues across 38 tumor types from PCAWG. *Butler*, a computational tool that facilitates large-scale genomic analyses on public and academic clouds, was created for large scale genomic analysis, where SHIROKANE, the supercomputer of Human Genome Center, was utilized as the computational resource from Japan. Butler enabled processing of a 725-terabyte cancer genome dataset from the PCAWG project in a time-efficient and uniform manner. On average, cancer genomes contained 4–5 driver mutations when combining coding and non-coding genomic elements; however, in around 5% of cases no drivers were identified, suggesting that cancer driver discovery is not yet complete.

c. Metagenome analysis of commensal microbiota

Sato N[3], Kakuta M, Hasegawa T, Yamaguchi R, Nakaji S[4], Okuno Y[3], Imoto S: [3]Kyoto University, [4]Hirosaki University

Cigarette smoking affects the oral microbiome, which is related to various systemic diseases. While studies that investigated the relationship between smoking and the oral microbiome by 16S rRNA amplicon sequencing have been performed, investigations involving metagenomic sequences are rare. We investigated the bacterial species composition in the tongue microbiome, as well as single-nucleotide variant (SNV) profiles and gene content of these species, in never and current smokers by utilizing metagenomic sequences. Among 234 never smokers and 52 current smokers, beta diversity, as assessed by weighted UniFrac measure, differed between never and current smokers (pseudo-$F = 8.44$, $R2 = 0.028$, $p = 0.001$). Among the 26 species that had sufficient coverage, the SNV profiles of Actinomyces graevenitzii, Megasphaera micronuciformis, Rothia mucilaginosa, Veillonella dispar, and one Veillonella sp. were significantly different between never and current smokers. Analysis of gene and pathway content revealed that genes related to the lipopolysaccharide biosynthesis pathway in Veillonella dispar were present more frequently in current smokers. We found that species-level tongue microbiome differed between never and current smokers, and 5 species from never and current smokers likely harbor different strains, as suggested by the difference in SNV frequency.

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Center for Gene and Cell Therapy

Division of Molecular and Medical Genetics
分子遺伝医学分野

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Assistant Professor	Yuji Tsunekawa, Ph.D.	助　教	博士(医学)	恒　川　雄　二
Project Assistant Professor	Hiromi Hayashita-Kinoh, Ph.D.	特任助教	博士(医学)	喜　納　裕　美

The Division of Molecular and Medical Genetics (DMMG) was generated in 2020, and we are focused on development of gene-addition and gene-editing therapy including viral vector preparation and purification, AAV vector-mediated gene therapy for Duchenne muscular dystrophy (DMD), and hematopoietic stem cell (HSC)-targeted gene therapy with lentiviral vectors. Our basic and translational efforts can allow to produce new genetic therapies for various neurologic, hematologic, and metabolic diseases.

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

AAV vectors are being used for *in vivo* gene therapy to cure various hereditary diseases including DMD. However, a detailed analysis of AAV-vector quality is required for *in vivo* gene therapy according to the recent safety regulation. We are conducting various research projects for (1) generation of an efficient and practical cell line to prepare AAV vectors, (2) improvement of large-scale preparation methods for AAV vectors, (3) development of analysis and purification methods among full, partial, and empty-genome particles in AAV vectors, and (4) evaluation of glycosylation on AAV capsids. In addition, we are planning to adapt these strategies to lentiviral vectors.

Innovation in AAV vector toward gene and cell therapies to treat neuromuscular diseases

DMD is caused by a mutation of the dystrophin gene in the X chromosome and induces progressive weakness and atrophy of both skeletal and heart muscle, thus resulting in motor disfunction, cardiomyopathy, respiratory failure, and early mortality. There is no known cure of DMD, and the current FDA-approved treatments including a corticosteroid (Deflazacort) and antisense oligonucleotides (Eteplirsen and Golodirsen) seem to be insufficient for cure. Therefore, we are developing AAV-based *in vivo* gene therapy which is potentially curative for DMD by adding a micro-dystrophin gene or an exon skipping molecule. We have recently published a preclinical evaluation of AAV-based *in vivo* gene therapy in a DMD model dog to improve heart functions with a micro-dystrophin gene delivery. We improved the efficacy by co-injection of mesenchymal stem cells (MSCs), allowing for elongation of transgene expression as well as reduction of AAV-vector dose. We are planning a pilot trial of MSC-based cell therapy in DMD patients.

Development of HSC-targeted gene therapy with lentiviral vectors

HSC-targeted gene therapy with lentiviral vectors can cure various inherited diseases, including immunodeficiencies, hemoglobinopathies, and metabolic diseases. In HSC gene therapy, patient CD34+ HSCs are harvested and genetically modified with lentiviral vectors to deliver a therapeutic gene, followed by HSC transplantation back to the patient.

This strategy can be applicable for most patients due to no requirement of histocompatible donor, and it allows for one-time cure of diseases due to the life-long efficiency of lentiviral HSC therapy. We are planning to develop various HSC gene therapies for immunodeficiencies as well as other hematopoietic and metabolic diseases with a collaboration to National Center for Child Health and Development.

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Invited Professor	Koji Tamada, M.D., Ph.D.	教授(委嘱)	博士(医学)	玉	田 耕 治
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The Center for Gene & Cell Therapy (CGCT) was generated in 2014 at IMSUT hospital, playing a crucial role in hematopoietic stem cell (HSC) transplantation and gene therapy. We are focused on development of gene and cell therapy, regenerative therapy, and ethical, legal and social implications (ELSI) research targeting malignant, chronic, and hereditary diseases including oncolytic virus therapy, engineered T-cell therapy, AAV vector-mediated gene therapy, and HSC-targeted gene therapy.

Initiation of gene and cell therapy trials in hemophilia and Parkinson disease

We have generated a consortium including CGCT and Center for Stem Cell Biology and Regenerative Medicine to develop a research center for gene and cell therapy, regenerative therapy, and ELSI research in the near future. In this consortium, we started a combined meeting which provided a seminar regarding advances of HSC-targeted gene-addition and gene-editing therapy. Currently, we are planning to perform AAV-mediated gene therapy trials for hemophilia B as well as Parkinson disease. Hemophilia B is a bleeding disorder, caused by a factor IX deficiency with a genetic mutation. Replacement therapy with recombinant factor IX is effective; however, it must be continued for a life-long with a high cost. AAV-mediated in vivo gene therapy is curative for long-term in Hemophilia B by adding a therapeutic factor IX gene. In contrast, Parkinson disease is a brain disorder caused by a loss of dopamine-producing cells in the brain. L-dopa treatment can reduce the symptoms of Parkinson disease; however, it should be continued for a life-long, and the disease remains progressive. AAV-mediated in situ gene therapy can allow for phenotypic correction in Parkinson disease by adding a neuroprotective molecule glial cell line-derived neurotrophic factor (GDNF). Both AAV vector-mediated gene therapies should be promising candidates to develop a curative strategy.

Development of an oncolytic virus therapy for malignant glioma

Dr. Todo's group in the CGCT developed an oncolytic herpes simplex virus type 1 (G47Δ), designed to replicate only in cancer cells. Dr. Todo's group started a phase II, investigator-initiated clinical trial of G47Δtargeting glioblastoma in 2015. In the intermediate analysis, 1-year survival rate after G47Δ therapy (92.3%) was much higher than the preset control value (15%) based on meta-analysis. Adverse events included fever, vomiting, lymphope-

nia, and nausea. Based on the high efficacy and safety shown in this phase II study, G47Δ awaits an approval as a new drug for malignant glioma, and expected to be the first oncolytic virus product in Japan.

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Laboratory Animal Research Center

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

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Genome engineering technologies achieve a "revolution" in life science and medical science. These techniques allow us to manipulate genes of interest for several purposes. Using those technologies, we have developed many useful strains in mice and rats. We are now focusing on generating "humanized animals" or "immunodeficient animals". These valuable animals can be used for xenotransplantation of human cells/tissues including human iPS cells. We are also developing therapeutic strategies such as cell therapy and gene therapy with genome editing tools.

Generation of Severe Combined Immunodeficiency Rats

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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.

Development strategies of effective treatment for human cancers with CRISPR-Cas3 system

Tomoaki Fujii, Jinxi Wang, Ken Mochizuki, Kazuto Yoshimi, and Tomoji Mashimo

The genome editing system could be a powerful

genetic tools for development of an editing around the world. However, CRISPR-Cas9 system sometimes induces off-target, mosaic mutations, and small indels which cause unexpected phenotypes. Therefore, there are often limitations in medical applications of CRISPR-Cas9 system. Recently, we have reported that genome editing using CRISPR-Cas3 system is possible in human cells. This novel genomic editing system rarely induces off-target and mosaic mutations. We aim for the development of a safe and effective therapy for human cancers with the use of the CRISPR-Cas3 system.

We focus on chimeric antigen receptor T (CAR-T) cell therapy, which is an effective cancer immunotherapy. However, the standard strategy for producing CAR-T cells is expensive due to using autologous T cells. We investigated whether CRISPR-Cas3 system induces a genetic modifications on the genes in Jurkat cells, a human acute T-cell Leukemia cell line. We further investigated whether CRISPR-Cas3 system induces genetic modifications on genes involved in graft-versus-host disease and immune rejection in Jurkat cells. As a result of this experiment, it causes loss of function of the target genes in its cells. This result indicates that CRISPR-Ca s3 system could be a genetic tool for generating allogenic CAR-T cells.

Photoactivatable Cre knock-in mice for spatiotemporal control of genetic engineering in vivo

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Although the Cre-loxP recombination system has been extensively used to analyze gene function in vivo, spatiotemporal control of Cre activity is a critical limitation for easy and precise recombination. We established photoactivatable-Cre (PA-Cre) knock-in mice at safe harbor locus for the spatial and temporal regulation of Cre recombinase activity. The mice showed Cre recombination activity in a whole-body following light exposure for only 1 h. Almost no leaks of Cre recombination activity were detected in the knock-in mouse under natural light conditions. Spot irradiation could induce locus-specific recombination noninvasively, enabling us to compare phenotypes on the left and right sides in the same mouse. Furthermore, long-term irradiation using an implanted wireless LED substantially improved Cre recombination activity, especially in the brain. These results demonstrated that the PA-Cre knock-in mice can facilitate spatiotemporal control of genetic engineering and promise a useful resource to elucidate gene function in vivo with Cre-loxP.

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Laboratory Animal Research Center

Animal Center
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The Laboratory Animal Research Center (LARC) was founded in 1965 as the first modern animal facility in Japan. Currently about 15,500 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 and IVIS imaging system. In 2020, 465 researchers from 49 laboratories are engaged in this facility with about 15,500 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 embryo for reducing number of using animals and laboratory space. In addition, it is useful for making back up of the strains. In 2020, 47 strains of embryos and 33 strains of sperm were stored, and 130 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 1970) has a long history originating from the branch office of the Institute for Infectious Disease which was established in 1902. We have made great achievements in filariasis eradication from this island and prevention of Habu bites. Currently, we are maintaining the colonies of New World monkeys, and aiming to overcome endemic infectious diseases in the tropical and subtropical regions through infection experiments using primates.

Reproduction of squirrel monkeys and owl monkeys

The squirrel monkey (*Saimiri boliviensis*) and the owl monkey (*Aotus lemurinus griseimenbra*) are widely distributed in the tropical rainforest in Central and South America. The advantage of using both species for medical researches resides in its small size and gentle behavior. Squirrel monkeys and owl monkeys are phylogenetically close to each other, and both are very famous as the best of malaria model in primates. In this laboratory, squirrel monkeys have a breeding season between winter and early spring. They are polygamy. Their puberty is 3-4 years old in females and 4-5 years old in males. Their gestation period is about 150 days. In contrast, owl monkeys are annual breeding animals. They are monogamy. Their puberty is 3 years old for both sex. Their gestation period is about 130 days. Four newborns were given in reproductive groups of squirrel monkeys in 2020, and two of them were nursed by laboratory staffs due to neglect from their mothers. On the other hand, owl monkeys have become male-only colonies, and breeding has stopped at present.

Research using non-human primates

Our laboratory is the unique International Joint Usage and Research Center capable of conducting infection experiment using squirrel monkeys, owl monkeys, and cynomolgus monkeys. Until two years ago, our facility had equipped BSL3 animal experimental rooms for monkeys, but we shut down these rooms due to aging equipments and buildings. Instead, we are currently renovating animal experimental rooms up to BSL2, which specializes in conducting infection experiments in small monkeys. We are working with collaborators from several institutions to develop an experimental squirrel monkey infection model to assess the anti-malarial activity of new compounds and vaccines. In 2020, during unstable social situation continued under COVID19 epidemic, we focused on in vitro experiments which we were able to conduct, and succeeded in producing two experimental strains of Plasmodium falciparum (NF54 and IC1) adapted to squirrel monkey erythrocytes.

Publications

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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. High-resolution proteomic analysis of EGF-regulated ubiquitination dynamics in human cancer cells

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

Protein ubiquitination is one of the most prevalent post-translational modifications (PTMs) and plays critical roles in regulating protein degradation, signal transduction and DNA repair in cooperation with other PTMs such as phosphorylation and acetylation. Recent mass spectrometry-based proteomics coupled with efficient enrichment technologies for each type of the modified peptides has enabled us to identify precise modification sites and measure their quantitative changes on a global scale. Our previous lysine-modification proteomic analyses of thirteen representative human cancer cell lines led us to identify thousands of ubiquitination (Ub) and acetylation (Ac) sites in total and revealed that their system-wide modification status was mutually different at the cellular network level. In this study, we further applied SILAC (Stable Isotope Labeling by Amino acids in Cell culture) for quantitative description of EGF-dependent lysine-modification site dynamics in HeLa cells in a time-resolved manner. Through integration of large-scale SILAC-encoded data on six time points upon EGF stimulation, we successfully quantified approximately 1,000 kinds of Ub-sites as well as 700 kinds of Ac-sites and found that one-third of these Ub-modified molecules, including several EGF signaling effectors, were subjected to downregulation by proteasomal inhibition.

2. Proteome-wide analysis of lysine acetylation and ubiquitination reveals critical signaling regulation in cancer cells

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

Post-translational modifications (PTMs), such as phosphorylation, ubiquitination and acetylation, are known to be widely involved in the regulation of various biological processes through extensive diversification of each protein function at the cellular network

level. Previous functional analyses of cancer cell signaling under a variety of experimental conditions revealed many of the key molecules and their associated protein modifications in relation to each type of cancer. In order to systematically discover critical modulators from diversified signaling molecules, we have developed a high-resolution mass spectrometry-based proteomics platform for integrative identification and quantification of multiple post-translational modifications from various types of cancer cells. Our large-scale proteomic analysis enabled us to identify more than 5,000 kinds of ubiquitinated sites and 1,600 kinds of acetylated sites from representative human cancer cell lines, leading to identification of approximately 900 novel lysine modification sites in total. Very interestingly, 236 lysine residues derived from 141 proteins were found to be modified with both ubiquitination and acetylation. As a consequence of the subsequent motif extraction analyses, glutamic acid (E) was found to be highly enriched at the position (-1) for the lysine acetylation sites, whereas the same amino acid was relatively dispersed along the neighboring residues of the lysine ubiquitination sites.

3. System-wide perturbation of the proteome and phosphoproteome dynamics in glioblastoma stem cells through mTOR signaling inihibition

Hiroko Kozuka-Hata, Tomoko Hiroki, Ryo Koyama-Nasu[1], Kouhei Tsumoto, Jun-ichiro Inoue, Tetsu Akiyama[1] and Masaaki Oyama; [1]Laboratory of Molecular and Genetic Information, Institute for Quantitative Biosciences, The University of Tokyo

As glioblastoma is the most common and aggressive brain tumor with poor prognosis, systematic elucidation of signaling networks causally linked to the tumorigenesis is very crucial for developing more effective treatments for this intractable cancer. In our previous study, we applied a high-resolution mass spectrometry-based proteomics technology in combination with SILAC quantitative methods to understand EGF-dependent phosphoproteome dynamics in patient-derived glioblastoma stem cells. We demonstrated that the phosphorylation levels of the representative mTOR signaling molecules such as RPS6 and PRAS40 were dramatically up-regulated upon EGF stimulation. As EGFR signaling has been reported to play a pivotal role in regulating the maintenance of cancer stem cells, we next carried out mTOR inhibitor-dependent signaling perturbations to unravel stemness-related pathways at the network level.

In the present study, we identified a total of 3,726 proteins including 49 up-regulated and 436 down-regulated factors by Torin 1 treatment. Interestingly, we found that one of the well-known cancer stem cell markers was significantly down-regulated through mTOR signaling inhibition. Our in-depth phosphoproteome analysis also led to identification of 6,250 unique phosphopeptides derived from 2,221 proteins and unveiled a variety of dynamic changes regarding phosphorylation levels of cancer and neural stem cell markers in a comprehensive manner. The integrative view of the mTOR inhibitor-dependent proteome and phosphoproteome dynamics in glioblastoma stem cells presents us with further prospects towards understanding previously unrecognized regulations at the system level.

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

Hiroko Kozuka-Hata, Masato Suzuki[2], Kotaro Kiga[2], Shinya Tasaki, Jun-ichiro Inoue, Tadashi Yamamoto[3], Chihiro Sasakawa[2] and Masaaki Oyama: [2]Division of Bacterial Infection, Department of Microbiology and Immunology, IMSUT, [3]Division of Oncology, Department of Cancer Biology, IMSUT

The signal transduction system within a cell regulates complex biological events in response to bacterial infection. The previous analyses of cell signaling in Helicobacter pylori-infected gastric epithelial cells have revealed that CagA, a major virulence factor of Helicobacter pylori, is delivered into cells via the type IV secretion system and perturbs signaling networks through the interaction with the key signaling molecules such as SHP-2, Grb2, Crk/Crk-L, Csk, Met, and ZO-1. Although the biological activity of tyrosine-phosphorylated CagA has intensively been studied, system-wide effects of the virulence factor on cellular signaling have yet to be analyzed. Here we performed time-resolved analyses of phosphoproteome and CagA-interactome in human gastric AGS cells by CagA-positive/negative Helicobacter pylori infection. Our highly sensitive nanoLC-MS/MS analyses in combination with the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) technology defined CagA-dependent perturbation of signaling dynamics along with a subset of CagA-associated possible modulators on a network-wide scale. Our result indicated that the activation level of the phosphotyrosine-related signaling molecules in AGS cells was suppressed overall in the presence of CagA during Helicobacter pylori infection. As Helicobacter pylori infection plays pivotal roles in the progression of gastric diseases including carcinogenesis, a comprehensive and fine description of the signaling dynamics would serve as a fundamental platform to theoretically explore for the potential drug targets through analyzing the regulatory mechanisms at the system-level.

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

Masaaki Oyama, Hiroko Kozuka-Hata, Sumio Sugano[4], Tadashi Yamamoto[3] and Jun-ichiro Inoue: [4]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo

In parallel with the human genome projects, human full-length cDNA data has also been intensively accumulated. Large-scale analysis of their 5'-UTRs revealed that about half of these had a short ORF upstream of the coding region. Experimental verification as to whether such upstream ORFs are translated is essential to reconsider the generality of the classical scanning mechanism for initiation of translation and define the real outline of the human proteome. Our previous proteomics analysis of small proteins expressed in human K562 cells provided the first direct evidence of translation of upstream ORFs in human full-length cDNAs (Oyama et al., Genome Res, 14: 2048-2052, 2004). In order to grasp an expanded landscape of the human short ORFeome, we have performed an in-depth proteomics analysis of human K562 and HEK293 cells using a two-dimensional nanoLC-MS/MS system. The results led to the identification of eight protein-coding regions besides 197 small proteins with a theoretical mass less than 20 kDa that were already annotated coding sequences in the curated mRNA database. In addition to the upstream ORFs in the presumed 5'-untranslated regions of mRNAs, bioinformatics analysis based on accumulated 5'-end cDNA sequence data provided evidence of novel short coding regions that were likely to be translated from the upstream non-AUG start site or from the new short transcript variants generated by utilization of downstream alternative promoters. Protein expression analysis of the *GRINL1A* gene revealed that translation from the most upstream start site occurred on the minor alternative splicing transcript, whereas this initiation site was not utilized on the major mRNA, resulting in translation of the downstream ORF from the second initiation codon. These findings reveal a novel post-transcriptional system that can augment the human proteome via the alternative use of diverse translation start sites coupled with transcriptional regulation through alternative promoters or splicing, leading to increased complexity of short protein-coding regions defined by the human transcriptome (Oyama et al., Mol Cell Proteomics, 6: 1000-1006, 2007).

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

Toshiyuki Fujita[5], Hiroko Kozuka-Hata, Yutaro Hori[5], Jun Takeuchi[5], Takeo Kubo[5], and Masaaki Oyama: [5]Department of Biological Sciences, Graduate School of Science, The University of Tokyo

The honeybee (*Apis mellifera* L.) uses various chemical signals produced by the worker exocrine glands to maintain the functioning of its colony. The roles of worker postcerebral glands (PcGs), thoracic glands (TGs), and mandibular glands (MGs) and the functional changes they undergo according to the division of labor from nursing to foraging are not as well studied. To comprehensively characterize the molecular roles of these glands in workers and their changes according to the division of labor of workers, we analyzed the proteomes of PcGs, TGs, and MGs from nurse bees and foragers using shotgun proteomics technology. We identified approximately 2000 proteins from each of the nurse bee or forager glands and highlighted the features of these glands at the molecular level by semiquantitative enrichment analyses of frequently detected, gland-selective, and labor-selective proteins. First, we found the high potential to produce lipids in PcGs and MGs, suggesting their relation to pheromone production. Second, we also found the proton pumps abundant in TGs and propose some transporters possibly related to the saliva production. Finally, our data unveiled candidate enzymes involved in labor-dependent acid production in MGs.

<Group II>

Biomolecular recognition is based on collective and specific non-covalent interactions between discrete biological molecules. Our laboratory studies a variety of 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. Two Secreted Proteoglycans, Activators of Urothelial Cell-Cell Adhesion, Negatively Contribute to Bladder Cancer Initiation and Progression

Papadaki V, Asada K, Watson JK, Tamura T, Leung A, Hopkins J, Dellett M, Sasai N, Davaapil H, Nik-Zainal S, Longbottom R, Nakakido M, Torii R, Veerakumarasivam A, Kaneko S, Sagoo MS, Murphy G, Mitani A, Tsumoto K, Kelly JD, Hamamoto

R and Ohnuma SI.

Osteomodulin (OMD) and proline/arginine-rich end leucine repeat protein (PRELP) are secreted extracellular matrix proteins belonging to the small leucine-rich proteoglycans family. This study found that OMD and PRELP were specifically expressed in umbrella cells in bladder epithelia, and their expression levels were dramatically downregulated in all bladder cancers from very early stages and various epithelial cancers. The overexpression of OMD in bladder cancer cells strongly inhibited the anchorage-independent growth and tumorigenicity in mouse xenograft studies. On the other hand, we found that in the bladder epithelia, the knockout mice of OMD and/or PRELP gene caused partial EMT and a loss of tight junctions of the umbrella cells and resulted in formation of a bladder carcinoma in situ-like structure by spontaneous breakdowns of the umbrella cell layer. These data indicate that OMD and PRELP are endogenous inhibitors of cancer initiation and progression by controlling EMT. OMD and/or PRELP may have potential for the treatment of bladder cancer.

2. Structural Basis for the Binding Mechanism of Human Serum Albumin Complexed with Cyclic Peptide Dalbavancin

Ito S, Senoo A, Nagatoishi S, Ohue M, Yamamoto M, Tsumoto K and Wakui N.

This study presents the crystal structure of HSA complexed with dalbavancin, a clinically used cyclic peptide. Small-angle X-ray scattering and isothermal titration calorimetry experiments showed that the HSA-dalbavancin complex exists in a monomeric state; dalbavancin is only bound to the subdomain IA of HSA in solution. Structural analysis and MD simulation revealed that the swing of Phe70 and movement of the helix near dalbavancin were necessary for binding. The flip of Leu251 promoted the formation of the binding pocket with an induced-fit mechanism; moreover, the movement of the loop region including Glu60 increased the number of noncovalent interactions with HSA. These findings may support the development of new cyclic peptides for clinical use, particularly the elucidation of their binding mechanism to HSA.

3. Highly sensitive HPLC analysis and biophysical characterization of N-glycans of IgG-Fc domain in comparison between CHO and 293 cells using FcγRIIIa ligand

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

The regulation of the N-glycans of IgG-Fc domain is one of the key factors to maintain the safety and efficacy of antibody drugs. We used the mutant FcγRIIIa, which is produced in Escherichia coli and is therefore not glycosylated, as an affinity reagent to analyze the N-glycans of monoclonal antibodies expressed in Expi293 and ExpiCHO cells. The monoclonal antibodies expressed in these cells showed very different chromatograms, because of differences in terminal galactose residues on the IgG-Fc domains. We also carried out kinetic and thermodynamic analyses to understand the interaction between monoclonal antibodies and the mutant FcγRIIIa. Expi293 cell-derived monoclonal antibodies had higher affinity for the mutant FcγRIIIa than those derived from ExpiCHO cells, due to slower off rates and lower binding entropy loss. Collectively, our results suggest that the FcγRIIIa column can be used to analyze the glycosylation of antibodies rapidly and specifically.

4. Structure-based design and discovery of novel anti-tissue factor antibodies with cooperative double-point mutations, using interaction analysis

Chiba S, Tanabe A, Nakakido M, Okuno Y, Tsumoto K and Ohta M.

The generation of a wide range of candidate antibodies is important for the successful development of drugs that simultaneously satisfy multiple requirements. To find cooperative mutations and increase the diversity of mutants, an in silico double-point mutation approach, in which 3D models of all possible double-point mutant/antigen complexes are constructed and evaluated using interaction analysis, was developed. Starting from an antibody with very high affinity, four double-point mutants were designed in silico. Two of the double-point mutants exhibited improved affinity or affinity comparable to that of the starting antibody. The successful identification of two active double-point mutants showed that a cooperative mutation could be found by utilizing information regarding the interactions. The individual single-point mutants of the two active double-point mutants showed decreased affinity or no expression. These results suggested that the two active double-point mutants cannot be obtained through the usual approach i.e. a combination of improved single-point mutants. In addition, a triple-point mutant, which combines the distantly located active double-point mutation and an active single-point mutation collaterally obtained in the process of the double-point mutation strategy, was designed. The triple-point mutant showed improved affinity. This finding suggested that the effects of distantly located mutations are independent and additive. The double-point mutation approach using the interaction analysis of 3D structures expands the design repertoire for mutants, and hopefully paves a way for the identification of cooperative multiple-point muta-

tions.

5. Unique Electronic Structures of the Highly Ruffled Hemes in Heme-Degrading Enzymes of Staphylococcus aureus, IsdG and IsdI, by Resonance Raman and Electron Paramagnetic Resonance Spectroscopies

Takahashi S, Nambu S, Matsui T, Fujii H, Ishikawa H, Mizutani Y, Tsumoto K and Ikeda-Saito M.

Staphylococcus aureus uses IsdG and IsdI to convert heme into a mixture of staphylobilin isomers, 15-oxo-β-bilirubin and 5-oxo-δ-bilirubin, formaldehyde, and iron. The highly ruffled heme found in the heme-IsdI and IsdG complexes has been proposed to be responsible for the unique heme degradation products. We employed resonance Raman (RR) and electron paramagnetic resonance (EPR) spectroscopies to examine the coordination and electronic structures of heme bound to IsdG and IsdI. Heme complexed to IsdG and IsdI is coordinated by a neutral histidine. The trans ligand is hydroxide in the ferric alkaline form of both proteins. In the ferric neutral form at pH 6.0, heme is six-coordinated with water as the sixth ligand for IsdG and is in the mixture of the five-coordinated and six-coordinated species for IsdI. In the ferrous CO-bound form, CO is strongly hydrogen bonded with a distal residue. The marker lines, ν2 and ν3, appear at frequencies that are distinct from other proteins having planar hemes. The EPR spectra for the ferric hydroxide and cyanide states might be explained by assuming the thermal mixing of the d-electron configurations, (dxy)2(dxz,dyz)3 and (dxz,dyz)4(dxy)1. The fraction for the latter becomes larger for the ferric cyanide form. In the ferric neutral state at pH 6.0, the quantum mechanical mixing of the high and intermediate spin configurations might explain the peculiar frequencies of ν2 and ν3 in the RR spectra. The heme ruffling imposed by IsdG and IsdI gives rise to unique electronic structures of heme, which are expected to modulate the first and subsequent steps of the heme oxygenation.

6. Polymeric Nanocarriers with Controlled Chain Flexibility Boost mRNA Delivery In Vivo through Enhanced Structural Fastening

Miyazaki T, Uchida S, Nagatoishi S, Koji K, Hong T, Fukushima S, Tsumoto K, Ishihara K, Kataoka K and Cabral H.

Nanocarriers loading mRNA via polyion complexation with block catiomers into core-shell micellar structures are promising systems for enhancing mRNA delivery. Engineering the interaction between mRNA and catiomers through polymer design can promote the development of mRNA-loaded micelles (mRNA/m) with increased delivery efficiency. Particularly, the polycation chain rigidity may critically affect the mRNA-catiomer interplay to yield potent poly(glycidylbutylamine) (PEG-PGBA) and PEG-poly(L-lysine) (PEG-PLL) is studied. PEG-PGBA allows more than 50-fold stronger binding to mRNA than the relatively more rigid PEG-PLL, resulting in mRNA/m with enhanced protection against enzymatic attack and polyanions. mRNA/m from PEG-PGBA significantly enhances mRNA in vivo bioavailability and increased protein translation, indicating the importance of controlling polycation flexibility for forming stable polyion complexes with mRNA toward improved delivery.

7. 64Cu-labeled minibody D2101 visualizes CDH17-positive gastric cancer xenografts with short waiting time

Fujiwara K, Akiba H, Tsuji AB, Sudo H, Sugyo A, Nagatsu K, Zhang MR, Iwanari H, Kusano-Arai O, Kudo S, Kikuchi C, Tsumoto K, Momose T, Hamakubo T and Higashi T.

We previously reported In-labeled anti-cadherin17 (CDH17) IgG visualized CDH17-positive gastric cancer xenografts. Unfortunately, a long waiting time was required to obtain high-contrast images due to long blood retention (blood half-life: 26 h). To accelerate blood clearance, we have developed anti-CDH17 minibody (D2101 minibody) and evaluated the pharmacokinetics in gastric cancer mouse models. Cu-D2101 minibody exhibited higher tumor-to-blood ratios at earlier time points than those of the radiolabeled parental IgG. Cu-D2101 minibody has potential as an immunoimaging agent for CDH17-positive tumors.

8. Dual-Sensitive Nanomicelles Enhancing Systemic Delivery of Therapeutically Active Antibodies Specifically into the Brain

Xie J, Gonzalez-Carter D, Tockary TA, Nakamura N, Xue Y, Nakakido M, Akiba H, Dirisala A, Liu X, Toh K, Yang T, Wang Z, Fukushima S, Li J, Quader S, Tsumoto K, Yokota T, Anraku Y and Kataoka K.

Delivering therapeutic antibodies into the brain across the blood-brain barrier at a therapeutic level is a promising while challenging approach in the treatment of neurological disorders. Here, we present a polymeric nanomicelle (PM) system capable of delivering therapeutically effective levels of 3D6 antibody fragments (3D6-Fab) into the brain parenchyma for inhibiting Aβ aggregation. PM assembly was achieved by charge-converting 3D6-Fab through pH-sensitive citraconylation to allow complexation with reductive-sensitive cationic polymers. Brain targeting was achieved by functionalizing the PM surface with glucose molecules to allow interaction with recycling glucose transporter (Glut)-1 proteins. Consequently,

41-fold enhanced 3D6-Fab accumulation in the brain was achieved by using the PM system compared to free 3D6-Fab. Furthermore, therapeutic benefits were obtained by successfully inhibiting Aβ1-42 aggregation in Alzheimer's disease mice systemically treated with 3D6-Fab-loaded glucosylated PM. Hence, this nanocarrier system represents a promising method for effectively delivering functional antibody agents into the brain and treating neurological diseases.

9. Engineering Stability, Viscosity, and Immunogenicity of Antibodies by Computational Design

Kuroda D and Tsumoto K.

In recent years, computational methods have garnered much attention in protein engineering. A large number of computational methods have been developed to analyze the sequences and structures of proteins and have been used to predict the various properties. Antibodies are one of the emergent protein therapeutics, and thus, methods to control their physicochemical properties are highly desirable. However, despite the tremendous efforts of past decades, computational methods to predict the physicochemical properties of antibodies are still in their infancy. Experimental validations are certainly required for real-world applications, and the results should be interpreted with caution. Among the various properties of antibodies, we focus in this review on stability, viscosity, and immunogenicity, and we present the current status of computational methods to engineer such properties.

10. Discovery of chemical probes that suppress Wnt/β-catenin signaling through high-throughput screening

Yamaguchi K, Nagatoishi S, Tsumoto K and Furukawa Y.

Aberrant activation of the Wnt/β-catenin signaling pathway has been observed in a wide range of human tumors. Deregulation of the pathway is closely linked to various aspects of human carcinogenesis such as cell viability, regulation of cell cycle, epithelial-mesenchymal transition, and maintenance of stemness. In addition, recent studies have disclosed the involvement of Wnt signaling in immune evasion of tumor cells. The accumulation of β-catenin in the nucleus is a common feature of cancer cells carrying defects in the pathway, which leads to the continuous activation of T-cell factor (TCF)/LEF transcription factors. Consequently, a genetic program is switched on, leading to the uncontrolled growth, prolonged survival, and acquisition of mesenchymal phenotype. As β-catenin/TCF serves as a signaling hub for the pathway, β-catenin/TCF-dependent transcriptional activity is a relevant readout of the pathway. To date, a wide variety of synthetic TCF/LEF reporters has been developed, and high-throughput screening (HTS) using these reporters has made significant contributions to the discovery of Wnt inhibitors. Indeed, HTS led to the identification of chemical probes targeting porcupine, a membrane bound O-acyltransferase, and CREB-binding protein, a transcriptional coactivator. This review focuses on various screening strategies for the discovery of Wnt inhibitors and their mode of action to help the creation of new concepts for assay/screening methods.

11. GPC1 specific CAR-T cells eradicate established solid tumor without adverse effects and synergize with anti-PD-1 Ab

Kato D, Yaguchi T, Iwata T, Katoh Y, Morii K, Tsubota K, Takise Y, Tamiya M, Kamada H, Akiba H, Tsumoto K, Serada S, Naka T, Nishimura R, Nakagawa T and Kawakami Y.

Current xenogeneic mouse models cannot evaluate on-target off-tumor adverse effect, hindering the development of chimeric antigen receptor (CAR) T cell therapies for solid tumors, due to limited human/mouse cross-reactivity of antibodies used in CAR and sever graft-versus-host disease induced by administered human T cells. We have evaluated safety and antitumor efficacy of CAR-T cells targeting glypican-1 (GPC1) overexpressed in various solid tumors. GPC1-specific human and murine CAR-T cells generated from our original anti-human/mouse GPC1 antibody showed strong antitumor effects in xenogeneic and syngeneic mouse models, respectively. Importantly, the murine CAR-T cells enhanced endogenous T cell responses against a non-GPC1 tumor antigen through the mechanism of antigen-spreading and showed synergistic antitumor effects with anti-PD-1 antibody without any adverse effects in syngeneic models. Our study shows the potential of GPC1 as a CAR-T cell target for solid tumors and the importance of syngeneic and xenogeneic models for evaluating their safety and efficacy.

12. How the protonation state of a phosphorylated amino acid governs molecular recognition: insights from classical molecular dynamics simulations

Kawade R, Kuroda D and Tsumoto K.

Physicochemical properties of proteins are controlled mainly by post-translational modifications such as amino acid phosphorylation. Although molecular dynamics simulations have been shown to be a valuable tool for studying the effects of phosphorylation on protein structure and dynamics, most of the previous studies assumed that the phosphate group

was in the unprotonated (PO2−3PO32-) state, even though the protonation state could in fact vary at physiological pH. In this study, we performed molecular dynamics simulations of four different protein-phosphorylated peptide complexes both in the PO2−3PO32- and PO_3 H^- states. Our simulations delineate different dynamics and energetics between the two states, suggesting importance of the protonation state of a phosphorylated amino acid in molecular recognition.

13. Methodology for Further Thermostabilization of an Intrinsically Thermostable Membrane Protein Using Amino Acid Mutations with Its Original Function Being Retained

Yasuda S, Akiyama T, Nemoto S, Hayashi T, Ueta T, Kojima K, Tsukamoto T, Nagatoishi S, Tsumoto K, Sudo Y, Kinoshita M and Murata T.

We develop a new methodology best suited to the identification of thermostabilizing mutations for an intrinsically stable membrane protein. The recently discovered thermophilic rhodopsin, whose apparent midpoint temperature of thermal denaturation Tm is measured to be ~91.8 °C, is chosen as a paradigmatic target. In the methodology, we first regard the residues whose side chains are missing in the crystal structure of the wild type (WT) as the "residues with disordered side chains," which make no significant contributions to the stability, unlike the other essential residues. We then undertake mutating each of the residues with disordered side chains to another residue except Ala and Pro, and the resultant mutant structure is constructed by modifying only the local structure around the mutated residue. This construction is based on the postulation that the structure formed by the other essential residues, which is nearly optimized in such a highly stable protein, should not be modified. The stability changes arising from the mutations are then evaluated using our physics-based free-energy function (FEF). We choose the mutations for which the FEF is much lower than for the WT and test them by experiments. We successfully find three mutants that are significantly more stable than the WT. A double mutant whose Tm reaches ~100 °C is also discovered.

14. System-Wide Analysis of Protein Acetylation and Ubiquitination Reveals a Diversified Regulation in Human Cancer Cells

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

Post-translational modifications are known to be widely involved in the regulation of various biological processes, through the extensive diversification of each protein function at the cellular network level. In order to unveil the system-wide function of the protein lysine modification in cancer cell signaling, we performed global acetylation and ubiquitination proteome analyses of human cancer cells, based on high-resolution nanoflow liquid chromatography-tandem mass spectrometry, in combination with the efficient biochemical enrichment of target modified peptides. Our large-scale proteomic analysis enabled us to identify more than 5000 kinds of ubiquitinated sites and 1600 kinds of acetylated sites, from representative human cancer cell lines, leading to the 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. Our pathway analysis also indicated that the protein translational control pathways, such as the eukaryotic initiation factor 2 (EIF2) and the ubiquitin signaling pathways, were highly enriched in both of the acetylation and ubiquitination proteome data at the network level. This report provides the first integrative description of the protein acetylation and ubiquitination-oriented systematic regulation in human cancer cells.

15. Per-Residue Program of Multiple Backbone Dihedral Angles of β-Peptoids viaBackbone Substitutions

Morimoto J, Kim J, Kuroda D, Nagatoishi S, Tsumoto K and Sando S.

Unique folded structures of natural and synthetic oligomers are the most fundamental basis for their unique functions. N-Substituted β-peptides, or β-peptoids, are synthetic oligomers with great potential to fold into diverse three-dimensional structures because of the existence of four rotatable bonds in a monomer with highly modular synthetic accessibility. However, the existence of the four rotatable bonds poses a challenge for conformational control of β-peptoids. Here, we report a strategy for per-residue programming of two dihedral angles of β-peptoids, which is useful for restricting the conformational space of the oligomers. The oligomer was found to form a unique loop conformation that is stabilized by the backbone rotational restrictions. Circular dichroism and NMR spectroscopic analyses and X-ray crystallographic analysis of the oligomer are presented. The strategy would significantly facilitate the discovery of many more unique folded structures of β-peptoids.

16. 111 In-labeled anti-cadherin17 antibody D2101 has potential as a noninvasive imaging probe for diagnosing gastric cancer and lymph-node metastasis

Fujiwara K, Tsuji AB, Sudo H, Sugyo A, Akiba H, Iwanari H, Kusano-Arai O, Tsumoto K, Momose T, Hamakubo T and Higashi T.

Cadherin-17 (CDH17) is a transmembrane protein that mediates cell-cell adhesion and is frequently expressed in adenocarcinomas, including gastric cancer. CDH17 may be an effective diagnostic marker for the staging of gastric cancer. Here, we developed an 111In-labeled anti-CDH17 monoclonal antibody (Mab) as an imaging tracer and performed biodistribution and single-photon emission computed tomography (SPECT)/computed tomography (CT) imaging studies using mice with CDH17-positive gastric cancer xenografts. Our 111In-anti-CDH17 Mab D2101 depicted CDH17-positive gastric cancer xenografts in vivo and has the potential to be an imaging probe for the diagnosis of primary lesions and lymph-node metastasis in gastric cancer.

17. Technical Capabilities and Limitations of Optical Spectroscopy and Calorimetry Using Water-Miscible Solvents: The Case of Dimethyl Sulfoxide, Acetonitrile, and 1,4-Dioxane.

Hirano A, Nagatoishi S, Wada M, Tsumoto K, Maluf KN and Arakawa T.

In drug development, water-miscible solvents are commonly used to dissolve drug substances. Typical routine procedures in drug development include dilution of the stock drug solution into an aqueous solution containing target macromolecules for drug binding assays. However, water-miscible solvents impose some technical limitations on the assays on account of their light absorption and heat capacity. Here, we examined the effects of the dilution of 3 water-miscible solvents, that is, dimethyl sulfoxide, acetonitrile, and 1,4-dioxane, on the baseline stability and signal/noise ratio in circular dichroism spectroscopy, isothermal titration calorimetry, and differential scanning calorimetry. Dimethyl sulfoxide and 1,4-dioxane affect the signal/noise ratio of circular dichroism spectra at typically used concentrations due to their light absorbance. The water-miscible solvents generate interfering signals in the isothermal titration calorimetry due to their mixing heat. They show negative or positive slope in the differential scanning calorimetry. Such interfering effects of the solvents are reduced by appropriate dilution according to the analytical techniques. Because the water-miscible solvents have solubilization capacity for alkyl chain moieties and aromatic moieties of chemicals, drug substances containing these moieties can be dissolved into the solvents and then subjected to the analyses to examine their interactions with target proteins after appropriate dilution of the drug solutions.

18. Generation of biparatopic antibody through two-step targeting of fragment antibodies on antigen using SpyTag and SpyCatcher

Akiba H, Takayanagi K, Kusano-Arai O, Iwanari H, Hamakubo T and Tsumoto K.

Biparatopic fragment antibodies can overcome deficiencies in avidity of conventional antibody fragments. Here, we describe a technology for generating biparatopic antibodies through two-step targeting using a pair of polypeptides, SpyTag and SpyCatcher, that spontaneously react to form a covalent bond between antibody fragments. In this method, two antibody fragments, each targeting different epitopes of the antigen, are fused to SpyTag and to SpyCatcher. When the two polypeptides are serially added to the antigen, their proximity on the antigen results in covalent bond formation and generation of a biparatopic antibody. We validated the system with purified recombinant antigen. Results in antigen-overexpressing cells were promising although further optimization will be required. Because this strategy results in high-affinity targeting with a bipartite molecule that has considerably lower molecular weight than an antibody, this technology is potentially useful for diverse applications.

<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 connectivity of actual neural cells morphologically was to collect ultrathin serial sections and observe them in transmission electron microscope (TEM). But the technical difficulties discouraged us from such a troublesome procedure. Recent progress in scanning electron microscope (SEM) equipment allowed us to develop a new method to observe ultrathin TEM sections in SEM (thin section scanning electron microscopy: TSSEM). To observe thin TEM sections, we have developed new sample staining methods to enhance electron contrast. To collect huge number of serial sections stably and efficiently, we

have been developing new equipment and techniques. By using this equipment, it became possible to cut more than 1000 serial sections of less than 30 nm thickness much easier. We have analyzed about 500 serial thin sections of zebrafish retinal outer plexiform layer by this method and succeeded in tracing thin processes of bipolar cells into the photoreceptor terminals.

Aside from getting 3D information, TSSEM method provide us precise information of much wider areas of thin sections more effectively and more easily. Such studies are currently in progress.

2. Collaborative and supportive works as electron microscope core-laboratory

This group is also engaged in collaborative researches using electron microscope. We offer supports for the research projects those need electron microscopic analysis. The services available in this group are the conventional thin section transmission electron microscopy, immuno-electron microscopy, negative staining techniques and scanning electron microscopy. By using these individual technique or combination of some of these we can offer direct visual evidence that cannot be acquired by other methods. This year, 18 projects in 12 laboratories were performed as core-laboratory works.

a. Thin section transmission electron microscopy

Thin section transmission electron microscopy is the most widely used technique to observe the inner structure of cells and tissues. In this method, samples are fixed and embedded in epoxy resin, thin sections with about 70 nm thickness are cut and observed in the electron microscope. In case of immuno-electron microscopy, thin sections are obtained by similar procedure and the antigen epitopes exposed on the surface of the sections are marked by sequential reaction with appropriate primary antibodies and colloidal gold labeled secondary antibodies. This year, thin section electron microscopy combined with immuno-electron microscopy were used in many collaborative works.

a-1. Ultrastructural analysis of entry and assembly of Herpes Simplex Virus

We have been performing several studies with research groups in Dr. Kawaguchi[1]'s laboratory: [1]Division of Molecular Virology, Department of Microbiology and Immunology, regarding the infection/replication processes of herpes simplex virus (HSV). Thin section electron microscopy has been used to analyze the function of viral proteins in trans-nuclear membrane processes of the newly formed viruses. By analyzing the virus forming processes in some mutant host cells, we could analyze viral proteins as well as candidate host molecules those may be involved in the trans-nuclear process of the HSV.

a-2. Roles of membrane lipids in development and maintenance of photoreceptor outer-segment

We have been performing several studies also with research groups in Dr. Watanabe[2]'s laboratory: [2]Project Division of Molecular and Developmental Biology. This year, we analyzed the composition of phospholipids in individual cell types in developing mouse retina under physiological and pathological conditions and checked with electron microscopic data. With the combination of cell sorting and mass spectrometry analysis, most of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) in retina are included in photoreceptor cells. When compared with the electron microscopic data in pathological conditions, PC and PE composition are dramatically changed before photoreceptor cell degeneration are apparent, suggesting that changes in PC and PE composition in photoreceptor cells may lead to the photoreceptor degeneration. (ref. Hamano *et al*) Another project is also running with Dr. Watanabe's laboratory.

Some other collaborative research works using thin section electron microscopy and/or immuno-electron microscopy were performed with Dr. Noda[3], [3]Laboratory of Ultrastructural Virology, Department of Virus Research, Institute of Frontier Life and Medical Sciences, Kyoto University, Dr. Sasou[4], [4]Division of Mucosal Immunology, Dr. Eguchi[5], (ref. Eguchi *et al*) in [5]Division of Genetics, Dr. Nakahara[6] (ref. Mochizuki *et al*) in [6]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. Ishii[7] in [7]Division of Vaccine Science, Laboratory of Adjuvant Innovation. The same techniques are also used in the research with Dr. Chou[8], in [8]Department of Research and Development, Life Innovation Center, LIFEBANK Japan, Inc. Negative staining techniques are also used to visualize virus like particles in the research with Dr. Kurokawa[4] and Dr. Yoshida[9] in [9]Project Division of Advanced Biopharmaceutical Science.

c. Conventional scanning electron microscopy

Conventional scanning electron microscopy is a technique used to examine the surface structure of the cells, tissues or other non-biological materials. The collaborative works using scanning electron micros-

copy were done with Dr. Nakamura[10], in [10] Division of Cell Signaling and Molecular Medicine, concerning the morphology of cilia.

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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 the Institute of Biophysics (IBP) and Institute of Microbiology (IM), which belong to the Chinese Academy of Sciences (CAS), a large national institution consisting of more than 100 research institutes all over China. IMSUT has dispatched Jin Gohda to IM as a principal investigator (PI). Along with his Chinese staffs, PI is conducting basic and translational studies of HIV, MERS coronavirus, dengue virus and SARS-CoV-2. In 2015 IMSUT has set up another project laboratory in Tokyo, whose studies complement those in Beijing. IMSUT is also conducting a joint research program on avian influenza virus between Yoshihiro Kawaoka at IMSUT and Hualan

Chen at the Harbin Veterinary Research Institute (HVRI) of Chinese Academy of Agricultural Sciences. The activities in Beijing and Harbin are supported by Mitsue Hayashi of the Beijing Project Office.

This project, making the most of the opportunity of collaboration with the highly advanced Chinese institution, aims to translate our basic studies into practical use in future. During the course of the collaboration the project intends to train and educate young Chinese and Japanese scientists for the future generation and hopes to contribute to the friendship between the two peoples.

PROJECT LABORATORIES AND PROGRAM

Y. Kawaguchi (Director of Research Center for Asian Infectious Diseases; Project Director) manages the Center and the AMED-supported Project, which includes the domestic and overseas laboratories and program. He coordinates their activities and decides the direction of research. He and his group conduct studies of molecular virology and immunology of herpes virus in the Research Center for Asian Infectious Diseases.

a. Project Laboratory at IMSUT and Joint Laboratory at IMCAS

Many enveloped viruses, such as HIV-1, flavivirus, herpes simplex virus, and coronavirus, are pathogenic and of clinical importance. J. Gohda's and Y. Kawaguchi's groups are conducting a basic research on the development of antiviral therapy for infectious diseases caused by enveloped viruses.

Severe acute respiratory syndrome coronavirus 2 (SRAS-CoV-2) is the causative virus of Coronavirus disease 2019 (COVID-19), which has spread worldwide since the first case was reported in China in December 2019. The rapid development of antiviral drugs and vaccine against SARS-CoV-2 infection is needed for bringing an ongoing pandemic of COVID-19 to an end. J. Gohda and his group established a dual split protein-based fusion assay for SARS-CoV-2 spike (S) protein to evaluate the antiviral activities of compounds and antibodies against SARS-CoV-2. Using the fusion assay, they found that an existing Japanese pancreatitis drug, nafamostat strongly prevents viral entry of SARS-CoV-2 by inhibiting a serine protease, TMPRSS2, which is crucial for membrane fusion between SARS-CoV-2 and its target cells. Furthermore, the fusion assay for Zika virus envelop (E) protein identified that an antimalarial drug, atovaquone inhibits E protein-mediated membrane fusion. Atovaquone blocked in vitro infection with Zika virus as well as dengue viruses, indicating that atovaquone is a clinical drug candidate to treat illness caused by flavivirus infection.

J. Gohda and his group are currently trying to identify compounds which block the release of HIV-1 viral particles from infected cells. They established the detection system for quantification of HIV-1 viral particles by using an HIV-1 vector with HiBiT-sequence inserted in a gag gene. In this system, viral particles in culture supernatant of infected cells with HIV-1 derived from the vector can be easily detected and quantified by a NanoLuc assay. They now identified 14 drugs that probably block the release of viral particles, through drug library screening using the system. Y. Kawaguchi and his group showed that phosphatidylethanolamine (PE) in cell membrane is crucial for herpes simplex virus 1 (HSV-1) envelopment in the cytoplasm of infected cells and for viral replication and pathogenicity *in vivo*. Furthermore, they also discovered that meclizine, which is an inhibitor of a key enzyme for PE biosynthesis, phosphate cytidylyltransferase 2, ethanolamine (Pcyt2), dramatically reduced HSV-1 replication and pathogenicity in mice. These suggest not only that PE biosynthesis can be a target for the development of novel antiviral drugs for HSV-1, but also that an old antihistamine, meclizine can be a repositioned drug for HSV-associated diseases.

b. Joint Laboratory at IBPCAS

The Joint Laboratory at IBPCAS was closed in March 2020. However, the research collaboration and academic exchange between IMSUT and IBPCAS is still ongoing.

c. Collaborative research program with HVRI

At the end of 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) was detected in Wuhan, China, that spread rapidly around the world, with severe consequences for human health and the global economy. In China, highly pathogenic avian influenza (HPAI) H5N1 virus transmitted to humans in 1997; since 2013, low pathogenic avian influenza A H7N9 viruses have caused sporadic infections in humans; and in 2016, HPAI H7N9 viruses emerged raising concerns of a pandemic. For these reasons, HVRI (Director, Zhigao Bu) has been conducting collaborative research on influenza virus, SARS-CoV-2, and other emerging viruses from all over Asia.

HVRI focuses on avian influenza viruses that are circulating in Chinese wild waterfowl, domestic poultry, and swine. Specifically, Y. Kawaoka and his group study type A influenza viruses and SARS-CoV-2 viruses with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral surveillance.

Their major findings this year include: (1) Prolonged treatment of immunocompromised influenza patients with viral neuraminidase (NA) inhibitors is required, because the immune system of such patients fails to eradicate the viruses. The Kawaoka group attempted to eradicate influenza virus from the

respiratory organs of nude mice, which is a model of immunocompromised hosts, by using combination therapy of the viral polymerase inhibitor favipiravir and monoclonal antibodies (mAbs) against the receptor-binding site (RBS) and stem of viral hemagglutinin (HA). Although monotherapy or combination therapy with two antivirals suppressed virus replication, they failed to eradicate viruses from nude mice. In contrast, triple combination therapy of favipiravir plus anti-Stem and anti-RBS mAbs completely blocked virus replication in nude mice, resulting in virus clearance. Triple combination approaches should be considered for the treatment of human immunocompromised patients with severe influenza. (2) The Kawaoka group assessed the replicative ability and pathogenesis of SARS-CoV-2 isolates in Syrian hamsters. SARS-CoV-2 isolates replicated efficiently in the lungs of hamsters, causing severe pathological lung lesions following intranasal infection. SARS-CoV-2–infected hamsters mounted neutralizing antibody responses and were protected against subsequent rechallenge with SARS-CoV-2. Moreover, passive transfer of convalescent serum to naïve hamsters efficiently suppressed the replication of the virus in the lungs even when the serum was administrated 2 days post-infection of the serum-treated hamsters. Collectively, these findings demonstrate that this Syrian hamster model will be useful for understanding SARS-CoV-2 pathogenesis and testing vaccines and antiviral drugs.

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 young investigators to challenge new fields of bio-medical sciences.

Frontier Research Unit

Protein phosphorylation and dephosphorylation are among the most important intracellular signaling mechanisms, and are mediated, respectively, by protein kinases and protein phosphatases. We study various aspects of cellular signal transduction with a particular emphasis on the role and regulation of protein phosphorylation and dephosphorylation in cellular stress responses, using yeast cells.

1. Osmostress enhances activating phosphorylation of Hog1 MAP kinase by mono-phosphorylated Pbs2 MAP2K

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Signaling by the conserved mitogen-activated protein kinase (MAPK) family is a major cellular mechanism through which eukaryotic cells respond to various extracellular stimuli. All MAPKs are activated through a three-tier kinase cascade, composed of a MAPK, a MAPK kinase (MAP2K) and a MAPKK kinase (MAP3K). Distinct MAP3Ks activated by a specific stimulus phosphorylate and thus activate a cognate MAP2K, which then phosphorylates and activates a downstream MAPK. Activated MAPKs regulate pertinent adaptive responses, such as gene expression, cell cycle progression, and apoptosis.

MAPK cascades are highly conserved from yeast to mammalian species, so much so that the mammalian MAPK p38 can functionally complement the yeast MAPK Hog1. A MAPK signal transduction pathway commonly comprises, in addition to the core MAPK cascade, an upstream transmembrane receptor or sensor that detects specific extracellular stimuli, and downstream MAPK substrate molecules (effectors) both in the cytoplasm and in the nucleus. Several different MAPK pathways often co-exist within a cell. In yeast, for example, four MAPKs (Slt2/Mpk1, Kss1, Fus3, and Hog1) are expressed in a cell. If inappropriate crosstalk occurred between two MAPK cascades, a stimulus aimed at activation of only one of these cascades could incite irrelevant or even detrimental responses.

Different MAPKs in a species are highly homologous to each other, and so are MAP2Ks. Thus, prevention of inappropriate crosstalk between MAPK cascades requires elaborate mechanism for any MAPK cascade, but its difficulty can be most clearly exemplified by the MAPK cascades in yeast, in which three different MAPK cascades with different specifi-

cities use the same MAP3K Ste11. The MAPK Hog1 is activated by hyperosmotic stress through the High Osmolarity Glycerol (HOG) pathway, and orchestrates an array of osmoadaptive 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 comprises 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 activates the functionally redundant MAP3Ks Ssk2 and Ssk22 (Ssk2/22). Activated Ste11 and Ssk2/22 phosphorylate the MAP2K Pbs2, and phosphorylated Pbs2 then activates Hog1.

Two other yeast MAPKs Fus3/Kss1 are activated by the mating pheromones through Ste11 and the MAP2K Ste7. Although the mating pheromones activate Ste11, they do not activate Hog1. Commonly, the absence of pheromone-to-Hog1 crosstalk is explained by the pathway insulation model, which posits that a scaffold protein holds several components of one pathway close together, so that signal flows only within that pathway. To prevent crosstalk, however, the scaffold proteins must hold kinases for significantly longer than the half-lives of their activities, which could be several minutes or longer. Because scaffold complexes are typically not so stable, additional mechanisms other than scaffolding of signaling complexes are likely to be necessary to effectively prevent crosstalk.

This year, we found that mono-phosphorylated Pbs2 cannot phosphorylate Hog1 unless the reaction between Pbs2 and Hog1 is enhanced by osmostress. 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. We also found that the rapid and transient Hog1 activation kinetics at mildly high osmolarities and the slow and prolonged activation kinetics at severely high osmolarities are both caused by a common feedback mechanism.

2. Activating phosphorylation sites of Ser-514 and Thr-518 in the yeast Pbs2 MAP2K are differentially regulated by MAP3Ks and phosphatases

Kazuo Tatebayashi

The family of mitogen-activated protein kinases (MAPKs) are major intracellular signal transducers in eukaryotic cells, and are associated with many human diseases. Each MAPK is activated in a three-tiered kinase cascade composed of a MAPK kinase kinase (MAPKKK or MAP3K), a MAPK kinase (MAPKK or MAP2K), and a MAPK. In the canonical model of the MAPK cascades, an activated MAP3K activates a cognate MAP2K by phosphorylating two conserved serine (Ser) and/or threonine (Thr) residues in the flexible activation loop of the MAP2K. In turn, an activated MAP2K activates a cognate MAPK by phosphorylating the conserved Thr and tyrosine (Tyr) residues in the latter's activation loop. While it is established that MAP3Ks phosphorylates two conserved sites in MAP2Ks, it is unclear whether phosphorylation of both sites is required for MAP2K activation or whether phosphorylation of only one site is sufficient.

This year, we found that the MAP3Ks Ste11 and Ssk2/22 differentially phosphorylate Pbs2 in the yeast HOG pathway. Although activated Ste11 and Ssk2/22 were believed to phosphorylate the MAP2K Pbs2 at Ser-514 and Thr-518 (S514 and T518), we showed that Ste11 phosphorylates only one activating phosphorylation site (T518) in Pbs2, whereas the MAP3Ks Ssk2/Ssk22 can phosphorylate both S514 and T518 under optimal osmostress conditions. In addition, we identified a phosphatase that specifically dephosphorylates phosphorylated T518, but not phosphorylated S514.

Publication

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We are challenging to cure intractable hematological disorders such as leukemia and lymphoma with the aid of hematopoietic stem cell transplantation (HSCT). Our major stem cell source for recipients without suitable family donors is unrelated cord blood, with which no less than 20 adult patients receive cord blood transplantation (CBT) annually. Since 1998, we have performed around 400 cases of CBT, which appears a distinguished experience in the world.

Recent advances in identification of tumor-specific therapeutic targets resulted in a series of rationally designed therapeutic agents. In the field of hematological malignancies, we have already experienced remarkable clinical efficacies of molecular targeted drugs including tyrosine kinase inhibitors for Philadelphia-chromosome positive leukemia, monoclonal antibodies (MAb) for $CD20^+$ B cell lymphoma and $CCR4^+$ adult T cell leukemia/lymphoma (ATL), and proteasome inhibitors, immunomodulatory drugs for multiple myeloma (MM), respectively. Additionally, novel therapeutic modalities including anti-CD319 and anti-CD38 MAb are available for MM. We extensively apply these molecular targeted therapies for in- and out-patients. Furthermore, our department is one of the hub facilities in Japan for clinical practice and clinical research in ATL and Langerhans cell histiocytosis (LCH), both of which are rare and intractable tumors.

1. Artificial intelligence (AI)-guided precision medicine approach to hematological malignancies.

Yokoyama K[1], Yokoyama N[2, 6], Nakamura S[3], Ogawa M[3], Takei T[3], Kobayashi M[3], Ando S[1], Kondo K[1], Mizusawa M[1], Isobe M[1], Tanoue S[1], Kawamata T[1], Makiyama J[1], Konuma T[1], Kato S[1], Imai Y[1], Takahashi S[1, 3], Shimizu E[4], Yamaguchi R[4], Imoto S[5], Furukawa Y[2, 6], Miyano S[4], Tojo A[1, 3]

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Next generation sequencing (NGS) of cancer genome is now becoming prerequisite for accurate diagnosis and proper treatment in clinical oncology (Precision oncology). While the genomic regions for NGS expand from a certain set of genes to whole exome or whole genome, the resulting sequence data becomes incredibly enormous, and then makes it quite laborious to translate the genomic data into medicine, so-called annotation and curation. We organized a clinical sequencing team and established a bidirectional (bed to bench and bench to bed) system to integrate clinical and genomic data in blood cancers. We also started a collaborative research with IBM Japan to adopt artificial or augmented intelligence (AI), Watson for Genomics (WG), to the pipeline of medical informatics. Genomic DNA was prepared from cancer cells as well as normal tissues (buccal swab) in each patient and subjected to NGS. Sequence data was analyzed using an in-house semi-automated pipeline in combination with WG, which was used to identify candidate driver mutations and relevant pathways, from which applicable drug information was deduced. Until now, we have analyzed more than 400 patients in total with hematological malignancies including AML, MDS, MPN, et al., and could obtain many informative findings. Although actionable mutations are quite insufficient for clinical practice mainly due to the lack of available molecular-targeted agents, our preliminary results indicate that AI can be a promising support tool for precision medicine.

2. High prevalence of left ventricular non-compaction and its impact on chemotherapy-related cardiac dysfunction in patients with hematological diseases

Hirano M[1, 2], Kimura K[3, 4, 5], Ishigaki T[3], Nojima M[6], Daimon M[5, 7], Morita H[5], Takenaka K[7], Xu B[5], Sawada N[5], Hirokawa M[5], Komuro I[5], Morisaki T[4], Yotsuyanagi H[4], Kawamata T[1, 2], Yokoyama K[1, 2], Konuma T[1, 2], Kato S[1, 2], Yasui H[1, 2], Nagamura-Inoue T[1, 2], Uchimaru K[1, 8], Takahashi S[1, 2], Imai Y[1, 2], Tojo A[1, 2].

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Recent progress in chemotherapy has prolonged the survival of patients with hematological diseases but has also increased the number of patients with chemotherapy-related cardiac dysfunction (CTRCD). However, the causes of individual variations and risk factors for CTRCD have yet to be fully elucidated. Methods and Results:Consecutive echocardiograms of 371 patients were retrospectively evaluated for the presence of left ventricular (LV) non-compaction (LVNC). Individual LV ejection fraction (LVEF) outcome estimates were made using bivariate linear regression with log-transformed duration Akaike information criterion (AIC) model fitting. The prevalence of LVNC was 6-fold higher in patients with hematological diseases than in those with non-hematological diseases (12% vs. 2%; risk ratio 6.1; 95% confidence interval [CI] 2.0, 18.2). Among patients with hematological diseases, the ratio of myeloid diseases was significantly higher in the group with LVNC ($P = 0.031$). Deterioration of LVEF was more severe in patients with than without LVNC (-14.4 percentage points/year [95% CI -21.0, -7.9] vs. -4.6 percentage points/year [95% CI -6.8, -2.4], respectively), even after multivariate adjustment for baseline LVEF, background disease distributions, cumulative anthracycline dose, and other baseline factors.

3. Clinical features and outcomes of adult Langerhans cell histiocytosis

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Langerhans cell histiocytosis (LCH) is a neoplastic disorder characterized by clonal expansion of

CD1a + CD207 + myeloid dendritic cells. Since LCH is a rare disease and its incidence is less frequent in adults than children, clinical features and prognosis of adult LCH is poorly documented. We retrospectively reviewed 56 adult LCH patients who were referred to IMSUT Hospital from 2005 to 2018. Median age at diagnosis was 41.5 and there was a slight female predominance (57%). The median time between disease onsets to diagnosis was 7.5 months, however, there was a wide variety and the average was 22.4 months. Forty-one % had a single organ involvement and 59% had multiple organ involvement. Overall, most frequently affected organ was bone (61%), and lung (30%) and skin (25%) followed. Twenty-six patients received Special C regimen formulated by the Japan LCH Study Group, and 21 patients observed partial response or better. One patient died during the treatment, and other 25 patients are alive to date. Median progression-free survival has not yet been reached despite a median follow-up of 35.5 months. Immunohistochemistry analysis revealed that 39% was positive for *BRAF*-V600E, which was lower than the previous reports from North America and Europe. Since adult LCH is quite rare and the symptoms vary among patients, the diagnosis and treatment is generally delayed. Physicians should be aware of this disorder, particularly as new opportunities emerge for treatment.

Next, we xamined 13 patients who underwent endoscopic examinations: an upper gastrointestinal (GI) endoscopy alone in 5, lower GI endoscopy alone in 3, and both in 5 patients. A gastric lesion (1 case), colonic lesion (1 case), and both gastric and rectal lesions (1 case) were detected. These three cases showed multiple organ involvement without gastrointestinal symptoms or increased uptake on PET-CT. Endoscopy revealed small erosions without specific features; histological examinations were required for diagnosis. The three patients were treated with Special-C regimen; in two cases, the clinical condition remained stable for several years post-treatment. One case showed recurrence 1 year 7 months after treatment, and chemotherapy was re-administered. Although gastrointestinal LCH lesions are rare, they were more common than expected in our cases of multisystem LCH.

4. Optimal treatment strategy with nilotinib for patients with newly diagnosed chronic-phase chronic myeloid leukemia based on early achievement of deep molecular response ($MR^{4.5}$): the phase 2, multicenter N-Road study.

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For patients who have chronic myeloid leukemia (CML), one of the primary treatment options is administration of nilotinib 300 mg twice daily (BID). In previous studies which compared outcomes associated with nilotinib or imatinib treatment, nilotinib achieved a higher rate of deep molecular response (MR). We conducted a phase II, open-label, multicenter study to investigate an intrapatient nilotinib dose-escalation strategy for patients with newly diagnosed chronic-phase (CP) CML based on early $MR^{4.5}$ achievement. The primary study endpoint was achievement of $MR^{4.5}$ by 24 months following the initiation of nilotinib 300 mg BID. Fifty-three patients were enrolled, 51 received nilotinib, and 37 completed the treatment. An increase in the nilotinib dose (to 400 mg BID) was allowed when patients satisfied our criteria for no optimal response at any time point. The median (range) dose intensity was 600 (207-736) mg/day. Of 46 evaluable patients, 18 achieved an optimal response and 28 did not. Of the latter, nine patients underwent dose escalation to 400 mg BID, and none achieved $MR^{4.5}$. The remaining 19 patients could not undergo dose escalation, 12 (63%) because of adverse events (AEs), and 7 (37%) for non-AE related reasons. Four of these patients achieved $MR^{4.5}$. The $MR^{4.5}$ rate by 24 months was 45.7%. The progression-free, overall and event-free survival were each 97.6%. No new safety concerns were observed. Our findings support the use of continuous nilotinib at a dose of 300 mg BID for newly diagnosed patients with CML-CP.

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Imai Y. Latest Development in Multiple Myeloma. *Cancers (Basel).* 12(9):2544, 2020

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Founded in 1981, IMSUT hospital started HIV clinic in 1986, and 1269 HIV-infected patients visited us by 2020. Currently, 548 patients in total are under our clinical management. Besides HIV infection, we have been treating patients with other infection such as hepatitis and malaria. Since the emergence of COVID-19, we started to treat COVID-19 patients, and more than 260 patients admitted to our hospital in 2020. (66/70 words)

1. Treatment of COVID-19 in IMSUT hospital

Eisuke Adachi, Makoto Saito[1], Hiroyuki Nagai, Shinya Yamamoto, Michiko Koga[1], Lay Ahyoung Lim, Kazuhiko Ikeuchi, Takeya Tsutsumi[1], Hiroshi Yotsuyanagi[1]
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Since the beginning of 2020, there have been the outbreak of coronavirus disease 2019 (COVID-19) in Japan, and we started to treat COVID-19 patients at the IMSUT Hospital in February. There were more than 260 patients admitted to the hospital in 2020. Most of the patients had mild severity of COVID-19, but 15% of them had moderate severity who needed non-invasive oxygen administration. (Several patients became exacerbated during the hospitalization and were transferred to other hospitals.) To detect the presence or absence of SARS-CoV-2 in COVID-19-suspected patients, we performed about 650 PCR-tests for about 390 COVID-19-suspected patients referred to our hospital by the public health centers. In addition to these cases, we performed totally about 2000 PCR tests including screening tests in 2020.

2. Treatment of HIV infection in IMSUT hospital: Statistical characteristics of HIV infected patients in IMSUT hospital this year

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

Sixteeen new patients with HIV-1 infection visited to our hospital this year (from January 1 to December 31, 2020), and 548 patients in total are under medical management in our outpatient clinic. The total number of HIV-infected in-patients during 2020 was 18. In 2020, the burden of COVID-19 medical care was added, but the number of HIV-infected patients treated did not change (Fig. 1). Anti-retroviral therapy (ART) has been introduced to 543 HIV-infected patients in

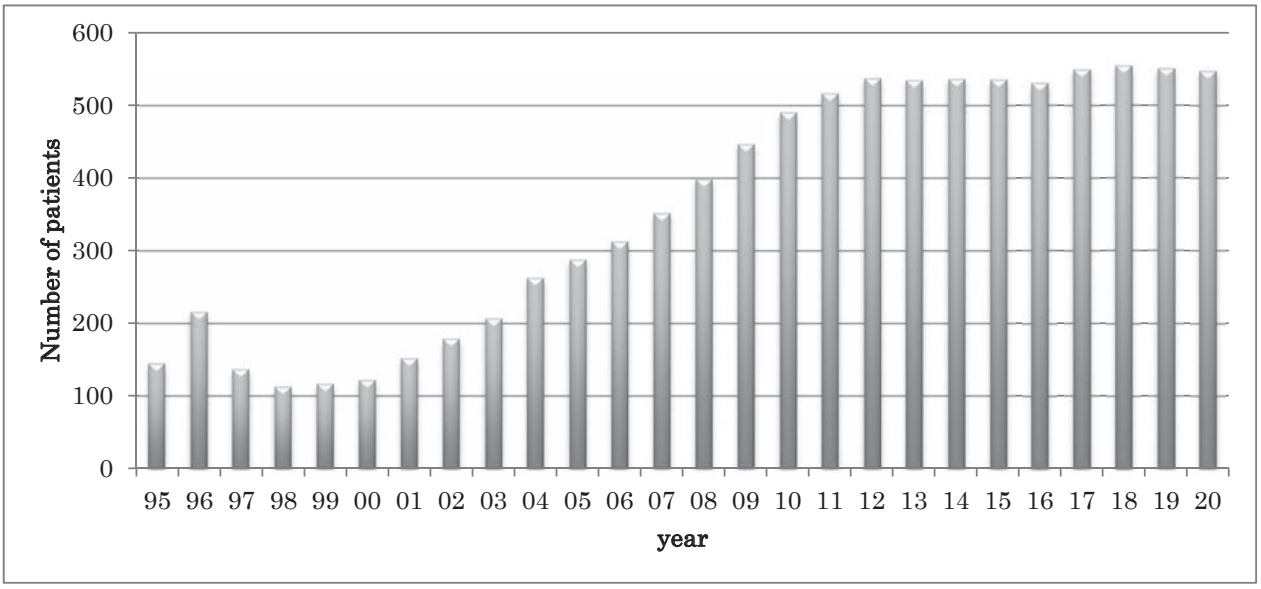

Figure 1. Number of HIV-infected outpatients in IMSUT Hospital

our hospital, and most of their HIV viral loads have been well controlled. After one year of ART, the viral loads become less than 100 copies/ml in 97.8% of HIV-infected patients in our outpatient clinic, presumably underscored by the change in the method of quantitative HIV-RNA assay. Consequently, the patients are able to maintain good condition as long as they keep excellent drug adherence rates. The clinical management of HIV-infected patients have been changing from how to treat opportunistic infections into how to control patients with ART.

3. Pre and post-travel treatment and clinical research of tropical diseases in IMSUT hospital

Makoto Saito[1], Eisuke Adachi, Michiko Koga[1], Lay Ahyoung Lim, Kazuhiko Ikekuchi, Tadashi Kikuchi, Takashi Odawara, Takeya Tsutsumi[1], Hiroshi Yotsuyanagi[1]:

The pandemic of COVID-19 has had unprecedented impact of our life: global transport and travelling is one of the most affected areas. The decline of international travelling has changed our pre- and post-travel clinic. While pre-travel consultations and visits of returned travelers get fewer, there have been a huge demand of pre-travel PCR testing, which is required to enter majority of the countries. Our hospital started providing pre-travel PCR testing service in mid-May, which was one of the earliest in Japan.

For the tropical and parasitic diseases, dozens of important medicines essential for treatment of them are not licensed in Japan. Research Group on Chemotherapy of Tropical Diseases, Research on Publicly Essential Drugs and Medical Devices, Grant from Japan Agency for Medical Research and Development had been established to cope with this situation. We are the medical institution of the research group using these orphan drugs if needed, and colleting clinical data.

4. Treatment of hepatitis in IMSUT hospital:

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

About 300 HIV-non-infected patients with liver diseases such as viral hepatitis and NAFLD are under medical management in our outpatient clinic. Several patients were introduced from outside for the treatment of chronic hepatitis C with direct acting anti-virals (DAA) and successfully achieved the sustained viral response (SVR). In addition, we treated HIV-infected patients who developed acute hepatitis C with DAAs, who achieved SVR.

5. Clinical trial of ebola virus disease vaccine and research of influenza virus in IMSUT hospital

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

The clinical study of the ebola virus disesase vaccine is progressive in cooperation with the Division of Virology and the Center for Translational Research in IMSUT hospital. And the influenza virus study has also proceeded in cooperation with the Division of Virology in IMSUT hospital.

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IMSUT Hospital

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Senior Assistant Professor	Noritada Yoshikawa, M.D., D.M.Sc.	講　師	博士（医学）	吉　川　賢　忠
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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

Hirotoshi Tanaka, Motohisa Yamamoto, Noritada Yoshikawa, Hiroki Yamazaki, Erika Matsubara

Rheumatologists at our division provide state-of-the-art diagnosis and treatment for systemic autoimmune diseases (total number of patients were approximately 5,000 per year). Our physicians have active basic and clinical research projects and also are involved in training of rheumatology specialists.

Rheumatologic services offered at IMSUT Hospital include:

- Outpatient consultations
- Outpatient specialty care for patients with rheumatic diseases
- Hospital consultations
- Diagnostic and therapeutic intra-articular and soft tissue injections and aspirations
- Diagnostic ultrasonography
- Education on rheumatologic diseases and treatments
- Clinical trials

2. Development of novel therapeutic approaches for rheumatic disorders

Hirotoshi Tanaka, Noritada Yoshikawa, Motohisa Yamamoto, Hiroki Yamazaki, Erika Matsubara, Masaaki Uehara, Akiko Souta-Kuribara, Mayu Nishimura

Although biologic agents targeting cytokine networks or specific lymphocyte subsets have significant advantages in the therapy of rheumatic diseases, glucocorticoids have been still used clinically for decades as potent anti-inflammatory and immunosuppressive agents to treat various rheumatic disorders. Nevertheless, their use is severely hampered by the risk of developing side effects. Therefore, efforts to understand the complex mechanisms underlying function of glucocorticoids and glucocorticoid receptor (GR) are ongoing.

Prolonged glucocorticoid treatment for rheumatic disorders accelerates skeletal muscle atrophy known as glucocorticoid-induced myopathy. To overcome this issue, we have studied precise mechanisms of glucocorticoid-induced myopathy and revealed that a mutually exclusive crosstalk between GR and

mTOR, a serine/threonine protein kinase that regulates protein synthesis, autophagy, and transcription, coordinately regulates catabolic and anabolic metabolism in skeletal muscle, and that, in glucocorticoid-induced myopathy, catabolic pathway is drastically activated over anabolic one by GR. Administration of branched-chain amino acids (BCAA) ameliorates such glucocorticoid-induced muscle atrophy via activation of mTOR and suppression of GR in animal model and in patients with rheumatic disorders. We are now challenging to establish a novel approach to improve the efficacy of BCAA administration for glucocorticoid-induced myopathy in patients with rheumatic disorders.

3. Development of novel modalities optimizing metabolic condition and body composition targeting transcriptional apparatus

Hirotoshi Tanaka, Hiroki Yamazaki, Masaaki Uehara, Mayu Nishimura, Noritada Yoshikawa, Motohisa Yamamoto, Akiko Souta-Kuribara, Erika Matsubara

(i) Development of novel therapeutic modalities against metabolic syndrome targeting the skeletal muscle-liver-fat signalling axis

We investigated that the critical importance of the interaction of GR and mTOR in the regulation of tissue metabolism-volume coupling in skeletal muscle. We further investigated that glucocorticoid effects in muscle mass may control adipose tissue metabolism via liver; GR-driven skeletal muscle degradation produces alanine, which negatively modulates hepatic gene expression of lipolytic hormone fibroblast growth factor 21, which also suggests the presence of glucocorticoid-driven metabolic communication among various tissues. However, it remains unknown how a variety of tissue-specific glucocorticoid effects are systemically integrated for coordinated regulation of systemic metabolism. Both Cushing's mouse model and leptin-deficient ob/ob mice exhibited metabolic syndrome involving central obesity, fatty liver, and impaired glucose tolerance, as expected. We revealed that glucocorticoid excess alters metabolic phenotype and body composition involving possible communication among skeletal muscle, liver, and adipose tissue, and that muscle specific knockout of GR (GRmKO) mitigated such metabolic unhealthy phenotype by blunting metabolic inter-organ communication. Targeting the skeletal muscle-liver-fat signalling axis involving glucose-alanine cycle, therefore, would be a novel approach for treatment of patients with obesity, diabetes, and metabolic syndrome.

(ii) Clarification of the effects of GR and sex hormone receptors on gender differences of gene transcription and metabolic regulation in the skeletal muscle

Skeletal muscle not only functions as a locomotory system but also maintains whole-body metabolism. Sex differences in such skeletal muscle morphology and function have been documented, however, their underlying mechanisms remain elusive. We evaluated the related contributions of the GR to sex differences of gene expression profile in skeletal muscle using GRmKO mice. We revealed that although the genes expressed in skeletal muscle may be predominantly sex-independent, sex-dominant genes may relate to sex difference in energy metabolism and the immune system and could be controlled by the GR. In addition, we created ER (estrogen receptor)αmKO, GR/ERα double mKO, AR (androgen receptor) mKO, and GR/AR double mKO, and found that each receptor was involved in regulation of body composition and its sexual dimorphism. Moreover, at least in skeletal muscle, functional crosstalk between GR and ERα and between GR and AR were existed and such crosstalk might regulate plasticity of metabolic regulation in skeletal muscle. Understanding impacts of sex on skeletal muscles exert a beneficial influence on not only skeletal muscle biology but also strategies for treating diseases including various types of muscle atrophy and metabolic syndrome.

(iii) Clarification of the effect of ageing for regulation of energy storage in skeletal muscle and adipose tissues

Ageing is accompanied by major changes in body composition that can negatively affect functional status in older adults, including a progressive decrease in muscle mass, strength, and quality, accompanied by an increase in fat mass. Such loss of muscle mass and increase of fat mass have recently been termed sarcopenic obesity, which is a high-risk geriatric syndrome related to functional impairment, increased mortality and reduction in quality of life. Because GRmKO shows the opposite phenotype against sarcopenic obesity, analyzing the effect of aging for regulation of energy storage in skeletal muscle and adipose tissue in GRmKO contributes to resolve biological significance of functional communication among multiple organs and the mechanisms of sarcopenic obesity. We revealed that GRmKO was resistant to age-related loss of muscle volume, gain of fat mass, and dysregulation of triglyceride. We further investigated that the association between different metabolic changes in several organs in an aged-mouse model was blunted in GRmKO, suggesting that age-related metabolic dysregulation/inter-organ relationship could be affected skeletal muscle GR. Recently, we revealed that aging skeletal muscle in male mice shows lower grip strength and fiber type changes, both of which can be inhibited by an omega-3 fatty acid, ei-

cosapentaenoic acid (EPA) supplementation irrespective of muscle mass alteration. Further understanding of glucocorticoid signaling in aged-condition and its relationship with nutrition might provide novel methods to overcome metabolic dysregulation at old-age life stage.

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

Hirotoshi Tanaka, Motohisa Yamamoto, Noritada Yoshikawa, Hiroki Yamazaki, Erika Matsubara

IgG4-related disease is a new disease concept, established this century. As a chronic fibro-inflammatory disorder, IgG4-related disease is characterized by elevated serum levels of IgG4 and abundant infiltration of IgG4-bearing plasma cells into and fibrosis of the involved organs. Whether the disorder is an autoimmune disease remains unclear; nevertheless, consultation with rheumatologists regarding patients with IgG4-related disease is increasing owing to the various organ dysfunction involved and the abnormal immune responses observed. We tackle elucidating the pathogenesis of IgG4-related disease and developing novel treatments. At first, we established a new registry system for the patients with IgG4-related disease (TOMMOROW registry), and started to enroll IgG4-related disease patients. We cooperate with national policies, and also provide the data to Rare Disease Data Registry of Japan (RADDAR-J), which was established by AMED. We will organize the clinical figures of IgG4-related disease and develop more accurate diagnostic and therapeutic approach by a TOMORROW registry. Furthermore, using the obtained blood and tissue samples, we will carry out multi-omics analysis. We will link the results to the individual clinical data, and promote personalized medicine that predicts therapeutic response and prognosis using artificial intelligence.

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Senior Assistant Professor	Yasuo Matsubara, M.D., D.M.Sc.	講　師	博士(医学)	松　原　康　朗
Project Senior Assistant Professor	Yasuki Hijikata, M.D., D.M.Sc	特任講師	博士(医学)	土　方　康　基
Project Assistant Professor	Koichi Kimura, M.D., D.M.Sc.	特任助教	博士(医学)	木　村　公　一

The division of general medicine was founded in 2017 taking over the department of advanced medical science. Our aim is to practice total human medical care at IMSUT hospital conducting exploratory clinical research. The members specialize in gastroenterology, hepatology, oncology, cardiology, endocrinology/metabolism. We have just started our new project in general medicine.

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

Matsubara Y., Hirata Y.

Some patients fail to respond first- and second-line *Helicobacter pylori* (*H. pylori*) eradication therapy, but third-line eradication is not always done. Meanwhile, penicillin allergy patients do not take routine eradication medicines because insurance coverage regimens in japan include penicillin. In IMSUT, *H. pylori* out-patient clinic, we give eradication therapy for these patients at their own expense, and high rates of successful eradication have been achieved. In addition, we have established effective fourth-line rescue therapy, which is now used for patients who failed with sitafloxacin containing third-line regimen. We also perform clinical studies to unveil the mechanisms of rare 'non-Hp' gastritis, which include autoimmune gastritis and eosinophilic gastroenteritis.

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

Matsubara Y., Hirata Y.

About 600 cases of upper gastrointestinal endoscopy and about 200 cases of colonic endoscopy were performed from January 1 to December 31, 2020, while examinations were restricted due to covid-19. We have diagnosed relatively rare disease (e.g. infectious disease, malignancy, other disease) in patients with immune dysfunction. We also performed a prospective study collaborated with Yamaguchi University, in which the fecal immunochemical test for hemoglobin, fecal DNA testing of TWIST1 methylation, and colonoscopy were performed on patients with or without colorectal neoplasia.

3. Abdominal ultrasonography in IMSUT Hospital

Tsutsumi T

Four hundred forty-five cases of abdominal ultrasonography were performed from January to December in 2020. Various pathologically abnormal lesions were detected such as liver tumors or pancreas tumors in some cases who were subsequently diagnosed as hepatocellular carcinoma or pancreas cancer. We also performed Fibroscan® for 242 cases with viral hepatitis or HIV infection.

4. Treatment of patients with advanced cancer.

Hijikata Y.

Patients with various types of cancer were treated by standard therapy including chemotherapy, molecular target drugs, immune checkpoint blockade, surgery and radiation therapy. Some of them were treated using next-generation sequencing to guide cancer therapy. By the help of special patient support team undergirded by conference twice a week, overall survival of our patients was longer than respectively reported overall survival. Importantly, it looked like they enjoyed their stay in our hospital. We actively make use the patient's cancer genome data to introduce the best treatment for patient refractory to standard treatment after the approval of our ethical committee. (Hijikata Y, et al. Successful clinical sequencing by molecular tumor board in an elderly patient with refractory Sézary syndrome. JCO Precision Oncology no. 4 (2020) 534-560.)

5. Diagnosis and management of patients with genetic vascular diseases.

Takayuki Morisaki

Patients and family members with genetic vascular diseases including connective tissue disorders like Marfan syndrome, Loeys-Dietz syndrome and related diseases were diagnosed by taking their history, physical examination, imaging including echo-cardiography and genetic examination. These patients were followed-up and managed also by doctors in other medical institutions. Study to identify novel pathogenic genes for genetic vascular diseases was being performed.

6. Clinical studies of echocardiography, muscular dystrophy, animal experiments, and multicenter studies in collaboration with other facilities.

Koichi Kimura

We performed several clinical studies regarding echocardiography and heart failure in collaboration with the echocardiography laboratory of The University of Tokyo Hospital. 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), 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. Other animal experiments using dogs and knockout mice were performed in collaboration with National Center of Neurology and Psychiatry (Tokyo).

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IMSUT Hospital

Department of Applied Genomics
ゲノム診療科

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Associate Professor	Tsuneo Ikenoue M.D., Ph.D.	准教授　医学博士	池　上　恒　雄

Our department has been working on the application of human genome information in clinics. As clinical services in IMSUT Hospital, we provide genetic counseling, genetic tests for human malignancies such as leukemia and cancer, and a surveillance program for patients with hereditary colorectal cancer. In addition, we have been carrying out two research projects; 1) determination of genetic alterations in human tumors, and elucidation of the mechanisms underlying their development, and 2) clinical sequence for the implementation of genomic medicine

1. Genetic test of human neoplasms

Nozomi Yusa, Yoichi Furukawa

As a part of clinical service, we have performed genetic analysis of human neoplasms such as leukemia and colorectal cancer. In 2020, a total of 499 genetic analyses were performed in our department. The results were utilized for the precise classification of neoplasms, evaluation of disease status, selection of therapeutic drugs, and evaluation of the response to treatment.

2. Genetic counseling and related activities

Yoichi Furukawa, Yoshinori Murakami, Yataro Daigo, Tsuneo Ikenoue, Koichiro Yuji, Makoto Hirata, Reiko Sada, Mitsuko Nakazawa, Momoyo Ohki[1], Yoshinari Miyamoto[2], Masae Ono[3], Masahiko Suzuki[4], Mayumi Tamari[4], Toshihiro Tanaka[5], Shiro Ikegawa[6], Hidewaki Nakagawa[6], Natsuko Watanabe[7], Ai Yoshihara[7], Toru Akiyama[8]: [1]Bunkyo University, [2]National Center for Global Health and Medicine, [3]Tokyo Teishin Hospital, [4]Jikei Medical University, [5]Tokyo Medical and Dental University, [6]Center for Integrative Medical Sciences, RIKEN, [7]Ito Hospital, [8]Jichi Medical University.

We provided genetic counseling and genetic tests to clients who visited our counseling clinic. In 2020, we had a total of ten counseling cases including hereditary breast and ovarian cancer, Peutz–Jeghers syndrome, Loeys-Dietz syndrome, myotonic dystrophy, myelodysplastic syndrome, and gastric polyposis. In the counseling, we provided appropriate information about hereditary diseases to the clients and took their psychological care in collaboration with a clinical psychologist. Genetic testing was performed in cases with informed consent after thoughtful discussion about its merit and demerit.

Systematic surveillance programs are provided for the clients susceptible for hereditary tumors.

3. Application of liquid-based genetic diagnosis for the screening of endometrial cancer

Kiyoko Takane[1], Kiyoshi Yamaguchi[1], Tsuneo Ikenoue, Yoichi Furukawa
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We have conducted a study to elucidate the usefulness of liquid-based genetic diagnosis (LBGDx) for screening of endometrial cancer (EC) in collaboration with Department of Obstetrics and Gynecology, Sapporo Medical University. Although liquid-based cy-

tology (LBC) has increased the sensitivity of cytological diagnosis of EC compared with conventional smear cytology, the sensitivity of LBC for the detection of EC is between 70% and 96% and remains unsatisfactory. To investigate the efficacy of genetic testing in the screening of EC, we analyzed pathogenic mutations in a total of 195 LBC samples including 34 ECs by target sequencing using Cancer Hotspot Panel comprising of 50 cancer-related genes. As a result, we identified pathogenic mutations in 27 of the 34 ECs, showing the sensitivity of 79%. On the other hand, the sensitivity of endometrial cancer by LBC alone was 71% (24/34 ECs). Importantly, LBGDx detected seven out of ten EC cases that were negative for cytology. These results suggest that LBGDx should contribute to the increase of sensitivity in the screening of EC.

4. Clinical sequencing for the implementation of genomic medicine

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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, Health Intelligence Center, and Advanced Clinical Research Center, we have been carrying on two projects; the first, genetic diagnosis of patients suspected of hereditary cancer, and the second, implementation of precision medicine for patients with rare or intractable cancer.

In the first project, we have applied NGS technology for molecular diagnostics of hereditary colon cancer syndromes such as familial adenomatous polyposis, polymerase proofreading-associated polyposis, and Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer syndrome). In our previous study, we performed genetic analysis of Lynch syndrome using the Sanger sequencing method and multiplex ligand-dependent probe amplification, and identified several structural variations (SVs) in the DNA mismatch repair (MMR) genes. Since detection of SVs using short-read NGS is a challenging work, we tested whether Oxford Nanopore MinION, a long read-sequencer, detects four SVs in the MMR gene. The long-read nanopore sequencing successfully identified all four SVs including three large deletions and one duplication. These data suggest that long read-sequencing will be help the identification of pathogenic SVs in patients with hereditary diseases.

In the second project, we opened an outpatient clinic in IMSUT hospital for the consultation of patients with rare or intractable cancer. In 2020, a total of 18 patients visited our clinic. After written informed consent was obtained from the patients, they were enrolled in the study of genetic analysis and interpretation of genomic data using IBM Watson for Genomics (WfG); genetic alterations in their tumors were determined by NGS and the data were subsequently analyzed by WfG. The results of WfG including predicted driver mutations and suggested actionable drugs were discussed in the Tumor Board meeting of this project, which is held every two weeks.

Publications

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Department of Radiological Technology
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Associate Professor	Akira Kunimatsu, M.D., D.M.Sc.	准教授	博士(医学)	國松　聡
Head Radiologic Technologist	Tomio Inoshita, RT	放射線技師長		井下富夫

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.

Application of artificial intelligence with deep learning to radiological imaging

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We are applying deep learning technique, which is one of the artificial intelligence strategies, to radiological imaging diagnosis including central neural system, musculoskeletal, abdominal regions. In this year, we published articles which revealed that, by using deep learning, (a) the bone mineral density (BMD) of lumbar vertebrae could be predicted from unenhanced abdominal computed tomography (CT) images and (b) patients with Parkinson's disease (PD) could be differentiated from healthy controls based on parameter-weighted connectome matrix derived from diffusion-weighted MRI. The estimated BMD values, according to the convolutional neural network model, were significantly correlated with the BMD values obtained with dual-energy X-ray absorptiometry (DXA) ($r = 0.852$ ($p < 0.001$) and 0.840 ($p < 0.001$) for the internal and external validation datasets, respectively). Using the model, osteoporosis was diagnosed with the area under the receiver operating characteristic curve (AUC) of 0.965 and 0.970 for the internal and external validation datasets, respectively. The diffusion kurtosis imaging-, neurite orientation dispersion and density imaging-, and g-ratio-weighted connectome matrices showed moderate performance (AUC = 0.895, 0.801, and 0.836, respectively) in discriminating PD patients from healthy controls, and neural circuit disorders including those between the basal ganglia on one side and the cerebellum on the contralateral side were visualized with gradient-weighted class activation mapping technique.

Exploring the functional effect of Gd deposition to brain: a mice experimental study

Hiroyuki Akai, Haruto Sugawara, Koichiro Yasaka, Akira Kunimatsu, Minoru Tsuji[5], Kuni Ohtomo, Osamu Abe, Shigeru Kiryu:
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We performed the present study to investigate whether the Gd deposition to brain structure has any functional effect on mice behavior. We used 36 female BALB/c mice, and they were injected with three test agents: Group A: phosphate-buffered saline (PBS, as the control group), Group B: gadoteridol, and Group C: gadodiamide and each study group contained 12 mice. Injections of the test agents were repeated twice a week over a period of 8 weeks. T1-weighted MRI scans were repeated (at 0, 2, 4, 6, and eighth week) to observe the Gd deposition to the mice brain. Additional MRI scans were performed during the treatment-free period in two mice from each group (i.e., at 0, 2, 4, 6, 8, 10, 12, and 14th week). Since the behavioral examinations were performed in a different institution from the institution that MRI scans were performed, the latter scans were performed to ensure that the brain's Gd deposition status is unchanged during the behavioral examinations. As a result, Group A and B showed no signal changes during the MRI scans, where Group C showed an apparent signal increase on the bilateral dentate nucleus, suggesting Gd deposition. For the two mice from each group that was performed MRI until the 14th week, the MRI signal intensity was constant after the 8th week. Now, we are performing mice behavioral examinations to check if any behavior change can be observed.

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IMSUT Hospital

Department of Palliative Medicine / Advanced Clinical Oncology

緩和医療・先端臨床腫瘍科

Professor	Arinobu Tojo, M.D., D.M.S		教　授	医学博士	東　條　有　伸
Project Senior Assistant Professor	Yasuki Hijikata, M.D., PhD.		特任講師	博士(医学)	土　方　康　基
Assistant Professor	Tetsuya Ito, M.D., PhD.		助　教	博士(医学)	伊　藤　哲　也

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], Kiyoshi Yamaguchi[2], Tsuneo Ikenoue[2], Seiya Imoto[3], Yasuo Matsubara[4], Kazuaki Yokoyama[5], Hiroshi Yotsuyanagi[4], Yoichi Furukawa[2], Arinobu Tojo[1,5]

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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], Yasuki Hijikata[1], Noriko Fujiwara[1], Arinobu Tojo[1,2]

[1] Dept. of Palliative Medicine / Advanced Clinical Oncology, IMSUT Hosp.[2] Dept. of Hematology/On-

cology, IMSUT Hosp.

Patients with life-threatening illness including cancer and their families are facing challenges, that interfere with their quality of life.

Regardless of the stage of the disease, we aim to address problems of patients and families, whether physical, psychological, social or spiritual, and eventually improve their quality of life under multidisciplinary collaboration.

At the same time, we will conduct research activities to build evidence on palliative medicine and disseminate new findings.

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IMSUT Hospital

Department of Diagnostic Pathology
病理診断科
Department of Pathology
病理部

Associate Professor Yasunori Ota, M.D., Ph.D.

准教授 医学博士 大 田 泰 徳

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.

1.Effusion-based lymphoma (EBL) in Japan.

Effusion-based lymphoma is found in pleura or ascites and usually lack of evidence for nodular lesion. Conventional findings about EBL are bad clinical course and many patients are infected by HIV. However, some of Japanese patients were not infected HIV and good clinical course. We reported some case reports about EBL in Japan and are going to promote multi-institutional joint research in Japan. We therefore conducted a retrospective study of 95 patients with EBL, regardless of HHV8 status, in Japan. Of 69 patients with EBL tested for HHV8, a total of 64 were negative. The median age of patients with primary HHV8-negative EBL at diagnosis was 77 years (range, 57-98 years); all 58 tested patients were negative for HIV. Primary HHV8-negative EBL was most commonly diagnosed in pleural effusion (77%). Expression of at least 1 pan B-cell antigen (CD19, CD20, or CD79a) was observed in all cases. According to the Hans algorithm, 30 of the 38 evaluated patients had nongerminal center B-cell (non-GCB) tumors. Epstein-Barr virus–encoded small RNA was positive in 6 of 45 patients. In 56 of 64 HHV8-negative patients, systemic therapy was initiated within 3 months after diagnosis. Cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) or CHOP-like regimens with or without rituximab (n = 48) were the most common primary treatments. The overall response and complete response rates were 95% and 73%, respectively. Three patients did not progress without systemic treatment for a median of 24 months. With a median 25-month follow-up, the 2-year overall survival and progression-free survival rates were 84.7% and 73.8%. Sixteen patients died; 12 were lymphoma-related deaths. Thus, most EBL cases in Japan are HHV8-negative and affect elderly patients. The non-GCB subtype is predominant. Overall, primary HHV8-negative EBL exhibits a favorable prognosis after anthracycline-based chemotherapy.

Publications

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IMSUT Hospital

Department of Surgery
外科

Professor	Dai Shida, MD, PhD	教　授	博士(医学)	志　田　　大
Associate Professor	Susumu Aiko, MD, PhD	准教授	博士(医学)	愛　甲　　丞
Associate Professor	Masaru Shinozaki, MD, PhD	准教授	博士(医学)	篠　崎　　大
Senior Assistant Professor	Giichiro Tsurita, MD, PhD	講　師	博士(医学)	釣　田　義一郎
Clinical Senior Assistant Professor	Kentaro Yazawa, MD, PhD	病院講師	博士(医学)	谷　澤　健太郎
Assistant Professor	Tomohiro Kurokawa, MD, PhD	助　教	博士(医学)	黒　川　友　博
Assistant Professor	Yuka Ahiko, MD	助　教		阿　彦　友　佳

The mission of our department is to provide surgical treatment for various gastrointestinal diseases, such as colorectal cancers, gastric cancers, and inflammatory bowel diseases. Since the participation of Dr. Shida and Dr. Ahiko in September 2020, we have mainly performed laparoscopic surgery (i.e., colectomy, low anterior resection, gastrectomy) instead of open surgery for these diseases. We also treat acute appendicitis and cholecystitis, and perform diagnostic and therapeutic gastrointestinal endoscopy.

1. Introduction

Professor Shida and Dr. Ahiko newly joined in September 2020. And Dr. Aiko also newly joined in November 2020. We specialize in the treatment of gastrointestinal cancers, especially surgical treatment of colorectal cancer and gastric cancer. Colorectal cancer can be completely cured by more than 70% of patients when appropriate surgery is performed, even if it is stage III cancer. As qualified surgeons (endoscopic surgical skill qualification system) of the Japan Society for Endoscopic Surgery (Dr. Shida and Dr. Aiko), we are actively performing minimally invasive surgery with less physical burden of patients. In addition, Dr. Shinozaki specializes in the treatment of inflammatory bowel diseases. All the staff will propose the most suitable treatment method according to the medical condition and will do our best to treat the patients.

2. Treatment for gastrointestinal malignancy

Colorectal cancers and gastric cancers are what we mainly treat. For rectal cancer, in order to improve the QOL (quality of life) after surgery as much as possible, we select not only autonomic nerve-sparing surgery but also anus-sparing surgery if the cancer can be sufficiently resected. For gastric cancer, we select the surgical method with policy of 'leaving the remaining stomach as much as possible', because stomach surgery limits the amount of food that patients eat after surgery which leads to weight loss and weakness. As qualified surgeons (endoscopic surgical skill qualification system) of the Japan Society for Endoscopic Surgery (Dr. Shida and Dr. Aiko), we are actively performing minimally invasive surgery, that is, laparoscopic surgery.

<Sep 2020 – Dec 2020>
Laparoscopic gastrectomy: n = 1
Laparoscopic ileocecal resection: n = 1
Laparoscopic right hemicolectomy: n = 2
Laparoscopic sigmoidectomy: n = 1
Laparoscopic high anterior resection: n = 2
Laparoscopic low anterior resection: n = 3
Laparoscopic ultra-low anterior resection: n = 2

3. Treatment for inflammatory bowel disease

We treat inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. As board certified surgeons in gastroenterology (Dr. Shia, Dr. Shinozaki, Dr. Aiko, Dr. Tsurita, Dr.Yazawa), laparoscopic surgery for ulcerative colitis is performed (mainly by Dr. Shinozaki).
<Sep 2020 – Dec 2020>
Laparoscopic total colectomy with ileal pouch anal anastomosis: n = 1

4. Surgical treatment for benign diseases

We also treat a variety of benign diseases such as acute appendicitis, cholecystitis, and colonic diverticulitis.
<Sep 2020 – Dec 2020>
Laparoscopic right hemicolectomy: n = 1
Laparoscopic sigmoidectomy: n = 1
Laparoscopic cholecystectomy: n = 2
Open appendectomy (small incision): n = 3

5. Endoscopic examination and treatment

Under cooperation with Department of General Medicine (Dr. Matsubara Y. and Dr. Hirata Y.), we performed many cases of upper gastrointestinal endoscopy and colonoscopy.

6. Preparing for Robotic Surgery

Currently, we are preparing for robotic rectal surgery for rectal tumors such as rectal cancer and rectal GIST. Robotic rectal surgery is scheduled to begin in the spring of 2021 in our hospital.

Publications

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IMSUT Hospital

Department of Anesthesia
麻酔科

Associate Professor Ryo Orii, M.D. , Ph.D.
Assistant Professor Miho Asahara, M.D. , Ph.D.

准教授 博士(医学) 折井 亮
助 教 博士(医学) 浅原美保

Our clinical practice and clinical studies have been focused on (1) anesthetic management in patients undergoing major surgery including joint arthroplastic surgery for hemophilia patients, variable surgical procedures for translational researches (2) assessment of functional failure of the internal valve of anesthesia machine (3) assessment of reliability of cardiac output measurements (4) risk management of medical electronic devices in Research Hospital.

1. Anesthetic management for carrier hemophilia.

Hemophilia is X-linked gene disease with the activity abnormality of the coagulation factor. The hemophilia A is caused by factor VIII abnormality, and the hemophilia B is caused by factor Ⅸ abnormality. Careful hemostatic management is required in perioperative care of the hemophilic patients. It is usually recommended that we perform coagulation factor replacement therapy and hemostatic monitoring.

We experienced anesthesia management of the orthopedic surgery of patients with hemophilia B that underwent living-donor liver transplantation for cirrhosis due to the hepatitis C virus this time. We carried out hemostatic monitoring and perioperative management, but did not require coagulation factor replacement therapy. There were no complications such as postoperative bleeding and infection.

Female hemophilia patients are often not informed as carriers themselves, and there is a possibility that medical practice may be performed without recognizing them as hemophilia patients. We experienced anesthesia of female hemophilia patients and safety managed anesthesia with appropriate hemostatic management.

2. Assessment of functional failure of the internal valve applying maximum and positive end-expiratory pressure of anesthesia machine

Equipment-related complications, whatever its cause, should be prevented by checking the breathing system prior to general anesthesia. We found irregularities with some of the anesthesia machines at our department, which was related to a ventilator-related problem that recurred after application of positive end-expiratory pressure (PEEP) during general anesthesia.

The issue with the PEEP/Pmax valve, which can lead to changes in flow and pressure during mechanical ventilation, could go unnoticed because the valve is encased inside the breathing circuit, and requires disassembly for close inspection. Our findings highlight the importance of keeping the anesthetic circuit, including the internal components of the PEEP/Pmax valve, free of unexpected contamination through more thorough preventive maintenance cycles.

3. Assessment of reliability of cardiac output measurements.

Knowing a patient's cardiac output (CO) could contribute to a safe, optimized hemodynamic control during surgery. Precise CO measurements can serve

as a guide for resuscitation therapy, catecholamine use, differential diagnosis, and intervention during a hemodynamic crisis. Despite its invasiveness and intermittent nature, the thermodilution technique via a pulmonary artery catheter (PAC) remains the clinical gold standard for CO measurements. LiDCO rapid™ (LiDCO, London, UK) and FloTrac/Vigileo™ (Edwards Lifesciences, Irvine, CA) are less invasive continuous CO monitors that use arterial waveform analysis. Anesthesiologists use FloTrac/Vigileo™ in our operating room.

We found both devices tended to underestimate the caluculated CIs when the CIs were relatively high. These proportional bias produced large parcentage errors in the present study.

4. Risk management of medical electronic devices.

We ourselves engage in preventive maintenance and care of the life support machines including instruments for mechanical ventilation or blood purification and defibrillator. 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.

Publications

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IMSUT Hospital

Department of Joint Surgery
関節外科

Senior Assistant Professor	Hideyuki Takedani, M.D. D.M.Sc.	講　師　博士(医学)	竹　谷　英　之
Assistant Professor	Kumiko Ono, M.D. D.M.Sc.	助　教　博士(医学)	大　野　久美子

Department of Joint Surgery was established in 2006. Our clinical mission is evaluation and treatment of hemophilic arthropathy. In Japan, many hospitals are able to control bleeding for haemophilia by concentrates, however there are few hospitals focus on surgical treatments except us. Many haemophilia patients come to our department from all over Japan. We evaluate their joint condition and function roentgenographically and physiotherapeutically and decide indication of surgical treatment. Many of patients will be performed joint arthroplasties and arthroscopic synovectomy to improve their quality of life. We researched how to control bleeding adequately during perioperative period as well.

As basic mission, we started the research for pathogenesis of hemophilic arthropathy, collaborated with the department of orthopedic surgery, the University of Tokyo. The aim of this research is to develop mesenchymal stem cell therapy for hemophilic arthropathy.

From 2006 to 2020, more than 238 surgical treatments for hemophilia included other coagulation diseases such as deficiency factor VII or Von Willebrand disease. Some of them have the deficiency factor antibody as well.

Publication 2020

1. Ono, K. and Takedani, H. Risk of deep venous thrombosis after total knee arthroplasty in patients with haemophilia A. Haemophilia 2020;
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IMSUT Hospital

Department of Surgical Neuro-Oncology
脳腫瘍外科

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任准教授	博士(医学)	田　中　　実
Assistant Professor	Seisaku Kanayama, M.D.	助　教		金　山　政　作
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
Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	博士(医学)	伊　藤　博　崇

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, innovative therapy such as oncolytic virus therapy is applied whenever possible. 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, was performed in patients with glioblastoma from 2015 to 2020, and is ongoing in patients with olfactory neuroblastoma or malignant pleural mesothelioma. 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 the one-year survival rate of the 13 patients who completed the one-year follow-up assessment was 92.3%. The independent data monitoring committee (IDMC) recommended terminating the trial because the one-year survival rate was tremendously higher than the set control value based on meta-analysis of historical data. A new drug application (NDA) for G47Δ for malignant glioma has been submitted to the Ministry of Health, Labour and Welfare in December 2020. It is expected that G47Δ be approved as the first oncolytic virus drug in Japan in 2021.

A clinical study of G47Δ in patients with progressive olfactory neuroblastoma

Olfactory neuroblastoma is an uncommon malignant neuroectodermal tumor, which is thought to originate from the olfactory membrane of the sinonasal tract. Patients should receive aggressive treatment with combined treatment such as surgery, radiation therapy, and chemotherapy because there is no effective treatment once it recurs: An aggressive en bloc resection, with combined radiation therapy was recommended. We have been conducting a phase I clinical trial of G47Δ in patients with progressive olfactory neuroblastoma since August 2013. The key inclusion criteria are histologically confirmed recurrent olfactory neuroblastoma despite previous or ongoing radiation therapy, age 18 or older, a measurable tumor lesion (≥1cm) on gadolinium-enhanced T1-MRI of the brain, life expectancy of at least 3 months, a performance-status of 0-2 and adequate organ function. In this protocol patients with advanced disease (eg, Kadish stage C) are covered. G47Δ will be repeatedly inoculated to the residual tumor innasal cavity every 4 weeks until tumor progression or excessive toxicity occurred. The primary endpoint is safety, and the secondary endpoints include efficacy analysis.

A clinical study of G47Δ in patients with progressive malignant pleural mesothelioma

Malignant pleural mesothelioma is a rare asbestos-induced malignancy with an estimated incidence of approximately 2,000 new cases diagnosed in Japan. Worldwide, nearly 80% of mesothelioma deaths occur in ten countries, with Japan, the United Kingdom, and the United States being in the top three. It is expected to continue to increase over the next several decades. Median survival ranges from 9 to 18 months and correlates with stages. Radiotherapy can be used for different indications in mesothelioma: palliation, as a preventive treatment, and as part of multimodality treatment. Combination doublet chemotherapy of cisplatin, with either pemetrexed or raltitrexed, has shown a more prolonged survival compared with cisplatin alone in randomized phase III trials. Carboplatin is an acceptable alternative to cisplatin and may be better tolerated in the elderly population. We have been conducting a phase I clinical trial of G47Δ for malignant pleural mesothelioma. The key inclusion criteria are histologically confirmed malignant pleural mesothelioma that is 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 is 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 is irrelative. A fixed dose of G47Δ will be administered into the pleural cavity every 4 weeks, maximum 6 times. The primary endpoint is safety, and the secondary endpoints include efficacy analysis.

A phase 1/2 clinical trial of a recombinant herpes simplex type 1 with human IL-12 expression, T-hIL12, in patients with malignant melanoma

Malignant melanoma is a tumor produced by the malignant transformation of melanocytes. Melanocytes are derived from the neural crest; consequently, melanomas, although they usually occur on the skin, can arise in other locations where neural crest cells migrate, such as the gastrointestinal tract and brain. The 5-year relative survival rate for patients with stage 0 melanoma is 97%, compared with about 10% for those with stage IV disease. We started a phase 1/2 clinical trial of T-hIL12 in patients with malignant melanoma since January 2020 jointly with Shinshu University. T-hIL12 is a G47Δ-based recombinant herpes simplex type I with IL-12 expression. This IL-12-mediated antitumor immunity could be T-cell-mediated. The main inclusion criteria in phase 1 are 1) histologically confirmed malignant melanoma with stage 3 or 4, 2) patients who have at least one metastatic skin lesion with 10 mm or larger (the longest diameter), or at least one metastatic lymph node with 15 mm or larger (the shortest axis), 3) patients who were administered with anti-PD-1 antibody, or targeted molecular drugs, 4) the size and distribution of all the metastatic lesions are recognized with clinical findings including imaging studies (CT, MRI), 5) age >=20 years, 6) more than 30 days have passed

from the previous treatment, 7) Eastern Cooperative Oncology Group (ECOG) performance Status (PS) of 0-2, 8) patients without severe disorders (severe myelosuppression, liver dysfunction, chronic renal dysfunction), whereas in phase 2 they are eight items, which are defined in the same way as in the phase 1 except for 3) of phase1. The 3rd inclusion criterion of phase2 is 3) patients who have not been administered with anti-PD-1 antibody or targeted molecular drugs. T-hIL12 will be administered into the tumor of skin or lymph node metastases in patients with advanced stage of malignant melanoma. The assigned dose will be repeatedly inoculated into the metastases 2 or 4 times, with an interval of 14 (14 -28) days. The primary endpoint in phase 1 is safety, and in phase 2 a response rate (RECIST 1.1).

Routine activities

Patients with brain tumors are treated by four neurosurgeons. A total of 23 operation were carried out in 2020 including 5 gliomas and 13 malignant pleural mesotheliomas. Standard craniotomies and image guided stereotactic biopsies of deep seated lesions, as well as high-tech brain tumor resections have been performed. The high-tech equipment regularly used in brain tumor resection surgeries includes an operative microscope, a 3-D neuro-navigation system, intraoperative motor evoked potential (MEP and SEP) recording, intraoperative ultrasonography and an ultrasonic surgical aspirator.

Patients with newly diagnosed malignant glioma have been treated with high dose or standard dose radiation therapy and concomitant chemotherapy. Temozolomide was administered to glioma patients during radiation therapy followed by a maintenance therapy every 28 days for as long as possible. The overall survival of patients with glioblastoma was 30.3 months (95% confidence interval, 24.5-36.1 months. The five-year overall survival rate was 26.5%.

Recurrent malignant glioma patients are treated with innovative non-standard therapies whenever possible. Recurrent glioma patients who have small lesions, receive extended field stereotactic radiosurgery. To enhance the efficacy of stereotactic radiosurgery (SRS), the irradiation field is enlarged to include as many tumor cells invasive to the surrounding tissue as possible. We demonstrated 93% local control in patients who received 20 Gy to a 0.5-1.0 cm extended field SRS compared to 47% of patients who were treated with 20 Gy to the gadolinium-enhancing margin only.

Treatment of primary central nervous system lymphoma

Primary central nervous system lymphoma patients will first undergo biopsy for pathological diagnosis. In addition to the standard therapy regimen using high-dose methotrexate followed by radiotherapy, an advanced treatment regimen utilizing rituximab, methotrexate, procarbazine, and vincristine (R-MPV) therapy followed by consolidation whole-brain radiation therapy has been used as a treatment option.

IMSUT Hospital

Department of Urology
泌尿器科

Professor	Haruki Kume, M.D., Ph.D.		教　授	博士(医学)	久　米　春　喜
Project Senior Assistant Professor	Sayuri Takahashi, M.D., Ph.D.		特任講師	博士(医学)	高　橋　さゆり
Project Assistant Professor	Akihiro Naito, M.D., Ph.D.		特任助教	博士(医学)	内　藤　晶　裕

In July 2020, the department of Urology was established to expand the clinical function of ISMUT hospital. We set up an out-patient clinic, urological examinations such as cystoscopy and uroflowmetry, general urological surgery including laparoscopic surgery, and successfully introduced robotic-surgery, which is the latest surgery for urological cancer. Furthermore, we have been conducting basic research on castration resistant prostate cancer using the method of molecular and cell biology.

1. Establishment of an out-patient clinic and public relations activities

The leadership of the University of Tokyo launched the expansion project to establish the department of Urology at IMSUT Hospital and also introduce robotic and laparoscopic surgery.

To recruit patients, we planned to accept all patients with urological symptoms such as benign prostatic hyperplasia, overactive bladder, and other conditions in addition to patients diagnosed with malignant tumors in need of robotic or laparoscopic surgery referred to the hospital from other healthcare facilities. We also registered prescription drugs in the urological field. In addition, we introduced a portable ultrasonography apparatus for screening, a portable ultrasound bladder scanner, a uroflowmeter to assess the urinary tract function, and chain-cystography to assess cystocele, and a fiber cystoscope system with the installation of a urology exam table for diagnosis of bladder tumors. After educating medical staff and arranging the electronic medical records ordering system for urological exams, we were able to efficiently practice ambulatory care without wait times.

Regarding public relations activities, we established our own homepage to introduce and publicize the details of our guiding principles and expertise. We also designed the homepage in English to help foster medical tourism. We sent notification letters regarding the inauguration of our department to 450 hospitals and clinics. This resulted in visits from clinics and hospitals and the establishment of partnerships with twelve facilities.

We saw a total of 293 patients at the out-patient clinic and 84 new patient referrals from outside facilities in the first six months.

2. Start-up of urological surgery and the latest surgery for overactive bladder

We purchased medical equipment and registered disposable materials which included a complete laparoscopic system, transurethral resection apparatus, ureteroscopy, electrosurgical and tissue fusion generators, and sets of conventional open surgery.

The Ministry of Health and Welfare approved the botulinum toxin injection to the bladder muscle by cystoscope as a treatment of overactive bladders in 2020. We conducted training and received certification, educated nurses, and optimized the patient flow to undergo out-clinic surgery.

We successfully performed a total of 66 surgeries, including robotic surgery, in the first six months, 23 prostate biopsies under anesthesia, 13 transurethral

resections of bladder tumors or prostates, six ureteral stentings, five castrations, six scrotal or penile surgeries, one laparoscopic nephrectomy, and one botulinum toxin injection.

3. Introduction of robotic surgery and education for medical staff

Urological staff members relocated to IMSUT Hospital in July 2020. However, there was a long delay in supplying the da Vinci Xi system which is an integrated technology for robot-assisted surgery. We contacted with Intuitive Surgical, Inc., and urged speedy import of the system from the United States after conclusion of the contract between Tokyo University and the company. Medical engineers and nurses visited outside hospitals and completed the e-learning and off-site training provided by Intuitive, Inc. We set up the peripheral equipment, organized the kick-off meeting with the all parties concerned on October 22nd, installed the da Vinci Xi system on November 3rd, and performed the first robot-assisted prostatectomy on November 10th after the sequential simulation. By the end of the year, we successfully completed five robotic surgeries.

4. Basic research

Prostate tumor cells are androgen dependent, so that hormonal therapy such as LH-RH antagonist, agonist, and anti-androgen drugs are often used to treat prostate cancer. However, it is well known to turn into CRPC (castration-resistant prostate cancer) in a few years. Previously, we reported that non-canonical Wnt signaling promotes growth of prostate cancer using androgen receptor mutant conditional mice. The Wnt signaling pathways (canonical and non- canonical) regulate crucial aspects of embryonic development. We found that Wnt5a promotes proliferation of cancer cells directly through non-canonical Wnt signals and by increasing secretion of growth factors and expression of cancer related genes including immune system. We clinically experience corticosteroid drug suppress progression of CRPC. Now, we are researching molecular mechanisms of prostate cancer in the point of view of immunotherapy to CRPC by the method of molecular and cell biology.

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IMSUT Hospital

Department of Medical Informatics
医療情報部

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Senior Assistant Professor	Hiroyuki Akai, M.D., D.M.Sc.	講　師	博士(医学)	赤井宏行
Assistant Professor	Koichiro Yasaka, M.D., D.M.Sc.	助　教	博士(医学)	八坂耕一郎

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

Akira Kunimatsu, Hiroyuki Akai, Koichiro Yasaka

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

Akira Kunimatsu, Hiroyuki Akai, Koichiro Yasaka

"Community-based integrated care systems" is a keyword for the Japanese healthcare system in this decade. IMSUT hospital belongs to its community-based healthcare provider network and we continuously improve infrastructure for cooperation in the network.

Our hospital information system has been renewed since 2017. We hope that the latest electronic healthcare record system will help to refer patients from hospital to clinic and from clinic to hospital in the network.

IMSUT Hospital

Department of Cell Processing and Transfusion

セルプロセッシング・輸血部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		病院教授	博士(医学)	長村 登紀子
Assistant Professor	Kazuaki Yokoyama, M.D., Ph.D.		助 教	博士(医学)	横山 和明
Assistant Professor	Toyotaka Kawamata, M.D., Ph.D.		助 教	博士(医学)	川俣 豊隆

Our department was established in 1990, in order to manage the transfusion medicine and the cell processing for hematopoietic stem cell transplantation. In addition to the transfusion-related works, our department has supported translational researches and managed IMSUT-Cell Resource Center (IMSUT-CRC), which has been established in 1997. Recent our projects include Research Cord Blood Bank (RCBB), as National BioResource Project (NBRP) supported by AMED (MEXT) and CB and umbilical cord-derived mesenchymal stromal cell (UC-MSC) banking for clinical use supported by AMED (MHLW). We have been conducting an investigator-initiated clinical trial; the administration of umbilical cord-derived MSCs for treatment-resistant severe acute graft-versus-host disease since 2018 to 2020. We also explore the clinical application of UC-MSC for cerebral palsy.

1. Transfusion medicine and related tests

Abe Y, Ogami K, Hiratak K, Yokoyama K, Kawamata T, Nagamura-Inoue T

In a part of Transfusion test and control, we control the blood transfusion products including concentrated Red Blood cells, Platelets, and Frozen plasma, and do blood typing, irregular antibodies test, and cross matching test. There are many patients with blood disease including hematopoietic stem cell transplantation. We carefully do the blood typing test, because the blood type of the patient transit to the donor type. We also collect the autologous blood for autologous transfusion for the patients with Hemophilia.

2. Apheresis for Peripheral Blood Stem Cell mobilization and collection, CAR-T, and dendritic cells therapy:

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

For autologous peripheral blood Stem Cell Transplantation (PBSCT), we perform the apheresis for the patients with myeloma and malignant lymphoma after mobilization by G-CSF with or without new CXCR-4 inhibitor, Plerixafor. We evaluate the efficacy of mobilization by testing HPC and CD34 positive cells in peripheral blood on the day of apheresis and processing products. We perform the mobilization and apheresis for the patients out of IMSUT hospital by request. We also apheresis to obtain the lymphocyte (CD3-positive cells) for the construction of CAR-T and monocytes for dendritic cell therapy.

3. Therapeutic application of Umbilical cord-derived mesenchymal stromal cells to the severe acute graft-versus-host –disease(aGVHD).

Nagamura-Inoue T, Takahashi A, Hori A, Okada M, Yamamoto Y, Nagamura F, Konuma T, Kato S, Saito Y, Takahashi S, Tojo A

Umbilical cord (UC) is a rich source of mesenchymal stromal cells (MSCs). MSCs have self-renewal capacity, multi-lineage differentiation potential and the ability to migrate toward sites of inflammation or injury, where MSCs control the inflammation and repair the damaged tissues. UC-MSCs harbored the immunosuppressive effects. Even 3rd party donor UC-MSCs suppress the activated T cells stimulated by allogeneic dendritic cells, through IDO, PGE2, and HGF etc. We succeeded to manufacture the clinical-grade UC-MSCs (IMSUT-CORD). Since 2018, July, we started the UC- MSCs treatment for severe acute graft-versus-host disease (GVHD), as the investigator initiated clinical trial (IIT) and the IIT is now still undergoing.

4. Therapeutic application of UC-MSCs to the cerebral palsy.

Sei K, Yamamoto Y, Mukai T, Takahashi A, Nagamura-Inoue T.

In the previous study, we demonstrated UC-MSCs have neurogenic differentiation potential and migration ability towards injured neuronal cells in vitro. We also established neonatal intraventricular hemorrhage (IVH) mice model, one of neonatal brain injuries and found that the intravenous injection of UC-MSCs improved behavioral outcome in IVH, by restoring periventricular reactive gliosis, hypomyelination, and periventricular cell death in vivo. Transplanted UC-MSCs migrated towards injured brain, but disappeared three weeks after injection. Interestingly, human brain-derived neurotrophic factor (BDNF) and hepatocyte growth factor (HGF) were elevated in the serum, cerebrospinal fluid and brain tissue of UC-MSCs injected mice. These results suggest that UC-MSCs ameliorate neuronal injury followed by functional improvement by secretion of neurotrophic factors such as BDNF and HGF rather than neuronal differentiation and eternal cell replacement, and that intravenous injection of UC-MSCs may be feasible treatment for neonatal brain injuries.

5. Research human Cord Blood Cell Bank / National BioResource Project (NBRP) (IMSUT-Cell Resource Center):

Izawa Y, Natori M, Nagaya N., Takahashi A, Hori A, Nagamura-Inoue T,

"Research Cord Blood Bank" was established in 2004, supported by MEXT for the development of the medicine including Regenerative Medicine, immunological cell therapy, infection research, modified gene cell therapy, and drug discovery. Since 2012, July, this project has been incorporated in National BioResource Project (NBRP). The research CB bank provides processed and cryopreserved CB units (Nucleated cells, mononuclear cells, and CD34 + cells), to world-wide researchers via RIKEN Bioresource Center. Visit our website http: //www.nbrp.jp/. More than 500 samples / year, we supply to the researchers.

6. Institute of Medical Science, University of Tokyo-Cell Resource Center (IMSUT-CRC):

Takahashi A, Shimazu T, Hori A, Okada M, Mori Y, Ichimura S, Nagamura-Inoue T

To promote the cell therapy in translational researches, IMSUT-Cell Resource Center (IMSUT- CRC) has been established in 1997 (originally called as Room for Clinical Cellular Technology (RCCT)). Until now, the following projects had implemented; 1) CB cell processing for banking (1997-2008) (for Tokyo Cord Blood Bank, Research cord blood stem cell bank, and related sibling donors), 2) Dendritic cell therapies (1998-2001), 3) Regenerative therapy of alveolar bone derived from bone marrow mesenchymal cells (2005-2011), 4) Gene therapy for renal cancer (1998), 5) CB and UC-MSCs banking (IMSUT-CORD) (2012-present), and 6) aAVC-WT1 cell therapy(2017~present), (7)Dendritic cell therapy using DC pulsed with neoantigen(2020-present).

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

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IMSUT Hospital

Surgical Center
手術部

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士（医学）	藤　堂　具　紀
Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任准教授	博士（医学）	田　中　　実

IMSUT hospital provides seamless support for translational research. Our mission is the management and operation of the surgical center to achieve a safe and organized environment where surgical procedures can be performed in high quality. A da Vinci surgical system (da Vinci Xi), a robotic technology that allows surgeons to perform minimally invasive procedures, was introduced in November 2020, and Robot-assisted Radical Prostatectomies (RARP) for prostate cancer started.

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. Medical engineer staffs increased accordingly, and a ME Division was newly established in the Surgical Center. Three of four operating rooms are maintained at a NASA class 10,000 clean level. One operating room is 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 2020

Total number of operations	149
Planned operations	141

Emergency operations	8	Epidural	0
		Local	48
General anesthesia	80	Others	0
Spinal	21		

IMSUT Hospital

Department of Laboratory Medicine
検査部

Professor	Arinobu Tojo, M.D., Ph.D.	教　授	医学博士	東　條　有　伸
Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	病院教授	博士(医学)	長　村　登紀子
Assistant Professor	Tomohiro Ishigaki, M.D., Ph.D.	助　教	博士(医学)	石　垣　知　寛

The department of laboratory medicine consists of seven divisions: clinical hematology, biochemistry/serology, microscopy, pathology, bacteriology, physiology, and TR verification laboratory.

Clinical laboratory tests are necessary for all clinical practice steps including diagnosis of diseases, evaluation of stages, determination of treatments, and assessment after therapy. Our department engages in most of the clinical laboratory examinations in our hospital under stringent quality control and provides investigational laboratory analysis in collaboration with many other departments.

To facilitate translational research projects in this research hospital, we had established a special division named TR verification laboratory. This division has contributed to evaluating the safety of experimental therapeutic approaches and biopharmaceutical products for clinical trials.

As a central medical department, we are also taking part in many clinical trials and researches conducted in our hospital.

1. Introduction of clinical laboratory tests for SARS-CoV-2 detection, and urgent environmental arrangement to prevent the infection in clinical laboratory including physiological laboratory.

Bacteriology team, Physiology team, Tomohiro Ishigaki, and Tokiko Nagamura-Inoue.

Due to the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapid and accurate diagnosis of the infection (COVID-19) is essential for clinical practice. Our clinical laboratory takes an important role in the tests for detection. The current standard method for diagnosing SARS-CoV-2 infection is the real-time reverse transcriptase-polymerase chain reaction (RT-PCR). To analyze much more clinical samples and report the results faster, we have applied BD MAX™ system, which is a fully-integrated automated platform that performs nucleic acid extraction and real-time PCR, to clinical laboratory. After sufficient validation and quality control, we have established protocols according to the standard method of the National Institute of Infectious Diseases. We are reporting the results of various clinical samples within about 2 hours. After the PCR assays, we have introduced SARS-CoV-2 antigen tests, and are about to introduce IgG/IgM antibody tests for deeper analysis for SARS-CoV-2 infection.

The virus could easily spread in confined spaces like a physiological laboratory. Respiratory function tests and some physiological tests are at high risk for infection. Therefore, we also advanced environmental arrangement to prevent infection in a physiological laboratory.

2. Immunophenotypic analysis of cerebrospinal fluid reveals concurrent development of ATL in the CNS of a HAM/TSP patient.

Tomohiro Ishigaki (corresponding) and Arinobu Tojo.

Both adult T-cell leukemia/lymphoma (ATL) and human T-cell leukemia virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) can be induced by HTLV-1, but concurrent development has been rarely reported. We reported the case of CNS ATL in a HAM/TSP patient. Our quantitative flow-cytometric analysis of cell populations in the cerebrospinal fluid (CSF) revealed that the CSF cells were classified as aggressive ATL; these cells exhibited a more progressed phenotype than those in peripheral blood (PB). On the other hand, HAM/TSP disease activity was estimated to be low. Based on our analysis, we made a diagnosis of acute-type ATL, which unusually developed in the central nervous system at initial onset prior to systemic progression. Immunophenotypic characterization of CSF and PB is valuable for differential diagnosis and understanding disease status in cases with a challenging diagnosis.

3. High Prevalence of Left Ventricular Non-Compaction and Its Effect on Chemotherapy-Related Cardiac Dysfunction in Patients With Hematological Diseases.

Koichi Kimura (corresponding), Tomohiro Ishigaki (co-first), Tokiko Nagamura-Inoue, and Arinobu Tojo.

Recent progress in chemotherapy has prolonged the survival of patients with hematological diseases, but has also increased the number of patients with chemotherapy-related cardiac dysfunction (CTRCD). However, the causes of individual variations and risk factors for CTRCD have yet to be fully elucidated. We retrospectively evaluated consecutive echocardiograms of 371 patients for the presence of left ventricular (LV) non-compaction (LVNC). The prevalence of LVNC was 6-fold higher in patients with hematological diseases than in those with non-hematological diseases (12% vs. 2%; risk ratio 6.1). Among patients with hematological diseases, the ratio of myeloid diseases was significantly higher in the group with LVNC. Deterioration of LVEF was more severe in patients with than without LVNC (-14.4 percentage points/year vs. -4.6 percentage points/year, respectively), even after multivariate adjustment. We elucidated LVNC is relatively prevalent in patients with hematological diseases (particularly myeloid diseases) and can be one of the major risk factors for CTRCD. Detailed cardiac evaluations including LVNC are recommended for patients undergoing chemotherapy.

4. Establishment and introduction of new management system of clinical laboratory examinations for translational researches (TR).

TR verification laboratory team, Tomohiro Ishigaki, and Arinobu Tojo.

Our department also functions as a safety-monitoring laboratory by examining the safety of new investigational therapeutic approaches. TR verification laboratory was funded by the Ministry of Education, Culture, Sports, and Technology (MEXT). We are examining the safety of bio-cellular materials for Translational Research (TR) clinical applications, such as gene therapies, viral therapies, and cell therapies, under GMP-based standards. After 10-years of manual management, we established a new management system for TR tests and introduced it to clinical laboratory practice with the database server and portable terminals. The introduction had many merits and enabled the following: (1) low-cost introduction compared with general systems on sale, (2) improvement of work efficiency, (3) simultaneous sharing, (4) prevention of human errors, and (5) deeper recommendation from the sequential view of results.

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

Hiroyuki Shingyochi and Clinical laboratory members (clinical hematology, biochemistry/serology, and bacteriology team)

We are taking part in other clinical trials and researches led by other departments in our hospital. Our laboratory members contributed to 9 clinical investigations and trials conducted in this hospital, including a trial of new vaccination and a trial of treatment for COVID-19.

We also contributed to many basic and clinical studies, such as clinical laboratory application of detection kit for *E. histolytica*, cell population analysis after cord blood transplantation, immunological analysis after SARS-CoV-2 infection, and so on.

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

Center for Translational Research was reorganized from Division of Clinical Trial Safety Management in 2014. Support for the conduct of clinical trials, especially for sponsor-investigator clinical trial based on Translational Research (TR) is our major mission. Our roles on TR varies from the advice for acquiring intellectual property, assistance for planning study design and writing protocol to the data confirmation by Case Report Form which is managed by Translational Research Coordinator (TRC) and the quality assurance of TRs by monitoring/audit. To protect the participants into TR and to conduct TR scientifically and ethically appropriately, we have organized TRC, which consists nurse, pharmacist, clinical laboratory technologist, dietitian, and clinical psychotherapist.

1. Promotion of Translational Research at IMSUT Hospital

All members of staff.

We have an unwavering commitment to deliver novel therapies through the conduct of translational research. To advance basic research findings into clinical application, we offer investigators the following services:

1)planning research and development (R & D) strategies, including selecting target diseases, planning product designs, and clarifying development pathways;
2)offering opportunities to consult an appointed patent attorney about acquisition and maintenance of intellectual property rights as well as patent strategies;
3)providing information necessary in preclinical phase of R & D, such as information on drug regulatory affairs and preclinical studies;
4)encouraging investigators to consult regulatory advisors of Pharmaceuticals and Medical Devices Agency (PMDA) in a timely manner;
5)participating in investigator-regulator meetings to help investigators deal with issues pointed out in the meetings;
6)advising on clinical trial design so that feasible and scientifically appropriate trials are conducted;
7)reviewing clinical study protocols, consent forms, and related documents in prior to Institutional Review Board examination to ensure the quality of clinical trials conducted at IMSUT Research Hospital;
8)assigning Translational Research Coordinators (TRCs) to each translational research project in the clinical trial phase; TRCs help patients participating in clinical trials to understand study protocols and to cope with negative emotions including fear, confusion, and depression; TRCs assist investigators.

2. Statistics and Quality control in Clinical Trials

Masanori Nojima, Motoki Amai, Mitsumi Tokunaga, Fumitaka Nagamura

We have planned and performed data management, monitoring, and statistical works in clinical tri-

als.

[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 2020, we supported four sponsor-investigator clinical trials by site management as well as project management. These four clinical trials were: oncolytic virus for malignant melanoma, peptide therapy for after rejection of non-small cell lung cancer, phase II clinical trial with novel gene-induced adjuvant cells for acute myelogenous leukemia, and umbilical cord derived mesenchymal stromal cells for severe acute graft-versus-host disease.

5. Statistical consulting

Masanori Nojima

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

Publications

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IMSUT Hospital

Center for Antibody and Vaccine Therapy
抗体・ワクチンセンター

Professor	Hirotoshi Tanaka, M.D., D.M.Sc.	教　授	医学博士	田　中　廣　壽
Professor	Kouhei Tsumoto, Ph.D.	教　授	博士(工学)	津　本　浩　平
Project Professor	Yataro Daigo, M.D., D.M.Sc.	特任教授	博士(医学)	醍　醐　弥太郎
Project Associate Professor	Satoru Nagatoishi, Ph.D.	特任准教授	博士(生命科学)	長門石　　暁
Senior Assistant Professor	Noritada Yoshikawa, M.D., D.M.Sc.	講　師	博士(医学)	吉　川　賢　忠
Project Senior Assistant Professor	Atsushi Takano, M.D., D.M.Sc.	特任講師	博士(医学)	高　野　　淳

Our center was established in April 2012, in the memory of Professor Shibasaburo Kitasato, the founder and the first director of our institute, because the year 2012 was 120th anniversary of our institute which was built in 1892. Professor Kitasato was keen to utilize 'serum therapy' for patients with infectious diseases and actually developed therapeutic sera from horses. Now, we can use monoclonal antibodies to cytokines and their receptors, growth factor receptors, cellular kinases, for treatment of autoimmune diseases and cancer. The aim of this center is to develop novel immunological therapy for patients with various diseases including cancers and autoimmune diseases. Moreover, attractive clinical trials are also ongoing in collaboration with research groups in IMSUT.

Tanaka Group

1. Clinical activities in IMSUT Hospital

Hirotoshi Tanaka, Motohisa Yamamoto, Noritada Yoshikawa, Hiroki Yamazaki,
Erika Matsubara*: *Department of Rheumatology and Allergy, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo

Rheumatologists at our division provide state-of-the-art diagnosis and treatment for systemic autoimmune diseases (total number of patients were approximately 5,000 per year). Our physicians have active basic and clinical research projects and also are involved in training of rheumatology specialists.

Rheumatologic services offered at IMSUT Hospital include:

- Outpatient consultations
- Outpatient specialty care for patients with rheumatic diseases
- Hospital consultations
- Diagnostic and therapeutic intra-articular and soft tissue injections and aspirations
- Diagnostic ultrasonography
- Education on rheumatologic diseases and treatments
- Clinical trials

2. Translational Research and Clinical Trial of Division of Rheumatology

See the section of Department of Rheumatology and Allergy, IMSUT Hospital.

Publications

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10. Fujisawa Y, Mizushima I, Yamada K, Yamamoto M, Saeki T, Matsui S, Tsuge S, Hara S, Ito K, Fujii H, Takahashi H, Nomura H, Kawa S, Kawano M. Hypocomplementemia is related to elevated serum levels of levels of IgG subclasses other than IgG4 in Igg4-related kidney disease. Mod Rheumatol. 2020 Jan 13: 1-8. doi: 10.1080/14397595.2019.1709942.

Daigo Group

1. Novel therapeutic target discovery for solid cancers

Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Yoshinori Murakami, Phung Manh Thang, Kayo Daigo, Tomoyuki Igarashi, Masako Nakamura, Tsevegjav Bayarbat, Zhu Ming, Mbugua Regina Wachuka

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

targets.

2. Development of therapeutic cancer vaccine

Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Koichiro Yuji, Hiroshi Yasui, Giichiro Tsurita, Kohzoh Imai, Yoshihide Fujiyama, Kazumasa Ogasawara

Using the systematic screening system shown above, we identified concoantigens which were overexpressed in the majority of cancers derived from lung, esophagus and urinary bladder and essential for the growth and/or survival of cancer cells, as targets for therapeutic cancer vaccine treatment against various solid cancers. We screened dozens of 9- or 10-amino-acid epitope peptides recognized by human HLA-A*0201 and/or A*2402-restricted CTL by enzyme-linked immunospot (ELISPOT) assay. In IMSUT Hospital and its collaborative hospitals, International Conference on Harmonization (ICH) - Good Clinical Practice (GCP)-based clinical study using the combination of some of these peptides derived from oncoantigens in patients with lung cancer is now being conducted. In addition, new type of peptides-pulsed DC vaccination therapy is under development.

3. Integrated genomics-based discovery of new biomarkers for cancer immunotherapy and development T cell receptor-engineered T cell (TCR-T) therapy

Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Koichiro Yuji, Hiroshi Yasui, Giichiro Tsurita, Yoshihide Fujiyama, Kazumasa Ogasawara, Yusuke Nakamura

Immune responses play a critical role in various disease conditions including cancer. Although various immunotherapies are being developed, predictive biomarkers for the choice of effective therapy are urgently required. Using systematic cancer genomics approach on clinical materials obtained from cancer patients treated with cancer vaccine, peptides-pulsed DC vaccination therapy, or immune checkpoint inhibitors, we are clarifying how molecular profiles of cancers can be used to identify biomarkers for predicting clinical outcomes. For example, there has not been a rapid, sensitive, comprehensive, and quantitative analysis method to examine T-cell or B-cell immune responses, therefore we developed a new approach to characterize tumor mutation burdens and T cell receptor (TCR) repertoire by sequencing millions of cDNA of exomes of cancer related genes as well as TCRα and β chains in combination with a newly-developed algorithm. Using samples from lung cancer patients, we are developing detailed information of neoantigen profiles of lung cancer patients and their TCR repertoire and are also developing T cell receptor-engineered T cell (TCR-T) therapy. This newly developed next generation sequencing (NGS) platform can be applied to better understand immune responses in many disease areas including immune disorders, allergies, and organ transplantations as we as development of new type of immunotherapies.

4. Detection of neoantigen-reactive T cell clones based on the clonal expansion using next-generation sequencing of TCRβ complementarity-determining region 3

Yataro Daigo, Hidetoshi Sumimoto, Koji Teramoto, Atsushi Takano

Development of mechanism-driven biomarkers for immune checkpoint inhibitors in cancer immunotherapy requires sensitive and efficacious assays to identify tumor antigen (Ag)-specific T cells. We demonstrated the concept for a sensitive method to determine Ag-reactive T cell clones based on clonal expansion using model neoantigens rather than cytokine production. Sequential increase in T cell clonal frequencies following Ag stimulation was detected by NGS of TCRβ complementarity-determining region 3 (CDR3), with a higher sensitivity than that of ELISPOT assay by 100-fold. The TCRβ CDR3 sequences could represent useful markers to track dynamic changes during immunotherapy. The TCRβ NGS-based method could represent a novel platform both for the development of new biomarkers as well as several therapeutic options.

5. Molecular characterization of tumor microenvironment molecules as diagnostic and therapeutic targets

Yataro Daigo, Koji Teramoto, Hidetoshi Sumimoto, Tomoyuki Igarashi, Atsushi Takano

Tumor microenvironment is supposed to be involved in tumor progression and drug resistance. To identify molecules that play crucial roles in cancer cells as well as tumor stromal cells such as cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) and apply them for the development of new molecular therapies and/or biomarkers, we are characterizing various immune checkpoint molecules and cytokines in a variety of solid cancer tissues and cell lines using cell-based assays and clinical cancer materials such as tumor tissues and/or blood samples from patients with lung, breast, colon, or ovarian cancers. Studies on molecular pathological role of these molecules are in progress, however, some of them are likely to be associated with malignant potential of cancer cells.

6. Biphasic prognostic significance of PD-L1 expression in patients with non-small cell lung cancer

Yataro Daigo, Koji Teramoto, Tomoyuki Igarashi

Programmed cell death-ligand 1 (PD-L1) expression on tumor cells is induced by interferon-γ, suggesting the induction of an anti-tumor immune response, while binding of PD-L1 to programmed cell death 1 (PD-1) triggers an immune checkpoint pathway that contributes to tumor growth. The data suggest the clinical significance of PD-L1 expression might vary with tumor progression in non-small-cell lung cancer (NSCLC). We performed immunohistochemical analysis of PD-L1 in tumor specimens from 228 patients who underwent surgery for stage I-IIIA NSCLC. In stage I, postoperative relapse-free survival (RFS) was significantly prolonged in patients with a high PD-L1 expression compared to a low PD-L1 score. A multivariate analysis confirmed that a high PD-L1 score was a prognostic factor of longer RFS. On the other hand, in stages II and IIIA, patients with a high PD-L1 expression were likely to suffer from postoperative tumor recurrence. The data indicate that in early-stage NSCLC, high tumor PD-L1 expression status represents a biomarker to predict good prognosis after surgery and may reflect the induction of an antitumor immune response, whereas in locally advanced NSCLC, tumor PD-L1 expression may reflect the execution of an immune checkpoint pathway and predicts the incidence of postoperative tumor recurrence.

7. Identification of lung cancer susceptibility loci by genome-wide association studies.

Yataro Daigo, Atsushi Takano

To identify new susceptibility loci associated with lung cancer risk, we imputed data from genome-wide association studies (GWAS) of lung cancer. In our meta-analysis, we are identifying new loci that achieved genome-wide significance, marked by single nucleotide polymorphism (SNP). In addition, we performed a GWAS with 212,453 Japanese individuals across 42 diseases. We detected 320 independent signals in 276 loci for 27 diseases, with 25 novel loci. Among east Asian-specific missense variants, we identified and replicated p.V326A of *POT1* that was associated with lung cancer by analyzing independent Japanese cohorts. The results extend the catalog of regions associated with lung cancer risk and highlight the potential of genetic susceptibility alleles as a new biomarker for cancer risk prediction and prevention.

8. Scientific Platform of Supporting Cohort Study and Biospecimen Analysis

Yataro Daigo, Atsushi Takano, Koji Teramoto, Kohzoh Imai, Jun-ichiro Inoue, Yoshinori Murakami

To support life science researchers in the field of basic life science, cancer diagnostics and therapeutics, we are collecting cancer and corresponding normal tissues, serum, plasma, and peripheral blood mononuclear cell (PBMC) from patients with solid cancers originated from 30 organs. To date, we collected 76,000 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 contributed to their clinical application and publications in international journals.

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Nagatoishi Group

Various types of antibodies have been approved for therapeutic use and currently examined in clinical development. Therefore, developments of technology for the discovery and optimization of high-potency antibodies have been improved and have greatly increased to find the specific and stable antibody with desired biological properties. Biophysical analyses of a therapeutic antibody, particularly those of protein interaction and stability, are recognized as one of the critical procedures in the development of biopharmaceuticals, which would be assessed as an essential step to develop next-generation antibodies. The development of analytical methods with quantitative and high-sensitive detection of antigen interaction, protein stability, and biological function of antibody, therefore, has been intriguing for the pharmaceutic companies. In this division, we study biophysical analyses of various antibodies to propose a new strategy for the development of the next-generation antibody.

1. Structural Basis for the Binding Mechanism of Human Serum Albumin Complexed with Cyclic Peptide Dalbavancin

Ito S, Senoo A, Nagatoishi S, Ohue M, Yamamoto M, Tsumoto K, Wakui N.

Here, we present the crystal structure of HSA complexed with dalbavancin, a clinically used cyclic peptide. Small-angle X-ray scattering and isothermal titration calorimetry experiments showed that the HSA-dalbavancin complex exists in a monomeric state; dalbavancin is only bound to the subdomain IA of HSA in solution. Structural analysis and MD simulation revealed that the swing of Phe70 and movement of the helix near dalbavancin were necessary for binding. The flip of Leu251 promoted the formation of the binding pocket with an induced-fit mechanism; moreover, the movement of the loop region including Glu60 increased the number of noncovalent interactions with HSA. These findings may support the development of new cyclic peptides for clinical use, particularly the elucidation of their binding mechanism to HSA.

2. Highly sensitive HPLC analysis and biophysical characterization of N-glycans of IgG-Fc domain in comparison between CHO and 293 cells using FcγRIIIa ligand

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

Quality control of monoclonal antibodies is challenging due in part to the diversity of post-translational modifications present. The regulation of the N-glycans of IgG-Fc domain is one of the key factors to maintain the safety and efficacy of antibody drugs. The FcγRIIIa affinity column is an attractive tool for the precise analysis of the N-glycans in IgG-Fc domain. We used the mutant FcγRIIIa, which is produced in Escherichia coli and is therefore not glycosylated, as an affinity reagent to analyze the N-glycans of monoclonal antibodies expressed in Expi293 and ExpiCHO cells. The monoclonal antibodies expressed in these cells showed very different chromatograms, because of differences in terminal galactose residues on the IgG-Fc domains. We also carried out kinetic and thermodynamic analyses to understand the inter-

action between monoclonal antibodies and the mutant FcγRIIIa. Expi293 cell-derived monoclonal antibodies had higher affinity for the mutant FcγRIIIa than those derived from ExpiCHO cells, due to slower off rates and lower binding entropy loss. Collectively, our results suggest that the FcγRIIIa column can be used to analyze the glycosylation of antibodies rapidly and specifically.

3. Polymeric Nanocarriers with Controlled Chain Flexibility Boost mRNA Delivery In Vivo through Enhanced Structural Fastening

Miyazaki T, Uchida S, Nagatoishi S, Koji K, Hong T, Fukushima S, Tsumoto K, Ishihara K, Kataoka K, Cabral H.

Messenger RNA (mRNA) shows high therapeutic potential, though effective delivery systems are still needed for boosting its application. Nanocarriers loading mRNA via polyion complexation with block catiomers into core-shell micellar structures are promising systems for enhancing mRNA delivery. Engineering the interaction between mRNA and catiomers through polymer design can promote the development of mRNA-loaded micelles (mRNA/m) with increased delivery efficiency. Particularly, the polycation chain rigidity may critically affect the mRNA-catiomer interplay to yield potent poly(glycidylbutylamine) (PEG-PGBA) and PEG-poly(L-lysine) (PEG-PLL) is studied. PEG-PGBA allows more than 50-fold stronger binding to mRNA than the relatively more rigid PEG-PLL, resulting in mRNA/m with enhanced protection against enzymatic attack and polyanions. mRNA/m from PEG-PGBA significantly enhances mRNA in vivo bioavailability and increased protein translation, indicating the importance of controlling polycation flexibility for forming stable polyion complexes with mRNA toward improved deliverynanocarriers, yet its effect remains unknown. Herein, the influence of polycation stiffness on the performance of mRNA/m by developing block complementary catiomers having polycation segments with different flexibility, that is, poly(ethylene glycol)-.

4. Discovery of chemical probes that suppress Wnt/β-catenin signaling through high-throughput screening

Yamaguchi K, Nagatoishi S, Tsumoto K, Furukawa Y.

Aberrant activation of the Wnt/β-catenin signaling pathway has been observed in a wide range of human tumors. Deregulation of the pathway is closely linked to various aspects of human carcinogenesis such as cell viability, regulation of cell cycle, epithelial-mesenchymal transition, and maintenance of stemness. In addition, recent studies have disclosed the involvement of Wnt signaling in immune evasion of tumor cells. The accumulation of β-catenin in the nucleus is a common feature of cancer cells carrying defects in the pathway, which leads to the continuous activation of T-cell factor (TCF)/LEF transcription factors. Consequently, a genetic program is switched on, leading to the uncontrolled growth, prolonged survival, and acquisition of mesenchymal phenotype. As β-catenin/TCF serves as a signaling hub for the pathway, β-catenin/TCF-dependent transcriptional activity is a relevant readout of the pathway. To date, a wide variety of synthetic TCF/LEF reporters has been developed, and high-throughput screening (HTS) using these reporters has made significant contributions to the discovery of Wnt inhibitors. Indeed, HTS led to the identification of chemical probes targeting porcupine, a membrane bound O-acyltransferase, and CREB-binding protein, a transcriptional coactivator. This review focuses on various screening strategies for the discovery of Wnt inhibitors and their mode of action to help the creation of new concepts for assay/screening methods.

5. Methodology for Further Thermostabilization of an Intrinsically Thermostable Membrane Protein Using Amino Acid Mutations with Its Original Function Being Retained

Yasuda S, Akiyama T, Nemoto S, Hayashi T, Ueta T, Kojima K, Tsukamoto T, Nagatoishi S, Tsumoto K, Sudo Y, Kinoshita M, Murata T.

We develop a new methodology best suited to the identification of thermostabilizing mutations for an intrinsically stable membrane protein. The recently discovered thermophilic rhodopsin, whose apparent midpoint temperature of thermal denaturation Tm is measured to be ~91.8 °C, is chosen as a paradigmatic target. In the methodology, we first regard the residues whose side chains are missing in the crystal structure of the wild type (WT) as the "residues with disordered side chains," which make no significant contributions to the stability, unlike the other essential residues. We then undertake mutating each of the residues with disordered side chains to another residue except Ala and Pro, and the resultant mutant structure is constructed by modifying only the local structure around the mutated residue. This construction is based on the postulation that the structure formed by the other essential residues, which is nearly optimized in such a highly stable protein, should not be modified. The stability changes arising from the mutations are then evaluated using our physics-based free-energy function (FEF). We choose the mutations for which the FEF is much lower than for the WT and test them by experiments. We successfully find three mutants that are significantly more stable than the WT. A double mutant whose Tm reaches

~100 °C is also discovered.

6. Per-Residue Program of Multiple Backbone Dihedral Angles of β-Peptoids viaBackbone Substitutions

Morimoto J, Kim J, Kuroda D, Nagatoishi S, Tsumoto K, Sando S.

Unique folded structures of natural and synthetic oligomers are the most fundamental basis for their unique functions. N-Substituted β-peptides, or β-peptoids, are synthetic oligomers with great potential to fold into diverse three-dimensional structures because of the existence of four rotatable bonds in a monomer with highly modular synthetic accessibility. However, the existence of the four rotatable bonds poses a challenge for conformational control of β-peptoids. Here, we report a strategy for per-residue programming of two dihedral angles of β-peptoids, which is useful for restricting the conformational space of the oligomers. The oligomer was found to form a unique loop conformation that is stabilized by the backbone rotational restrictions. Circular dichroism and NMR spectroscopic analyses and X-ray crystallographic analysis of the oligomer are presented. The strategy would significantly facilitate the discovery of many more unique folded structures of β-peptoids.

7. Technical Capabilities and Limitations of Optical Spectroscopy and Calorimetry Using Water-Miscible Solvents: The Case of Dimethyl Sulfoxide, Acetonitrile, and 1,4-Dioxane.

Hirano A, Nagatoishi S, Wada M, Tsumoto K, Maluf KN, Arakawa T.

In drug development, water-miscible solvents are commonly used to dissolve drug substances. Typical routine procedures in drug development include dilution of the stock drug solution into an aqueous solution containing target macromolecules for drug binding assays. However, water-miscible solvents impose some technical limitations on the assays on account of their light absorption and heat capacity. Here, we examined the effects of the dilution of 3 water-miscible solvents, that is, dimethyl sulfoxide, acetonitrile, and 1,4-dioxane, on the baseline stability and signal/noise ratio in circular dichroism spectroscopy, isothermal titration calorimetry, and differential scanning calorimetry. Dimethyl sulfoxide and 1,4-dioxane affect the signal/noise ratio of circular dichroism spectra at typically used concentrations due to their light absorbance. The water-miscible solvents generate interfering signals in the isothermal titration calorimetry due to their mixing heat. They show negative or positive slope in the differential scanning calorimetry. Such interfering effects of the solvents are reduced by appropriate dilution according to the analytical techniques. Because the water-miscible solvents have solubilization capacity for alkyl chain moieties and aromatic moieties of chemicals, drug substances containing these moieties can be dissolved into the solvents and then subjected to the analyses to examine their interactions with target proteins after appropriate dilution of the drug solutions.

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Case of Dimethyl Sulfoxide, Acetonitrile, and 1,4-Dioxane. J. Pharm. Sci. 109:524-531, 2020.

IMSUT Hospital

Therapeutic Vector Development Center
治療ベクター開発センター

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The Therapeutic Vector Development Center (TVDC), formerly named Core Facility for Therapeutic Vectors, was established in 2002 as the first facility in Japanese academia for the clinical-grade production of viral or cellular vectors. TVDC is designed to support clinical trials that require the production of recombinant viral vectors, genetic modification, and/or ex vivo manipulation of patient-derived tissues or cells under current Good Manufacturing Practice (cGMP) conditions.

Maintenance of the Standard Operating Procedures (SOPs)

The cGMP compliance is maintained by the regularly-revised SOPs that document all the elements of laboratory works, including both tangible and intangible factors like equipment, facility design, personnel, etc.

ISO certification

The management system of TVDC was re-qualified as ISO9001-certified in 2020, which has been regularly performed by an independent organization to meet the requirement for ISO9001 standard.

Validation of TVDC

The TVDC consists of two units; 1) Vector Unit, the primary suite for viral vector production and ex vivo transduction; 2) Cell Unit, the suite for cell processing capable of generating therapeutic cells such as dendritic cells for immunotherapy and gene therapy. Each unit has two independent compartments kept as a Class 10,000 clean level. The facility and equipment are regularly validated by the SOPs to fulfill the cGMP standard.

Production of clinical grade oncolytic HSV-1

Multiple lots of clinical-grade oncolytic herpes simplex virus type 1 (HSV-1) have been produced in the Vector Unit by the laboratory specialists of the Division of Innovative Cancer Therapy.

Oncolytic MV project

A clinical-grade oncolytic measles virus (MV) was produced by the Laboratory Animal Research Center and stored in the Vector Unit

IMSUT Hospital

IMSUT CORD
臍帯血・臍帯バンク

Clinical Professor　Tokiko Nagamura-Inoue, M.D., PhD.　病院教授　博士（医学）　長　村　登紀子

IMSUT CORD is the umbilical cord blood (CB) and cord (UC) derived cell bank. It has been established in IMSUT hospital, since 2016. The aim of IMSUT CORD is to collect, process/culture, cryopreservation, stock, and release the CB and UC/UC-derived cells including mesenchymal stromal cells (MSCs) for clinical and research use. We have released CB and UC-MSCs to the researchers by material transfer agreements to accelerate the translational researches in the fields of immunotherapy, regenerative medicine, disease specific drug discovery. Since 2018, July, we started to release the UC-MSCs products (namely IMSUT-CORD) for the investigator initiated clinical trial of the treatment of severe acute graft-versus-host disease (GVHD), which was ended in 2020, October. In 2020, we additionally firstly shipped the UC-MSCs for the clinical trial for COVID-19-related ARDS, which has been conducted by the company. We are also preparing the products for the treatment of cerebral palsy.

1. Umbilical Cord Blood and Cord/Cord-derived mesenchymal stromal cells banking (IMSUT CORD):

Nagamura-Inoue T, Takahashi A, Hori A, Yamamoto Y, Iwasawa I, Nagaya N, Miharu Y, Ogami K, Saito Y, Nagamura F, Tojo A

Umbilical cord (UC) is a rich source of mesenchymal stromal cells (MSCs). The UC-derived MSCs (UC-MSCs) possess many advantageous features, (1) ease of collection, storage, and transport; (2) abundant sources with high proliferation capacity, (3) multipotency to differentiate into various tissue cells including osteoblast, chondroblast, adipocyte, and neurons; (4) low immunogenicity with significant immunosuppressive ability, (5) tissue repair potency, (6) migration ability toward the inflammatory or injured sites, subsiding the inflammation and repairing the damaged tissues, and (7) no donor age-dependent variations.

We established a cord blood/cord bank at the IMSUT hospital (IMSUT CORD) to collect cord blood (CB) and UC after informed consent from mother in collaborate obstetrics and frozen UC and manufactured into master cells and product cells for research and clinical use..

For clinical use, we have introduced the serum-free process in whole manufacture.

To keep the quality control, we introduced ISO9001:2015 into our bank and received accreditation and were certified it on June, 18, 2018. Complying ISO9001, GCTP standard, and other related guidelines, we constructed quality manuals and standard operation procedure (SOP) by many documentations. We have transferred the technology of manufacturing and tests methods to the companies, where they shall follow our techniques and apply the clinical trials, including the therapies for acute GVHD, cerebral palsy, and acute respiratory distress syndrome (ARDS) as a complication of COVID-19.

2. Establishing a stable supply model system of perinatal appendage-derived cells as the resource of allogeneic cell therapies:

Nagamura-Inoue T, Takahashi A, Kamisato A, Hori A, Yamamoto Y, Iwasawa I, Nagaya N, Miharu Y, Ogami K, Saito Y, Nagamura F

We constructed the review system and associated SOP and application forms for research and clinic. Actually using the review system, two applications were approved by review board and IMSUT CORD have started manufacturing to ship UC-MSCs to the companies, which shall conduct the clinical trials for COVID-19-related ARDS and cerebral palsy.

In the future, we will ship the master cells as a source of final products, and the companies may plan to acquire the manufacturing and marketing approval using UC-MSCs.

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

Publications

1) Nagamura-Inoue T, Nagamura F., Umbilical Cord Blood and Cord tissue bank as a source of allogeneic use, IntechOpen, 2020, March 11, DOI: 10.5772/intechopen.91649.

IMSUT Hospital

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看護部

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看護師長		亀田史絵

Department of Nursing seeks to provide high-quality nursing care and contribute to the team approach to patient centered care to meet diversified needs, along with changes in social circumstances and with the progress of medical science. In 2020, we focused on two areas: responding to covid-19 and implementing the Shirokane-Hongo Special Enhancement Project.

One of our missions is "Making a difference in patient outcome provided by nursing care." As nurses, we provide optimal care so that patients may receive quality treatment. Patients should be able to live valuable and meaningful life. As healthcare providers, we make an effort to prevent infection, pressure ulcer and other complications. We also do our best for patient safety and quality of life.

In 2020, in response to the outbreak of covid-19, the infection control department led to strengthen infection prevention measures and established a system for accepting inpatients as a nursing department. In late March, the first wave arrived, and the ward dedicated to patients with the novel coronavirus infection (mild to moderate) and the general patient ward were partly different, and inpatients were accepted. In June, screening of general inpatients was started, and a dedicated screening bed was set up in one ward. Initially, nurses in dedicated wards were confused by the response of patients with unknown viral diseases, and nurses in general wards were confused by the response of patients in multiple departments that had been less involved until now. Gradually, the nurses regained their calm and returned to the original state which provided appropriate nursing to the patient according to the function of each ward. With the arrival of the second and third waves again, and a rapid increase in the number of affected patients, the number of nurses who tested positive for COVID-19 did not occur, and the past year passed without any hospital infection. In December, he was awarded the 2019-2020 President's Award for Business Reform along with the Infection Control Team as part of his efforts to prevent the spread of covid-19.

In 2019, we launched the Shirokane-Hongo Special Enhancement Project in collaboration with the University Hospital for management improvement. At the beginning of the year, the project had scalled

due to the covid-19 pandemic, but from June, the opening of new urology departments, the introduction of robotic surgery, the resumption of palliative medicine and the adoption of patients for palliative care, and the expansion of radiological examinations began to move. The Nursing Department worked on the following three points in cooperation with other departments. 1) With regard to the establishment of a new urology department, preparations were made from the nursing point of view for the establishment of outpatient clinics, the system for accepting inpatients, and the introduction of robotic surgery. 2) In Order to strengthen medical cooperation with university hospitals and community medical institutions, a new regional medical cooperation charge was established as a nursing unit in August. 3) In order to make the project a success, six nurses were added to the operating room, two nurses were deployed in the radiological photography room, and three nurses were deployed in the community medical cooperation room. Then, the head nurse summarized the nursing system of urological patients and palliative care target patients in the study group, and the deputy nurse director planned the study meeting and spread the knowledge of medical care and nursing to the staff. It is expected that this result will be reflected in nursing practice in the future.

Publication

谷井真弓．高坂久仁子．久原みな代．一色裕美．吉井栄子．藤原紀子．「臨床におけるリサーチナース導入への取り組み～ワーキンググループ設立と看護基準作成のプロセス～」看護管理．第30巻．第11号．2020. 11. 10発行．P. 1016-1020

Conference Presentation

上山美香，永田早津紀，小粥美香．左大腿骨人工骨頭置換術後に認知機能の低下がみられた患者の支援．第53回　東京都看護協会看護研究学会．東京．2020. 1. 18

小野谷厚子．効果的な経路別感染対策を目指しパンフレットを用いた患者指導．第35回日本環境感染学会・学術集会．横浜．2020. 2. 14-15

亀田史絵．リンクナースの活動につなげる環境ラウンドのシステム構築に向けて～リンクナース主体による自部署の改善活動の支援～第35回日本環境感染学会・学術集会．横浜 2020. 2. 14-15

Hisahara M. Kogayu.M. Nurse Manager training with competency-based approach. The 6th International Nursing Research Conference of World Academy of Nursing Science. Osaka.Japan.2020.2.28-29（Web開催）

成田初子．教訓を風化させない取り組み ―離床センサーの確実な作動確認―．第6回日本医療安全学会学術総会．東京．2020. 3. 5-7（Web開催）

山岸康子．造血幹細胞移植後のOral　Assessment Guide　スコアによる口腔粘膜障害の変動．第42回日本造血幹細胞移植学会総会．東京．2020. 3. 5-7（開催中止）

Nakano.T. Ueyama.M. Kogayu.M. Taketani.H. Effectiveness of re-education for hemophilia patients with joint dysfunction using leaflets. WFH Virtual Summit2020

小粥美香．小澤昌子．谷井真弓．野地有子．竹谷英之．成人血友病患者の実態とニーズの把握．第42回日本血栓止血学会学術集会（誌上発表）

上山美香．小児の血友病における，一般病棟での入院・手術にあたっての支援．第42回日本血栓止血学会学術集会．大阪．2020. 6. 18-20（誌上発表）

上山美香．ヘムライブラを導入目的で入院した患者への看護師の指導．第42回日本血栓止血学会学術集会．大阪．2020. 6. 18-20（誌上発表）

一色裕美．小澤昌子．都留由香里．リンツビヒラ希.粕谷文子．久原みな代．吉井栄子．部署を運営する上で，副看護師長と看護師長が発揮するコンピテンシーの関連の検討．第24回日本看護管理学会学術集会．2020. 8. 28-29（Web開催）

小粥美香．福田あかり．看護過程を活用した外来看護．24回日本看護管理学会学術集会．2020. 8. 28-29（Web開催）

清水祐美．仲井あや．中村伸枝．発達上の課題がある子どもとクラスメイトの年齢に応じた社会性獲得にむけた保育所での支援．千葉県看護学会．第26回学術集会．2020. 9. 5（Web開催）

中村明日美．青木美紀子．島袋林秀．大西涼子．中澤光子．Scope and Standards of Practice Genetics/Genomics Nursing　～2006年版と2016年版の比較～日本遺伝看護学会 第19回学術大会．2020. 9. 19-20.（Web開催）

IMSUT Hospital

Department of Pharmacy
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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.

1) Iimura Y, Kurokawa T, Nojima M, Kanemoto Y, Yazawa K, Tsurita G, Kuroda S. Potential thiamine deficiency and neurological symptoms in patients receiving chemotherapy for gastrointestinal cancer. Int J Clin Pharmacol Ther. 58: 139-145, 2020.

2) Yasu T, Iimura Y, Momo K, Kuroda S. Potential thiamine deficiency in elderly patients with gastrointestinal cancer undergoing chemotherapy. Int J Clin Pharmacol Ther. 58: 174-176, 2020.

3) Ono K, Momo K, Yasu T, Kiyomi A, Sugiura M, Kuroda S. Optimization of Preparation, and Stability of Indomethacin Mouth Wash for Oral Mucositis Caused by Cancer Therapy. Gan To Kagaku Ryoho. 47: 1465-1470, 2020.

4) Yasu T, Momo K, Horii M, Yokoyama K, Ono K, Kiyomi A, Sugiura M, Kuroda S. Effect of Indomethacin Mouthwash on Pain Due to Chemotherapy-Induced Oral Mucositis. J Palliat Med. 23: 886-887, 2020.

5) Yasu T, Momo K, Kuroda S, Kawamata T. Fluconazole Increases Ibrutinib Concentration. Am J Ther. 27: e620-e621, 2020

IMSUT Hospital

Department of AIDS Vaccine Development
エイズワクチン開発担当

Professor	Tetsuro Matano, M.D., D.M.Sc.		教授（委嘱）	博士（医学）	俣野哲朗
Visiting Associate Professor	Ai Kawana-Tachikawa, D.M.Sc.		客員准教授	博士（医学）	立川（川名）愛

We are working on Microbiology and Immunology to elucidate the immune mechanism for retroviral control in vivo. In particular, we are studying virus-host immune interaction and viral evolution using non-human primate models and human clinical samples derived from African and Asian countries as well as Japan. Furthermore, we are developing vaccines using Sendai virus vectors eliciting antibody and/or cytotoxic T lymphocyte responses targeting pathogens including HIV-1, HTLV-1, and SARS-CoV-2.

1. A novel immunogen selectively eliciting CD8+ T cells but not CD4+ T cells targeting immunodeficiency virus antigens.

Hiroshi Ishii[1], Kazutaka Terahara[2], Takushi Nomura[1], Akiko Takeda[1], Midori Okazaki[1], Hiroyuki Yamamoto[1], Tsuyoshi Tokusumi[3], Tsugumine Shu[3], Tetsuro Matano: [1]AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; [2]Department of Immunology, National Institute of Infectious Diseases, Tokyo, Japan; [3]ID Pharma Co., Ltd., Ibaraki, Japan

Induction of effective $CD8^+$ T-cell responses is an important HIV vaccine strategy. Several promising vaccine delivery tools have been developed, and immunogen optimization is now crucial for effective T-cell induction. Conventional T cell-based vaccines have been designed to induce virus-specific $CD4^+$ T as well as $CD8^+$ T cells. However, it has been indicated that induction of HIV-specific $CD4^+$ T cells, preferential targets for HIV infection, by vaccination may accelerate viral replication after HIV exposure. In this study (publication #4), we presented a novel immunogen to selectively induce $CD8^+$ T cells but not $CD4^+$ T cells targeting viral antigens. The immunogen, CaV11, was constructed by tandem connection of overlapping 11-mer peptides spanning SIV Gag capsid and Vif. Prime-boost immunization with DNA and Sendai virus (SeV) vectors expressing CaV11 efficiently induced Gag/Vif-specific $CD8^+$ T-cell responses with inefficient Gag/Vif-specific $CD4^+$ T-cell induction in rhesus macaques. None of the macaques exhibited the enhancement of acute viral replication after an intravenous high-dose SIV challenge, which was observed in those immunized with DNA and SeV expressing the whole Gag protein in our previous study. SeV-specific helper responses were considered to contribute to effective Gag/Vif-specific $CD8^+$ T-cell induction by vaccination. This immunogen design could be a promising method for selective induction of effective anti-HIV $CD8^+$ T-cell responses.

2. Therapeutic vaccine-mediated Gag-specific CD8+ T-cell induction under anti-retroviral therapy augments anti-virus efficacy of CD8+ cells in simian immunodeficiency virus-infected macaques.

Midori Nakamura-Hoshi[1], Yusuke Takahara[1], Saori Matsuoka[1], Hiroshi Ishii, Sayuri Seki[1], Takushi Nomura, Hiroyuki Yamamoto, Hiromi Sakawaki[4], Tomoyuki Miura[4], Tsuyoshi Tokusumi, Tsugumine Shu, Tetsuro Matano: [4]Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

Anti-retroviral therapy (ART) can inhibit HIV proliferation but not achieve virus eradication from HIV-infected individuals. Under ART-based HIV control, virus-specific $CD8^+$ T-cell responses are often reduced. In this study (publication #6), we investigated the impact of therapeutic vaccination inducing virus-specific $CD8^+$ T-cell responses under ART on viral control in a macaque AIDS model. Two groups of rhesus macaques, Groups I and II, received ART from week 12 to 32 after SIV infection. Group II macaques were vaccinated with SeV vectors expressing SIV Gag and Vif at weeks 26 and 32, and Gag/Vif-specific $CD8^+$ T-cell responses were enhanced and became predominant. All macaques controlled viremia during ART but showed viremia rebound after ART cessation. Analysis of *in vitro* $CD8^+$ cell ability to suppress replication of autologous lymphocytes-derived SIVs found augmentation of anti-SIV efficacy of $CD8^+$ cells after vaccination. In Group II, the anti-SIV efficacy of $CD8^+$ cells post-ART was correlated positively with Gag-specific $CD8^+$ T-cell frequencies and inversely with rebound viral loads. These results indicate that Gag-specific $CD8^+$ T-cell induction by therapeutic vaccination can augment anti-virus efficacy of $CD8^+$ cells, which may be insufficient for functional cure but contribute to more stable viral control under ART.

3. Inefficient Tax-specific T-cell responses in mice after syngeneic transplantation with *tax*-transgenic mouse-derived adult T-cell leukemia cells.

Midori Nakamura-Hoshi, Tadaki Suzuki[5], Akira Ainai[5], Hideki Hasegawa[5], Hiroshi Ishii, Tetsuro Matano: [5]Department for Pathology, National Institute of Infectious Diseases, Tokyo, Japan

Adult T-cell leukemia (ATL) is induced by chronic latent infection of human T-cell leukemia virus type 1 (HTLV-1). HTLV-1 Tax is an oncogenic factor and can be a target for host T-cell responses. However, Tax expression *in vivo* is little in ATL cells and the impact of Tax-specific T-cell responses on ATL progression remains unclear. In this study (publication #2), we examined Tax-specific T-cell responses in C57BL/6 mice after syngeneic transplantation with *tax*-transgenic mouse-derived ATL (mATL) cells. We first confirmed that cellular *tax* cDNAs are mostly maintained and detectable in the spleen three weeks after mATL cell transplantation. The mATL cell transplantation did not induce significant Tax-specific T-cell responses. Mice immunized with DNA and adenovirus vectors expressing Tax elicited Tax-specific $CD4^+$ T-cell responses but showed no enhancement of the responses or reduction in cellular *tax* cDNA levels after mATL cell transplantation. This study provides an animal model for analyzing the interaction between ATL cells and host immune responses and indicates limited impact of Tax-specific T-cell responses on ATL cell proliferation.

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2. Nakamura-Hoshi, M., Suzuki, T., Ainai, A., Hasegawa, H., Ishii, H. and Matano, T. Inefficient Tax-specific T-cell responses in mice after syngeneic transplantation with *tax*-transgenic mouse-derived adult T-cell leukemia cells. Jpn. J. Infect. Dis. 73:221-225, 2020.
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IMSUT Distinguished Professor Unit

Division of Stem Cell Therapy
幹細胞治療部門

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Project Assistant Professor	Hideki Masaki, Ph.D.	特任助教　博士（理学）	正　木　英　樹

We are working on uncovering new diseases, elucidating the causes of disease, and developing therapeutic modalities by connecting the knowledge and methodology of basic science including immunology, molecular biology, cell biology, and developmental engineering with clinical medicine. Our ultimate goal is to contribute to establishing new frontiers of stem cell therapy and to make clinical applications of stem cells a reality.

1. Generation of functional organs using a cell competitive niche in intra-and inter-species chimeras

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We have been trying to create human organ in chimeric animal body by using blastocyst complementation technology. As a proof-of-concept study, interspecies organ generation via blastocyst complementation has succeeded in rodents, but not yet in evolutionally more distant species, such as human-mouse or human-pig chimeras. Early developmental arrest hinders the formation of highly chimeric fetuses. We demonstrate that the deletion of insulin-like growth factor 1 receptor (Igf1r) in mouse embryos creates a permissive "cell-competitive niche" in several organs, significantly augmenting both mouse intraspecies and mouse/rat interspecies donor chimerism that continuously increases from embryonic day 11 onward, sometimes even taking over entire organs within intraspecies chimeras. Since Igf1r deletion allows the evasion of early developmental arrest, interspecies fetuses with high levels of organ chimerism can be generated via blastocyst complementation. This observation should facilitate donor cell contribution to host tissues, resulting in whole-organ generation via blastocyst complementation across wide evolutionary distances.

2. Cas9-AAV6 gene correction of beta-globin in autologous HSCs to improve sickle cell disease erythropoiesis

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A year ago, we reported a simple platform for the expansion of functional mouse HSCs ex vivo for more than 1 month under fully defined albumin-free condi-

tions. Taking advantage of this ex vivo expansion system, we performed CRISPR/Cas9-mediated beta-globin (*HBB*) gene correction of Sickle Cell Disease (SCD) patient-derived hematopoietic stem cells (HSCs) in rodents as a model of gene therapy. Although several Cas9-based *HBB*-correction approaches have been proposed, functional correction of *in vivo* erythropoiesis has not been investigated. We used a humanized globin-cluster SCD mouse model to study Cas9-AAV6-mediated *HBB*-correction in functional HSCs within the context of autologous transplantation. We discover that long-term multipotent HSCs can be gene corrected ex vivo and stable hemoglobin-A production can be achieved *in vivo* from *HBB*-corrected HSCs following autologous transplantation. We observed a direct correlation between increased *HBB*-corrected myeloid chimerism and normalized *in vivo* RBC features, but even low levels of chimerism resulted in robust hemoglobin-A levels. Moreover, this study offers a platform for gene editing of mouse HSCs for both basic and translational research.

Publications

1. Nishimura T, Suchy FP, Bhadury J, Igarashi KJ, Charlesworth CT, Nakauchi H. Generation of Functional Organs Using a Cell Competitive Niche in Intra-and Inter-species Rodent Chimeras. Cell Stem Cell, 28(1), 141-149.
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1. Innate and adaptive immune cells regulate Paneth cell granule formation and α-defensin secretion

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The gastrointestinal tract is constantly exposed to numerous foreign antigens. Intestinal epithelial cell layer acts as a first line of defense and is divided into villi and crypt regions. In the crypts, epithelial stem cells and Paneth cells are preferentially located. Paneth cells release granules containing a variety of antimicrobial peptides as a major part of the host innate immune system. α-defensin is most abundant and highly bactericidal peptide specifically produced by Paneth cells.

It has been known that crypts are surrounded by immune cells. Type3 innate lymphoid cells located beneath of crypts preferentially produce Interleukin 22 (IL-22) known as innate immune signaling. We found that IL-22 promotes the differentiation of Paneth cells with matured granules containing α-defensin. We further found that granule release of Paneth cells is regulated by acquired immune signaling via membrane trafficking system.

Our results indicate that the cell fate and function of Paneth cells are dually regulated by innate and adaptive immune cells for the production and secretion of α-defensin in gastrointestinal tract. α-defensin plays a crucial role for the creation and maintenance of intestinal homeostasis, thus we concluded that the mutual interaction of Paneth cells and immune cells provide healthy intestinal environment.

2. Cytochrome c released from intra-tissue opportunistic bacteria induce apoptosis of host cells.

Naoko Shibata[1,2], Jun Kunisawa[2-6], Masahiro Ando[6], Masato Hosokawa[7], Shumpei Horii[1,8], Koji Hosomi[3], Haruko Takeyama[1,6,7,8], and Hiroshi Kiyono[2,9,10,]

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Our intestinal tracts are continuously exposed to various antigens, including dietary food materials, commensal bacteria, and pathogenic bacteria. The mucosal immune systems developed for the elimination of pathogenic bacteria and tolerance to dietary antigens and symbiotic bacteria. On the other hand, the deteriorations of bacterial compositions cause gut-related immune diseases such as food allergies and inflammatory bowel diseases. Therefore, it is becoming more critical to understand the interactions between intestinal bacteria and the mucosal immune system in both steady- and symbiotically disrupted states. Peyer's patches (PPs) are one of the primary gut-associated lymphoid tissues in the small intestine.

We previously identified *Alcaligenes* as a symbiont within dendritic cells (DCs) in the Peyer's patches. *Alcaligenes* contributes to IgA production via the appropriate activation of DCs and the impaired symbiosis induces inflammatory responses; however, it remains to be investigated how the symbiotic relationship is maintained. Here, we show that *Alcaligenes* changed the morphology from rod- to filament-shape, which was induced via quorum-sensing-dependent manner. The changes associated with the increase and release of cytochrome c, an apoptosis-inducing factor against eukaryotes, and consistently induced the apoptosis of host DCs. These findings indicated that the functional changes of *Alcaligenes* associated with their morphology can be a regulator of symbiotic communication between *Alcaligenes* and DCs.

3. Development of Nanogel-based nasal vaccine against RSV infection.

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Respiratory syncytial virus (RSV) is a leading cause of lower respiratory infections in children under 5 years of age. High-risk children (e.g., those with congenital heart diseases) can develop severe, sometimes fatal, complications. In terms of vaccine development, the inadequate protection provided by previous reported vaccines and the risk of vaccine-enhanced diseases (VED; e.g., eosinophilic pneumoniae) have thus far prevented their use in a clinical setting. Despite these issues, the development of an effective and safe vaccine is expected from clinical needs, and is an important issue from a public health perspective.

We have developed a novel nasal vaccine against RSV, targeting an ectodomain of RSV and using a cationic cholesteryl-group-bearing pullulan (cCHP)-nanogel as a vaccine delivery system. Furthermore, we have previously revealed the protective effects of this vaccine against RSV in viral challenge tests, and demonstrated that VED was not induced by administration of this vaccine in mice. This year, we have analyzed the protective mechanisms of this nasal vaccine against RSV.

We have evaluated the neutralizing activity of antigen (Ag) -specific IgG in serum and Ag-specific IgA in nasal wash against RSV, and found that Ag-specific IgG had no direct neutralizing ability, but interestingly, Ag-specific IgA had direct neutralizing activity against RSV. Moreover, we found that Ag-specific IgG may eliminate RSV by antibody-dependent cellular cytotoxity (ADCC) by assessing the immune response and performing challenge tests in FcγKO mice. We are currently studying the method of constructing hybridoma to purify Ag-specific monoclonal IgG and synthesize multimeric IgA using gene conversion technology in order to elucidate the mechanisms of neutralizing ability of Ag-specific IgA. Additionally, we have adjusted the intranasal immunization protocol of this vaccine for neonatal mice and obtained preliminary data that antigen-specific immunity can be induced in neonatal mice as well as in adult mice.

These results provide promising evidence for the protective efficacy and safety of this novel nasal vaccine against RSV.

Corporate Sponsored Research Program

Project Division of Molecular and Developmental Biology

再生基礎医科学国際研究拠点寄付研究部門

Project Professor　Sumiko Watanabe, Ph.D.

特任教授　博士（医学）　渡　辺　すみ子

Our long-term goal is to understand the molecular mechanisms which coordinately regulate differentiation and maintenance of neural retina. Recently we are focusing on failure of maintenance of differentiated cells, that ultimately lead to neural degeneration. In addition, how immune cells, such as microglia, affect pathogenesis of central nervous system is important issue. For this purpose we use models ranging from iPS, mouse, monkey, to clinical samples.

The neural retina is a part of the central nervous system (CNS), and regeneration of the retina from retinal stem cells or other sources by transplantation is a critical issue from both clinical and neurobiological points of view. Although reports of successful regeneration of the CNS from neural stem cells (NSC) have appeared in the literature, such has not been the case for the vertebrate neural retina. Furthermore, the nature of retinal stem cells has not been clarified, making it difficult to attempt regeneration of the retina. Based on the techniques and knowledge that have been accumulated through work on of haematopoietic systems in our laboratory, we attempt to identify mammalian retinal stem cells and following developmental processes by revealing the expression pattern of cell surface proteins. We found that various CD antigens mark spatiotemporally distinct populations of retinal cells, and genes specifically expressed in such populations has been revealed by microarray analyses. Various signaling molecules, transcriptional factors, and epigenetic modification are under investigation for their roles on retinal development. Projects, which gave major findings during 2020 are as follows.

Mapping membrane lipids in the developing and adult mouse retina under physiological and pathological conditions using mass spectrometry

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Membrane phospholipids play pivotal roles in various cellular processes, and their levels are tightly regulated. In the retina, phospholipids had been scrutinized because of their distinct composition and requirement in visual transduction. However, how lipid composition changes during retinal development remains unclear. We aimed to identify phospholipid composition during mouse retinal development and degeneration by using liquid chromatography–mass spectrometry (LC-MS). We assessed the dynamic changes in the levels of two main glycerophospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), in the developing mouse retina under

physiological and pathological conditions. The total levels of PC and PE increased during retinal development, and individual lipid species exhibited distinct level changes. The amount of very-long-chain (VLC) PC and PE increased dramatically in the late stages of retinal development, which is consistent with previous reports that mature photoreceptor outer segments contain VLC. The mRNA levels of *Elovl2* and *Elovl4*, genes encoding enzymes essential for the synthesis of VLC polyunsaturated fatty acids, increased in developing photoreceptors, and the expression patterns were retinal cell type specific. Cell sorting based on CD73 expression followed by LC-MS revealed distinct changes in PC and PE levels in CD73-positive rod photoreceptors and CD73-negative retinal cells, suggesting cell type specific lipid metabolism and roles. Finally, using the $NaIO_3$- induced photoreceptor degeneration model, we identified photoreceptor-specific changes in PC and PE levels from 1 day after $NaIO_3$ administration. This stage, no apparent morphological impairment of the outer segment of photoreceptors was displayed, suggesting that changing of lipid composition precedes the degeneration. In conclusion, our findings provide insight into the dynamic changes in PC and PE levels in the developing and adult mouse retina under physiological and pathological conditions. Furthermore, we provide evidence that cell sorting followed by LC-MS is a promising approach for investigating the relevance of lipid homeostasis in the function of different retinal cell types.

RasV12 expression in microglia initiates retinal inflammation and induces photoreceptor degeneration

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Microglia is central nervous system specific tissue resident macrophage like cells. The role of activated retinal microglia in driving retinal degeneration has been implicated in a number of *in vivo* disease models. Here, we investigated the primary consequences of microglial activation by the specific expression of constitutively active Ras in microglia in a transgenic mouse model prior to the onset of any degenerative changes in the retina. For that purpose, the double transgenic lines *CAG-LSL-RasV12-IRES-EGFP; Cx3cr-1*^{CreER} (Cx3cr1-RasV12 mice) and *CAG-LSL-EGFP*; Cx-3cr1^{CreER} (control mice) were generated. The expression of RasV12 was induced in microglia by tamoxifen administration, and the retinas were examined by immunohistochemistry of frozen sections, RT-qPCR, and live imaging. We confirmed that RasV12 expression in retinal microglial cells promoted cell proliferation, cytokine expression, and phagocytosis. RasV12-expressing microglia migrated towards both the inner and outer layers of the retina. Examination of GFAP expression revealed activation of Müller glia was induced in the retina of C3cr1-RasV12 mice. We also observed loss of the photoreceptors in the outer nuclear layer (ONL) in close proximity to microglial cells. However, no significant neurodegeneration was detected in the inner nuclear layer (INL) or ganglion cell layer (GCL). The morphology of RasV12-expressing microglia in the GCL and INL retained more ramified features compared with the predominantly-amoeboid morphology found in outer retinal microglia. The expression of RasV12 is sufficient to activate microglia and led to photoreceptor degeneration, which is in contrast to that the neurons in inner side of the retina were not damaged by the RasV12 activated microglia. These results suggested that microenvironment cues may modulate the microglial phenotypic features and effects of microglial activation.

H3K27me3 demethylase UTX regulates the differentiation of a subset of bipolar cells in the mouse retina

Daisy Umutoni, Toshiro Iwagawa, Asano Tsuhako, Hiroaki Honda4 Makoto Aihara5, Sumiko Watanabe: 4 Field of Human Disease Models, Major in Advanced Life Sciences and Medicine, Institute of Laboratory Animals, Tokyo Women's Medical University. 5 Department of Ophthalmology, University of Tokyo

The molecular mechanisms that determine retinal cell fate and maturation have been extensively investigated in retina. Papers showing the roles of transcription factors in retinal development have received considerable attention. Moreover, there is increasing clarity regarding the contributions of epigenetic modifications (e.g., DNA methylation and histone modifications) to retinal development. Di- and tri-methylation of lysine 27 on histone 3 (H3K27me2/3) is a critical gene repression mechanism. Trimethylation of histone H3 at lysine 27 (H3K27me3) is a critical mediator of transcriptional gene repression, and *Jmjd3* and *Utx* are the demethylases specific to H3K27me3. We previously showed that downregulation of the H3K27 demethylase, Jumonji domain-containing protein 3 (JMJD3), resulted in a reduced number of protein kinase C (PKC)α-positive rod ON-bipolar cells. In this work, we focused on the role of another H3K27 demethylase, ubiquitously transcribed tetratricopeptide repeat X chromosome (UTX), in retinal development. UTX was expressed in the retinal progenitor cells of the embryonic mouse retina and was observed in the inner nuclear layer during late retinal development and in the mature retina. The short hairpin RNA-mediated knockdown of *Utx* in a mouse retinal explant led to a reduced number of PKCα-positive

rod ON-bipolar cells. However, other retinal subtypes were unaffected by this knockdown. Using a retina-specific knockout of *Utx* in mice, the in vivo effects of UTX downregulation were examined. Again, the number of PKCα-positive rod-ON bipolar cells was reduced, and no other apparent phenotypes, including retinal progenitor proliferation, apoptosis, or differentiation, were observed. Finally, we examined retina-specific *Utx* and *Jmjd3* double knockout mice and found that although the number of rod ON-bipolar cells was reduced, no additional effects from the loss of *Utx* and *Jmjd3* were observed. Taken together, our data show that UTX contributes to retinal differentiation in a lineage-specific manner.

Jmjd3 plays pivotal roles in the proper development of early-born retinal lineages: amacrine, horizontal, and retinal ganglion cells

Toshiro Iwagawa1, Hiroaki Honda4, and Sumiko Watanabe

Using an *in vitro* retinal explant culture system, we previously revealed the role of *Jmjd3* in the development of rod bipolar cells; however, the roles of *Jmjd3* in the development of early-born retinal cells are unknown due to limitations concerning the use of retinal explant culture systems. In this study, we investigated the roles of *Jmjd3* in the development of early-born retinal cells by using retina specific knokout mouse model by crossing a *Jmjd3*-floxed mouse with a *Dkk3*-Cre mouse. We examined retina-specific conditional *Jmjd3* knockout (Jmjd3-cKO) mice using immunohistochemistry and quantitative reverse transcription PCR and JMJD3 binding to a target locus by chromatin immunoprecipitation analysis. We observed reductions in amacrine cells (ACs) and horizontal cells (HCs), as well as lowered expression levels of several transcription factors involved in the development of ACs and HCs in the Jmjd3-cKO mouse retina. JMJD3 bound the promoter regions of these transcription factors. Notably, an elevated number of retinal ganglion cells (RGCs) was observed at embryonic stages, while RGCs were moderately reduced at later postnatal stages in the Jmjd3-cKO retina. We also observed reduced expression of *Eomes*, which is required for the maintenance of RGCs, as well as lower H3K27me3 level and lower JMJD3 binding in the promoter region of *Eomes* in RGC-enriched cells. The results indicated that *Jmjd3* has critical roles in the development of early-born retinal subtypes, and suggested biphasic roles of *Jmjd3* in RGC production and maintenance.

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Social Cooperation Research Program

Project Division of RNA Medical Science
RNA 医科学社会連携研究部門

Project Associate Professor　Masaki Takahashi, Ph.D.　特任准教授　博士（理学）　高　橋　理　貴

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 1014-15 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. RaptRanker: *in silico* RNA aptamer selection from HT-SELEX experiment based on local sequence and structure information.

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Aptamers are short single-stranded RNA/DNA molecules that bind to specific target molecules. Aptamers with high binding-affinity and target specificity are identified using an *in vitro* procedure called high throughput systematic evolution of ligands by exponential enrichment (HT-SELEX). However, the development of aptamer affinity reagents takes a considerable amount of time and is costly because HT-SE-

LEX produces a large dataset of candidate sequences, some of which have insufficient binding-affinity. Here, we present RNA aptamer Ranker (RaptRanker), a novel *in silico* method for identifying high binding-affinity aptamers from HT-SELEX data by scoring and ranking. RaptRanker analyzes HT-SELEX data by evaluating the nucleotide sequence and secondary structure simultaneously, and by ranking according to scores reflecting local structure and sequence frequencies. To evaluate the performance of RaptRanker, we performed two new HT-SELEX experiments, and evaluated binding affinities of a part of sequences that include aptamers with low binding-affinity. In both datasets, the performance of RaptRanker was superior to Frequency, Enrichment and MPBind. We also confirmed that the consideration of secondary structures is effective in HT-SELEX data analysis, and that RaptRanker successfully predicted the essential subsequence motifs in each identified sequence.

2. A G-quadruplex-forming RNA aptamer binds to the MTG8 TAFH domain and dissociates the leukemic AML1-MTG8 fusion protein from DNA

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MTG8 (RUNX1T1) is a fusion partner of AML1 (RUNX1) in the leukemic chromosome translocation t(8;21). The AML1-MTG8 fusion gene encodes a chimeric transcription factor. One of the highly conserved domains of MTG8 is TAFH which possesses homology with human TAF4 [TATA-box binding protein-associated factor]. To obtain specific inhibitors of the AML1-MTG8 fusion protein, we isolated RNA aptamers against the MTG8 TAFH domain using systematic evolution of ligands by exponential enrichment. All TAF aptamers contained guanine-rich sequences. Analyses of a TAF aptamer by NMR, CD, and mutagenesis revealed that it forms a parallel G-quadruplex structure in the presence of K^+. Furthermore, the aptamer could bind to the AML1-MTG8 fusion protein and dissociate the AML1-MTG8/DNA complex, suggesting that it can inhibit the dominant negative effects of AML1-MTG8 against normal AML1 function and serve as a potential therapeutic agent for leukemia.

3. Nucleic acid ligands act as a PAM and agonist depending on the intrinsic ligand binding state of P2RY2.

Masaki Takahashi, Ryo Amano, Michiru Ozawa[1], Anna Martinez[1], Kazumasa Akita[1], Yoshikazu Nakamura[1]: [1]RIBOMIC Inc., Minato-ku, Tokyo

G-protein coupled receptors (GPCRs) play diverse roles in physiological processes, and hence the ligands to modulate GPCRs have served as worthwhile molecules in biological and pharmacological approaches. However, the exploration of novel ligands for GPCR still remains an arduous challenge. In this study, we report a method for the discovery of nucleic acid ligands against GPCRs by advanced RNA aptamer screening technology that employs a virus-like particle (VLP) exposing GPCR of interest and integrates high-throughput sequencing and bioinformatics. An array of biochemical analyses coupled with cell-based assay revealed that one of the aptamers raised against purinergic receptor P2Y2 (P2RY2), a GPCR, exhibits an activation potency to unliganded receptor and prohibits a further receptor activation by endogenous ligand, behaving like a partial agonist. However, the aptamer enhances the activity of intrinsic ligand-binding P2RY2, thereby acting as a positive allosteric modulator (PAM) to liganded receptor. Our findings demonstrate that the nucleic acid aptamer conditionally exerts PAM and agonist effects on GPCRs depending on their intrinsic ligand binding state. These results indicate the validity of our VLP-based aptamer screening targeting GPCR and re-emphasize the high potential of nucleic acid ligands for exploring the GPCR activation mechanism and therapeutic applications.

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1 Ishida R, Adachi T, Yokota A, Yoshihara H, Aoki K, Nakamura Y, Hamada M.: RaptRanker: *in silico* RNA aptamer selection from HT-SELEX experiment based on local sequence and structure information, Nucleic Acids Res., 48(14):e82 (2020)

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Social Cooperation Research Program

Project Division of International Advanced Medical Research

国際先端医療社会連携研究部門

Project Associate Professor　Koichiro Yuji, M.D., Ph.D.　特任准教授　博士（医学）　湯　地　晃一郎

The mission of the Project Division is to apply changes in advanced medical research at the Institute of Medical Science at the University of Tokyo (IMSUT). Our activities include field research in which innovative medicine will be implemented; cross-disciplinary education of physicians, researchers, and professionals; collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development; and establishing projections of the future healthcare system of Japan, which will be the first fully fledged aged society.

Implementing advanced medical research at IMSUT

Yuji, K.

The Project Division was established in November 2014. Our mission is to contribute to the progress of advanced medical research at IMSUT; to perform field research in which innovative medicine will be implemented; and to further the cross-disciplinary education of physicians, researchers, and professionals. Our future plans include collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development.

Projections on the future healthcare system in Japan, the first fully fledged aged society

Yuji, K.

Japan is rapidly becoming a fully fledged aged society, and the increasing dependence of the elderly population is a significant concern. We have simulated both the supply and demand features of Japan's future healthcare system.

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Corporate Sponsored Research Program

Project Division of Fundamental Study on Cutting Edge of Genome Medicine

先端ゲノム医療の基盤研究寄付研究部門

Professor	Arinobu Tojo, M.D., D.M.Sc.	教　授	医学博士	東　條　有　伸
Project Associate Professor	Hiroshi Yasui, M.D., D.M.Sc.	特任准教授	博士（医学）	安　井　　寛

Our major goal is to realize advanced genomic medicine. Major advancements in genome analysis have recently been reported from researchers around the globe, along with improvements in next-generation sequencing, leading to an era where genomic information can be collected and analyzed at low cost and in a short period of time. Thus, it is necessary to establish a framework for developing genome analysis while expanding our understanding of general society, academia, and medical associations, etc., in order to identify different diseases, such as hereditary diseases or non-genetic diseases.
In our department, comprehensive basic research on advanced genome medicine has been realized through our multidisciplinary collaborations among scientific experts; the Ethical, Legal, and Social Implications (ELSI) program; specimen preservation; electronic medical records access; and personal information protection.

1. Japan-US Comparative study for the promotion of the cancer genomic medicine in Japan

Hiroshi Yasui, Mikiko Suzuki, Arinobu Tojo

Regarding the spread of cancer genomic medicine Japan is behind not only Western countries but also China and Korea. We study to compare the current situation and the future prospects of cancer genomic medicine in Japan and the United States in order to contribute to design a policy to promote dissemination and uniformization of cancer genomic medicine for cancer patients in Japan.

2. Construction of infrastructure for research on advanced genome medicine

Hiroshi Yasui, Mikiko Suzuki, Megumi Isobe, Arinobu Tojo

In order to establish a framework for developing genome analysis while expanding our understanding of diseases, including hereditary and nongenetic diseases, we are using comprehensive approaches to advanced genome medicine. These approaches include addressing various issues, such as multidisciplinary collaborations among scientific experts; the Ethical, Legal, and Social Implications (ELSI) program; management of specimen preservation, clinical information, and personal information protection for genomic medicine as well as biobanking. Our mission also includes enhancement of social acceptance for genomic medicine.

3. Program for supporting biospecimen analysis for the diagnosis and treatment of hematological malignancies

Hiroshi Yasui, Arinobu Tojo, Kaoru Uchimaru[1], Toshiki Watanabe[2]
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[2]IMSUT hospital, The Institute of Medical Science, The University of Tokyo

To support cancer scientists in promoting translational research and genome medicine, we have established a platform for supporting cohort studies and biospecimen analysis. Under this program, we are collecting and managing clinical materials, including tumor cells, serum, and peripheral blood mononuclear cells from patients at high risk of hematologic malignancies as well as patients with blood cancer. We provide support for obtaining and/or analyzing biomaterials, as requested by researchers, and contribute to their clinical studies and publications.

4. Development of novel immunodiagnostics for graft-versus-host disease

Hiroshi Yasui, Asako Kobayashi, Reika Li, Takahiro Asatsuma, Aobulikasimu Aikebaier, Mikiko Suzuki, Arinobu Tojo

Novel immunodiagnostics to analyze immune function is important for the evaluate the activity of autoimmune diseases as well as development of cancer immunotherapy. We study to develop novel immunodiagnostics to evaluate activities of immune cells in patients with allogenic hematopoietic stem cell transplantation to diagnose severity of graft-versus-host disease.

It will be also expected to contribute the development of the novel cancer immunotherapy in hematologic malignancies.

5. Investigator-initiated clinical trials under an Investigational New Drug application for the development of novel cancer therapeutics and biomarkers

Hiroshi Yasui, Fumitaka Nagamura[1], Giichiro Tsurita[2], Kohzoh Imai[3], Arinobu Tojo:
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[2]Department of Surgery, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo
[3]Office of Support for Platforms for Advanced Technologies and Research Resources, The Institute of Medical Science, The University of Tokyo

Genome medicine and genome research, including pharmacogenomics and pharmacogenetics, are important for developing novel therapeutic agents for cancer and incurable diseases and identifying biomarkers. Our research aims to develop efficient approaches for conducting investigator-initiated clinical trials under Investigational New Drug (IND) applications to promote translational research and discover biomarkers for prediction of the safety and efficacy of novel therapeutics through omics analyses, including genomics. We were conducting, supporting, summarizing or preparing three investigator-initiated clinical trials under INDs applications for the development of academic-oriented innovative anticancer drug especially novel cancer immunotherapy.

6. Support and management of translational research

Hiroshi Yasui, Arinobu Tojo

To promote translational research and genome medicine, we participate in the "Translational Research Network Program, Japanese Translational Research and Clinical Trials Core Centers" supported by the Japan Agency for Medical Research and Development, as members of the Translational Research Advancement Center of the University of Tokyo. The aim of the program is to promote translational research and investigator-led clinical trials aiming for practical applications of basic studies in academia, managing the assessment of scientific seeds and intellectual property rights, and therefore promoting the development of advanced medical arts, including genome medicine.

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Social Cooperation Research Program

Project Division of Advanced Biopharmaceutical Science

先進的バイオ医薬品学社会連携研究部門

Professor	Hirotoshi Tanaka, Ph.D.	教　授	医学博士	田　中　廣　壽
Professor	Kouhei Tsumoto, Ph.D.	教　授	博士(工学)	津　本　浩　平
Project Associate Professor	Satoru Nagatoishi, Ph.D.	特任准教授	博士(生命科学)	長門石　　曉

Various types of antibodies have been approved for therapeutic use and currently examined in clinical development. Therefore, developments of technology for the discovery and optimization of high-potency antibodies have been improved and have greatly increased to find the specific and stable antibody with desired biological properties. Biophysical analyses of a therapeutic antibody, particularly those of protein interaction and stability, are recognized as one of the critical procedures in the development of biopharmaceuticals, which would be assessed as an essential step to develop next-generation antibodies. The development of analytical methods with quantitative and high-sensitive detection of antigen interaction, protein stability, and biological function of antibody, therefore, has been intriguing for the pharmaceutic companies. In this division, we study biophysical analyses of various antibodies to propose a new strategy for the development of the next-generation antibody.

1. Structural Basis for the Binding Mechanism of Human Serum Albumin Complexed with Cyclic Peptide Dalbavancin

Ito S, Senoo A, Nagatoishi S, Ohue M, Yamamoto M, Tsumoto K, Wakui N.

Here, we present the crystal structure of HSA complexed with dalbavancin, a clinically used cyclic peptide. Small-angle X-ray scattering and isothermal titration calorimetry experiments showed that the HSA-dalbavancin complex exists in a monomeric state; dalbavancin is only bound to the subdomain IA of HSA in solution. Structural analysis and MD simulation revealed that the swing of Phe70 and movement of the helix near dalbavancin were necessary for binding. The flip of Leu251 promoted the formation of the binding pocket with an induced-fit mechanism; moreover, the movement of the loop region including Glu60 increased the number of noncovalent interactions with HSA. These findings may support the development of new cyclic peptides for clinical use, particularly the elucidation of their binding mechanism to HSA.

2. Highly sensitive HPLC analysis and biophysical characterization of N-glycans of IgG-Fc domain in comparison between CHO and 293 cells using FcγRIIIa ligand

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

Quality control of monoclonal antibodies is challenging due in part to the diversity of post-translational modifications present. The regulation of the N-glycans of IgG-Fc domain is one of the key factors to maintain the safety and efficacy of antibody drugs.

The FcγRIIIa affinity column is an attractive tool for the precise analysis of the N-glycans in IgG-Fc domain. We used the mutant FcγRIIIa, which is produced in Escherichia coli and is therefore not glycosylated, as an affinity reagent to analyze the N-glycans of monoclonal antibodies expressed in Expi293 and ExpiCHO cells. The monoclonal antibodies expressed in these cells showed very different chromatograms, because of differences in terminal galactose residues on the IgG-Fc domains. We also carried out kinetic and thermodynamic analyses to understand the interaction between monoclonal antibodies and the mutant FcγRIIIa. Expi293 cell-derived monoclonal antibodies had higher affinity for the mutant FcγRIIIa than those derived from ExpiCHO cells, due to slower off rates and lower binding entropy loss. Collectively, our results suggest that the FcγRIIIa column can be used to analyze the glycosylation of antibodies rapidly and specifically.

3. Polymeric Nanocarriers with Controlled Chain Flexibility Boost mRNA Delivery In Vivo through Enhanced Structural Fastening

Miyazaki T, Uchida S, Nagatoishi S, Koji K, Hong T, Fukushima S, Tsumoto K, Ishihara K, Kataoka K, Cabral H.

Messenger RNA (mRNA) shows high therapeutic potential, though effective delivery systems are still needed for boosting its application. Nanocarriers loading mRNA via polyion complexation with block catiomers into core-shell micellar structures are promising systems for enhancing mRNA delivery. Engineering the interaction between mRNA and catiomers through polymer design can promote the development of mRNA-loaded micelles (mRNA/m) with increased delivery efficiency. Particularly, the polycation chain rigidity may critically affect the mRNA-catiomer interplay to yield potent poly(glycidylbutylamine) (PEG-PGBA) and PEG-poly(L-lysine) (PEG-PLL) is studied. PEG-PGBA allows more than 50-fold stronger binding to mRNA than the relatively more rigid PEG-PLL, resulting in mRNA/m with enhanced protection against enzymatic attack and polyanions. mRNA/m from PEG-PGBA significantly enhances mRNA in vivo bioavailability and increased protein translation, indicating the importance of controlling polycation flexibility for forming stable polyion complexes with mRNA toward improved deliverynanocarriers, yet its effect remains unknown. Herein, the influence of polycation stiffness on the performance of mRNA/m by developing block complementary catiomers having polycation segments with different flexibility, that is, poly(ethylene glycol)-.

4. Discovery of chemical probes that suppress Wnt/β-catenin signaling through high-throughput screening

Yamaguchi K, Nagatoishi S, Tsumoto K, Furukawa Y.

Aberrant activation of the Wnt/β-catenin signaling pathway has been observed in a wide range of human tumors. Deregulation of the pathway is closely linked to various aspects of human carcinogenesis such as cell viability, regulation of cell cycle, epithelial-mesenchymal transition, and maintenance of stemness. In addition, recent studies have disclosed the involvement of Wnt signaling in immune evasion of tumor cells. The accumulation of β-catenin in the nucleus is a common feature of cancer cells carrying defects in the pathway, which leads to the continuous activation of T-cell factor (TCF)/LEF transcription factors. Consequently, a genetic program is switched on, leading to the uncontrolled growth, prolonged survival, and acquisition of mesenchymal phenotype. As β-catenin/TCF serves as a signaling hub for the pathway, β-catenin/TCF-dependent transcriptional activity is a relevant readout of the pathway. To date, a wide variety of synthetic TCF/LEF reporters has been developed, and high-throughput screening (HTS) using these reporters has made significant contributions to the discovery of Wnt inhibitors. Indeed, HTS led to the identification of chemical probes targeting porcupine, a membrane bound O-acyltransferase, and CREB-binding protein, a transcriptional coactivator. This review focuses on various screening strategies for the discovery of Wnt inhibitors and their mode of action to help the creation of new concepts for assay/screening methods.

5. Methodology for Further Thermostabilization of an Intrinsically Thermostable Membrane Protein Using Amino Acid Mutations with Its Original Function Being Retained

Yasuda S, Akiyama T, Nemoto S, Hayashi T, Ueta T, Kojima K, Tsukamoto T, Nagatoishi S, Tsumoto K, Sudo Y, Kinoshita M, Murata T.

We develop a new methodology best suited to the identification of thermostabilizing mutations for an intrinsically stable membrane protein. The recently discovered thermophilic rhodopsin, whose apparent midpoint temperature of thermal denaturation Tm is measured to be ~91.8 °C, is chosen as a paradigmatic target. In the methodology, we first regard the residues whose side chains are missing in the crystal structure of the wild type (WT) as the "residues with disordered side chains," which make no significant contributions to the stability, unlike the other essential residues. We then undertake mutating each of the residues with disordered side chains to another resi-

due except Ala and Pro, and the resultant mutant structure is constructed by modifying only the local structure around the mutated residue. This construction is based on the postulation that the structure formed by the other essential residues, which is nearly optimized in such a highly stable protein, should not be modified. The stability changes arising from the mutations are then evaluated using our physics-based free-energy function (FEF). We choose the mutations for which the FEF is much lower than for the WT and test them by experiments. We successfully find three mutants that are significantly more stable than the WT. A double mutant whose Tm reaches ~100 °C is also discovered.

6. Per-Residue Program of Multiple Backbone Dihedral Angles of β-Peptoids viaBackbone Substitutions

Morimoto J, Kim J, Kuroda D, Nagatoishi S, Tsumoto K, Sando S.

Unique folded structures of natural and synthetic oligomers are the most fundamental basis for their unique functions. N-Substituted β-peptides, or β-peptoids, are synthetic oligomers with great potential to fold into diverse three-dimensional structures because of the existence of four rotatable bonds in a monomer with highly modular synthetic accessibility. However, the existence of the four rotatable bonds poses a challenge for conformational control of β-peptoids. Here, we report a strategy for per-residue programming of two dihedral angles of β-peptoids, which is useful for restricting the conformational space of the oligomers. The oligomer was found to form a unique loop conformation that is stabilized by the backbone rotational restrictions. Circular dichroism and NMR spectroscopic analyses and X-ray crystallographic analysis of the oligomer are presented. The strategy would significantly facilitate the discovery of many more unique folded structures of β-peptoids.

7. Technical Capabilities and Limitations of Optical Spectroscopy and Calorimetry Using Water-Miscible Solvents: The Case of Dimethyl Sulfoxide, Acetonitrile, and 1,4-Dioxane.

Hirano A, Nagatoishi S, Wada M, Tsumoto K, Maluf KN, Arakawa T.

In drug development, water-miscible solvents are commonly used to dissolve drug substances. Typical routine procedures in drug development include dilution of the stock drug solution into an aqueous solution containing target macromolecules for drug binding assays. However, water-miscible solvents impose some technical limitations on the assays on account of their light absorption and heat capacity. Here, we examined the effects of the dilution of 3 water-miscible solvents, that is, dimethyl sulfoxide, acetonitrile, and 1,4-dioxane, on the baseline stability and signal/noise ratio in circular dichroism spectroscopy, isothermal titration calorimetry, and differential scanning calorimetry. Dimethyl sulfoxide and 1,4-dioxane affect the signal/noise ratio of circular dichroism spectra at typically used concentrations due to their light absorbance. The water-miscible solvents generate interfering signals in the isothermal titration calorimetry due to their mixing heat. They show negative or positive slope in the differential scanning calorimetry. Such interfering effects of the solvents are reduced by appropriate dilution according to the analytical techniques. Because the water-miscible solvents have solubilization capacity for alkyl chain moieties and aromatic moieties of chemicals, drug substances containing these moieties can be dissolved into the solvents and then subjected to the analyses to examine their interactions with target proteins after appropriate dilution of the drug solutions.

<Group III>

Publications

1. Ito, S., Senoo, A., Nagatoishi, S., Ohue, M., Yamamoto, M., Tsumoto, K., and Wakui, N. Structural Basis for the Binding Mechanism of Human Serum Albumin Complexed with Cyclic Peptide Dalbavancin. J. Med. Chem. 63:14045-14053, 2020
2. Kosuge, H., Nagatoishi, S., Kiyoshi, M., Ishii-Watabe, A., Tanaka, T., Terao, Y., Oe, S., Ide, T., and Tsumoto, K. Highly sensitive HPLC analysis and biophysical characterization of N-glycans of IgG-Fc domain in comparison between CHO and 293 cells using FcγRIIIa ligand. Biotechnol. Prog. 36:e3016, 2020.
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7. Hirano, A., Nagatoishi, S., Wada, M., Tsumoto, K., Maluf, K.N., and Arakawa, T. Technical Capabilities and Limitations of Optical Spectroscopy and Calorimetry Using Water-Miscible Solvents: The Case of Dimethyl Sulfoxide, Acetonitrile, and 1,4-Dioxane. J. Pharm. Sci. 109:524-531, 2020.

Social Cooperation Research Program

Project Division of Cancer Biomolecular Therapy
がん生体分子治療社会連携研究部門

Project Professor	Hideaki Tahara, M.D., Ph.D.	特任教授　医学博士	田　原　秀　晃
Project Associate Professor	Hiroaki Uchida, M.D., Ph.D.	特任准教授　博士（医学）	内　田　宏　昭

Our division has been conducting basic research projects for development of innovative cancer therapy using immunologic and gene therapy approaches. The reagents, modalities, and concepts developed in this division have been clinically applied as translational research projects. We believe that bidirectional information exchange between the bench and the bedside would be one of the most important requirements for the successful development of novel and effective therapies.

I. Development of cancer immunotherapy using the blockade of MFG-E8

Mika Uematsu-Hamada, Yu Mizote[1], Miho Kudo, Hiroaki Uchida, Hideaki Tahara ([1]Department of Cancer Drug Discovery and Development Osaka International Cancer Institute)

The secreted protein, milk fat globule-EGF factor 8 (MFG-E8), stimulates disease progression through coordinated αvβ3 integrin signaling in tumor and host cells. MFG-E8 enhances tumor cell survival, invasion, and angiogenesis, and contributes to local immune suppression.

We have shown that systemic MFG-E8 blockade cooperates with cytotoxic chemotherapy, molecularly targeted therapy, and radiation therapy to induce destruction of various types of established mouse tumors. The combination treatments evoke extensive tumor cell apoptosis that is coupled to efficient dendritic cell cross-presentation of dying tumor cells. Our previous findings suggest that systemic MFG-E8 blockade might intensify the antitumor activities of existing therapeutic regimens through coordinated cell-autonomous and immune-mediated mechanisms also in human. In order to further investigate these findings in mouse tumor systems, we have developed multiple means to abrogate the functions of MFG-E8 antibodies specific to the mouse MFG-E8. Our recent projects include the investigation on the significance of tumor-derived and non-tumor derived MFG-E8, which has been implicated in our histological examination on human samples and MFG-E8 gene knockout mice. Furthermore, we are now seeking the opportunities of developing this agent for clinical application.

II. Development of fully retargeted herpes simplex virus (HSV) vectors for oncolytic virotherapy

Hiroaki Uchida, Hitomi Ikeda, Tomoko Shibata, Takuma Suzuki, Fumihiro Nagata, Yasuhiko Sasaki, Naoki Kabasawa, Rintaro Hayase, Naoya Nagata, Natsuki Matsumoto, Yukinari Kato[2], Hideaki Tahara ([2]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine)

Herpes simplex virus (HSV) vectors are promising agents for oncolytic virotherapy. Uchida established a fully retargeted HSV platform that mediates virus entry exclusively via tumor-associated antigens in the lab of Prof. Joseph Glorioso at the University of Pittsburgh. Entry of HSV is initiated by the binding of glycoprotein D (gD) to one of its receptors, herpesvirus entry mediator (HVEM) or nectin-1. This interaction results in a conformational change in gD, triggering sequential activation of gH and gB to execute fusion

between the viral envelope and cell membranes. We inserted single-chain antibodies (scFv) against a number of different cell surface molecules such as epidermal growth factor receptor (EGFR), carcinoembryonic antigen (CEA), and epithelial cell adhesion molecule (EpCAM), into the retargeted HSV platform that encodes a gD ablated for binding to natural receptors and a gB containing entry-enhancing mutations we previously identified. As a result, we observed specific virus entry into cells expressing the cognate target antigen for each of the retargeted constructs. Our results indicate the adaptability of our system to different targeting ligands, leading to a new generation of broadly applicable and effective oncolytic HSV vectors. Furthermore, we introduced syncytial mutations into the gB and/or gK genes of gD-retargeted HSVs and found that gD retargeting does not abolish the hyperfusogenic activity of syncytial mutations and that these mutations do not eliminate the dependence of HSV entry and spread on a specific gD-receptor interaction. These observations suggest that syncytial mutations may be valuable for increasing the tumor-specific spreading of retargeted oncolytic HSV vectors. We have found that syncytium formation in tumors is associated with more potent antitumor effects *in vivo*. We have also found that our retargeted oncolytic HSV vectors can exert robust antitumor effects when administered not only intratumorally but also intravenously. Additionally, we are developing novel oncolytic vectors that are retargeted to tumor-associated antigens that have been shown to be expressed specifically on cancer cells.

III. Establishment of highly functional monoclonal antibodies through novel screening methods for targeted cancer therapy

Hiroaki Uchida, Hitomi Ikeda, Tomoko Shibata, Nanami Hayashi, Miki Yamaguchi[3], Hideaki Tahara ([3]Department of Molecular Medicine, Research Institute for Frontier Medicine, Sapporo Medical University School of Medicine)

Monoclonal antibodies (mAbs) have become an established therapeutic modality in clinical oncology. In order to identify cell-surface molecules that may be useful for targeting various types of cancers, our group established a unique screening approach that employs an adenoviral vector harboring fiber proteins engineered to bind antibodies, Adv-FZ33. This approach led to the successful identification of an array of potential target molecules for cancer treatment. Immunotoxins (antibody-drug conjugates; ADC) are a promising class of cancer therapeutics composed of a cytotoxic agent linked covalently to a cancer-targeted antibody. To systematically hunt for cell-surface molecules that may be efficiently targeted by immunotoxins, our group created another method for screening highly functional cancer-targeted mAbs and cognate antigens. The receptor-binding domain of the Diphtheria toxin (DT) was replaced with the antibody-binding domain (3C) derived from the Streptococcal protein G. The resultant mutated toxin protein (DT-3C) was used for selection of mAbs for specific cell killing activity as components of immunotoxins. Our novel screening system is advantageous in that the selected antibodies bind to intact cancer cells and get internalized efficiently, which has been critically required for therapeutic applications but elusive thus far. Furthermore, we have successfully taken advantage of some of these in-house monoclonal antibodies for development of novel fully retargeted HSV vectors. Additionally, we have created an HSV-based probe for screening of Abs that could mediate HSV entry by recognition of unknown receptors. We have found that one of the Abs selected by this screening method is capable of mediating HSV entry when incorporated into gD as an scFv. Interestingly, the antigen recognized by the Ab has been found to be a molecule that is known as a growth factor expressed and shed from cancer cells. This was unexpected because this molecule has been commonly investigated as a soluble "ligand" that acts as a growth factor, and thus not as a membrane-bound "receptor". Thus, we expect that this novel Ab-screening system may lead to a new generation of RR-oHSV vectors.

Publications

1. Kiho, Miyazato., Hideaki, Tahara., and Yoshihiro, Hayakawa. Anti-metastatic effects of thalidomide by inducing the functional maturation of peripheral NK cells. Cancer Sci. 111:2770–2778, 2020.

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

Professor	Yoshinori Murakami, M.D., Ph.D.	教　授	医学博士	村　上　善　則
Project Professor	Takayuki Morisaki, M.D., Ph.D.	特任教授	医学博士	森　崎　隆　幸

Most human diseases, including cancer and common diseases, develop and progress by the combinations and interactions of genetic background, acquired environmental exposures, life-style factors and aging. Therefore, in order to promote the healthy life of citizens, it is a prerequisite to identify health risks of individuals both at the time of birth and later in life and to provide them with appropriate approaches to disease prevention when necessary. For this purpose, the Project Division of Genomic Medicine and Disease Prevention was started on July 1, 2019 in cooperation with Nippon Telegram and Telephone Cooperation (NTT). The goal of our project is to develop personalized and precision prevention of diseases by integrating genomic information, health records and life-style data into a new predictive program of disease prevention for the healthy life of individuals.

1.Towards the development of personalized and precision prevention of diseases on the basis of genomic information.

Atsuko Hiraishi[1], Takayuki Morisaki[1], Masaru Koido[1], Momoko Horikoshi[2], Yoichiro Kamatani[3], Toru Suzuki[4], Yoshinori Murakami[1]; [1]Project Division of Genomic Medicine and Disease Prevention, The Institute of Medical Science, the University of Tokyo. [2]Laboratory for Endocrinology, Metabolism and Kidney Diseases, RIKEN Centre for Integrative Medical Sciences. [3]Laboratory of Complex Trait Genomics, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, the University of Tokyo, Tokyo, [4]Cardiovascular Research Center, Glenfield Hospital, University of Leicester, England.

Project Division of Genetic Medicine and Disease Prevention was established to obtain scientific evidence to develop a new predictive program for disease prevention for healthy lives of individuals by integrating genomic information into the classic health-related information, such as individual health records, life-style data and age.

For this purpose, we designed and launched the "GenoVision" project in collaboration with NTT Life Science, Corp. In this project, the employees of NTT group who got periodic physical examinations and provided an informed consent are recruited to a comprehensive survey program of individual SNPs. The purposes of this program are as follows. 1) To return the results of the selected SNPs to participants, which are directly involved in the activities of important biological enzymes for maintaining the healthy lives of individuals. 2) To obtain basic information for the research on polygenic risk scores for establishing a next-generation approach to promoting healthy life based on the genomic information of individuals.

Thus, numerous relevant publications were searched comprehensively and the candidate SNPs were selected to meet the following three criteria. 1)

The SNPs are directly involved in the enzymatic functions or expression. 2) The enzymatic activities are directly involved in pathological or unhealthy phenotypes. 3) The enzymatic dysfunction and the resultant unhealthy phenotype are shown to be mitigated by behavioral changes. According to these criteria, SNPs at the *MTHFR* gene affecting the activity of methylenetetrahydrofolate reductase for folic acid production and SNPs at the *ADH1B* and the *ALDH2* genes affecting the activity of alcohol dehydrogenase and aldehyde dehydrogenase, respectively, for alcohol metabolism were selected as the initial targets of this project. Recruitment of participants started in June 2020 and about 13,000 individuals are going to be recruited by the end of March, 2021. The individual SNPs are being analyzed and a portion of the results have been obtained.

The utilization of the clinically significant genomic information in possible lifestyle improvement for individuals and its validity from the viewpoint of ethical, legal and social issues (ELSI) are being investigated in collaboration with medical doctors in several hospitals, including Center for Disease Prevention, NTT Medical Center, Tokyo. The integration of genomic information of these SNPs into health records and re-evaluation of disease risks of individuals are also being examined.

Furthermore, a novel approach to estimate disease risks of individuals based on a large number of SNPs information through poly genic risk score (PRS) model is being investigated (Ref. Sakaue S, Kamatani Y, Murakami Y, Okada Y *et al*., *Nature Medicine*, 16:542-548, 2020). The power and potentials as well as the limitation of PRS analysis in assessment of disease risks and disease prevention will be elucidated.

Dean's Office

Project Coordination Office
プロジェクトコーディネーター室

Professor Makoto Nakanishi, M.D., Ph.D.

教 授 医学博士 中 西 真

Our major missions are to coordinate institutional projects, enhance the mutual cooperation, alliance among teaching and research staff, administration staff, and technical staff, in order to execute the activities in our institute effectively. For these purposes, we carry out several tasks such as planning for new institutional research programs and symposiums, fundraising, supporting international students and researchers, outreach activities, providing academic advice to administration staff, and other projects directed by the dean.

1. Support for the management of institutional projects

Kiyomi Nakagawa, Yoko Udagawa

We served as a secretariat of institutional projects implemented by the Institute of Medical Science, the University of Tokyo (IMSUT) and supported their management. The projects supported are as follows:
- "Studies to Control Emerging, Re-emerging and Imported Infectious Diseases to Be Conducted in International Collaboration Sites in China" supported by Japan program of Infectious Diseases Research and Infrastructure from Japan Agency for Medical Research and Development (AMED)
- "World-leading Innovative Graduate Study Program for Life Science and Technology (WINGS-LST)" supported by the Doctoral Program for World-leading Innovative & Smart Education from Japan Society for the Promotion of Science (JSPS)

2. International Joint Usage/Research Center Program of MEXT

Junko Tsuzuku

IMSUT has been authorized by MEXT as Joint Usage/ Research Center in 2009 and began its activity in 2010. The center's main activity is to implement joint research projects that diverse universities and research organizations can apply to join and organize academic gathering such as international symposia, and meetings for young researchers as well as to publish activity reports on our website. The Project Coordination Office executed these activities, edited documents pertaining to various investigations, and submitted evaluation reports requested by MEXT in collaboration with the Research Promotion Team, Research Support Division, Administration Office. In November 2018, IMSUT has been reauthorized as International Joint Usage/Research Center by MEXT. In this capacity, we will continue our utmost efforts to expand this program from the domestic to the international level.

3. Data acquisition about research and educational activities of IMSUT

Kiyomi Nakagawa, Ayako Miyake

We collected and stocked data using an original format to construct a data system available any time for evaluation, submission of various reports, public relation activities, and basic data for application of external funding.

4. Others

Kiyomi Nakagawa, Ayako Miyake, Yoko Udagawa

a. Educational activities:
- Support for the call for application and selection of the Outstanding Student Publication Award of IMSUT

b. International activities:
- Support for conclusion and renewal of MOUs
- Support for delegation and management of international exchange events, "East Asia Joint Symposium" and "International Symposium of the Institute Network for Biomedical Sciences"
- Translation of documents and manuscripts
- Support for foreign researchers in English
- Support for reception of overseas visitors
- Support for management of the University of Tokyo New York Office, Inc. and its event organization
- Planning and running of get-together party for international students and foreign researchers

c. Public relations:
- Support for information update of IMSUT website
- Edition of brochures of IMSUT (Japanese and English version) and support for edition of the Annual Report

d. Support for Evaluation work:
- National university corporation evaluation
- Self-review and self-evaluation of IMSUT
- External review of IMSUT

Dean's Office

Research Platform Office
学術研究基盤支援室

Chair and Senior Professor	Jun-ichiro Inoue, Ph.D.	特命教授・室長	薬学博士　井　上　純一郎
Vice chair and Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教授・副室長	博士(医学)　武　川　睦　寛

"Platforms for Advanced Technologies and Research Resources" (platform.umin.jp/) was launched in fiscal year (FY) 2016 under the new framework of the Grant-in-Aid for Scientific Research on Innovative Areas by the Ministry of Education, Culture, Sports, Science and Technology (MEXT). It consists of six platforms, of which four platforms are supporting researches in life science. They are the platforms that have been developed from and strengthened the previous program "Support Programs for Three Fields in Life Sciences (Cancer, Genome and Brain Sciences)" conducted between FY 2010 and 2015. "Platforms for Advanced Technologies and Research Resources" aims to establish the academic research support platforms to efficiently support various needs of the researchers in grants-in-aid. It also aims to work in close cooperation with the relevant core bodies such as Inter-University Research Institutes and Joint Usage/ Research Centers. This office mainly plays the role of the representative secretariat of the "Committee on Promoting Collaboration in Life Sciences" that is an academic collaborative foundation and cooperates with the four platforms mentioned above. The objective is to contribute the further development of the academic research in Japan through providing the cutting-edge technologies and biological resources to the individual researchers on life science KAKENHI (Grants-in-Aid for Scientific Research). We also aim to promote cooperation among researchers across support functions and cross-disciplinary, as well as human resource development. To achieve the goal, the General Management Group was organized to facilitate a close cooperation between four platforms comprising 54 universities and 23 research institutions nationwide which provide 81 support functions. This office was established in this Institute as Dean's Office in 2016 in order to strengthen the flexible management. Further, we hold several Management Board Meetings in which 17 members participated: four platform representatives and 13 board members, to construct a cooperative system to facilitate a cross-over support functions and to provide technical support with the universities and research institutions nationwide.

Management of "Committee on Promoting Collaboration in Life Sciences" and the two platforms: Advanced Animal Model Support (AdAMS) and Cohort Study and Biospecimen Analysis (CoBiA):

Yuri Takei, M.A., Jun Saito, Eriko Shibata, Tomoko Fujita, Hiroshi Abe, Mutsuhiro Takekawa and Jun-ichiro Inoue

The following activities have been performed under the management of this office in 2020.

1. Planning and organization of the budgetary allocation.
2. One-stop service for applicants through the home page.
3. Organization of the events for developing young scientists and interdisciplinary researches.
4. Holding public symposiums on the relation of life science and society.
5. Holding the explanatory meeting for possible applicants.
6. Conducting public relations activities such as posting the committee's banner on home page of various scientific meetings.
7. Creating a video to promote our activities and upload it on YouTube.
8. Facilitating cooperative networks between our platforms and other domestic or international groups that support life science researches.

Dean's Office

International Affairs Office
国際学術連携室

Professor Makoto Nakanishi, M.D., Ph.D.

教 授 医学博士 中 西 真

International Affairs Office consists of two parts: one concerned with public relations and the other with language assistance. The office is responsible for public relations activities strategy and publishes new information about a variety of scientific research of IMSUT on its official website and social media. The office also works towards increasing IMSUT's international presence by issuing press releases both in Japanese and English, holding press conferences, and editing public relations magazines of IMSUT. For its part in language assistance, the staff contributes to the creation of a favourable environment for international members of IMSUT by providing Japanese-English (or vice versa) language support including translation of various notices and documents.

1. Publication of Press Releases on IMSUT research results

Asako Shimizu

The office issued press releases on various new findings from IMSUT, including SARS-CoV-2 and COVID-19, and distributed Japanese press releases to media institutions and science journalists strategically. The office also disseminated English press releases to the global community of science journalists through the official website, social media such as Twitter and Facebook, along with the international public relations website "Eurek Alert!".

2. Launch 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 launched the first issue of IMUST's PR magazine "PLATINUM STREET TIMES" featuring IMSUT's research achievements on COVID-19 in December 2020. The magazine will be published twice a year, in June and December.

3. Dissemination of IMSUT official events on the website

Asako Shimizu

Details of various academic events at IMSUT, such as the 47th IMSUT founding commemorative symposium and the online English seminar series 2020 hosted by the IMSUT International Joint Usage/Research Center Project, were actively reported by the office on the official website and social media.

4. Language Assistance

Kazuyo Ohara

Under the COVID-19 pandemic, the International Affairs Office worked closely with the administrative staff to provide international members of IMSUT with the Dean's messages, official announcements, and relevant information in English where necessary. Our language support also contributed to the compilation for materials for the external review of IMSUT in 2020. Looking ahead, the office will actively promote the internationalization at IMSUT and a favourable environment for international members of IMSUT by offering tailored language services.

Dean's Office

BioBank Japan
バイオバンク・ジャパン

Professor	Yuji Yamanashi, Ph.D.	教　授	理学博士	山　梨　裕　司
Professor	Koichi Matsuda, M.D., Ph.D.	連携教授	博士(医学)（大学院新領域創成科学研究科）	松　田　浩　一
Project Professor	Takayuki Morisaki, M.D., Ph.D.	特任教授	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 260,000 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: Terao, C, Suzuki, A, Momozawa, Y, Akiyama, M, Ishigaki, K, Yamamoto, K, Matsuda, K,Murakami, Y, McCarroll, SA, Kubo, M, Loh, PR, Kamatani, Y. Chromosomal alterations among age-related haematopoietic clones in Japan. Nature. 584:130-135, 2020.

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4: Yokomichi, H, Mochizuki, M, Hirata, M, Nagai, A, Kojima, R, Horiuchi, S, Ooka, T, Akiyama, Y, Shinohara, R, Miyake, K; BioBank Japan P, Yamagata, Z. All-cause and cardiovascular disease mortality in underweight patients with diabetic nephropathy: BioBank Japan Cohort. J. Diabetes. Investig. 2020. doi:10.1111/jdi.13483. Epub ahead of print. PMID: 33340268.

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Dina, C, Naggara, O, Gentric, JC, Shotar, E, Eugène, F, Desal, H, Winsvold, BS, Børte, S, Johnsen, MB, Brumpton, BM, Sandvei, MS, Willer, CJ, Hveem, K, Zwart, JA, Verschuren, WMM, Friedrich, CM, Hirsch, S, Schilling, S, Dauvillier, J, Martin, O; HUNT All-In Stroke; China Kadoorie Biobank Collaborative Group; BioBank Japan Project Consortium; ICAN Study Group; CADISP Group; Genetics and Observational Subarachnoid Haemorrhage (GOSH) Study investigators; International Stroke Genetics Consortium (ISGC), Jones, GT, Bown, MJ, Ko, NU, Kim, H, Coleman, JRI, Breen, G, Zaroff, JG, Klijn, CJM, Malik, R, Dichgans, M, Sargurupremraj, M, Tatlisumak, T, Amouyel, P, Debette, S, Rinkel, GJE, Worrall, BB, Pera, J, Slowik, A, Gaál-Paavola, EI, Niemelä, M, Jääskeläinen, JE, von Und Zu Fraunberg, M, Lindgren, A, Broderick, JP, Werring, DJ, Woo, D, Redon, R, Bijlenga, P, Kamatani, Y, Veldink, JH, Ruigrok, YM. Genome-wide association study of intracranial aneurysms identifies 17 risk loci and genetic overlap with clinical risk factors. Nat. Genet. 52:1303-1313, 2020.

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15: Shirai, Y, Honda, S, Ikari, K, Kanai, M, Takeda, Y, Kamatani, Y, Morisaki, T, Tanaka, E, Kumanogoh, A, Harigai, M, Okada, Y. Association of the R-PA3-UMAD1 locus with interstitial lung diseases complicated with rheumatoid arthritis in Japanese. Ann. Rheum. Dis. 79:1305-1309, 2020.

SCIENTIFIC MEETINGS & SEMINARS

47th IMSUT Founding Commemorative Symposium Neo-Immunology on infection, allergy and cancer

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「感染症、アレルギー及び癌に関するネオ免疫学」というテーマで講演をお願いした。

日　時：令和 2 年 11 月 27 日（金）　13：00 ～ 17：00
会　場：Zoom によるオンライン開催

Ken Ishii（Division of Vaccine Science, Department of Microbiology and Immunology , IMSUT）
Adjuvant for vaccine and immunotherapy; classical immunology versus machine learning

Hiroshi Kawamoto（Laboratory of Immunology, Institute for Frontier Life and Medical Sciences, Kyoto University）
Regeneration of T cells using the iPS cell technology: Development of “off-the-shelf T cells” for cancer immunotherapy

Cevayir Coban（Division of Malaria Immunology, Department of Microbiology and Immunology , IMSUT）
Host-Plasmodium interactions at tissue level

Satoshi Uematsu（Department of Immunology and Genomics, Osaka City University Graduate School of Medicine/ Division of Innate Immune Regulation, International Research and Development Center for Mucosal Vaccine , IMSUT）
Development of new treatments for dysbiosis-related diseases

Cezmi A. Akdis（Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Christine Kühne-Center for Allergy Research and Education (CK-CARE), Davos, Switzerland）
The epithelial barrier hypothesis for the development of allergic and autoimmune diseases

学友会セミナー

（令和２年１月～令和２年 12 月）

1 月 8 日　演題：　Development of deep learning models for genomic data: novel applications for short-read structural variant detection and long-read basecalling
　　演者：　張　　耀中

1 月 10 日　演題：　Organoid modeling of the hepatic–hematopoietic symbiotic relationship during human fetal liver development
　　演者：　聶　　運中

1 月 14 日　演題：　造血幹細胞を生体外で増幅させる試み
　　演者：　山崎　　聡

1 月 20 日　演題：　医療・医学研究に関する「告知」の研究　―遺伝性腫瘍に関する家族内での情報共有を事例に
　　演者：　李　　怡然

1 月 20 日　演題：　ロイコトリエン受容体と炎症性疼痛
　　演者：　浅原　美保

1 月 23 日　演題：　中胚葉系組織を制御する因子の解析
　　演者：　藤井　智明

1 月 24 日　演題：　Waivers of Informed Consent for Research: A Legal and Historical Review and Consideration of Emerging Practices
　　演者：　Jon F Merz

1 月 27 日　演題：　健康医療ビッグデータ解析に対する統計科学的アプローチ
　　演者：　片山　琴絵

1 月 30 日　演題：　次世代シークエンシングデータを用いた迅速な細胞安全性評価
　　演者：　朴　聖俊

2 月 7 日　演題：　Wnt シグナルによる新たな分子制御機構の解明と分子標的治療薬の開発
　　演者：　山口 貴世志

2 月 18 日　演題：　多能性幹細胞からの臓器創出の現状と今後の展望
　　演者：　山口　智之

4 月 3 日　演題：　エンベロープウイルスの膜融合阻害剤の探索とその作用機序の解明
　　演者：　山本　瑞生

6 月 25 日　演題：　腹水濾過濃縮再静注法の緩和ケアにおける位置づけ
　　演者：　伊藤　哲也

6 月 25 日　演題：　Computational intractability as an ally and adversary in genomic privacy, analysis, and engineering
　　演者：　Robert Daniel Barish

7 月 29 日　演題：　高齢者大腸癌の術前全身状態評価における CONUT score の有用性

演者：　阿彦　友佳

7 月 31 日　演題：　dsRNA センサー TLR3 の緻密な応答制御機構

演者：　佐藤　亮太

8 月 28 日　演題：　哺乳類の初期胚・生殖細胞発生における保存性と多様性

演者：　小林　俊寛

9 月 3 日　演題：　安全な上部消化管手術に向けての取り組み

演者：　愛甲　　丞

9 月 4 日　演題：　Molecular control of regulatory T cell development and function by the transcription factor Foxp3 and T cell receptor signals

演者：　堀　　昌平

9 月 4 日　演題：　Chronic interferon-γ stimulation impairs memory CD8 T cell maintenance

演者：　瀬戸口　留可

10 月 5 日　演題：　単純ヘルペスウイルスの病態発現機構

演者：　加藤　哲久

11 月 12 日　演題：　がんを中心とした様々な疾患に関わるゲノム異常の研究

演者：　髙橋　数冴

11 月 26 日　演題：　インフルエンザウイルス及び熱帯ウイルス感染症に対する研究

演者：　前村　　忠

12 月 1 日　演題：　肝炎ウイルスの増殖と病原性に関与する宿主細胞性因子

演者：　後藤　　覚

12 月 21 日　演題：　慢性腎臓病における細胞老化の役割

演者：　荒谷　紗絵

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たてられている。2020 年には、「ビッグデータ解析と AI を活用した新たな医科学研究」というテーマの下で次のようなセミナーが行われた。

ビッグデータ解析と AI を活用した新たな医科学研究

	月　日	講師名		演　　題
1	4 月 6 日	谷内江　望	東京大学先端科学技術研究センター 准教授	Recording cellular events in DNA
2	4 月 13 日	渋谷　哲朗	東京大学医科学研究所 医療データ情報学分野 教授	ビッグデータ・バイオインフォマティクス
3	4 月 20 日	北野　宏明	特定非営利活動法人システム・バイオロジー研究機構 会長	Nobel Turing Challenge: Creating the Engine for Scientific Discovery
4	4 月 27 日	岡田　随象	大阪大学大学院医学系研究科 教授	遺伝統計学で迫る疾患病態解明とゲノム創薬
5	5 月 11 日	鎌谷　洋一郎	東京大学新領域創成科学研究科 教授	全ゲノム関連解析による複雑形質の理解
6	5 月 25 日	岡本　青史	株式会社 富士通研究所 人工知能研究所 所長	企業における人工知能研究の現状と未来
7	6 月 1 日	榊原　　彰	日本マイクロソフト株式会社 執行役員 最高技術責任者	医療 AI の現状と展望～ Microsoft Research Healthcare Next を 中心に
8	9 月 28 日	清水　昭伸	東京農工大学 教授	深層学習を用いた医用画像のコンピュータ支援診断
9	10 月 5 日	曽我　朋義	慶應義塾大学先端生命科学研究所 教授	メタボローム解析とがん研究
10	10 月 12 日	奥野　恭史	京都大学大学院医学研究科 教授	医療・創薬における AI の現状と可能性
11	10 月 19 日	岡田　眞里子	大阪大学蛋白質研究所 教授	オミクスと数理モデルを用いた細胞解析法
12	10 月 26 日	井元　清哉	東京大学医科学研究所 健康医療インテリジェンス分野 教授	人工知能とゲノムビッグデータ解析
13	11 月 9 日	山口 類	愛知県がんセンター 分野長	臨床シークエンスのための人工知能を活用した情報解析基盤の開発

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２年 12 月 12 日（土）　９：15 ～ 16：40
　　令和２年 12 月 13 日（日）　９：30 ～ 16：40
場　所：Zoom によるオンラインでの開講

教員および題目

12 月 12 日（土）

講　師　名		題　　目
中西　　真	癌・細胞増殖部門 癌防御シグナル分野	医科研紹介
根岸　貴子	国際粘膜ワクチン開発研究センター 粘膜バリア学分野	骨の代謝と骨疾患
井元　清哉	ヒトゲノム解析センター 健康医療インテリジェンス分野	ゲノム研究の新次元
尾山　大明	疾患プロテオミクスラボラトリー	プロテオミクスが解き明かすがん細胞情報伝達機構
舘林　和夫	遺伝子解析施設 フロンティア研究領域	細胞が環境ストレスに応答する仕組み

12 月 13 日（日）

講　師　名		題　　目
伊藤　博崇	先端医療研究センター 先端がん治療分野	脳神経外科治療におけるウイルス療法とその位置づけ
堤　　武也	先端医療研究センター 感染症分野	最近のウイルス感染症における医学研究の貢献
小檜山　康司	感染・免疫部門 ワクチン科学分野	ワクチンのサイエンス
真下　知士	実験動物研究施設 先進動物ゲノム研究分野	ゲノム編集技術とヒト化動物について

ANNUAL REPORT 2020

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