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Annual Report

2019

Preface

It is our pleasure to present Annual Report 2019 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.

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 128 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 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 22 international joint research projects in fiscal year 2019. 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 2019. 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 2020

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, 2019〉

Department of Microbiology and Immunology　感染・免疫部門

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Professor	Yoshihiro Kawaoka, D.V.M., Ph.D.	教　授	獣医学博士　河　岡　義　裕
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ワクチン科学分野

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シークエンスデータ情報処理分野

Laboratory of Genome Database
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		(新領域創成科学研究科)		
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Laboratory Animal Research Center　実験動物施設

IMSUT Hospital　附属病院

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Assistant Professor	Hiroki Yamazaki, M.D., D.M.Sc.	助　教	博士(医学)	山　崎　広　貴

Department of General Medicine ····· 212

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Department of Applied Genomics ····· 215

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Department of Radiology ····· 217

放射線科

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Assistant Professor	Koichiro Yasaka, M.D., D.M.Sc.	助　教	博士(医学)	八　坂　耕一郎

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放射線管理室

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IT サービス室

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写真室

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遺伝子組換え・微生物研究支援室

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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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顕微鏡コアラボラトリー

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IMSUT 臨床フローサイトメトリー・ラボ

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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	Tokiko Watanabe, D.V.M., Ph.D.	特任准教授	博士(獣医学)	渡　邉　登喜子
Project Associate Professor	Seiya Yamayoshi, D.V.M., Ph.D.	特任准教授	博士(医学)	山　吉　誠　也
Assistant Professor	Kiyoko Iwatsuki-Horimoto, D.V.M., Ph.D.	助　教	博士(獣医学)	岩附(堀本)研子
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
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 and Ebola virus infections as models. Interactions between viral and host gene products during viral replication cycles determine the consequences of infection (i.e., the characteristics of disease manifestation, whether limited or widespread); hence, our research has centered on such interactions in these viral infections.

1. Baloxavir marboxil treatment of nude mice infected with influenza A virus.

Kiso M, Yamayoshi S, Murakami J, Kawaoka Y:

Immunocompromised patients infected with influenza virus require prolonged treatment with neuraminidase inhibitors, because these patients are not able to eradicate the virus from the respiratory tract, leading to the emergence of drug-resistant mutant viruses. Here, we examined the efficacy of baloxavir marboxil in nude mice, which are immunologically deficient. Daily treatment with a suboptimal dose of baloxavir marboxil increased the survival time of the virus-infected nude mice but did not clear the virus from their respiratory organs, resulting in gradual body weight loss after termination of treatment. Despite the prolonged baloxavir marboxil treatment, few resistant mutants were detected.

2. 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[2], Dutta J[3], Khan Z[3], Kriti D[3], van Bakel H[3], Tokita A[5], Hagiwara H[5], Izumida N[5], Kuroki H[6], Nishino T[5], Wada N[5], Koga M[7], Adachi E[8], Jubishi D, Hasegawa H[9], Kawaoka Y:[1]Department of Pathology, National Institute of Infectious Diseases, Japan. [2]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, USA. [3]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, USA. [5]Members of the Tokyo Pediatric Association Public Health Committee, Japan. [6]Sotobo Children's Clinic, Japan. [7]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Japan.

[8]Department of Infectious Diseases and Applied Immunology, IMSUT Hospital of the Institute of Medical Science, University of Tokyo, Japan. [9]Influenza Virus Research Center, National Institute of Infectious Diseases, Japan.

Here we report the isolation of the influenza A/H1N1 2009 pandemic (A/H1N1pdm) and A/H3N2 viruses carrying an I38T mutation in the polymerase acidic protein-a mutation that confers reduced susceptibility to baloxavir marboxil-from patients before and after treatment with baloxavir marboxil in Japan. These variants showed replicative abilities and pathogenicity that is similar to those of wild-type isolates in hamsters; they also transmitted efficiently between ferrets by respiratory droplets.

3. Influenza Virus Polymerase Mutation Stabilizes a Foreign Gene Inserted into the Virus Genome by Enhancing the Transcription/Replication Efficiency of the Modified Segment.

Furusawa Y, Yamada S, Lopes TJS[2], Dutta J[3], Khan Z[3], Kriti D[3], van Bakel H[3], Kawaoka Y:

We previously attempted to establish a reporter influenza virus by inserting the gene for the Venus fluorescent protein into the NS segment of influenza A/Puerto Rico/8/34 (PR8, H1N1) virus to yield WT-Venus-PR8. Although the inserted Venus gene was deleted during serial passages of WT-Venus-PR8, we discovered that the PB2-E712D mutation stabilizes the Venus gene. Here, we explored the mechanisms by which Venus gene deletion occurs and how the polymerase mutation stabilizes the Venus gene. Deep sequencing analysis revealed that PB2-E712D does not cause an appreciable change in the mutation rate, suggesting that the stability of the Venus gene is not affected by polymerase fidelity. We found by using quantitative real-time PCR that WT-Venus-PR8 induces high-level interferon beta (IFN-β) expression. The induction of IFN-β expression seemed to result from the reduced transcription/replication efficiency of the modified NS segment in WT-Venus-PR8. In contrast, the transcription/replication efficiency of the modified NS segment was enhanced by the PB2-E712D mutation. Loss of the Venus gene in WT-Venus-PR8 appeared to be caused by internal deletions in the NS segment. Moreover, to further our understanding of the Venus stabilization mechanisms, we identified additional amino acid mutations in the virus polymerase complex that stabilize the Venus gene. We found that some of these amino acids are located near the template exit or the product exit of the viral polymerase, suggesting that these amino acids contribute to the stability of the Venus gene by affecting the binding affinity between the polymerase complex and the RNA template and product.

4. A humanized MDCK cell line for the efficient isolation and propagation of human influenza viruses.

Takada K, Kawakami C[10], Fan S[2], Chiba S[2], Zhong G[2], Gu C[2], Shimizu K[10], Takasaki S, Sakai-Tagawa Y, Lopes TJS[2], Dutta J[3], Khan Z[3], Kriti D[3], van Bakel H[3], Yamada S, Watanabe T, Imai M, Kawaoka Y: [10]Yokohama City Institute of Public Health, Japan

Here, we developed hCK, a Madin-Darby canine kidney (MDCK) cell line that expresses high levels of human influenza virus receptors and low levels of avian virus receptors. hCK cells supported human A/H3N2 influenza virus isolation and growth much more effectively than conventional MDCK or human virus receptor-overexpressing (AX4) cells. A/H3N2 viruses propagated in hCK cells also maintained higher genetic stability than those propagated in MDCK and AX4 cells.

5. Antigenic drift originating from changes to the lateral surface of the neuraminidase head of influenza A virus.

Yasuhara A, Yamayoshi S, Kiso M, Sakai-Tagawa Y, Koga M[7], Adachi E[8], Kikuchi T[11], Wang IH, Yamada S, Kawaoka Y: [11]AIDS Research Center, National Institute of Infectious Diseases, Japan.

Influenza viruses possess two surface glycoproteins, haemagglutinin and neuraminidase (NA). Although haemagglutinin plays a major role as a protective antigen, immunity to NA also contributes to protection. The NA protein consists of a stalk and a head portion, the latter of which possesses enzymatic NA (or sialidase) activity. Like haemagglutinin, NA is under immune pressure, which leads to amino acid alterations and antigenic drift. Amino acid changes accumulate around the enzymatic active site, which is located at the top of the NA head. However, amino acid alterations also accumulate at the lateral surface of the NA head. The reason for this accumulation remains unknown. Here, we isolated seven anti-NA monoclonal antibodies (mAbs) from individuals infected with A(H1N1)pdm09 virus. We found that amino acid mutations on the lateral surface of the NA head abolished the binding of all of these mAbs. All seven mAbs activated Fcγ receptor (FcγR)-mediated signalling pathways in effector cells and five mAbs possessed NA inhibition activity, but the other two did not; however, all seven protected mice from lethal challenge infection through their NA inhibition activity and/or FcγR-mediated antiviral activity. Serological analysis of individuals infected with A(H1N1)pdm09 virus revealed that some possessed or acquired the anti-NA-lateral-surface antibodies following infection. We also found antigenic drift on the lateral surface of the NA head of isolates from 2009 and

2015. Our results demonstrate that anti-lateral-surface mAbs without NA inhibition activity can provide protection by activating FcγR-mediated antiviral activity and can drive antigenic drift at the lateral surface of the NA head. These findings have implications for NA antigenic characterization in that they demonstrate that traditional NA inhibition assays are inadequate to fully characterize NA antigenicity.

Publications

1. Kato-Miyashita S, Sakai-Tagawa Y, Yamashita M, Iwatsuki-Horimoto K, Ito M, Tokita A, Hagiwara H, Izumida N, Nishino T, Wada N, Koga M, Adachi E, Jubishi D, Yotsuyanagi H, Kawaoka Y, Imai M. Antigenic variants of influenza B viruses isolated in Japan during the 2017–2018 and 2018–2019 influenza seasons. Influenza Other Respi Viruses. in press
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感染遺伝学分野

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Immune cells express multiple Toll-like receptors (TLRs) that are concomitantly activated by a variety of pathogen products derived from microbes and viruses. Recent reports have indicated that losing the balance of TLRs responses result in autoimmune diseases. Nucleic acid-sensing (NA-sensing) TLRs sense bacterial and viral NAs, but also host-derived NAs. To avoid excessive immune responses for host-derived NAs, there must exist regulatory mechanisms coordinating the expression, the localization and the function of TLRs. Our research focuses on the regulatory mechanisms controlling pathogenic ligand recognition by TLRs and the identification of endogenous ligands.

1. Increased cross-presentation by Rab7a deficient dendritic cells causes type 2 autoimmune hepatitis

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Type 2 autoimmune Hepatitis (Type 2 AIH) is characterized by progressive inflammatory responses in the liver. Pathogenetic mechanisms underlying type 2 AIH is largely unknown because its model mouse is not available. Rab7a is a small GTPase that is associated with TLR3 and controls its subcellular distribution. Here we show that a type 2 AIH-like disease spontaneously develops in mice with the Rab7a gene deleted in conventional dendritic cells (cDCs) by CD11c-Cre expression (Rab7a cKO mice). All the Rab7a cKO mice died by 41 weeks old. The serum levels of liver-specific enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated in Rab7a cKO mice, suggesting hepatocyte damage. Consistent with this, histological analyses revealed massive infiltration of inflammatory cells and fibrosis. Rab7a cKO mice showed systemic immune responses as evidenced by hypergammaglobulinemia and T cell activation, suggesting that Rab7a cKO mice suffer from AIH. Type 1 and 2 AIH are discriminated by autoantibody production to nuclear/mitochondrial/smooth muscle antigens or liver/kidney microsomal antigens (anti-LKM1), respectively. Rab7a cKO mice showed the production of the latter autoantibodies, but the former autoantibodies. These results indicate that Rab7a cKO mice suffered from type 2 AIH-like disease. This mouse is the first model of type 2 AIH. $CD8^+$DCs, known as professional cells for cross-presentation, accumulated in the liver of Rab7a cKO mice and highly expressed MHC class I, CD80 and TLR3. CD8 T cells were intensely activated and differentiated into cytotoxic T cells in the liver.

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. Although pathogenic roles of the acquired immune system are well established, a role of the innate immune system in T1D remains unclear. 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-/FcgR4+/TLR7+ monocytes (patrolling monocyte/macrophages) and the expression of FcgR4 on the subset were decreased by TLR7 deficiency. These results suggest that TLR7 drives disease progression through the activation of patrolling monocyte/macrophages in NOD mice.

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ウイルス病態制御分野

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To date, approximately 250 herpesviruses have been identified, affecting most animal species. These viruses are associated with a variety of diseases such as encephalitis, malignancy and mucocutaneous diseases in human and animals. The objective of our research is to understand the mechanisms by which herpesviruses replicate in cells and manifest diseases in their hosts. Our goal is to apply our fundamental findings for the development of anti-herpetic drugs and vaccines for the control of these viral infections.

1. Identification of the Capsid Binding Site in the Herpes Simplex Virus 1 Nuclear Egress Complex and Its Role in Viral Primary Envelopment and Replication

Kosuke Takeshima, Jun Arii, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato and Yasusi Kawaguchi

Binding of HSV-1 NEC to nucleocapsids has been thought to promote nucleocapsid budding at the inner nuclear membrane and subsequent incorporation of nucleocapsids into vesicles during nuclear egress of nucleocapsids. However, data to directly support this hypothesis have not been reported thus far. In this study, we have present data showing that two amino acids in the membrane-distal face of the HSV-1 NEC, which contains the putative capsid binding site based on the solved NEC structure, were in fact required for efficient NEC binding to nucleocapsids and for efficient incorporation of nucleocapsids into vesicles during primary envelopment. This is the first report showing direct linkage between NEC binding to nucleocapsids and an increase in nucleocapsid incorporation into vesicles during herpesvirus primary envelopment.

During nuclear egress of nascent progeny herpesvirus nucleocapsids, the nucleocapsids acquire a primary envelope by budding through the inner nuclear membrane of infected cells into the perinuclear space between the inner and outer nuclear membranes. Herpes simplex virus 1 (HSV-1) UL34 and UL31 proteins form a nuclear egress complex (NEC) and play critical roles in this budding process, designated primary envelopment. To clarify the role of NEC binding to progeny nucleocapsids in HSV-1 primary envelopment, we established an assay system for HSV-1 NEC binding to nucleocapsids and capsid proteins in vitro Using this assay system, we showed that HSV-1 NEC bound to nucleocapsids and to capsid protein UL25 but not to the other capsid proteins tested (i.e., VP5, VP23, and UL17) and that HSV-1 NEC binding of nucleocapsids was mediated by the interaction of NEC with UL25. UL31 residues arginine-281 (R281) and aspartic acid-282 (D282) were required for efficient NEC binding to nucleocapsids and UL25. We also showed that alanine substitution of UL31 R281 and D282 reduced HSV-1 replication, caused aberrant accumulation of capsids in the nucleus, and induced an accumulation of empty vesicles that were similar in size and morphology to primary envelopes in the

perinuclear space. These results suggested that NEC binding via UL31 R281 and D282 to nucleocapsids, and probably to UL25 in the nucleocapsids, has an important role in HSV-1 replication by promoting the incorporation of nucleocapsids into vesicles during primary envelopment.

2. Roles of the Interhexamer Contact Site for Hexagonal Lattice Formation of the Herpes Simplex Virus 1 Nuclear Egress Complex in Viral Primary Envelopment and Replication

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

The scaffolding proteins of several envelope viruses required for virion assembly form high-order lattice structures. However, information on the significance of their lattice formation in infected cells is limited. Herpesviruses acquire envelopes twice during their viral replication. The first envelop acquisition (primary envelopment) is one of the steps in the vesicle-mediated nucleocytoplasmic transport of nascent nucleocapsids, which is unique in biology. HSV-1 NEC, thought to be conserved in all members of the Herpesviridae family, is critical for primary envelopment and was shown to form a hexagonal lattice structure. Here, we investigated the significance of the interhexamer contact site for hexagonal lattice formation of the NEC in HSV-1-infected cells and present evidence suggesting that the lattice formation of the NEC hexamer has an important role in HSV-1 replication by regulating primary envelopment. Our results provide insights into the mechanisms of the envelopment of herpesviruses and other envelope viruses.

During the nuclear export of nascent nucleocapsids of herpes simplex virus 1 (HSV-1), the nucleocapsids acquire a primary envelope by budding through the inner nuclear membrane into the perinuclear space between the inner and outer nuclear membranes. This unique budding process, termed primary envelopment, is initiated by the nuclear egress complex (NEC), composed of the HSV-1 UL31 and UL34 proteins. Earlier biochemical approaches have shown that the NEC has an intrinsic ability to vesiculate membranes through the formation of a hexagonal lattice structure. The significance of intrahexamer interactions of the NEC in the primary envelopment of HSV-1-infected cells has been reported. In contrast, the contribution of lattice formation of the NEC hexamer to primary envelopment in HSV-1-infected cells remains to be elucidated. Therefore, we constructed and characterized a recombinant HSV-1 strain carrying an amino acid substitution in a UL31 residue that is an interhexamer contact site for the lattice formation of the NEC hexamer. This mutation was reported to destabilize the interhexamer interactions of the HSV-1 NEC. Here, we demonstrate that the mutation causes the aberrant accumulation of nucleocapsids in the nucleus and reduces viral replication in Vero and HeLa cells. Thus, the ability of HSV-1 to form the hexagonal lattice structure of the NEC was linked to an increase in primary envelopment and viral replication. Our results suggest that the lattice formation of the NEC hexamer has an important role in HSV-1 replication by regulating primary envelopment.

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Department of Microbiology and Immunology

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. ZBP1 governs the inflammasome-independent IL-1α and neutrophil inflammation that play a dual role in anti-influenza virus immunity.

Influenza A virus (IAV) triggers the infected lung to produce IL-1 and recruit neutrophil. Unlike IL-1β, however, little is known about IL-1α in terms of its mechanism of induction, action and physiological relevance to the host immunity against IAV infection. In particular, whether Z-DNA binding protein 1 (ZBP1), a key molecule for IAV-induced cell death, is involved in the IL-1α induction, neutrophil infiltration, and the physiological outcome have not been elucidated. Here we show in murine model that the IAV-induced IL-1α is mediated solely by ZBP1, in an NLRP3-inflammasome-independent manner, and is required for the optimal IL-1β production followed by the formation of neutrophil extracellular traps. During IAV infection, ZBP1 displays a dual role in anti-IAV immune responses mediated by neutrophil, resulting in either protective or pathological outcome in vivo. Thus, ZBP1-mediated IL-1α production is the key initial step of IAV-infected NETs, owing the duality of the consequent lung inflammation.

2. Cyclic GMP-AMP Triggers Asthma in an IL-33-Dependent Manner That Is Blocked by Amlexanox, a TBK1 Inhibitor.

Extracellular host-derived DNA, as one of damage associated molecular patterns (DAMPs), is associated with allergic type 2 immune responses. Immune recognition of such DNA generates the second messenger cyclic GMP-AMP (cGAMP) and induces type-2 immune responses; however, its role in allergic diseases, such as asthma, has not been fully elucidated. This study aimed to determine whether cGAMP could induce asthma when used as an adjuvant. We intranasally sensitized mice with cGAMP together with house dust mite antigen (HDM), followed by airway challenge with HDM. We then assessed the levels of eosinophils in the broncho-alveolar lavage fluid (BALF) and serum HDM-specific antibodies. cGAMP promoted HDM specific allergic asthma, characterized by significantly increased HDM specific IgG1 and total IgE in the serum and infiltration of eosinophils in the BALF. cGAMP stimulated lung fibroblast cells to produce IL-33 in vitro, and mice deficient for IL-33 or IL-33 receptor (ST2) failed to develop asthma enhancement by cGAMP. Not only Il-33 -/- mice, but also Sting -/-, Tbk1 -/-, and Irf3 -/- Irf7 -/- mice which

lack the cGAMP-mediated innate immune activation failed to increase eosinophils in the BALF than that from wild type mice. Consistently, intranasal and oral administration of amlexanox, a TBK1 inhibitor, decreased cGAMP-induced lung allergic inflammation. Thus, cGAMP functions as a type 2 adjuvant in the lung and can promote allergic asthma in manners that dependent on the intracellular STING/TBK1/IRF3/7 signaling pathway and the resultant intercellular signaling pathway via IL-33 and ST2 might be a novel therapeutic target for allergic asthma.

3. A unique nanoparticulate TLR9 agonist enables a HA split vaccine to confer FcγR-mediated protection against heterologous lethal influenza virus infection.

The development of a universal influenza vaccine that can provide a robust and long-lasting protection against a broader range of influenza virus strains is a global public health priority. One approach to improve vaccine efficacy is to use an adjuvant to boost immune responses to the target antigens; nevertheless, the role of adjuvants in the context of influenza vaccines is not fully understood.

We have previously developed the K3-schizophyllan (SPG) adjuvant, which is composed of nanoparticulated oligodeoxynucleotides K3, a TLR9 agonist, with SPG, a non-agonistic β-glucan ligand of Dectin-1. In this study, K3-SPG given with conventional influenza hemagglutinin (HA) split vaccine (K3-SPG HA) conferred protection against antigenically mismatched heterologous virus challenge. While K3-SPG HA elicited robust cross-reactive HA-specific IgG2c and CD8 T-cell responses, CD8 T-cell depletion had no impact on this cross-protection. In contrast, K3-SPG HA was not able to confer protection against heterologous virus challenge in FcRγ-deficient mice. Our results indicated that FcγR-mediated antibody responses induced by the HA antigen and K3-SPG adjuvant were important

for potent protection against antigenically mismatched influenza virus infection. Thus, we demonstrated that the K3-SPG-adjuvanted vaccine strategy broadens protective immunity against influenza and provides a basis for the development of next-generation influenza vaccines.

4. Combination and inducible adjuvants targeting nucleic acid sensors.

Innate immune sensing of nucleic acids derived from invading pathogens or tumor cells via pattern recognition receptors is crucial for mounting protective immune responses against infectious disease and cancer. Recently, discovery of tremendous amounts of nucleic acid sensors as well as identification of natural and synthetic ligands for these receptors revealed the potential of adjuvants targeting nucleic acid sensing pathways for designing efficacious vaccines. Especially, current data indicated that unique adjuvants targeting TLR9 and stimulator of interferon genes (STING)-dependent cytosolic nucleic acid sensing pathways along with the combinations of already existing adjuvants are promising candidates for this purpose. Here, we review current vaccine adjuvants targeting nucleic acid sensors and their modes of action.

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Department of Microbiology and Immunology

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

Professor　Cevayir Coban, M.D., Ph.D.

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Malaria is an infectious disease caused by Plasmodium parasites that often leads to severe complications such as cerebral malaria and death. Moreover, millions of people living in endemic areas suffer from asymptomatic- or post-malaria complications. In our lab, we focus on the elucidation of the mechanisms involved in the host and Plasmodium parasites interactions. Our final goal is to develop vaccines and vaccine modalities against malaria and other infectious disease.

1. B Cell-intrinsic MyD88 Signaling Controls IFN-γ-mediated Early IgG2c Class Switching in Response to a Particulate Adjuvant Synthetic Hemozoin.

Michelle Sue Jann Lee[1], Yayoi Natsume-Kitatani[2], Burcu Temizoz[3], Yukiko Fujita[1], Aki Konishi[1], Kyoko Matsuda[1], Yoshikatsu Igari[4], Toshihiro Tsukui[4], Kouji Kobiyama[3,5], Etsushi Kuroda[3,5], Motoyasu Onishi[5], Thomas Marichal[6], Wataru Ise[7], Takeshi Inoue[7], Tomohiro Kurosaki[7], Kenji Mizuguchi[2], Shizuo Akira[8], Ken J Ishii[4,5], and Cevayir Coban[1,9].: [1]Laboratory of Malaria Immunology, Immunology Frontier Research Center (IFReC), Osaka University, [2]Laboratory of Bioinformatics, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, [3]Laboratory of Vaccine Science, IFReC, Osaka University, [4]ZENOAQ, Nippon Zenyaku Kogyo Co. Ltd., Koriyama, [5]Laboratory of Adjuvant Innovation, Center for Vaccine and Adjuvant Research, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, [6]Laboratory of Cellular and Molecular Immunology, GIGA Institute, and Faculty of Veterinary Medicine, Liege University, Belgium, [7]Laboratory of Lymphocyte Differentiation, IFReC, Osaka University, [8]Laboratory of Host Defense, IFReC, Osaka University, [9]Current: Division of Malaria Immunology, The Institute of Medical Science (IMSUT), The University of Tokyo.

Synthetic hemozoin (sHZ) is a synthetic analog of *Plasmodium*-produced hemozoin and is produced from hemin as a water-insoluble black particle with adjuvant effects. Safe administration of sHZ has been demonstrated by our group in various animals such as mice, ferrets, dogs and monkeys and its production has been successfully optimized in a GLP-certified facility by ZENOAQ (Nippon Zenyaku Kogyo Co. Ltd.) (*Vaccine, 2016; Vaccine 2014; Vaccine, 2014; Hum Vaccin Immunother, 2013; Cell Host Microbe, 2010*). However, how it functions as adjuvant, has not been understood well. We showed by using T-dependent antigen NIPOVA and sHZ combination that MyD88 is required for early GC formation and enhanced antibody class-switch recombination (CSR). Using cell-type-specific MyD88 KO mice, we found that IgG2c class switching, but not IgG1 class switching, was controlled by B cell-intrinsic MyD88 signaling. Notably, IFN-γ produced by various cells including T cells, NK cells, and dendritic cells was the primary cytokine for IgG2c CSR and B-cell intrinsic MyD88 is required for IFN-γ production. Moreover, IFN-γ receptor (IFNγR) deficiency abolished sHZ-induced IgG2c production, while recombinant IFN-γ administration successfully rescued IgG2c CSR impairment in mice lacking B-cell intrinsic MyD88. Together, our results show that B cell-intrinsic MyD88 signaling is involved in the mode-of-action of certain particulate adjuvants such

as sHZ and this may enhance our specific understanding of how adjuvants and vaccines work.

2. Rapid Quantification of NETs *in vitro* and in Whole Blood Samples by Imaging Flow Cytometry

Patrick M. Lelliott[1], Masatoshi Momota[2,3], Michelle S. J. Lee[1], Etsushi Kuroda[2,3], Norifumi Iijima[2,3], Ken J. Ishii[2,3], and Cevayir Coban[1,4]: [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]Current: Division of Malaria Immunology, The Institute of Medical Science (IMSUT), The University of Tokyo.

Our approach to understand malaria-related pathologies is to use imaging techniques such as high resolution 11.7 T MRI or develop new imaging technologies. One of the new imaging techniques we developed recently is the quantification of neutrophil extracellular traps (NETs) in vitro and in vivo by using imaging flow cytometry. Neutrophil extracellular trap (NET) formation involves the release of DNA outside the cell to neutralize pathogens. Techniques such as live microscopy, flow cytometry, and intra vital imaging allow the characterization of NETs, but these either cannot be applied *in vivo*, lack specificity, or require invasive procedures. We developed an automated analysis method to rapidly acquire and characterize cells as NETs or NET precursors, as opposed to cells undergoing other forms of cell death, using imaging flow cytometry. NETs were maintained in solution using a novel 3D cell culture system in which cells are suspended at the interface of two liquids of different density. Critically, we identify NETs using an image analysis algorithm based on morphological data showing the extrusion of DNA beyond the cell boundaries. *In vitro,* we used this technique to demonstrate different requirements for NET formation in human and mouse neutrophils. We also measured NETs in whole blood during infection of mice with the malaria parasite *Plasmodium yoelii*. We expect this technique will provide a valuable approach to better understand the process of NET formation and its importance in disease.

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Book Chapter(s):

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Department of Cancer Biology

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

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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 and immunological responses of cancer. Genomic and epigenomic abnormalities involved in human tumors, including 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

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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 rather 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. We demonstrated that CADM1 significantly repressed the saturation density elevated by YAP1 overexpression in NIH3T3 cells and that CADM1 significantly promoted YAP1 phosphorylation on Ser 127 and downregulated YAP1-target gene expression at confluency in lung adenocarcinoma cell lines. Moreover, CADM1 was co–precipitated with multiple Hippo pathway components, including the core kinases MST1/2 and LATS1/2, suggesting the involvement of CADM1 in the regulation of the Hippo pathway through cell-cell contact. Furthermore, an immunohistochemical analysis of primary lung adenocarcinomas (n = 145) revealed that the histologically low-grade subtype frequently showed the membranous co–expression of CADM1. A subset analysis of disease-free survival revealed that the membranous co–expression of CADM1 and LATS2 was a favorable prognosis factor. These results indicate that the relationship between CADM1 and Hippo pathway core kinases at the cell membrane is important for suppressing the oncogenic role of YAP1 (1).

Next, in order to understand possible roles of cell membrane proteins in acquired resistance against molecular targeting drugs, we have adopted mathematical approaches to investigate gefitinib resistance of lung adenocarcinoma caused by *MET* amplification using lung adenocarcinoma HCC827-GR (gefitin-

ib resistant) cells. The molecular reactions involved in gefitinib resistance consisted of dimerization and phosphorylation of three molecules, EGFR, ErbB3, and MET, and were described by a series of ordinary differential equations. To perform a computer simulation, we quantified each molecule on the cell surface using flow cytometry and estimated unknown parameters by dimensional analysis. Our simulation showed that the number of active ErbB3 molecules is around a hundred-fold smaller than that of active MET molecules. Limited contribution of ErbB3 in gefitinib resistance by *MET* amplification is also demonstrated using HCC827-GR cells in culture experiments. Our mathematical model provides a quantitative understanding of the molecular reactions underlying drug resistance (2).

We are also investigating possible cross-talk 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 using molecule to cell, cell to cell and cell to organ interaction assay individually developed in our laboratory. We have found that most of IgCAMs interact one to several IgCAMs with high affinity, suggesting that these interactions play important roles in specific biological situation, including cancer metastasis and tumor immunology.

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

Takeshi Ito, Yumi Tsuboi, Yuki Kumagai, Tomoko Masuda, Daisuke Matsubara, Zenichi Tanei[2], and Yoshinori Murakami; [2]Department of Pathology, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology

CADM1 is overexpressed in adult T-cell leukemia (ATL) and small cell lung cancer (SCLC), conferring highly invasive or metastatic phenotypes characteristic to ATL or SCLC. To establish sensitive diagnostic tools of ATL or SCLC through detecting CADM1, monoclonal antibodies against the fragments of CADM1 overexpressed in ATL or SCLC are being generated and characterized in collaboration with scientists in the Institute of Advanced Science and Technology, the University of Tokyo (PCT/JP2019/011201). Detection systems of ATL and SCLC by these antibodies are being validated using the serum from patients of ATL and SCLC in collaboration with clinical oncologists in the University of Tokyo Hospital and National Cancer Research Center Hospital. These antibodies would be also promising to generate several therapeutic approaches, including radioisotope or drug-conjugated antibodies and chimeric antigen receptor-T cell therapy.

To unveil additional molecular mechanisms underlying multistage carcinogenesis and therapeutic response, genomic, epigenomic, and transcriptional alterations in key molecules in human tumorigenesis, including CADM1, were examined in colorectal, breast, prostate and other cancers in collaboration with others (3-5).

3. Genomic-epidemiological studies of various human diseases on the basis of Biobank Japan.

Yoshinori Murakami, Takayuki Morisaki, Atsuko Hiraishi, Makoto Hirata and Koichi Matsuda[3]; [3]Laboratory of Clinical Genome Sequencing, Graduate School of Frontier Sciences, The University of Tokyo

A large number of genomic DNA from normal peripheral lymphocytes as well as serum samples from around 267, 000 cases with 51 diseases was collected and preserved in BioBank Japan in the Institute of Medical Science, the University of Tokyo. These DNA samples, clinical information and typing information of more than 900, 000 SNPs provide valuable resources for genome-wide association study (GWAS) of complex traits and susceptibility of various human diseases, including gastric cancer, colorectal cancer, ovarian cancer, hypospadias and diabetes, in collaboration with a large collaborative study group of order-made medicine and basic and clinical geneticists in Japan (6-22).

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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.

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Department of Cancer Biology

Division of Genetics
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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 orchestrated by MuSK and by neural agrin, an extracel-

lular 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 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., 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 a pathogenic mutation of agrin in patients with congenital myasthenic syndromes (see below) did not show impaired ability to activate MuSK at least in vitro (*Am. J. Hum. Genet.*, 85: 155-167, 2009), the novel role of agrin may be relevant to pathogenicity of the mutation. We

are investigating minimal regions of agrin required for the role distinct from MuSK activation to dissect 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, D.[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 (CMS)) 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. We are investigating other defects in NMJ functions and detailed pathophysiology, including electrophysiology and ultrastructural physiology, in the Dok-7 KI mice in the absence or presence of potential therapeutic treatments. Also, we are investigating molecular pathways underlying NMJ defects and muscle weakness in Dok-7 KI mice.

4. *DOK7* gene therapy that enlarges NMJs.

Ueta, R., Tezuka, T., Okada, H.[1], Kasahara, Y.[1], Tomono, T.[1], Watanabe, Y., Sagara, H., Motomura, M., Beeson, D., Takeda, S.[2], Okada, T.[1], and Yamanashi, Y.: [1]Department of Biochemistry and Molecular Biology, Nippon Medical School. Weatherall Institute of Molecular Medicine, University of Oxford. [2]Department of Molecular Therapy, National Institute of Neuroscience.

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 likewise resulted in enlargement of NMJs as well as positive effects on motor activity and life span. These results suggest that therapies aimed at enlarging the NMJ may be useful for a range of neuromuscular disorders. Indeed, we have recently found that therapeutic administration of AAV-D7 is beneficial to other mouse models of neuromuscular disorders, including amyotrophic lateral sclerosis (ALS), a progressive, multifactorial motor neurodegenerative disease with severe muscle atrophy. We are further investigating the effects, including ultrastructural and electrophysiological ones, of AAV-D7 administration in other types of muscle weakness.

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

Inoue-Yamauchi, A., Jozawa, H., Arimura, S.[1], Shimura, E.[2], Hayata, T.[3], Ezura, Y.[4], Taguchi, Y.[5], Ueta, R., Oda, H.[6], Nakae, S.[2], Inoue, J.[5], Noda, M.[4], and Yamanashi, Y.: [1]Department of Biology and Biochemistry, University of Houston. [2]Laboratory of Systems Biology, IMSUT. [3]Department of Biological Signaling and Regulation, Faculty of Medicine, University of Tsukuba. [4]Department of Skeletal Molecular Pharmacology, Medical Research Institute, Tokyo Medical and Dental University. [5]Division of Cellular and Molecular Biology, IMSUT. [6]Department of Pathology, Tokyo Women's Medical University.

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 have 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 the molecular function of Dok-1/2/3 in bone homeostasis, 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., Arimura, S., 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. However, the role of C/EBPδ in intestinal inflammation remains unclear. We have recently demonstrated that C/EBPδ plays an essential role in suppressing DSS-induced colitis, likely by attenuating intestinal epithelial cell apoptosis. We are further investigating pathological roles of C/EBPδ in inflammatory diseases.

7. Omic analyses.

Ueta, R., Eguchi, T., Jozawa, H., Takada, Y.[1], Iemura, S.[2], Natsume, T.[3], Kozuka-Hata, H.[4], Oyama, M.[4], and Yamanashi, Y.: [1]Laboratory of Glyco-bioengineering, The Noguchi Institute, [2]Translational Research Center, Fukushima Medical University. [3]National Institute of Advanced Science and Technology, Molecular Profiling Research Center for Drug Discovery. [4]Medical Proteomics Laboratory, IMSUT.

To gain insights into signaling mechanisms underlying a variety of physiological and pathophysiological events, including NMJ formation, 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 glycomic and metabolomic analyses.

8. Screening of chemical compound and siRNA libraries.

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.

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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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Assistant Professor	Yoshikazu Johmura, Ph.D.	助　教	博士(薬学)	城　村　由　和

In response to genetic and epigenetic insults, normal human cells execute various cellular responses such as transient cell cycle arrest, apoptosis, and cellular senescence as an anti-tumorigenesis barrier. Our research interests are to elucidate the mechanisms underlying induction and regulation of cellular senescence. Our final goal is to develop innovative cancer therapies, prevention, and anti-aging strategies through regulating and/or eliminating senescent cells in vivo (senotherapy). 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. Mechanisms underlying maintenance of genomic and epigenomic integrities such as DNA methylation maintenance are also under investigation.

1. Regulatory mechanisms of aging by senescent cells and development of strategies that eliminate senescent cells *in vivo*

Yoshikazu Johmura, Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Dan Li, Wang Tehwei, Takehiro Yamanaka, Kisho Yokote, Narumi Suzuki, Satotaka Ohmori, Tomomi Kanai, Akane Kenjo, Yue Zhang, and Makoto Nakanishi:

Several lines of evidences have underpinned a prominent role of senescent cells in aging, life span and pathogenesis of age-associated disorders. Durable cessation of proliferation in response to any growth signals is an essential hallmark of senescence, but other characters including apoptosis resistance, mitochondrial dysfunction, and secretory phenotypes (SASP) are also notable. In addition to the above physiological aspects, senescent cells showed various unique physiological peculiarity in metabolic organelles. These characters are typically observed independent of senescence-inducting stimuli and thus might underlie common metabolic reorganizations that ensure their survival and physiological traits.

Accumulation of senescent cells strongly associates with a variety of age-related pathologies, such as atherosclerosis and type II diabetes. Recently, selective elimination of p16-positive senescent cells (senotherapy) by transgenic approach from progeroid and normally aged mice has been reported to improve the healthy lifespan and ameliorates the consequences of various age-related disorders. However, because of the characteristic heterogeneity of senescence *in vivo*, the compounds and molecular targets that effectively induce a lethality into all types of senescent cells have not yet been identified. In order to develop such drugs, we uncovered the metabolic vulnerability in senescence in which glutaminolysis was addicted for lysosome-mediated intracellular low pH. Inhibition of GLS1, a rate limiting enzyme of glutaminolysis, effectively induced senolysis both *in vitro* and *in vivo*. Most intriguingly, treatment of aged mice with GLS1 inhibitor ameliorated various forms of age- and senescence-associated organ dysfunction, such as age-associated glomeruloscrerosis, lung fibrosis, cardiac hypertrophy, and adipose tissue atrophy as well

as senescence-associated atherosclerosis. Our results suggest that cells in a senescent state require glutaminolysis, and its inhibition offers a promising strategy for inducing senolysis *in vivo*.

Although cellular senescence has been relatively well characterized *in vitro*, their physiological characters and actual contributions to aging and carcinogenesis *in vivo* are still largely unknown. In order to clarify these issues, we have established two mouse models. In one mouse model, p16-positive senescent cells are visualized by fluorescent labeling. Using these mice, we found that p16-positive senescent cells are detectable in all tissues, such as brain, heart, liver, lung, and kidney even from young-aged mice, and the number of senescent cells increases as mice grow older. In the other mouse model, senescence can be induced by ectopic expression of p53 fused with geminin degron, which is specifically functional during S to G2 phase. We found that these mice showed an acceleration of various age-associated phenotypes, such as glomerulosclerosis, lung fibrosis, liver lipidosis, and pancreas lipidosis. Using these mouse models, we are expecting to uncover the physiological characters of senescent cells and their contributions to aging and cancers *in vivo*.

2. p53 is essential for acquired stress resistance through upregulating basal autophagy

Yoshikazu Johmura, Narumi Suzuki, Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Dan Li, Wang Tehwei, Takehiro Yamanaka, Satotaka Ohmori, Tomomi Kanai, Akane Kenjo, Yue Zhang, Tamotsu Yoshimori[1], Tomohiko Ohta[2], and Makoto Nakanishi:
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Acquired stress resistance means that preconditioning to a low dose of stressors protects against a higher and harmful dose of the same or related insults. These responses are common phenomena in a broad range of yeast, plant, and animal models, and could be induced by a plethora of insults. For example, it is well known that dietary approaches, such as fasting or FMDs (fasting mimicking diets), have the potential to promote the protection of normal cells against chemotherapy, radiotherapy, and other treatments. However, despite of its widespread generality and involvement in a variety of biological processes, such as aging and evolution, the molecular mechanisms underlying are largely unknown.

Macroautophagy (hereafter referred to as autophagy) is one of the intracellular degradative processes through which knackered or damaged cytoplasmic materials including organelles and proteins are degraded within lysosomes. In addition to the function as a cleaner inside the cell, autophagy also plays a role in supplying nutrition and materials for cell survival under harmful conditions such as starvation. Although these two functions of autophagy as a cleaner and a supplier might fit the concept of "induced autophagy" and "basal autophagy", respectively, the physiological situation regulated by autophagy is not simple but complicated. For example, autophagy functions as both self-protecting and cell death-inducing mechanisms depending on the context. One such reason is that the factors regulating the level of basal autophagy are largely unexplored whereas those regulating induced autophagy are relatively well investigated. Nevertheless, there is an increasing awareness of the relationship between acquired resistance and autophagy because autophagy is an adaptive and pro-survival mechanism against various insults.

We demonstrate that p53 plays an essential role in this process through upregulating the level of basal autophagy. Activated p53 by preconditioning transcriptionally induces Fbxo22, which in turn upregulates transcription of TFEB through degradation of KDM4B within Myc-N-CoR suppressor complexes. Mitogen-induced AKT activation inhibits this process through phosphorylation of KDM4B. Fbxo22$^{-/-}$ mice appear die within 10 hours of delivery, presumably due to a very low level of basal autophagy. Fbxo22 transgenic mice driven by CAG promoter were highly resistant to a harmful insult without preconditioning, which was reversed by the treatment with autophagy inhibitor. Taken together, these results suggest that p53 determines the level of basal autophagy through Fbxo22 and is essential for acquired resistance to various stimli.

3. Regulation of maintenance DNA methylation by dual-mono ubiquitylated PAF15

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DNA cytosine methylation is a conserved epigenetic modification essential for embryonic development, transcriptional regulation, and genome stability. In higher eukaryotes, individual differentiated cells possess unique DNA methylation patterns that determine their own cellular phenotypes. Therefore, the DNA methylation pattern must be precisely maintained in coordination with DNA replication during S phase. We and others have recently reported that UHRF1-mediated dual mono-ubiquitylation of his-

tone H3 (H3Ub2) on lysine residues 14, 18 and 23 plays a role in the recruitment of DNMT1 and its enzymatic activation, ensuring the high fidelity of maintenance DNA methylation. We have recently identified another mechanism coupled with DNA replication machinery for the recruitment of DNMT1 to replicating chromatin in which UHRF1-mediated dual mono-ubiquitylation of PCNA-associated factor 15 (PAF15) plays an essential role. In *Xenopus* cell-free extracts, PAF15 accumulates on chromatin with dual mono-ubiquitylation (PAF15Ub2) in a PCNA-, UHRF1- and DNA replication-dependent manner. PAF15Ub2 interacts with DNMT1 with a structural mode similar to that of H3Ub2. Suppression of DNMT1 interaction with H3Ub2 in the extract shows that PAF15 and histone H3 have non-redundant roles in the recruitment of DNMT1 and subsequent DNA methylation. Consistent with this, in mammals, PAF15 is also subjected to UHRF1-dependent dual mono-ubiquitylation and is capable of binding to DNMT1. Mouse embryonic stem cells expressing PAF15 carrying substitutions of lysine to arginine at ubiquitylation sites demonstrate a marked reduction in the DNA methylation level. Together, our study reveals that PAF15 and histone H3 have non-redundant roles in the regulation of DNA methylation maintenance.

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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 learning 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. Behavioral abnormalities and impairments in GluA1 trafficking in Lmtk3 KO mice

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Accumulating evidence suggests that glutamatergic signaling and synaptic plasticity underlie one of a number of ways psychiatric disorders appear. The present study reveals a possible mechanism by which this occurs through highlighting the importance of lemur tyrosine kinase 3 (LMTK3) in the brain. Behavioral analysis of Lmtk3 knockout (KO) mice revealed a number of abnormalities that have been linked to psychiatric disease such as hyper-sociability, deficits in pre-pulse inhibition and cognitive dysfunction. Treatment with the antipsychotic clozapine suppressed these behavioral changes in Lmtk3 KO mice. As synaptic dysfunction is implicated in human psychiatric disease, we analyzed long-term potentiation (LTP) of excitatory synaptic transmission in Lmtk3 KO mice and found that its induction was severely impaired. Further investigation revealed abnormalities in trafficking of the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)-receptor subunit GluA1 after AMPA stimulation in Lmtk3 KO neurons, along with a reduction in GluA1 expression in the postsynaptic density. Therefore, we hypothesize that LMTK3 is an important factor involved in the trafficking of GluA1 during LTP, and that disruption of this pathway contributes to the appearance of behavior associated with human psychiatric disease in mice.

2. Analyses of the vanishing white matter disease model mouse

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Vanishing white matter disease (VWM) is an auto-

somal recessive neurological disorder caused by mutation(s) in any subunit of eukaryotic translation initiation factor 2B (eIF2B), an activator of translation initiation factor eIF2. VWM occurs with mutation of the genes encoding eIF2B subunits (EIF2B1, EIF2B2, EIF2B3, EIF2B4 and EIF2B5). However, little is known regarding the underlying pathogenetic mechanisms or how to treat patients with VWM. Here, we describe the identification and detailed analysis of a new spontaneous mutant mouse harboring a point mutation in the *Eif2b5* gene (p.Ile98Met). Homozygous Eif-2b5I98M mutant mice exhibited a small body, abnormal gait, male and female infertility, epileptic seizures and a shortened lifespan. Biochemical analyses indicated that guanine nucleotide exchange activity on eIF2 was decreased in the mutant eIF2B protein with the Eif2b5I98M mutation, and the level of the endoplasmic reticulum stress marker activating transcription factor 4 was elevated in the 1-month-old Eif-2b5I98M brain. Histological analyses indicated upregulated immunoreactivity of glial fibrillary acidic protein in the astrocytes of the Eif2b5I98M forebrain and translocation of Bergmann glia in the Eif-2b5I98M cerebellum, as well as increased mRNA expression of an endoplasmic reticulum stress marker, C/EBP homologous protein. Disruption of myelin and clustering of oligodendrocyte progenitor cells were also found in the white matter of the Eif2b5I98M spinal cord at 8 months old. Our data show that Eif-2b5I98M mutants are a good model for understanding VWM pathogenesis and therapy development.

3. Store-operated Ca^{2+} entry and insulin secretion mediated by GPR40 via the IP3R1/STIM1/Orai1 pathway in pancreatic β-cells

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The long-chain fatty acid receptor GPR40 plays an important role in potentiation of glucose-induced insulin secretion (GIIS) from pancreatic β-cells. Previous studies demonstrated that GPR40 activation enhances Ca^{2+} release from the endoplasmic reticulum (ER) by activating inositol 1,4,5-triphosphate (IP3) receptors. However, it remains unknown how ER Ca^{2+} release via the IP3 receptor is linked to GIIS potentiation. Recently, stromal interaction molecule (STIM) 1 was identified as a key regulator of store-operated Ca^{2+} entry (SOCE), but little is known about its contribution in GPR40 signaling. We show that GPR40-mediated potentiation of GIIS is abolished by knockdown of IP3 receptor 1 (IP3R1), STIM1 or Ca^{2+}-channel Orai1 in insulin-secreting MIN6 cells. STIM1 and Orai1 knockdown significantly impaired SOCE and the increase of intracellular Ca^{2+} by the GPR40 agonist fasiglifam. Furthermore, β-cell-specific STIM1 knockout mice showed impaired fasiglifam-mediated GIIS potentiation not only in isolated islets but also in vivo. These results indicate that the IP3R1/STIM1/Orai1 pathway plays an important role in GPR40-mediated SOCE initiation and GIIS potentiation in pancreatic β-cells.

Publications

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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. Epigenetic regulation of tissue-specific gene expression during embryonic lung development by a CtBP regulator MCRIP1.

Jane S. Weng, Takanori Nakamura, Hisashi Moriizumi, Hiroshi Takano[1], Ryoji Yao[1], and Mutsuhiro Takekawa: [1] Division of Cell Biology, Cancer Institute, Japanese Foundation for Cancer Research

Proper regulation of epigenetic states of chromatin is crucial to achieve tissue-specific gene expression during embryogenesis. The lung-specific gene products, surfactant proteins B (SP-B) and C (SP-C), are synthesized in alveolar epithelial cells and prevent alveolar collapse. Epigenetic regulation of these surfactant proteins, however, remains unknown. In vertebrates, the C-terminal-binding protein (CtBP) family proteins, CtBP1 and CtBP2, serve as core components of a nuclear transcriptional co-repressor complex that contains chromatin-modifying enzymes such as histone deacetylases and histone methyltransferases. Although CtBP proteins do not themselves directly bind to DNA, they can be recruited to promoter regions by interacting with DNA-binding transcriptional repressors that possess a consensus CtBP-binding motif (PxDLS or similar sequences). Thus, CtBP proteins form a bridge between these transcriptional repressors and chromatin-modifying enzymes, thereby altering the pattern of histone modifications to generate a repressive chromatin structure in the promoters of target genes. We have recently identified the MAPK-regulated co-repressor interacting protein 1 (MCRIP1) as a regulator of the co-repressor function of CtBP. MCRIP1 is a relatively small protein of 97 amino acids that possesses no other known structural domain apart from the C-terminal CtBP-binding motif (PxDLS). We demonstrated that MCRIP1 bound to CtBP, thereby competitively inhibiting the interaction of CtBP with ZEB1 in human epithelial MCF-10A cells and several other cell types. Thus, MCRIP1 prevents CtBP-mediated transcriptional repression of the ZEB1-target genes such as E-cadherin. As CtBP is involved in various biological phenomena, MCRIP1-mediated regulation of CtBP might also impact on a broad range of biological processes. However, the role of MCRIP1 in embryonic

development is totally unknown.

This year, to uncover the physiological function of MCRIP1 in vivo, we generated *Mcrip1*-knockout (KO) mice. The *Mcrip1*-KO mice died shortly after birth owing to respiratory failure that resembles human infant respiratory distress syndrome (RDS). Analysis of the neonatal mice revealed that the levels of the pulmonary surfactant proteins, SP-B and SP-C, which are essential for proper inflation of alveoli, were decreased in *Mcrip1*-KO lungs. We showed that MCRIP1 is highly expressed in lung epithelium during alveolar sac formation, and interferes with interactions of CtBP with the lung-enriched transcriptional repressors, Foxp1 and Foxp2, thereby preventing the recruitment of the CtBP co-repressor complex to the SP-B and SP-C promoters and maintaining them in an active chromatin state. Thus, MCRIP1 promotes the expression of the surfactant proteins by disrupting the CtBP1/2 and Foxp1/2-mediated epigenetic gene silencing in embryonic lungs. Our results demonstrated the critical role of MCRIP1 in lung development and function, and delineated a molecular mechanism that prevents cells from inappropriate epigenetic gene silencing to generate tissue-specific gene expression during organogenesis.

2. Role of stress granule assembly in cellular stress response and in carcinogenesis.

Daisuke Yoshioka, 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 a strategy to identify the molecules that reside in SGs, we discovered novel SG-components including nucleotide-binding proteins, cytoskeletal proteins, and signaling molecules (e.g., kinases, scaffold proteins, and transcriptional regulators). 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 development and progression of human cancers.

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

Seina Oe, Natusno Suzuki, Sho Tanaka, Yuji Kubota, and Mutsuhiro Takekawa

Sequential activation of protein kinases within MAPK cascades is an evolutionary-conserved mechanism of intracellular signaling among eukaryotes. 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 growth factors and mitogens, two relatively newly identified MAPKs, p38 and JNK, are preferentially activated by various environmental stresses (e.g., ultraviolet-light and g irradiation, oxidative stress, DNA-damaging reagents, osmotic stress, and pro-inflammatory cytokines). 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 crucial 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 substrate proteins, the identification of which is a prerequisite for

the understanding of regulatory mechanisms of critical biological phenomena. By developing a novel screening strategy using yeast Saccharomyces cerevisiae, we have isolated several new human MAPK substrate proteins from cDNA libraries. These substrates include regulatory molecules for the expression of growth-promoting genes and for centrosome duplication, and several Ser/Thr protein kinases that regulate inflammation and cell death. We confirmed that these molecules were indeed directly phosphorylated by one (or more) of the human MAPKs *in vitro* as well as *in vivo* in response to mitogenic and/or stress stimuli. Thus, these molecules are bona fide substrates of MAPKs. The biological functions of these novel substrate proteins are currently under investigation in our laboratory.

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

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

Mammalian cells are frequently exposed to a variety of environmental stresses, such as ultraviolet rays, ionizing radiation, genotoxins, and oxidative stress. In coping with the barrage of these and other stresses, multi-cellular eukaryotic organisms have developed a strategy as to how damaged cells will respond to stresses. In general, if the intensity of the damage is moderate, the cell will seek to repair the damage. If, however, the damage to a cell is too severe to be repaired, the affected cells are eliminated by apoptosis. This cell death reduces the risk to the organism as a whole, such as development of a cancer. Such a crucial decision between survival and death is, at least in part, mediated by the stress-activated p38 and JNK MAPK pathways (collectively called SAPK pathways). SAPKs are a group of serine/threonine protein kinases that convert extracellular stress stimuli into diverse cellular responses, including apoptotic cell death and cytokine production, through phosphorylation of specific target proteins. Perturbation of these critical signaling systems is involved in a variety of life-threatening diseases. Therefore, these signaling systems are of clinical importance. Recent progress in the identification of molecules that participate in the SAPK pathways has provided new insights, not only into the molecular basis of the cellular response to environmental stress, but also into the etiology of human diseases including cancer and inflammatory diseases.

The MTK1 MAPKKK is a selective mediator for SAPK signaling. Although its C-terminal kinase domain is homologous to other stress-activated MAPKKKs, its N-terminal regulatory domain has only limited similarity, suggesting that the regulatory mechanism of MTK1 is unique. Indeed, we have previously identified three GADD45 family proteins (GADD45a/b/g) as activators of MTK1. All GADD45 family genes are induced by various stress stimuli such as UV irradiation and DNA-damaging reagents, although the optimal stress stimuli for each gene are different. Enforced 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 under various stress conditions, and uncovered novel roles of MTK1 in DNA-damage response, immune reaction, 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.

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

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. It is well known that MAPK signaling pathways 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 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.

6. Regulation and role of protein O-GlcNAc modification Development of a novel method for the detection of O-GlcNAc-modified proteins by lectin affinity gel electrophoresis.

Hitomi Seki, Yuji Kubota, and Mutsuhiro Takekawa

O-linked β-N-acetyl-glucosamine modification (O-GlcNAcylation) is a relatively recently identified post-translational modification (PTM), in which a single-sugar N-acetylglucosamine (GlcNAc) is conjugated to the hydroxyl moiety of a serine or a threonine residue of cytoplasmic and nuclear proteins. Accumulating evidence has shown that O-GlcNAcylation affects various properties of proteins, including stability, subcellular localization, and catalytic activity, thereby regulating a wide range of biological processes. So far, it is well known that only two enzymes, i.e., O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), regulate this PTM. OGT catalyzes the addition of a GlcNAc moiety from a uridine diphosphate (UDP)-GlcNAc to target proteins, while OGA removes O-GlcNAc from the modified proteins. Thus, these two enzymes synergistically make O-GlcNAcylation a reversible and dynamic process. Interestingly, since O-GlcNAcylation as well as phosphorylation occurs at serine and threonine residues, these two types of PTMs are mutually exclusive on the same target site. Indeed, dynamic crosstalk between O-GlcNAcylation and phosphorylation has been demonstrated in various proteins that regulate critical biological signaling pathways. Despite recent advances in understanding the importance of O-GlcNAcylation in diverse physiological processes, regulatory mechanisms of protein O-GlcNAcylation remain to be elucidated.

We recently developed a novel electrophoretic method, termed wheat germ agglutinin (WGA)-SDS-PAGE, which separates O-GlcNAcylated and unmodified proteins, and enables the detection and quantification of O-GlcNAcylated proteins. Electrophoresis of cell lysates through a gel containing copolymerized WGA, a lectin from *Triticum vulgaris*, selectively induced retardation of the mobility of O-GlcNAcylated proteins, thereby allowing the simultaneous visualization of both the O-GlcNAcylated and the unmodified forms of proteins. This year, by using this method as well as comprehensive proteomic approaches, we successfully identified dozens of molecules that selectively interact with the O-GlcNAc modification enzymes in cells. The roles of these molecules in the regulation of protein O-GlcNAc modification are currently under investigation in our laboratory.

Publications

Weng JS, Nakamura T, Moriizumi H, Takano H, Yao R, and Takekawa M. MCRIP1 promotes the expression of lung-surfactant proteins in mice by disrupting CtBP-mediated epigenetic gene silencing. Commun Biol 2, Article No. 227 doi: 10.1038/s42003-019-0478-3, (2019)

久保田裕二，藤岡興，武川睦寛　O-GlcNAc化蛋白質の検出と定量的解析を可能にするレクチン親和性ゲル電気泳動法の開発と応用.生物物理化学　電気泳動63，41−45.

Human Genome Center

Laboratory of DNA Information Analysis
Laboratory of Sequence Analysis
Laboratory of Genome Database

DNA 情報解析分野
シークエンスデータ情報処理分野
ゲノムデータベース分野

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We are facing with biomedical big data comprising of ultra-high dimensional ultra-heterogeneous data. Our current mission is to develop computational/informatics strategy for medical informatics to implement personalized genomic medicine through genomics, systems biology and supercomputer.

1. Pan-Cancer Analysis of Whole Genomes

Pan-Cancer Analysis of Whole Genomes Consortium (Collaborators (1341)

Cancer is driven by genetic change, and the advent of massively parallel sequencing has enabled systematic documentation of this variation at the whole-genome scale. 1341 collaborators worked for the integrative analysis of 2,658 whole-cancer genomes and their matching normal tissues across 38 tumour types from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA). We describe the generation of the PCAWG resource, facilitated by international data sharing using compute clouds. 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. Chromothripsis, in which many clustered structural variants arise in a single catastrophic event, is frequently an early event in tumour evolution; in acral melanoma, for example, these events precede most somatic point mutations and affect several cancer-associated genes simultaneously. Cancers with abnormal telomere maintenance often originate from tissues with low replicative activity and show several mechanisms of preventing telomere attrition to criti-

cal levels. Common and rare germline variants affect patterns of somatic mutation, including point mutations, structural variants and somatic retrotransposition. A collection of papers from the PCAWG Consortium describes non-coding mutations that drive cancer beyond those in the TERT promoter; identifies new signatures of mutational processes that cause base substitutions, small insertions and deletions and structural variation; analyses timings and patterns of tumour evolution; describes the diverse transcriptional consequences of somatic mutation on splicing, expression levels, fusion genes and promoter activity; and evaluates a range of more-specialized features of cancer genomes

The supercomputer SHIROKANE of Human Genome Center performed computational analyses as one of the six centers for this project.

2. Systems Cancer Research and Systems Biology

a. Divergent lncRNA MYMLR regulates MYC by eliciting DNA looping and promoter-enhancer interaction

Kajino T[1], Shimamura T[1], Gong S[1], Yanagisawa K[1], Ida L[1], Nakatochi M[1], Griesing S[1], Shimada Y[1], Kano K[1], Suzuki M[1], Miyano S, Takahashi T[1,2]; [1]Nagoya University, [2]Aichi Cancer Center

Long non-coding RNAs (lncRNAs) function in a wide range of processes by diverse mechanisms, though their roles in regulation of oncogenes and/or tumor suppressors remain rather elusive. We performed a global search for lncRNAs affecting MYC activity using a systems biology-based approach with a K supercomputer and the GIMLET algorism based on local distance correlations. Consequently, *MYMLR* was identified and experimentally shown to maintain MYC transcriptional activity and cell cycle progression despite the low levels of expression. A proteomic search for *MYMLR*-binding proteins identified PCBP2, while it was also found that *MYMLR* places a 557-kb upstream enhancer region in the proximity of the *MYC* promoter in cooperation with PCBP2. These findings implicate a crucial role for *MYMLR* in regulation of the archetypical oncogene *MYC* and warrant future studies regarding the involvement of low copy number lncRNAs in regulation of other crucial oncogenes and tumor suppressor genes.

K computer at RIKEN performed the data analysis.

b. Virtual Grid Engine: a simulated grid engine environment for large-scale supercomputers

Ito S, Yadome M, Nishiki T[3], Ishiduki S[3], Inoue H[3], Yamaguchi R[2], Miyano S; [3]Fujitsu Limited

Supercomputers have become indispensable infrastructures in science and industries. In particular, most state-of-the-art scientific results utilize massively parallel supercomputers ranked in TOP500. However, their use is still limited in the bioinformatics field due to the fundamental fact that the asynchronous parallel processing service of Grid Engine is not provided on them. To encourage the use of massively parallel supercomputers in bioinformatics, we developed middleware called Virtual Grid Engine, which enables software pipelines to automatically perform their tasks as MPI programs.

We conducted basic tests to check the time required to assign jobs to workers by VGE. The results showed that the overhead of the employed algorithm was 246 microseconds and our software can manage thousands of jobs smoothly on the K computer. We also tried a practical test in the bioinformatics field. This test included two tasks, the split and BWA alignment of input FASTQ data. 25,055 nodes (2,000,440 cores) were used for this calculation and accomplished it in three hours.

We considered that there were four important requirements for this kind of software, non-privilege server program, multiple job handling, dependency control, and usability. We carefully designed and checked all requirements. And this software fulfilled all the requirements and achieved good performance in a large scale analysis.

c. A Bayesian model integration for mutation calling through data partitioning

Moriyama T, Imoto S[4], Hayashi S, Shiraishi Y[5], Miyano S, Yamaguchi R; [4]Health Intelligence Center, [5]National Cancer Center

Detection of somatic mutations from tumor and matched normal sequencing data has become among the most important analysis methods in cancer research. Some existing mutation callers have focused on additional information, e.g. heterozygous single-nucleotide polymorphisms (SNPs) nearby mutation candidates or overlapping paired-end read information. However, existing methods cannot take multiple information sources into account simultaneously. Existing Bayesian hierarchical model-based methods construct two generative models, the tumor model and error model, and limited information sources have been modeled.

We proposed a Bayesian model integration framework named as partitioning-based model integration. In this framework, through introducing partitions for paired-end reads based on given information sources, we integrate existing generative models and utilize multiple information sources. Based on that, we constructed a novel Bayesian hierarchical model-based method named as OHVarfinDer. In both the tumor model and error model, we introduced partitions for a set of paired-end reads that cover a mutation candi-

date position, and applied a different generative model for each category of paired-end reads. We demonstrated that our method can utilize both heterozygous SNP information and overlapping paired-end read information effectively in simulation datasets and real datasets. The software application is available from https: //github.com/takumorizo/OHVarfinDer.

d. Accurate and flexible Bayesian mutation call from multi-regional tumor samples

Moriyama T, Imoto S[4], Miyano S, Yamaguchi R[2]

We propose a Bayesian method termed MultiMuC for accurate detection of somatic mutations (mutation call) from multi-regional tumor sequence data sets. To improve detection performance, our method is based on the assumption of mutation sharing: if we can predict at least one tumor region has the mutation, then we can be more confident to detect a mutation in more tumor regions by lowering the original threshold of detection. We find two drawbacks in existing methods for leveraging the assumption of mutation sharing. First, existing methods do not consider the probability of the "No-TP (True Positive)" case: we could expect mutation candidates in multiple regions, but actually, no true mutations exist. Second, existing methods cannot leverage scores from other state-of-the-art mutation calling methods for a single-regional tumor. We overcome the first drawback through evaluation of the probability of the No-TP case. Next, we solve the second drawback by the idea of Bayes-factor-based model construction that enables flexible integration of probability-based mutation call scores as building blocks of a Bayesian statistical model. We empirically evaluate that our method steadily improves results from mutation calling methods for a single-regional tumor, e.g., Strelka2 and NeuSomatic, and outperforms existing methods for multi-regional tumors through a real-data-based simulation study. Our implementation of MultiMuC is available at https: //github.com/takumorizo/MultiMuC.

e. ALPHLARD-NT: Bayesian method for human leukocyte antigen genotyping and mutation calling through simultaneous analysis of normal and tumor whole-genome sequence data

Hayashi S, Moriyama T, Yamaguchi R, Mizuno S[6], Komura M, Miyano S, Nakagawa H[7], Imoto S[4]; [6]Kyusu University, [7]RIKEN

Human leukocyte antigen (HLA) genes provide useful information on the relationship between cancer and the immune system. Despite the ease of obtaining these data through next-generation sequencing methods, interpretation of these relationships remains challenging owing to the complexity of HLA genes. To resolve this issue, we developed a Bayesian method, ALPHLARD-NT, to identify HLA germline and somatic mutations as well as HLA genotypes from whole-exome sequencing (WES) and whole-genome sequencing (WGS) data. ALPHLARD-NT showed 99.2% accuracy for WGS-based HLA genotyping and detected five HLA somatic mutations in 25 colon cancer cases. In addition, ALPHLARD-NT identified 88 HLA somatic mutations, including recurrent mutations and a novel HLA-B type, from WES data of 343 colon adenocarcinoma cases. These results demonstrate the potential of ALPHLARD-NT for conducting an accurate analysis of HLA genes even from low-coverage data sets. This method can become an essential tool for comprehensive analyses of HLA genes from WES and WGS data, helping to advance understanding of immune regulation in cancer as well as providing guidance for novel immunotherapy strategies.

3. Cancer Genomics

a. Frequent mutations that converge on the NFKBIZ pathway in ulcerative colitis

Kakiuchi N[8], Yoshida K[8], Uchino M[9], Kihara T[9], Akaki K[8], Inoue Y[8], Kawada K[8], Nagayama S[10], Yokoyama A[8], Yamamoto S[8], Matsuura M[8], Horimatsu T[8], Hirano T[8], Goto N[8], Takeuchi Y[8], Ochi Y[8], Shiozawa Y[8], Kogure Y[8], Watatani Y[8], Fujii Y[8], Kim SK[8], Kon A[8], Kataoka K[8], Yoshizato T[8], Nakagawa MM[8], Yoda A[8], Nanya Y[8], Makishima H[8], Shiraishi Y[5], Chiba K[5], Tanaka H, Sanada M[8], Sugihara E[11], Sato TA[11], Maruyama T[12], Miyoshi H[8], Taketo MM[8], Oishi J[13], Inagaki R[8], Ueda Y[13], Okamoto S[8], Okajima H[8], Sakai Y[8], Sakurai T[8], Haga H[8], Hirota S[9], Ikeuchi H[9], Nakase H[8], Marusawa H[8], Chiba T[8], Takeuchi O[8], Miyano S, Seno H[8], Ogawa S[8,14]; [8]Kyoto University, [9]Hyogo College of Medicine, [10]Japanese Foundation for Cancer Research, [11]University of Tsukuba, [12]Akita University, [13]Sumitomo Dainippon Pharma, [14]Karolinska Institute

Clonal expansion in aged normal tissues has been implicated in the development of cancer. However, the chronology and risk dependence of the expansion are poorly understood. Here we intensively sequence 682 micro-scale oesophageal samples and show, in physiologically normal oesophageal epithelia, the progressive age-related expansion of clones that carry mutations in driver genes (predominantly *NOTCH1*), which is substantially accelerated by alcohol consumption and by smoking. Driver-mutated clones emerge multifocally from early childhood and increase their number and size with ageing, and ultimately replace almost the entire oesophageal epithelium in the extremely elderly. Compared with mutations in oesophageal cancer, there is a marked overrepresentation of *NOTCH1* and *PPM1D* mutations in physiologically normal oesophageal epithe-

lia; these mutations can be acquired before late adolescence (as early as early infancy) and significantly increase in number with heavy smoking and drinking. The remodelling of the oesophageal epithelium by driver-mutated clones is an inevitable consequence of normal ageing, which-depending on lifestyle risks-may affect cancer development.

Our DNA and RNA-sequence analysis pipe line Genomon (https: //github.com/Genomon-Project) on HGC supercomputer SHIROKANE played an important role in this study. We contributed to sequence data analysis and statistical methodology development using HGC supercomputer SHIROKANE.

b. Defective Epstein-Barr virus in chronic active infection and haematological malignancy

Okuno Y[15], Murata T[16], Sato Y[16], Muramatsu H[16], Ito Y[16], Watanabe T[16], Okuno T[16], Murakami N[16], Yoshida K[8], Sawada A[17], Inoue M[17], Kawa K[17], Seto M[18], Ohshima K[18], Shiraishi Y[5], Chiba K[5], Tanaka H, Miyano S, Narita Y[16], Yoshida M[16], Goshima F[16], Kawada JI[16], Nishida T[16], Kiyoi H[16], Kato S[15], Nakamura S[15], Morishima S[19], Yoshikawa T[20], Fujiwara S[21], Shimizu N[22], Isobe Y[23], Noguchi M[24], Kikuta A[25], Iwatsuki K[26], Takahashi Y[16], Kojima S[16], Ogawa S[8], Kimura H[16]; [15]Nagoya University Hospital, [16]Nagoya University Graduate School of Medicine, [17]Osaka Women's and Children's Hospital, [18]Kurume University School of Medicine, [19]University of the Ryukyus, [20]Fujita Health University School of Medicine, [21]National Research Institute for Child Health and Development [22]Tokyo Medical and Dental University, [23]St. Marianna University School of Medicine, [24]Juntendo University Urayasu Hospital, [25]Fukushima Medical University School of Medicine, [26]Okayama University Graduate School of Medicine

Epstein-Barr virus (EBV) infection is highly prevalent in humans and is implicated in various diseases, including cancer. Chronic active EBV infection (CAEBV) is an intractable disease classified as a lymphoproliferative disorder in the 2016 World Health Organization lymphoma classification. CAEBV is characterized by EBV-infected T/natural killer (NK) cells and recurrent/persistent infectious mononucleosis-like symptoms. Here, we show that CAEBV originates from an EBV-infected lymphoid progenitor that acquires DDX3X and other mutations, causing clonal evolution comprising multiple cell lineages. Conspicuously, the EBV genome in CAEBV patients harboured frequent intragenic deletions (27/77) that were also common in various EBV-associated neoplastic disorders (28/61), including extranodal NK/T-cell lymphoma and EBV-positive diffuse large B-cell lymphoma, but were not detected in infectious mononucleosis or post-transplant lymphoproliferative disorders (0/47), which suggests a unique role of these mutations in neoplastic proliferation of EBV-infected cells. These deletions frequently affected *Bam*HI A rightward transcript microRNA clusters (31 cases) and several genes that are essential for producing viral particles (20 cases). The deletions observed in our study are thought to reactivate the lytic cycle by upregulating the expression of two immediate early genes, *BZLF1* and *BRLF1*, while averting viral production and subsequent cell lysis. In fact, the deletion of one of the essential genes, *BALF5*, resulted in upregulation of the lytic cycle and the promotion of lymphomagenesis in a xenograft model. Our findings highlight a pathogenic link between intragenic EBV deletions and EBV-associated neoplastic proliferations.

Our DNA and RNA-sequence analysis pipe line Genomon on HGC supercomputer SHIROKANE played an important role in this study. We contributed to sequence data analysis and statistical methodology development using HGC supercomputer SHIROKANE.

c. Applications of Genomon for Cancer Genomics

All laboratory members and many collaborators

We have been developing an omics analysis pipeline Genomon for analyzing genome sequence data including RNA sequences. By collaborations with many cancer researchers, we contributed to data analyses using the supercomputer at Human Genome Center and K computer at RIKEN. Due to the limit of space, we list up our contributed papers: 2-3, 5, 8-11, 14, 20-24, 26, 28-32, 34, 36, 39, 43-47.

4. Contributions by System for Cancer Clinical Sequencing

We have developed a system for cancer clinical sequencing using HGC supercomputer and Genomon, and have been contributing to cancer genomic medicine at IMSUT Research Hospital.

a. Prognostic impact of circulating tumor DNA status post-allogeneic hematopoietic stem cell transplantation in AML and MDS

Nakamura S[27], Yokoyama K[27, 28], Shimizu E, Yusa N[28], Kondoh K[27], Ogawa M[27], Takei T[27], Kobayashi A[27], Ito M[27], Isobe M[27, 28], Konuma T[28], Kato S[28], Kasajima R[4], Wada Y[28], Nagamura-Inoue T6, Yamaguchi R, Takahashi S[27, 28], Imoto S[4], Miyano S, Tojo A[27, 28]; [27]Advanced Clinical Research Center, [28]Research Hospital

This study was performed to assess the utility of tumor-derived fragmentary DNA, or circulating tumor DNA (ctDNA), for identifying high-risk patients

for relapse of acute myeloid leukemia and myelodysplastic syndrome (AML/MDS) after undergoing myeloablative allogeneic hematopoietic stem cell transplantation (alloSCT). We retrospectively collected tumor and available matched serum samples at diagnosis and 1 and 3 months post-alloSCT from 53 patients with AML/MDS. After identifying driver mutations in 51 patients using next-generation sequencing, we designed at least 1 personalized digital polymerase chain reaction assay per case. Diagnostic ctDNA and matched tumor DNA exhibited excellent correlations with variant allele frequencies. Sixteen patients relapsed after a median of 7 months post-alloSCT. Both mutation persistence (MP) in bone marrow (BM) at 1 and 3 months post-alloSCT and corresponding ctDNA persistence (CP) in the matched serum (MP1 and MP3; CP1 and CP3, respectively) were comparably associated with higher 3-year cumulative incidence of relapse (CIR) rates (MP1 vs non-MP1, 72.9% vs 13.8% [P = .0012]; CP1 vs non-CP1, 65.6% vs 9.0% [P = .0002]; MP3 vs non-MP3, 80% vs 11.6% [P = .0002]; CP3 vs non-CP3, 71.4% vs 8.4% [P < .0001]). We subsequently evaluated whether subset analysis of patients with 3 genes associated with clonal hematopoiesis, *DNMT3A*, *TET2*, and *ASXL1* (DTA), could also be helpful in relapse prediction. As a result, CP based on DTA gene mutations also had the prognostic effect on CIR. These results, for the first time, support the utility of ctDNA as a noninvasive prognostic biomarker in patients with AML/MDS undergoing alloSCT.

b. The first case of elderly TCF3-HLF-positive B-cell acute lymphoblastic leukemia

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A 67-year-old man, who complained of persistent fever and general fatigue, was referred to our hospital. Peripheral blood examination showed that white blood cell (WBC) count was elevated to 9.2 × 10^9/L, with 27.5% myeloperoxidase-negative blasts. Anemia (hemoglobin, 9.2 g/dL) and thrombocytopenia (platelets, 43 × 10^9/L) were also observed. Lactate dehydrogenase was elevated to approximately four times the upper normal limit. The patient also presented with severe DIC, hypercalcemia, and mild renal dysfunction. Computed tomography (CT) revealed mild generalized lymphadenopathy without hepatosplenomegaly and osteolytic changes in bones. Bone marrow (BM) aspiration showed infiltration of myeloperoxidase-negative blasts in 86% of the counted nuclear cells. Flowcytometric analysis showed that these blast cells were positive for CD10, CD19, cytoplasmic CD79a, TdT, CD13, CD33, and HLA-DR. Cytogenetic analysis identified the chromosomal translocation t(17; 19)(q22; p13). Transcription of chimeric *TCF3-HLF* in leukemia cells was detected by reverse transcription polymerase chain reaction (RT-PCR). Hence, the patient was diagnosed with *TCF3-HLF*-positive B-ALL. He initially received standard chemotherapy for adult B-ALL, containing daunorubicin, cyclophosphamide, vincristine (VCR), L-asparaginase, and prednisolone. After the chemotherapy was administered, the leukemia cell count decreased rapidly, accompanied by amelioration of DIC and hypercalcemia. However, the response was transient, resulting in primary induction failure. The leukemia cells regrew with exacerbation of the complications. Four additional lines of conventional chemotherapies also failed to achieve durable remission. Thus, this case of leukemia appeared refractory to agents including glucocorticoids, L-asparaginase, anthracyclines, cyclophosphamide, VCR, cytarabine (Ara-C), etoposide, and methotrexate (MTX). At the end of the fifth chemotherapy, the patient lost consciousness due to intracerebral hemorrhage, followed by drastic growth of the leukemia cells and DIC. The patient died of leukemia five months after the initial diagnosis.

c. Development of an MSI-positive colon tumor with aberrant DNA methylation in a PPAP patient

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Polymerase proofreading-associated polyposis (PPAP) is a disease caused by germline variations in the *POLE* and *POLD1* genes that encode catalytic subunits of DNA polymerases. Studies of cancer genomes have identified somatic mutations in these genes, suggesting the importance of polymerase proofreading of DNA replication in suppressing tumorigenesis. Here, we identified a germline frameshift variation in the *POLE* gene (c.4191_4192delCT, p.Tyr1398*) in a case with multiple adenomatous polyps and three synchronous colon cancers. Interestingly, one of the colon cancers showed microsatellite instability-high (MSI-H) and another microsatellite stable. Immunohistochemical staining revealed that the MSI-H tumor cells lost the expression of MLH1 protein. Whole genome sequencing of the MSI-H tumor did not find pathogenic somatic mutations in mismatch repair genes but found frameshift mutations in the *TET* genes that catalyze 5-methylcytosine hydroxylation. Bisulfite sequencing of the tumor corroborated an increase in the number of hypermethylated regions including the *MLH1* promoter. These data indicate that PPAP patients might develop MSI-positive tumors

through epigenetic silencing of *MLH1*. These findings will contribute to comprehensive understanding of the molecular basis of tumors that involve deficiency of proofreading activity of DNA polymerases.

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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. A unified simulation model for understanding the diversity of cancer evolution

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Because cancer evolution underlies the therapeutic difficulties of cancer, it is clinically important to understand cancer's evolutionary dynamics. Thus far, a number of evolutionary processes have been proposed to be working in cancer evolution. However, no simulation model exists that can describe the different evolutionary processes in a unified manner. In this study, we constructed a unified simulation model for describing the different evolutionary processes and performed sensitivity analysis on the model to determine the conditions in which cancer growth is driven by each of the different evolutionary processes. Our sensitivity analysis has successfully provided a series of novel insights into cancer's evolutionary dynamics. For example, we found that, while a high neutral mutation rate shapes neutral intratumor heterogeneity (ITH) characterized by a fractal-like pattern, a stem cell hierarchy can also contribute to shaping the neutral ITH by apparently increasing the mutation rate. Although It has been reported that the evolutionary principle shaping ITH shifts from selection to accumulation of neutral mutations during colorectal tumorigenesis, our simulation demonstrated the possibility that this evolutionary shift arises from punctuated evolution, which is triggered by drastic evolutionary events conferring a marked fitness increase on one or a few cells. This result helps us to understand that each of the processes works not separately but simultaneously and continuously as a series of phases of cancer evolution.

2. Prediction of RNA tertiary structures and RNA-RNA/Protein interactions.

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Recent progress in molecular biology has revealed that many non-coding RNAs regulate gene expression or catalyze biochemical reactions in tumors and viruses, and then cause several critical diseases. The tertiary structure of RNA molecules and RNA-RNA/protein interaction sites are of increasing importance as potential targets for new medicines that treat a broad array of human diseases. In this study, we present a novel workflow to predict RNA tertiary structures and RNA-RNA or RNA-protein interactions using the KNIME environment, which enables us to assemble a combination of RNA-related analytical tools and databases. In this workflow, three analytical workflows for comprehensive structural analysis of RNA are available: (1) prediction of the tertiary structure of RNA; (2) prediction of the structure of RNA-RNA complexes and analysis of their interactions; and (3) prediction of the structure of RNA-protein complexes and analysis of their interactions. We demonstrated that the tertiary structure prediction of several kind of RNA aptamer drug, and performed docking calculations of the aptamer and its target proteins using a fragment of the interaction site of the aptamer. The affinity of aptamer-protein complex was evaluated using MMGB/SA method. The results provide valuable information for designing novel features of aptamer-protein complexes.

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1. Sato, K., Niida, A., Masuda, T., Shimizu, D., Tobo, T., Kuroda, Y., Eguchi, H., Nakagawa, T., Suzuki, Y. and Mimori, K. Multiregion Genomic Analysis of Serially Transplanted Patient-derived Xenograft Tumors. Cancer Genomics-Proteomics. 16:21-27, 2019
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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

Novel Risk Loci Identified in a Genome-Wide Association Study of Urolithiasis in a Japanese Population.

BACKGROUND: A family history of urolithiasis is associated with a more than doubling of urolithiasis risk, and a twin study estimating 56% heritability of the condition suggests a pivotal role for host genetic factors. However, previous genome-wide association studies (GWAS) have identified only six risk-related loci. METHODS: To identify novel urolithiasis-related loci in the Japanese population, we performed a large-scale GWAS of 11,130 cases and 187,639 controls, followed by a replication analysis of 2289 cases and 3817 controls. Diagnosis of urolithiasis was confirmed either by a clinician or using medical records or self-report. We also assessed the association of urolithiasis loci with 16 quantitative traits, including metabolic, kidney-related, and electrolyte traits (such as body mass index, lipid storage, eGFR, serum uric acid, and serum calcium), using up to 160,000 samples from BioBank Japan. RESULTS: The analysis identified 14 significant loci, including nine novel loci. Ten regions showed a significant association with at least one quantitative trait, including metabolic, kidney-related, and electrolyte traits, suggesting a common genetic basis for urolithiasis and these quantitative traits. Four novel loci were related to metabolic traits, obesity, hypertriglyceridemia, or hyperuricemia. The remaining ten loci were associated with kidney- or electrolyte-related traits; these may affect crystallization. Weighted genetic risk score analysis indicated that the highest risk group (top 20%) showed an odds ratio of 1.71 (95% confidence interval, 1.42 to 2.06) - 2.13 (95% confidence interval, 2.00 to 2.27) compared with the reference group (bottom 20%). CONCLUSIONS: Our findings provide evidence that host genetic factors related to regulation of metabolic and crystallization pathways contribute to the development of urolithiasis.

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. Four previous GWAS identi-

fied 23 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, LINC00485). Subgroup analysis indicated that 4 loci exhibited a statistically significant effect among multiple leiomyomas, and rs75228775 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 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.

2. Genes playing significant roles in human cancers

Integrated exome and RNA sequencing of dedifferentiated liposarcoma.

The genomic characteristics of dedifferentiated liposarcoma (DDLPS) that are associated with clinical features remain to be identified. Here, we conduct integrated whole exome and RNA sequencing analysis in 115 DDLPS tumors and perform comparative genomic analysis of well-differentiated and dedifferentiated components from eight DDLPS samples. Several somatic copy-number alterations (SCNAs), including the gain of 12q15, are identified as frequent genomic alterations. CTDSP1/2-DNM3OS fusion genes are identified in a subset of DDLPS tumors. Based on the association of SCNAs with clinical features, the DDLPS tumors are clustered into three groups. This clustering can predict the clinical outcome independently. The comparative analysis between well-differentiated and dedifferentiated components identify two categories of genomic alterations: shared alterations, associated with tumorigenesis, and dedifferentiated-specific alterations, associated with malignant transformation. This large-scale genomic analysis reveals the mechanisms underlying the development and progression of DDLPS and provides insights that could contribute to the refinement of DDLPS management.

Massively parallel sequencing of tenosynovial giant cell tumors reveals novel CSF1 fusion transcripts and novel somatic CBL mutations.

Tenosynovial giant cell tumor (TSGCT) is a rare neoplasm. Although surgical resection is the widely accepted primary treatment for TSGCT, recurrences are frequent, and patients' joint function may be severely compromised. Previous studies reported that CSF1-COL6A3 fusion genes were identified in approximately 30% of TSGCTs. The aim of our study was to comprehensively clarify the genomic abnormalities in TSGCTs. We performed whole exome sequencing in combination with target sequence validation on 34 TSGCT samples. RNA sequencing was also performed on 18 samples. RNA sequencing revealed fusion transcripts involving CSF1, including novel CSF1-VCAM1, CSF1-FN1 and CSF1-CDH1 fusions, in 13/18 (72%) cases. These fusion genes were validated by chromogenic in situ hybridization. All CSF1 fusions resulted in the deletion of CSF1 exon 9, which was previously shown to be an important negative regulator of CSF1 expression. We also found that 12 (35%) of the 34 TSGCT samples harbored CBL missense mutations. All mutations were detected in exons 8 or 9, which encode the linker and RING finger domain. Among these mutations, C404Y, L380P and R420Q were recurrent. CBL-mutated cases showed higher JAK2 expression than wild-type CBL cases (p = 0.013). CSF1 fusion genes and CBL mutations were not mutually exclusive, and both alterations were detected in six of the 18 (33%) tumors. The frequent deletion of CSF1 exon 9 in the fusion transcripts suggested the importance of this event in the etiology of TSGCT. Our results may contribute to the development of new targeted therapies using JAK2
inhibitors for CBL-mutated TSGCT.

INKA2, a novel p53 target that interacts with the serine/threonine kinase PAK4.

The p53 protein is a tumour suppressor and transcription factor that regulates the expression of target genes involved in numerous stress responses systems. In this study, we designed a screening strategy using DNA damage-induced mouse and human transcriptome data to identify novel downstream targets of p53. Our method selected genes with an induced expression in multiple organs of X-ray-irradiated p53 wild-type mice. The expression of inka box actin regulator 2 gene, known as Inka2, was upregulated in 12 organs when p53 expression was induced. Similarly, INKA2 was induced in a p53-dependent manner at both the mRNA and protein level in human cells treated with adriamycin. Reporter assays confirmed that p53 directly regulated INKA2 through an intronic binding site. The overexpression of INKA2 produced a slight decrease in cancer cell growth in the colony formation assay.Moreover, the analysis of The Cancer Genome Atlas (TCGA) data revealed a decreased INKA2 expression in tumour samples carrying p53 mutations compared with p53 wild-type samples. In addition, significantly higher levels of DNA methylation were observed in the INKA2 promoter in tumour samples, concordant with the reduced INKA2 expression in tumour tissues. These results demonstrate the potential of INKA2 as a cancer cell growth inhibitor. Furthermore, INKA2 protein interacts with the serine/threonine-protein kinase, p21 (RAC1) activated kinase (PAK)4, which phosphorylates β-catenin

to prevent ubiquitin-proteasomal degradation. As β-catenin was downregulated in a stable INKA2-expressing cell line, the findings of this study suggest that INKA2 is a novel, direct downstream target of p53 that potentially decreases cell growth by inhibiting the PAK4-β-catenin pathway.

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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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The mission of our laboratory is to conduct computational ("in silico") studies on the functional aspects of genome information. Roughly speaking, genome information represents what kind of proteins/RNAs are synthesized under which conditions. Thus, our study includes the structural analysis of the molecular function of each gene product as well as the analysis of its regulatory information, which will lead us to the understanding of its cellular role represented by the networks of inter-gene interactions.

1. Detecting microbial contaminants from next generation sequencing data and functional inference

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Microbial contamination poses a major difficulty for successful data analysis in biological and biomedical research. Computational approaches utilizing next-generation sequencing (NGS) data offer promising diagnostics to assess the presence of contaminants. However, as host cells are often contaminated by multiple microorganisms, these approaches require careful attention to intra- and interspecies sequence similarities. We presented a computational approach that rigorously investigates the genomic origins of sequenced reads, including those mapped to multiple species. Through the analysis of large-scale NGS samples, we estimated that 1000–100,000 contaminating microbial reads are detected per million host reads sequenced by RNA-seq. The microbe catalog we established included Cutibacterium as a prevalent contaminant, suggesting that contamination mostly originates from the laboratory environment. Importantly, by applying a systematic method to infer the functional impact of contamination, we revealed that host-contaminant interactions cause profound changes in the host molecular landscapes, as exemplified by changes in inflammatory and apoptotic pathways during Mycoplasma infection of lymphoma cells.

2. Analyzing ChIP-seq data of homeobox TF HHEX for studying its cooperation with mutant ASXL1 in myeloid leukemogenesis

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Additional sex combs-like 1 (ASXL1) mutations are frequently found in myeloid malignancies, but the mutant ASXL1 (ASXL1-MT) alone is insufficient to

develop myeloid leukemia. As a potential factor cooperating with ASXL1-MT, Hematopoietically Expressed Homeobox (HHEX) was identified by mutagenesis experiments and analyzed its binding preference through a ChIP-seq experiment. The results suggest that ASXL1-MT and HHEX upregulate MYB and ETV5 gene. Conversely, the depletion of HHEX reduced their expression levels. In addition, the luciferase reporter assay revealed that co-expression of ASXL1-MT and HHEX cooperatively enhanced the promoter activity of MYB. Furthermore, the depletion of Myb or Etv5 reduced colony-forming activity in cSAM and cRAM cells by promoting apoptosis or differentiation, respectively. Taken together, this study demonstrates that ASXL1-MT cooperates with HHEX to promote myeloid leukemogenesis through upregulating MYB and ETV5 genes.

3. Genome-wide screening of modulators for chromatin accessibilitySung-Joon Park, Kenta Nakai, and Yusuke Miyanari[5]

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Chromatin compaction is thought to be indispensable for packing and protecting DNA, and functions in various biological contexts. In particular, the open and closed chromatin dynamics is highly relevant to cell differentiation, aging, disease development, and so on. However, the regulation of chromatin dynamics still remains poorly understood. In this study, using eHAP cell line and CRISPR, we first conducted transposase-accessible chromatin with visualization (ATAC-see) experiments with eHAP in which potential regulatory genes were knocked out (KO). Simultaneously, we profiled the genome-wide change of chromatin accessibility in the KO samples using ATAC-seq assays. This approach detected several genes that significantly changed the chromatin accessibility in cellular and genomic levels. These genes are functionally diverse and may mediate the dynamics of chromatin structure through various pathways. We have analyzed the interplay between these potential modulators and phenotypes, which promotes understanding of the mechanism underlying chromatin regulation.

4. Analyzing the 3D chromatin organization coordinating with gene expression regulation in B-cell lymphoma

Luis Augusto Eijy Nagai, Sung-Joon Park, and Kenta Nakai

Eukaryotes compact chromosomes densely and non-randomly, forming three-dimensional structures. Alterations of the chromatin structures are often associated with diseases. In particular, aggressive cancer development from the disruption of the humoral immune system presents abnormal gene regulation accompanied by chromatin reorganizations. In B-cell lymphomas, a hallmark is the diverse chromosomal translocations, mostly involving transcriptionally deregulation in oncogenes. In this study, we focus on the chromatin dynamics in normal and abnormal B cell lymphocytes and investigate its functional impact on the regulation of oncogenes. We conducted an integrative approach using transcriptomic and epigenetic publicly available multi-omics data. Although the chromatin compartments were highly preserved among several cells, 5.2% of B-cell genes in the repressive compartment switched to the permissive compartment in lymphoma with increased expression levels. These genes are involved in B-cell lymphoma related biological processes, such as V(D)J recombination. We observed an inexpressive amount of significant inter-chromosomal interactions in normal cells. However, in B-cell lymphoma, 34% of the significant interactions were between distinct chromosomes. Surprisingly, about 23% of all interactions were only between chromosomes 7 and 17. Such higher interaction is typical between regions in cis-chromatin interaction proximity and may indicate a translocation provoked by the "contact first" hypothesis.

5. Search for a novel epigenetic code related to human embryonic stem cell natures

Yasuhisa Ishikawa, Sung-Joon Park, and Kenta Nakai

It is well known that epigenetic factors such as DNA methylation and histone modifications influence gene expression and cell-specific natures. Among them, it is reported that 5-hydroxymethylcytosine (5hmC), an oxidized form of DNA methylation, is highly accumulated in embryonic stem cells (ESCs). 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 forms 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 may influence the natures of human ESCs. As a result, a novel epigenetic code candidate consisting of 5hmC and two histone modifications were 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). This finding has the potential to help better understand the nature of ESCs and its relationship to epigenetic factors.

6. *In silico* identification of epigenetic crosstalk between histone modifications and intragenic microRNAs during adipogenesis of adipose-derived stem cells

Yukiyo Yamatani, Sung-Joon Park, and Kenta Nakai

The epigenome has become increasingly important in stem cell differentiation but has still incompletely understood in adipose-derived stem cells (ASCs). Here, we focus on the ASC differentiation and aim to dissect the impact of multiple epigenetic factors during the differentiation. We prepared public RNA-seq, ChiP-seq and microRNA-seq (miRNA-seq) datasets of human ASCs and adipocytes. We first conducted an integrated analysis for the identification of differentially expressed genes and miRNAs (DEGs and DEmiRs), the interpretation of gene functions and the detection of ChIP peaks at gene promoters. We showed rapid transcription at the middle stage of the differentiation. In this transcription event, we observed a part of DEGs with transiently up-regulation at the middle stage and down-regulation at the late stage. These DEGs did not have any adipogenesis-related function. To estimate the interaction between histone modifications and miRNAs involved in this late-stage specific down-regulation, we next investigated intragenic DEmiRs that could have those DEGs as targets and histone marks at promoters of their host genes. As a result, we found that two intragenic miRNAs, miR-133a-3p and miR-186-5p, were differentially up-expressed and their host genes, MIB1 and ZRANB2, were constitutively expressed through adipogenesis. Moreover, active histone marks (H3K4me3, H3K27ac, or H3K36me3) were increasingly enriched at MIB1 and ZRANB2 promoters during differentiation. These results suggest that histone modifications regulate intragenic miRNA expression with its host gene expression, which will gain insight into understanding the gene regulation process for normal differentiation by the crosstalk of multiple epigenetic factors.

7. Whole-genome sequencing analysis identifies recurrent structural alterations in esophageal squamous cell carcinoma

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Esophageal Squamous Cell Carcinoma is one of the most aggressive cancers, which is the eighth most common cancer type and the sixth most common cause of cancer-related death globally. The two major types of esophageal cancer are Esophageal Squamous Cell Carcinoma (ESCC) and Esophageal Adenocarcinoma (EAC). ESCC has most commonly occurred in the Asian region, such as Japan, China and India. EAC is prevalent in western countries. To provide a comprehensive mutational landscape, we performed whole-genome sequencing (WGS) analysis of ESCC in Japan. We analyzed biopsy specimens from 20 ESCC patients in a Japanese population before chemo- and/or radiation therapy. WGS analysis identified non-silent mutations of *TP53, ZNF750* and *FAT1* in ESCC. We profiled the mutational signature patterns in ESCC, which unveiled six mutation signatures and their association with environmental risk factors. In addition, we found recurrent structural variations in genes such as LRP1B, TTC28, CSMD1, PDE4D, and WWOX in around 25%-30% of the tumors. Furthermore, our somatic copy number alterations analysis uncovered recurrent amplification and deletion regions such as 3q26.33, 8p11.23, and 9p21.3. In summary, the multi-dimensional view of genomic alterations improves our understanding of the ESCC development at the molecular level and provides future therapeutic implications for ESCC in Japan.

8. *E Pluribus Cellulam*: A study of aggregated single-cell haematopoietic transcriptomes

Phit Ling Tan, Ashwini Patil, and Kenta Nakai

The increasing prevalence of single-cell transcriptomic technologies and analyses leads to an increase of public single-cell datasets that are made available to explore. However, single-cell transcriptome is well known for its sparsity and noise, which would interfere with the analysis. These interferences may result in loss of cell types while annotating the identities of the cells. The risk of losing rare cell types can be mitigated when multiple datasets of the same system are integrated in order to complement the sparsity of one another. In this study, six haematopoietic datasets that use different single-cell isolation and sequencing technologies are integrated with Mutual Nearest Neighbors. As a result, a rare and potentially novel subtype of dendritic progenitor cells is unveiled while studying the merged dataset. Similarly, rare plasma cells that are overshadowed by the expression of B cells also come to light when datasets are aggregated. In addition, this study is able to identify cells that were mis-annotated in the original study and restore

their true identities when integrated with other datasets. In conclusion, expressions of rare cell types that are masked in a single dataset are aggregated and revealed using integrated data. On the other hand, merging multiple datasets can cross-check cell identities in order to avoid mis-annotation. Furthermore, combining data can serve as a measure to annotate new single-cell dataset based on a reference atlas. Hence, integrating datasets should be a part of the common single-cell analysis pipeline, which is useful in annotating cell types if there is a well-defined dataset that can serve as a reference atlas.

9. OPAL+: Length-specific MoRF prediction in intrinsically disordered protein sequences

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Intrinsically disordered proteins (IDPs) contain long unstructured regions, which play an important role in their function. These intrinsically disordered regions (IDRs) participate in binding events through regions called molecular recognition features (MoRFs). Computational prediction of MoRFs helps identify the potentially functional regions in IDRs. In this study, OPAL+, a novel MoRF predictor, is presented. OPAL+ uses separate models to predict MoRFs of varying lengths along with incorporating the hidden Markov model (HMM) profiles and physicochemical properties of MoRFs and their flanking regions. Together, these features help OPAL+ achieve a marginal performance improvement of 0.4–0.7% over its predecessor for diverse MoRF test sets. This performance improvement comes at the expense of increased run time as a result of the requirement of HMM profiles.

10. Discovering MoRFs by trisecting intrinsically disordered protein sequence into terminals and middle regions

Ronesh Sharma[10,11], Alok Sharma[10,12], Ashwini Patil, and Tatsuhiko Tsunoda[12]

Molecular Recognition Features (MoRFs) are short protein regions present in intrinsically disordered protein (IDPs) sequences. MoRFs interact with structured partner protein and upon interaction, they undergo a disorder-to-order transition to perform various biological functions. Analyses of MoRFs are important towards understanding their function. Performance is reported using the MoRF dataset that has been previously used to compare the other existing MoRF predictors. The performance obtained in this study is equivalent to the benchmarked OPAL predictor, i.e., OPAL achieved AUC of 0.815, whereas the model in this study achieved AUC of 0.819 using TEST set. Achieving comparable performance, the proposed method can be used as an alternative approach for MoRF prediction.

Publication list

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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 achieve three major missions: public policy science studies of translational research and its impact on society; research ethics consultation for scientists to comply with ethical guidelines and to build public trust; and development of "minority-centered" scientific communication. By conducting qualitative and quantitative social science study and policy analysis, we facilitate discussion of challenges arising from advances in medical sciences.

1. Research ethics consultation and studies on ethical, legal, and social implications of stem cell research

Japan Agency for Medical Research and Development (AMED) has commissioned us to provide research ethics consultation to stem cell research since 2012. The program is called “research on the ethical, legal, and social implications related to regenerative medicine”. In order to make regenerative medicine more concrete, it is essential to promote research development with a definite focus on clinical applications and to establish a framework for clinical research at an early stage. We provided more than 70 consultations for stem cell researchers per year. Topics of those consultations include research design, informed consent, research ethics committees, return of research results, inclusion criterion of participants of first-in-human trials and governance of iPSC banking. We also organized interdisciplinary research groups to address the ethical, legal, and social implications (ELSI) related to regenerative medicine in a comprehensive manner, with a view to establishing a framework for ethical support and review of regenerative medicine.

2. Research ethics consultation and studies on ethical, legal, and social implications of cancer research

In order to create next-generation cancer therapies, this research program promotes research aimed at elucidating the biological properties of cancer, research based on patients’ clinical data, and research combining both aspects. Through this process, the program accelerates the development and practical application of diagnostic biomarkers for the prevention and early detection of cancer, and innovative drugs for cancer treatment. We provided a model form for information sheet, consent form and leaflet for research participants.

3. Research ethics consultation and studies on ethical, legal, and social implications of prospective cohort study of elderly people

Japan Prospective Studies Collaboration for Aging and Dementia (JPSC-AD) study is a collaborative prospective cohort study of approximately 10,000 elderly people from 8 newly-established community-based dementia cohort studies in Japan, in which the data is prospectively collected by using the pre-specified standardized protocol. Approximately 10,000 com-

munity-dwelling individuals aged 65 years or older at 8 sites of Japan are recruited in the baseline survey from 2016 to 2018, and followed up to detect incident cases of dementia for at least 5 years. In the baseline survey, the following data has been collected: lifestyle information (smoking habits, alcohol intakes, diet, physical activity, etc.), medical history, physical examination, blood test, and brain magnetic resonance imaging (MRI). Frozen samples of serum and plasma (−80℃) have been also stored. We provided several suggestions for protecting vulnerable research participants and created a short movie to remind them that their data has been collected and analyzed for this cohort study.

4. Survey on the perception of germline genome editing among the general public in Japan

Genome editing of human embryos could become a fundamental treatment approach for genetic diseases; however, a few technical and ethical issues need to be resolved before its application in clinical settings. Presently, the Japanese government has issued a statement prohibiting human germline editing and emphasizing the need for discussions that include a wide range of perspectives. However, current discussions tend to exclude the general public. Therefore, we conducted a survey of 10,881 general adults and 1044 patients in Japan who indicated that their disease conditions are related to their genetic makeup, and clarified their attitude toward this technology. The results clearly indicated that the Japanese people generally accepted the use of genome editing for disease-related genes, but many were concerned about the risks. In addition, many Japanese people did not understand the technology well. To improve awareness and understanding about genome editing, it is important that scientists and science communicators create opportunities for the public to participate in relevant discussions without harming vulnerable participants. It is also important to continuously track changes in the acceptance of genome editing by the public.

5. Ethical concerns on sharing genomic data including patients' family members

Platforms for sharing genomic and phenotype data have been developed to promote genomic research, while maximizing the utility of existing datasets and minimizing the burden on participants. The value of genomic analysis of trios or family members has increased, especially in rare diseases and cancers. However, current data-sharing policies have no specific safeguards or provisions for familial data sharing. A quantitative survey conducted on 10,881 general adults in Japan indicated that they expected stronger protection mechanisms when their family members' clinical and/or genomic data were shared together, as compared to when only their data were shared. A framework that respects decision-making and the right of withdrawal of participants, including family members, along with ensuring usefulness and security of data is needed. To enable this, we propose recommendations on ancillary safeguards for familial data sharing according to the stakeholders, namely, initial researchers, genomic researchers, data submitters, database operators, institutional review boards, and the public and participants. Families have played significant roles in genetic research, and its value is re-illuminated in the era of genomic medicine. It is important to make progress in data sharing while simultaneously protecting the privacy and interests of patients and families, and return its benefits to them.

6. Attitudes toward genomic tumor profiling tests in Japan

Genomic tumor profiling tests (GTPTs) to find molecular targeted drugs for patients with advanced cancer are being introduced into clinical settings, which may result in secondary germline findings. We conducted anonymous surveys with 757 cancer patients (CPs), 763 family members (FMs), and 3697 general adults (GAs) in Japan. Awareness of GTPTs was low in all groups, however, both CPs and FMs showed a higher degree of recognition in the benefits of GTPTs. FMs wanted information on germline findings to be shared more than the CPs. Since advanced CPs may have psychological burdens that make it difficult to express their opinions on their therapeutic options and sharing germline findings, GTPTs should be offered with advanced care planning for patients.

7. Ethical, legal, and social issues (ELSI) of artificial intelligence in healthcare

We launched a new multidisciplinary research team on ELSI in healthcare AI (artificial intelligence). The Ministry of Health and Welfare funds this project, and the patient organization (COML; Consumer Organization for Medicine and Law) has supported our discussion by adding patients' views. Main aims of the project are to clarify the regulatory characteristics of AI in healthcare and to illuminate public concerns about it. We have mainly adopted three approaches: analysis of ethical and regulatory discussion in Japan and other developed countries, group discussions for hearing responses from the public and patients, and nationwide questionnaire surveys of attitudes of physicians and the general public. We also made more than ten fictitious episodes on using AI in a clinical setting, so that the participants of the discussion can imagine how some aspects of AI could matter for patients and physicians. Our research results show the generally calm evaluation of the potential of AI, but also show the public expectation on physicians' unbiased judgement and varied response to "black box"

issues of AI in healthcare. Based on these works, we are writing a report showing points for ethical discussion of AI in healthcare.

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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. Human Pluripotent Stem Cell-Derived Tumor Model Uncovers the Embryonic Stem Cell Signature as a Key Driver in Atypical Teratoid/Rhabdoid Tumor

Yukinori Terada[1,2], Norihide Jo[1], Yoshiki Arakawa[2], Megumi Sakakura[1], Yosuke Yamada[1], Tomoyo Ukai[1], Mio Kabata[1], Kanae Mitsunaga[1], Yohei Mineharu[1], Sho Ohta, MasatoNakagawa[1], SusumuMiyamoto[2], Takuya Yamamoto[1,3,4], Yasuhiro Yamada[1,3] : [1] Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University [2] Department of Neurosurgery, Kyoto University Graduate School of Medicine [3] AMED-CREST, AMED [4] Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University

Atypical teratoid/rhabdoid tumor (AT/RT), which harbors SMARCB1 mutation and exhibits a characteristic histology of rhabdoid cells, has a poor prognosis because of the lack of effective treatments. Here, we establish human SMARCB1-deficient pluripotent stem cells (hPSCs). SMARCB1-deficient hPSC-derived neural progenitor-like cells (NPLCs) efficiently give rise to brain tumors when transplanted into the mouse brain. Notably, activation of an embryonic stem cell (ESC)-like signature confers a rhabdoid histology in SMARCB1-deficient NPLC-derived tumors and causes a poor prognosis. Consistently, we find the activation of the ESC-like gene expression signature and an ESC-like DNA methylation landscape in clinical specimens of AT/RT. Finally, we identify candidate genes that maintain the activation of the ESC-like signature and the growth of AT/RT cells. Collectively, SMARCB1-deficient hPSCs offer the human models for AT/RT, which uncover the role of the activated ESC-like signature in the poor prognosis and unique histology of AT/RT.

2. Cell-type dependent enhancer binding of the EWS/ATF1 fusion gene in clear cell sarcomas

Shingo Komura[1, 5], Kenji Ito[1], Sho Ohta, Tomoyo Ukai, Mio Kabata[1], Fumiaki Itakura[1], Katsunori Semi[1], Yutaka Matsuda[1, 6], Kyoichi Hashimoto[1], Hirofumi Shibata[1], Masamitsu Sone[1], Norihide Jo[1], Kazuya Sekiguchi [1, 7, 8], Takatoshi Ohno[5], Haruhiko Akiyama[5], Katsuji Shimizu[9], Knut Woltjen[1, 10], Manabu Ozawa , Junya Toguchida[1, 7, 8], Takuya Yamamoto[1,3, 4, 11], Yasuhiro Yamada[1, 4] : [5] Depart-

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Clear cell sarcoma (CCS) is a rare soft tissue sarcoma caused by the EWS/ATF1 fusion gene. Here, we established induced pluripotent stem cells (iPSCs) from EWS/ATF1-controllable murine CCS cells harboring sarcoma-associated genetic abnormalities. Sarcoma-iPSC mice develop secondary sarcomas immediately after EWS/ATF1 induction, but only in soft tissue. EWS/ATF1 expression induces oncogene-induced senescence in most cell types in sarcoma-iPSC mice but prevents it in sarcoma cells. We identify Tpp3-expressing cells in peripheral nerves as a cell-of-origin for these sarcomas. We show cell type-specific recruitment of EWS/ATF1 to enhancer regions in CCS cells. Finally, epigenetic silencing at these enhancers induces senescence and inhibits CCS cell growth through altered EWS/ATF1 binding. Together, we propose that distinct responses to premature senescence are the basis for the cell type-specificity of cancer development.

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Center for Experimental Medicine and Systems Biology

Laboratory of Innate Immunity
自然免疫研究分野

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

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

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

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

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

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

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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. Cytidine deaminase enables Toll-like receptor 8 activation by cytidine or its analogs.

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Toll-like receptor 8 (TLR8), a sensor for pathogen-derived single-stranded RNA (ssRNA), binds to uridine (Uri) and ssRNA to induce defense responses. We here show that cytidine (Cyd) with ssRNA also activated TLR8 in peripheral blood leukocytes (PBLs) and a myeloid cell line U937, but not in an embryonic kidney cell line 293T. Cyd deaminase (CDA), an enzyme highly expressed in leukocytes, deaminates Cyd to Uri. CDA expression enabled TLR8 response to Cyd and ssRNA in 293T cells. CDA deficiency and a CDA inhibitor both reduced TLR8 responses to Cyd and ssRNA in U937. The CDA inhibitor also reduced PBL response to Cyd and ssRNA. A Cyd analogue, azacytidine, is used for the therapy of myelodysplastic syndrome and acute myeloid leukemia. Azacytidine with ssRNA induced tumor necrosis factor-α expression in U937 and PBLs in a manner dependent on CDA and TLR8. These results suggest that CDA enables TLR8 activation by Cyd or its analogues with ssRNA through deaminating activity. Nucleoside metabolism might impact TLR8 responses in a variety of situations such as the treatment with nucleoside analogues.

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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. Identification of multiple male reproductive tract-specific proteins that regulate sperm migration through the oviduct in mice

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CRISPR/Cas9-mediated genome editing technology enables researchers to efficiently generate and analyze genetically modified animals. We have taken advantage of this game-changing technology to uncover essential factors for fertility. In this study, we generated knockouts (KOs) of multiple male reproductive organ-specific genes and performed phenotypic screening of these null mutant mice to attempt to identify proteins essential for male fertility. We focused on making large deletions (dels) within 2 gene clusters encoding cystatin (CST) and prostate and testis expressed (PATE) proteins and individual gene mutations in 2 other gene families encoding glycerophosphodiester phosphodiesterase domain (GDPD) containing and lymphocyte antigen 6 (Ly6)/Plaur domain (LYPD) containing proteins. These gene families were chosen because many of the genes demonstrate male reproductive tract-specific expression. Although *Gdpd1* and *Gdpd4* mutant mice were fertile, disruptions of *Cst* and *Pate* gene clusters and *Lypd4*resulted in male sterility or severe fertility defects secondary to impaired sperm migration through the oviduct.

While absence of the epididymal protein families CST and PATE affect the localization of the sperm membrane protein A disintegrin and metallopeptidase domain 3 (ADAM3), the sperm acrosomal membrane protein LYPD4 regulates sperm fertilizing ability via an ADAM3-independent pathway. Thus, use of CRISPR/Cas9 technologies has allowed us to quickly rule in and rule out proteins required for male fertility and expand our list of male-specific proteins that function in sperm migration through the oviduct.

2. RNA-binding protein Ptbp1 is essential for BCR-mediated antibody production

Hiroki Sasanuma, Manabu Ozawa, Nobuaki Yoshida

The RNA-binding protein polypyrimidine tract-binding protein-1 (Ptbp1) binds to the pyrimidine-rich sequence of target RNA and controls gene expression via post-transcriptional regulation such as alternative splicing. Although Ptbp1 is highly expressed in B lymphocytes, its role to date is largely unknown. To clarify the role of Ptbp1 in B-cell development and function, we generated B-cell-specific Ptbp1-deficient (P1BKO) mice. B-cell development in the bone marrow, spleen and peritoneal cavity of the P1BKO mice was nearly normal. However, the P1BKO mice had significantly lower levels of natural antibodies in serum compared with those of the control mice. To investigate the effect of Ptbp1 deficiency on the immune response *in vivo*, we immunized the P1BKO mice with T-cell-independent type-2 (TI-2) antigen NP-Ficoll and T-cell-dependent (TD) antigen NP-CGG. We found that B-cell-specific Ptbp1 deficiency causes an immunodeficiency phenotype due to defective production of antibody against both TI-2 and TD antigen. This immunodeficiency was accompanied by impaired B-cell receptor (BCR)-mediated B-cell activation and plasmablast generation. These findings demonstrate that Ptbp1 is essential for the humoral immune response.

3. PTBP1 contributes to spermatogenesis through regulation of proliferation in spermatogonia.

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Polypyrimidine tract-binding protein 1 (PTBP1) is a highly conserved RNA-binding protein that is a well-known regulator of alternative splicing. Testicular tissue is one of the richest tissues with respect to the number of alternative splicing mRNA isoforms, but the molecular role(s) of PTBP1 in the regulation of these isoforms during spermatogenesis is still unclear. Here, we developed a germ cell-specific *Ptbp1* conditional knockout (cKO) mouse model to investigate the role of PTBP1 in spermatogenesis. Testis weight in *Ptbp1 cKO mice was comparable to that in age-matched controls until 3 weeks of age; at ≥2 months old, testis weight was significantly lighter in cKO mice than in age-matched controls. Seminiferous tubules that exhibited degeneration in spermatogenic function were more evident in the 2-month-old Ptbp1* cKO mice than in controls. In addition, the early neonatal proliferation of spermatogonia, during postnatal days 1–5, was significantly retarded in *Ptbp1* cKO mice compared with that in controls. We also compared transcriptome or splicome in spermatogonia using spermatogonia culture model (germline stem cells, GSCs) by NGS. Interestingly, a group of genes of which expression was significantly different between *Ptbp1* KO GSCs or control was totally apart from a group of genes of which alternative splicing was changed between the genotypes. Furthermore, mRNA expression of *Nanos3*, known as an essential gene for primordial germ cell development and sustainable spermatogenesis, was significantly lower in the *Ptbp1* KO GSCs. We developed *Nanos3*$^{+/-}$;*Ptbp1*$^{+/-}$ mouse (double-hetero mouse) to compare spermatogenesis with either Nanos3$^{+/-}$ or Ptbp1$^{+/-}$ mouse (single-hetero mouse). Strikingly, no significant abnormality in spermatogenesis was observed in each single hetero mouse, whereas double-hetero mouse showed *Ptbp1* cKO like abnormal spermatogenesis. These data suggest that PTBP1 contributes for maintaining spermatogonial proliferation through regulation of Nanos3 expression.

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Center for Experimental Medicine and Systems Biology

Laboratory of Systems Biology
システムズバイオロジー研究分野

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Gene-modified mice are considered to be powerful tools for understanding of pathophysiological function of the targeted gene(s) in vivo. Our research focus is the understanding of pathogenesis of rejection and immune disorders such as allergy and autoimmunity using gene-modified mice.

IL-25 exacerbates autoimmune aortitis in IL-1 receptor antagonist-deficient mice

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IL-25, a member of the IL-17 family of cytokines, is known to enhance type 2 immune responses, but suppress type 3 (IL-17A)-mediated immune responses. Mice deficient in IL-1 receptor antagonist (*Il1rn*$^{-/-}$ mice) have excessive IL-1 signaling, resulting in spontaneous development of IL-1–, TNF– and IL-17A–dependent aortitis. We found that expression of *Il25* mRNA was increased in the aortae of *Il1rn*$^{-/-}$ mice, suggesting that IL-25 may suppress development of IL-1–, TNF– and IL-17A–dependent aortitis in *Il1rn*$^{-/-}$ mice by inhibiting type 3-mediated immune responses. However, we unexpectedly found that *Il25*$^{-/-}$*Il1rn*$^{-/-}$ mice showed attenuated development of aortitis, accompanied by reduced accumulation of inflammatory cells such as dendritic cells, macrophages and neutrophils and reduced mRNA expression of *Il17a* and *Tnfa*—but not *Il4* or *Il13*—in local lesions compared with *Il1rn*$^{-/-}$ mice. Tissue–, but not immune cell–, derived IL-25 was crucial for development of aortitis. IL-25 enhanced IL-1b and TNF production by IL-25 receptor–expressing dendritic cells and macrophages, respectively, at inflammatory sites of aortae of *Il1rn*$^{-/-}$ mice, contributing to exacerbation of development of IL-1–, TNF– and IL-17A–dependent aortitis in those mice. Our findings suggest that neutralization of IL-25 may be a potential therapeutic target for aortitis.

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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 been 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

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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.

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

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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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Advanced Clinical Research Center

Division of Molecular Therapy
分子療法分野

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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:
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 and Health Intelligence Center to establish a platform for precision medicine.

(2) Development of anti-cancer therapy using recombinant vaccinia virus:
Oncolytic virotherapy is an emerging type of cancer therapy in which a native or genetically modified virus selectively infects and replicates in the tumor and destroys tumor cells. The anti-tumor effects of oncolytic virus alone were generally insufficient in pre-clinical and clinical trials. Using genetic engineering, we loaded oncolytic viruses with foreign transgenes to increase the potency of the therapeutic effect.

(3) 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.

(4) 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. Therapeutic targeting of monokine production is a promising strategy to attenuate cytokine-release syndrome in CAR-T cell thrapy.

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Division of Molecular Therapy

Cancer immunotherapy using chimeric antigen receptor-armed T cells (CAR-T cells) have shown excellent outocomes in hematological malignancies. However, cytokine release syndrome (CRS), characterized by excessive activation of CAR-T cells and macrophages remains to be overcome. Steroid administration usually resolves signs and symptoms of CRS but abrogates CAR-T cell expansion and persistence. Tocilizumab, a humanized monoclonal antibody against interleukin-6 receptor (IL-6R), attenuates CRS without significant loss of CAR-T cell activities, while perfect rescue of CRS symptoms cannot be achieved by IL-6/IL-6R blockade. There is actual need for novel strategies to prevent or cure CRS. TO-207, an N-benzoyl-L-phenylalanine derivative compound, significantly inhibits inflammatory cytokine production in a human monocyte/ macrophage-specific manner. Here we tested TO-207 for its ability to inhibit cytokine production without impaired CAR-T cell function in a CRS-simulating co-culture system consisting of CAR-T cells, target leukemic cells and monocytes. To observe a precise pattern of cytokine release from CAR-T cells and monocytes, we first established a co-culture system that mimics CRS using K562/CD19 cells, 19-28z CAR-T cells, and peripheral blood $CD14^+$ cells. IFN-γ was produced exclusively from CAR-T cells, and TNF-α, MIP-1α, M-CSF, and IL-6 were produced from both CAR-T cells and monocytes, but monocytes were the major source of these cytokine production. MCP-1, IL-1β, IL-8, and IL-10 were released exclusively from monocytes. To observe the effect of drugs on cytokine production, prednisolone (PSL), TO-207, tocilizumab, and anakinra (an IL-1R antagonist) were added to the co-culture. PSL exhibited suppressive effects on TNF-α, IFN-γ, and MCP-1 production. Tocilizumab did not suppress these cytokines. Anakinra up-regulated IL-6 and IL-1β production, probably due to activation of negative feedback loops. Interestingly, TO-207 widely suppressed all of these monocyte-derived cytokines including TNF-α, IFN-γ, IL-6, IL-1β, MCP-1, IL-8, and GM-CSF. Next, we observed whether the cytokine inhibition by TO-207 attenuates killing effect of CAR-T cells. PSL attenuated killing effect of $CD4^+$ CAR-T cells and $CD8^+$ CAR-T cells toward K562/CD19 cells. In contrast, TO-207 did not exhibit any change in cytotoxicity of $CD4^+$ CAR-T cells and $CD8^+$ CAR-T cells. To determine whether the effect of PSL and TO-207 on cytotoxicity changes in the presence of $CD14^+$ monocytes, $CD14^+$ cells were added to the co-culture. In the absence of CAR-T cells, PSL induced a modest attenuation of cytotoxicity, whereas to the CAR-T cells, PSL exhibited a significant attenuation of cytotoxicity. TO-207 exhibited a minimal effect on cytotoxicity in the absence or presence of CAR-T cells. These results suggested that CAR-T cells play a major role in the cytotoxicity toward leukemia cells, and drugs that do not affect CAR-T cell functions, such as TO-207, maintain their cytotoxic effects on leukemia cells. In conclusion, our present co-culture model with K562/CD19 cells, 19-28z CAR-T cells, and $CD14^+$ monocytes accurately recapitulated killing effect and cytokine release profiles. IFN-γ was produced exclusively by CAR-T cells, but most of cytokines such as TNF-α, MIP-1α, M-CSF, IL-6, MCP-1, IL-1β, IL-8, and IL-10 were from $CD14^+$ monocytes/macrophages. Because killing effect was largely dependent on CAR-T cells while cytokine production was dependent on monocytes/macrophages, selective inhibition of pro-inflammatory cytokines from monocytes by TO-207 would be ideal for treatment of CAR-T-related CRS. These results encourage us to consider a clinical trial for the use of CRS.

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

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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 usually mismatch between patients and donors, and transplanted MSCs are eventually rejected by the host immunity. The knockout (KO) of HLA gene would unlock the HLA restriction and facilitate the development of universal cell therapy. For a longer retention of transplanted MSCs in the recipient, a genome editing to knockout HLA molecule was performed. As 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 on day 7. 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 successfully transduced into MSCs using a lentiviral vector. We are also attempting to insert the B2M/HLA-G fusion into MSCs with a genome editing method using CRIS-

PR/Cas9. To establish a sophisticated method by which efficient and safe gene KO and/or knock-in (KI) are carried out, we examined several methods using CRISPR/Cas9. While a high efficiency of KO could be achieved by the transfection of either a CRISPR/Cas9-expressing plasmid (pX330) or a mixture of sgRNA and Cas9 protein, the efficiency of KI was very low using a conventional electroporation of sgRNA, Cas9 protein, and donor DNA (in the form of plasmid). The limiting factor in KI seemed to be the cytotoxicity due to the large DNA size that was transfected as a DNA donor. We compared several different types of DNA donors, including plasmids with homology arms (HA) on both sides of the inserted gene, plasmids without HA on both sides, the linear double strand DNAs, and the single strand DNAs. We found that transfection of sgRNA/Cas9 with a plasmid that have sgRNA recognition sites on both sides of transgene (with no HAs) showed a relatively low cytotoxicity and a good KI efficiency (homology-independent transgene insertion). Using the method that we have confirmed, we will put forward our genome editing experiments for the development of a new cell therapy.

3. CD4$^+$CADM1$^+$ cell percentage predicts disease progression in asymptomatic HTLV-1 carriers and indolent adult T-cell leukemia/lymphoma.

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We recently took advantage of the universal expression of cell adhesion molecule 1 (CADM1) by CD4$^+$ cells infected with HTLV-1 and the downregulation of CD7 expression that corresponds with the oncogenic stage of HTLV-1-infected cells to develop a flow cytometric system using CADM1 versus CD7 plotting of CD4$^+$ cells. We risk-stratified HTLV-1 asymptomatic carriers (AC) and indolent adult T-cell leukemia/lymphoma (ATL) cases based on the CADM1$^+$ percentage, in which HTLV-1-infected clones are efficiently enriched. AC and indolent ATL cases were initially classified according to their CADM1$^+$ cell percentage. Follow-up clinical and flow cytometric data were obtained for 71 cases. In G1 (CADM1$^+$ ≤ 10%) and G2 (10% < CADM1$^+$ ≤ 25%) cases, no apparent clinical disease progression was observed. In G3 (25% < CADM1$^+$ ≤ 50%) cases, five out of nine (55.5%) cases progressed from AC to smoldering-type ATL. In G4 (50% < CADM1$^+$) cases, the cumulative incidence of receiving systemic chemotherapy at 3 years was 28.4%. Our results indicate that the percentage of the CD4$^+$ CADM1$^+$ population predicts clinical disease progression: G1 and G2 cases, including AC cases, are stable and considered to be at low risk; G3 cases, including advanced AC cases and smoldering-type ATL cases based on the Shimoyama criteria, are considered to have intermediate risk; and G4 cases, which are mainly indolent ATL cases, are unstable and at high risk of acute transformation.

4. Prognostic impact of circulating tumor DNA status post allogeneic hematopoietic stem cell transplantation in acute myeloid leukemia and myelodysplastic syndrome.

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This study was performed to assess the utility of tumor-derived fragmentary DNA, or circulating tumor DNA (ctDNA), for identifying high-risk patients for relapse of acute myeloid leukemia and myelodysplastic syndrome (AML/MDS) after undergoing myeloablative allogeneic hematopoietic stem cell transplantation (alloSCT). We retrospectively collected tumor and available matched serum samples at diagnosis and 1 and 3 months post-alloSCT from 53 patients with AML/MDS. After identifying driver mutations in 51 patients using next-generation sequencing, we designed at least 1 personalized digital polymerase chain reaction assay per case. Diagnostic ctDNA and matched tumor DNA exhibited excellent correlations with variant allele frequencies. Sixteen patients relapsed after a median of 7 months post-alloSCT. Both mutation persistence (MP) in bone marrow (BM) at 1 and 3 months post-alloSCT and corresponding ctDNA persistence (CP) in the matched serum (MP1 and MP3; CP1 and CP3, respectively) were comparably associated with higher 3-year cumulative incidence of relapse (CIR) rates (MP1 vs non-MP1, 72.9% vs 13.8% [P = .0012]; CP1 vs non-CP1, 65.6% vs 9.0% [P = .0002]; MP3 vs non-MP3, 80% vs 11.6% [P = .0002];

CP3 vs non-CP3, 71.4% vs 8.4% [$P < .0001$]). We subsequently evaluated whether subset analysis of patients with 3 genes associated with clonal hematopoiesis, *DNMT3A*, *TET2*, and *ASXL1* (DTA), could also be helpful in relapse prediction. As a result, CP based on DTA gene mutations also had the prognostic effect on CIR. These results, for the first time, support the utility of ctDNA as a noninvasive prognostic biomarker in patients with AML/MDS undergoing alloSCT.

5. Combined inhibition of HDAC and AKT as a strategy to overcome multi-drug resistance in patients with multiple myeloma

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Patients with multiple myeloma (MM) have multiple choices of therapy including monoclonal antibodies, proteasome inhibitors, and immunomodulatory drugs (IMiDs), whereas some patients still develop resistance to these drugs and require novel therapeutic modalities. Here, we focused on inhibition of HDAC and AKT to overcome drug resistance. Lenalidomide (Len) selectively binds to cereblon (CRBN), which mediates recruitment of specific substrates like IKZF1 to E3 ubiquitin ligase and subsequent degradation, resulting in downregulation of IRF-4 and c-Myc. Then, we developed Len-resistant myeloma cells by RNAi-mediated downregulation of CRBN. Treatment of these cells with HDAC inhibitors reduced IKZF1 mRNA, suggesting potential efficacy of HDAC inhibitors against CRBN-low expressing or mutated MM. According to the integrated database for expression profile and disease prognosis (GenomicScape, http://www.genomicscape.com), higher expression of MICA was significantly associated with better overall survival in MM. MICA is an NK cell-activating ligand and plays an important role in ADCC. We observed that ADCC activity of both daratumumab and elotuzumab against MM cells was enhanced in the presence of HDAC inhibitors, which was compatible with our previous data that HDAC inhibitors upregulated MICA mRNA expression via inhibition of IKZF1 (ASH2018 abstract #4435). We also observed that HDAC inhibitors upregulated MICA mRNA in CRBN-deficient cells, suggesting promise of the combination of HDAC inhibitors and monoclonal antibodies against Len-resistant MM. Len-resistance is also affected by phosphorylation status of GSK-3. PI3K/AKT pathway is frequently activated in MM cells, and AKT inactivates GSK-3 by direct phosphorylation, resulting in c-Myc stabilization. Enhanced phosphorylation of GSK-3 was observed in CRBN-deficient H929 (MM) cells after long-term culture with Len, and such a phosphorylation status of GSK-3 was correlated with less CRBN amount and higher Len concentration (Figure 1). Afuresertib, an AKT inhibitor, suppressed GSK-3 phosphorylation (p-GSK-3) with or without ACY-1215, an HDAC inhibitor, leading to a substantial decrease of c-Myc (Figure 2). On the other hand, CHIR 99021, a GSK-3 inhibitor, partially counteracted to cytotoxic effect of afuresertib on H929 cells (Figure 3). These results suggest that increased p-GSK-3 is involved in acquired Len-resistance, and that combined inhibition of HDAC and AKT can overcome Len-resistance through decreased p-GSK-3. Furthermore, we examined the efficacy of CUDC-907, a dual HDAC and PI3K inhibitor. CUDC-907 had a cytotoxic effect on the MM cell lines including those had low CRBN expression. Bortezomib, doxorubicin, and dexamethasone resistant MM cell lines were also sensitive to CUDC-907. CUDC-907 upregulated MICA mRNA expression, but downregulated IKZF1 mRNA expression. Treatment of RPMI-8226 cells with CUDC-907 enhanced the ADCC activity of daratumumab (Figure 4). Furthermore, CUDC-907 was effective on primary MM cells which were resistant to bortezomib and Len (Figure 5). Thus, dual inhibition of HDAC and AKT with or without monoclonal antibodies is a promising therapeutic approach to multi-drug resistant MM.

6. Genomic Analysis of therapy-related myeloid neoplasms and tracking of he founder clone by circulating tumor DNA (ctDNA)

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We retrospectively collected tumor samples, including bone marrow (BM), tumor-rich peripheral blood (PB), or alternatively, serum samples, at diagnosis and before diagnosis from 15 tMNs patients in our hospital. We subjected tumor DNA and control buccal swab DNA to comparative whole-exome sequencing (WES) and/or whole-genome sequencing (WGS). After identifying somatic driver mutations, we designed droplet digital PCR (ddPCR) assays for each mutation identified. All 15 patients had a history

of primary hematological malignancies (malignant lymphoma, n=9; acute leukemia, n=4; multiple myeloma, n=2) and had received prior chemotherapy and/or radiotherapy with or without autologous stem cell transplantation (ASCT). The median age at presentation of tMNs was 53 years (range, 6-74), and the median latent period between prior malignancy and tMNs was 45 months (range, 10-161). Conventional cytogenetic analysis revealed high incidence of complex karyotype (38.4%) and *MLL* rearrangement (30.7%). WES and/or WGS revealed that 93.3% (n=14/15) of the cases contained at least one putative driver mutation in 17 genes (median of 1 mutation per patient [range 1-4]). The most frequent mutations found in *TP53* and epigenetic modifier gene (*KMT2D*/*KDM6A*/*ASXL1*/*ASXL2*), mutated in 33% of the samples, followed by signal transduction proteins (*MPL*/*BRAF*/*FLT3-TKD*/*KRAS*, 26.7%). On the other hand, none of the patients with tMNs had mutations characteristic for *de novo*-AML or -MDS (e.g., *FLT3-ITD*, *NPM1*, or spliceosome factors). Together, the spectrum of driver mutations in our cohort was consistent with previous reports in tMNs. We tried to trace back mutant clone using BM and/or serum before diagnosis of tMNs in 2 patients. In UPN-5 who developed MDS-EB1 after ASCT for lymphoma, *ETV6* p.E153fs, a putative founder mutation of tMNs, was applied to liquid biopsy to trace back. *ETV6* ctDNA could be detected as early as 7 months prior to the development of MDS with variant allele frequency (VAF) of 0.06% (blue arrowhead in figure 1A). Most intriguingly, the proportion of *ETV6* ctDNA varied with or without G-CSF administration during the clinical course; VAF increase from 0 to 47.0% on G-CSF and decrease from 47.0 to 1.2% off G-CSF. In UPN-10 who had been clinically diagnosed as tMNs (MDS-EB2) after intensive chemotherapy for prior AML, not otherwise specified with normal karyotype, WGS combined with WES identified four driver mutations in BM at diagnosis of tMNs. Then, four driver mutations, *WT1* p.A365fs, *MLL* rearrangement, inv(3), and del(20q) were all applied to combined analysis of ctDNA and BM as well. Unexpectedly, we could find the presence of the founder clone, inv(3), with high allele burden in BM at initial diagnosis of AML-NOS with normal karyotype. On the contrary, we could not detect other three genes alterations until 4 months before diagnosis of tMNs. These findings would contribute to outline the genetic landscape of tMNs, and especially suggest the role of cytokine-related selective pressures after chemotherapy and of the potential pre-tMNs conditions in the pathogenesis of tMNs.

7. Utility of whole exome sequencing of ctDNA in drug-resistant and/or advanced phase chronic myeloid leukemia

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Despite the success of tyrosine kinase inhibitors (TKIs), some patients with chronic myeloid leukemia (CML) reveal drug resistance and/or progress to advanced phase, which is partly attributed to pre-existing or acquired genomic alterations, separate from the *BCR-ABL1* gene. Circulating tumor DNA (ctDNA) is now applied to a less-invasive and unbiased evaluation of cancer-associated mutations in various malignancies. Herein, we performed comparative whole exome sequencing of bone marrow (BM) and matched ctDNA from 10 patients with CML, most of which were TKI-resistant: chronic phase (CP, n=5); accelerated phase (AP, n=1); blast phase (BC, n=4). We identified seven mutations in two BC, one AP, and one CP patients. These include *ABL1 (2)*, *ASXL1* (3), *SETD2* (1), and *TP53* (1), among which six mutations were shared between BM and ~~matched~~ ctDNA, but the *TP53* mutation was solely detected in ctDNA. Intriguingly, all the patients with these mutations exhibited poor prognosis following TKI therapy (dead, n=3; relapse, n=1). Our results suggest that ctDNA may be comparable to BM in evaluating genomic alterations associated with TKI-resistance and/or disease progression in CML.

8. Granulocyte colony-stimulating factor-asociated aortitis in the Japanes Adverse Drug Event Report database.

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Granulocyte colony-stimulating factor (G-CSF) is the standard-of-care therapy for chemotherapy-associated neutropenia in patients with malignancies. Recent case reports have implied that G-CSF treatment may be associated with the development of aortitis, but the precise nature of the relationship is unclear. We investigated the association between G-CSF and risk for aortitis in patients with various malignancies. We performed an observational study of 102,014 subjects with malignant neoplasms documented in the Japanese Adverse Drug Event Report (JADER) database between April 2004 and February 2018. The ad-

justed odds ratio (OR) and 95% confidence interval (CI) for aortitis in patients treated and not treated with G-CSF were estimated using multivariate logistic regression with R software. Among the 102,014 subjects, 25 developed aortitis. Of the 3409 and 98,630 subjects treated and not treated with G-CSF, 16 (0.47% [95% CI; 0.27, 0.76]) and 9 (0.01% [0.00, 0.02]) developed aortitis, respectively. Multivariate logistic regression indicated an association between G-CSF and aortitis (adjusted OR 45.87 [19.16, 109.8], $p < 0.001$). The values for filgrastim, pegfilgrastim, and lenograstim were 0.25% (0.07, 0.63), 1.58% (0.79, 2.81), and 0.24% (0.05, 0.69), respectively. G-CSF treatment was associated with a signal of increased risk for aortitis among patients with malignant neoplasms. Three different G-CSF agents were associated with such risk, implying that it is a class effect. However, we do not recommend changing G-CSF prescriptions, because our results may have been influenced by the limitations of the JADER database and because the benefit of G-CSF treatment outweighs the potential risk.

9. T memory stem cells after allogeneic haematopoietic cell transplantation: unique long-term kinetics and influence of chronic graft-versus-host disease.

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T memory stem cells (TSCMs) are a subset of primitive T cells capable of both self-renewal and differentiation into all subsets of memory and effector T cells. Therefore, TSCMs may play a role in immune reconstitution and graft-versus-host disease (GVHD) in patients receiving allogeneic haematopoietic cell transplantation (HCT). We conducted a cross-sectional study to evaluate the proportions, absolute counts, phenotypes and functions of TSCMs in 152 adult patients without disease recurrence at least 12 months after undergoing HCT. $CD4^+$ TSCMs were negatively correlated with number of months after transplantation in HCT patients that received cord blood transplantation, but not in patients that received bone marrow transplantation or peripheral blood stem cell transplantation. The proportions and absolute counts of $CD4^+$ TSCMs and expression levels of inducible co-stimulator (ICOS) in $CD8^+$ TSCMs were significantly higher in patients with mild and moderate/severe cGVHD compared to patients without cGVHD. These data suggested that, more than 12 months after allogeneic HCT, the kinetics of $CD4^+$ TSCMs were dependent on the type of donor source, and further that $CD4^+$ TSCMs and ICOS levels in $CD8^+$ TSCMs were associated with cGVHD.

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Advanced Clinical Research Center

Division of Cellular Therapy
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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).

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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

Using human and mouse models for AML1-MTG8/ETO leukemia, we have been elucidating new molecular aspects in the pathogenesis and progression of acute myeloid leukemia (AML).

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. Interestingly, AML1-

ETO9a (AE9a) that lacks c-terminus of AML1-MTG8 is shown to possess leukemogenic potential in a mouse model of retroviral transduction-transplantation. Nonetheless, AE9a protein is barely expressed in t(8;21) cells and a recent report suggested that there was no impact on clinical outcome by AE9a. Now we have identified a new splicing variant that has significant ability to induce leukemia in mouse model. Mechanisms of leukemogenesis by it as well as clinical significance are currently under investigation.

5. Development of RUNX1-targeted therapy for AML

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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.

6. Understanding and Targeting TP53 in Myeloid Neoplasms

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 Hif1a and PD-L1, and inhibition of the Hif1a-PD-L1 axis sensitized AML cells to p53 activation. We also found that NK cells are important mediators of antileukemia immunity. Our study showed the potent activity of a p53-activating drug against AML, which is further augmented by antitumor immunity.

Decitabine is a DNA methyltransferase inhibitor and is considered a promising drug to treat myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) with p53 mutations. However, whether loss of p53 in fact increases the response of MDS/AML cells to decitabine remains unclear. We therefore assessed the role of p53 in MDS and AML cells treated with decitabine using mouse models for MLL-AF9-driven AML and mutant ASXL1-driven MDS/AML. CRISPR/Cas9-mediated depletion of p53 in MDS/AML cells did not increase, but rather decreased their sensitivity to decitabine. Forced expression of a dominant-negative p53 fragment (p53DD) in these cells also decreased their responses to decitabine, confirming that acute inhibition of p53 conferred resistance to decitabine in AML and MDS/AML cells. In contrast, MLL-AF9-expressing AML cells generated from bone marrow progenitors of *Trp53*-deficient mice were more sensitive to decitabine *in vivo* than their wild-type counterparts, suggesting that long-term chronic p53 deficiency increases decitabine sensitivity in AML cells. These data revealed a multifaceted role for p53 to regulate responses of myeloid neoplasms to decitabine treatment.

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

Yosuke Tanaka, Keiko Mikami, Susumu Goyama, Toshio Kitamura

CML-LSCs (Chronic myelogenous 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 for 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 for 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 with 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 concentrated 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 for imatinib. Moreover, we found that the CML-LSCs expressed PD-L1 at higher level than the CML progenitor cells. Combination therapy of imatinib and PD-L1 blocking antibody was more efficient for eliminating CML-LSCs than mono therapy of imatinib. These data exhibited that PD-L1 expression was linked to G0 status of CML-LSCs and their resistance for TKIs.

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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 current main subject is immunopathogenesis of HIV-1 infection. We are focusing on how cellular immune responses fight against to HIV-1 and how immune system is disrupted and develops AIDS. We are also working on viral pathogenesis in HIV-infected patients. We work together with the staffs in the Department of Infectious Diseases and Applied Immunology in the IMSUT hospital and apply the research results to the people living with HIV-1/AIDS. We are extending our research project to other viral diseases including viral hepatitis and associated morbidities, especially pathogenesis of co-infection with HIV and hepatitis A virus (HAV), hepatitis B virus (HBV), or hepatitis C virus (HCV).

1. Identification of vaccine escape mutants of HBV among blood donors.

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The number of patients infected with HBV dramatically decreased by successful prevention of mother-to-child transmission and post-transfusion infection, but horizontal transmission, especially of genotype A, is recently increasing. HBV vaccine is very useful for preventing infection, but about 10% of vaccinated adults cannot get an adequate neutralizing antibody. In addition, some vaccine-escape mutants (VEM) which cannot be controlled by vaccination have been identified. Considering these situations, a novel and more effective HBV vaccine are necessary and expected to be developed. In fact, some candidates which include preS as well as S region of HBV are under study. To develop a more effective vaccine, it is necessary to search sequence of this region of HBV derived from current patients. Thus far, we determined the genomic sequence of HBV derived from sera of HIV/HBV co-infected patients who visited IMSUT hospital, and found that some patients had HBV-VEM. Therefore, we proceeded to examine blood donors who were shown to be HBsAg-positive to investigate the presence of HBV-VEM also in non-immunocompromised HBV carriers. Thus far, we examined 34 samples and found the presence of HBV-VEM in some samples. We also observed interesting mutations and deletions of HBV genome in these blood donors.

2. Analysis of HIV-infected patients with acute infection of hepatitis A virus

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Since the end of 2017, in metropolitan areas in Japan, acute hepatitis A virus (HAV) infection is frequently observed in HIV-infected patients who are MSM (men who have sex with men). In IMSUT Hospital, we experienced 11 HIV-infected patients diagnosed as acute hepatitis by HAV in 2017-2018. In 1999-2000, we also experienced 5 HIV-infected patients (MSM) with hepatitis A. Since conditions of current HIV-infected patients have changed owing to recent progress in anti-HIV therapies, we compared clinical features of hepatitis A between 11 recent (2017-2018) patients and 5 former (1999-2000) patients. We found that peak levels of hepatic transaminases after the onset of hepatitis were significantly higher in the recent patients than in the former patients. This may be, at least partially, due to the higher CD4/CD8 ration in the recent patients reflecting the improved immune status. We also analyzed the HAV genome derived from some of the recent patients with informed consent and found the HAV strains were almost the same and slightly different from a formerly identified strain. The recent strains belonged to RIVM-HAV16-090 cluster that was prevalent in Taiwan where outbreak of HAV was observed before that in Japan. We are now preparing to perform a multicenter study including the identification of factors associated with severe hepatitis.

3. Investigation of prevalence of hepatitis A immunity and decision-tree analysis among HIV-MSM

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

The outbreak of hepatitis A among HIV-MSM has occurred in metropolitan areas in Japan around 2018. To prevent HAV infection, HA vaccine is useful for HIV-MSM without HAV immunity, but the level of HAV immunity among HIV-MSM in Japan is unknown. Therefore, we started this study to examine HAV immunity among HIV-MSM in IMSUT Hospital and applied the decision-tree analysis to explore the factors associated with the presence of IgG-HA in HIV-MSM. Overall, 378 HIV-MSM were examined for IgG-HA antibodies in 2017, before the outbreak of hepatitis A in Japan. After excluding 24 patients with history of HA vaccination, the data of 354 HIV-MSM were analyzed (median age 45 years, interquartile range 39-51 years). Of the 354 patients, 60 (16.9%) were positive for IgG-HA antibodies. The positivity rate increased with patients' age, and age (>63.5 years) was extracted as the most important variable by classification of the decision-tree algorithm. This study, conducted just before the HAV outbreak among MSM in Japan, showed that age was the most relevant factor in anti-HAV prevalence and that an extensive HAV vaccination program for HIV-MSM is urgently needed, particuraly for younger people.

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 few studies showing 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, Tomohiko Koibuchi[1], Eisuke Adachi[1], Tadashi Kikuchi[1], Takeya Tsutsumi, Hiroshi Yotsuyanagi

Progress in antiretroviral treatment has led to fewer virological failure cases, but 10%-20% 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 4.9%, ranging from 4.9% in 2018 to 11.7% in 2010. The prevalence of RS was significantly higher among cases who were male, Japanese, and men who have sex with men. Common mutations in both groups were M46I/L and T215 revertants. Furthermore, sequences with these mutations, K103N and D30N/N88D formed clusters on phylogenetic trees. It was suggested that HIV with these mutations have become circulating strains.

6. Study of HIV and the host genome database construction

Michiko Koga, Tomohiko Koibuchi[1], Eisuke Adachi[1], Tadashi Kikuchi[1,3], Takeya Tsutsumi, Hiroshi Yotsuyanagi, Ai Tachikawa-Kawana[3], Teiichiro Shiino[3], Tetsuro Matano[3]
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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

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To characterize HIV-associated gut microbiome in Japanese individuals, we analyzed stool samples from 103 individuals with chronic HIV infection and 63 uninfected controls. The microbiome was investigated by sequencing of the 16S rRNA V3-V4 regions on the Illumina MiSeq platform and data were analyzed using QIIME2 software. HIV-associated gut dysbiosis was characterized by reduced phylogenetic diversity and increased ratio of *Prevotella*. Further analysis is ongoing to obtain more detailed information on the bacterial taxa with differential abundance as well as the relationship between gut dysbiosis and HIV outcomes.

9. Exploration of drugs to restore mitophagy suppressed by hepatitis C virus

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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 it is possible that if Bnip3 function is restored by some chemicals, mitophagy will be recovered and ROS accumulation will be attenuated. To explore the candidate chemicals, we screened 1540 chemicals provided as FDA-approved drug library and also 1322 chemicals provided as Natural product library, and found some chemicals enhanced Bnip3 homodimerization. We are now going to administer these candidate chemicals to HCV core-transgenic mice to determine the effect in vivo. This study will lead to the future development of drugs for the prevention of HCC accompanied in HCV-infected patients.

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Advanced Clinical Research Center

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

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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 molecular targeted anticancer drugs 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 as a result of genetic alterations of the components such as *APC* and *CTNNB1* has been found in various types of cancers. This activation leads to the accumulation of β-catenin in the nucleus, and transactivation of the TCF/LEF family. Identification of target genes directly transactivated by the heterodimeric β-catenin/TCF transcriptional complex results in the better understanding of the role of Wnt/β-catenin signaling in carcinogenesis. These downstream genes of the transcriptional complex, referred to as "Wnt target genes", include proto-oncogene *c-myc*, cell cycle regulator *cyclin D1*, and stem cell marker *LGR5*. We have also identified the Wnt target genes such as *RNF43*, *SP5*, *CLDN1*, *ENC1*, *APCDD1*, and *FRMD5*. In contrast to these up-regulated genes, roles of down-regulated genes by the signaling remain largely unknown. Thus, we conducted transcriptome analysis using colorectal cancer cells introduced with β-catenin siRNAs or a dominant negative form of TCF7L2 (dnTCF7L2), and identified a set of genes down-regulated by the Wnt/β-catenin signaling. Notably, interferon-regulated proteins IFITs were included in the top 50 down-regulated genes. Through the analysis of *IFIT2* promoter, we found that *IFIT2* is transcriptionally regulated by interferon regulatory factor 1 (IRF1) that is destabilized by the Wnt/β-catenin signaling pathway. In addition, we uncovered that decreased activity of a deubiquitinase complex containing USP1 and UAF1 plays a crucial role in the degradation of IRF1 by the Wnt/β-catenin signaling. The identification of IRF1, a new class of Wnt target gene that is negatively regulated through the Wnt/β-catenin signaling, will not merely provide a novel insight into the regulatory mechanism of the pathway, but also 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. Recently, we developed a sensitive and specific cell-based reporter assay for the detection of the Wnt/β-catenin signaling activity. Taking advantage of this assay, we established a high-throughput screening system, and performed a screening of a chemical library containing 20,000 compounds. As a result, we have identified seven 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 started a study of structure-activity relationship (SAR) of these chemical 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* and homozygous deletion of *Pten* 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 are trying to establish novel mouse models of gastrointestinal, liver, and pancreatic cancer using mice carrying a cancer-associated mutant allele of the *Idh1/2* gene that is frequently mutated in human cancers. Intensive analysis of these models should provide better understanding of their carcinogenesis and facilitate the development of new therapies to these cancers.

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[2], Satoru Miyano[1,3], Rui Yamaguchi[4]: [1]Laboratory of Sequence Analysis, Human Genome Center, [2]Division of Health Medical Data Science, [3]Division of Health Medical Computational Science, Health Intelligence Center, IMSUT, [4]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute

Whole genome and whole exome sequencing have disclosed comprehensive catalogues of various types of human cancer, and identified driver genes and pathways associated with their carcinogenesis. Pseudomyxoma peritonei (PMP) is a rare disease exhibiting a distinct clinical feature caused by cancerous cells that produce mucinous fluid 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. Our previous analysis of 18 PMPs containing 10 low-grade tumors and 8 high-grade tumors in appendix determined that *KRAS* and/or *GNAS* mutations are common genetic features of PMP. Furthermore, we suggested that mutations in *TP53* and/or genes related to the PI3K-AKT pathway might provide malignant properties to PMP. To comprehensively understand genetic alterations in PMP, we extensively analyzed PMP tumors and matched normal colonic mucosa by the whole genome sequencing and RNA sequencing. Ongoing analysis of genetic and transcriptome data will facilitate the discovery of biomarkers of PMP, selection of effective anti-cancer drugs, and individualized 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], Tetsuo Shibuya[2], Satoru Miyano[1,2], Takanori Hasegawa[3], Seiya Imoto[3], Kazuaki Yokoyama[4], Arinobu Tojo[4], Koichiro Yuji[5], Rui Yamaguchi[6],: [1]Laboratory of DNA Information Analysis, [2]Laboratory of Sequence Analysis, Human Genome Center, [3]Division of Health Medical Data Science, Health Intelligence Center, [4]Division of Molecular Therapy, [5]Division of International Advanced Medical Research, Advanced Clinical Research Center, IMSUT, [6]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute

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 working on the genetic diagnosis of patients suspected of hereditary cancer, and the 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 non-

polyposis colorectal cancer syndrome). In our previous study, we performed genetic analysis of Lynch syndrome using the Sanger's 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, can detect 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-sequencers will be help the identification of pathogenic SVs in patients with hereditary diseases.

In the second project, we have been testing interpretation of genomic data using IBM Watson for Genomics (WfG). After written informed consent was obtained from the patients with colorectal, breast, uterine, gallbladder, pancreatic cancer, lymphoma, prostate cancer, liposarcoma, glioblastoma, and hepatoblastoma, they were enrolled in this study. 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.

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Advanced Clinical Research Center

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

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Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	博士(医学)	伊　藤　博　崇

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

Development of novel recombinant oncolytic HSV-1

With a steady increase in the number of deaths caused by cancer, there is an urgent need for novel therapeutics. Oncolytic virus therapy utilizing genetically engineered viruses not only destroy tumor cells in the course of tumor cell-specific viral replication, but also exhibit robust antitumor effect by eliciting systemic and specific antitumor immunity. HSV-1 is particularly useful for cancer therapy, because of following favorable characteristics; (1) a high specificity for tumor cells while maintaining safety to normal cells, (2) a high stability of viral genome, (3) a potent oncolytic activity in a wide variety of cancer, (4) a minimal impact of antiviral antibodies on cell-to-cell spread of virus, (5) antiviral drugs are available that can terminate therapy if necessary, and (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 require time-consuming processes to create a new recombinant HSV-1, our original system, T-BAC, enables quick and accurate generation of a new recombinant HSV-1 with desired transgenes inserted into a specific locus by utilizing BAC and two sets of recombinases (Cre/loxP and FLP/FRT). Using T-BAC, we generated human IL-12-expressing oncolytic HSV-1 (T-hIL2) that is currently used in the first-in-human clinical trial for malignant melanoma.

While developing G47Δ as the first-in-the-world, Japan-originated, oncolytic virus product for malignant brain tumors, we have meticulously accumulated pre-clinical data with the intention to expand the application of G47Δ to 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. Our research has revealed that G47Δ is universally effective for all types of solid tumors, and is expected as an innovative treatment for cancer in the near future.

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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 researches. 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 researches, we engage in research on Regulatory Science.

1. Assistance of Clinical Trials/TRs at Research Hospital

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

In Research 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 2014, we supported 4 investigator-initiated investigational new drug application (IND) clinical trials and 2 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 researches 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 2019, we supported 28 basic researches (19: other than IMSUT), 15 preclinical studies (3: other than IMSUT), and 11 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.

Publications

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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], Kazuhiko Koike[1]. [1]Department of Gastroenterology, The University of Tokyo

Our previous study has unveiled the important role of IL-33 in the carcinogenesis of bile duct. But the effect of this cytokine is largely unknown in the intestinal tract. To investigate the role of IL-33 in murine intestinal tract, we have established conditional IL-33 expression mouse line. Stomach specific gene modification line (TFF1-cre mouse) was crossed with lox-stop-lox IL-33 mouse (LSL-IL-33 mouse) to explore its functions in the stomach. TFF1-IL-33 mouse showed gastritis at 6wks after birth 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. We are currently investigating the characteristics and the mechanism of gastritis induced by IL-33.

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. Barrett's adenocarcinoma and squamous cell carcinoma are two major tumors found in human gastric SCJ. The origin of SCJ tumors and the process of tumorigenesis are largely unknown. Using mouse models and lineage tracing, 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]. [2]Division of Gastroenterology, Institute for Adult Diseases, Asahi Life Foundation

Disturbance of intestinal homeostasis is associated with the development of inflammatory bowel disease (IBD). Intestinal homeostasis is governed by multiple factors of host and luminal contents like microbes and food antigens. Among host factors, genetics and immunity have been intensively studied, but little is known about the role of hormonal and neural responses in the development of IBD. Here 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, indicating of attenuated colitis. To investigate the mechanism of nicotine action, we applied in-vitro organoid-dendritic cell co-culture system. Intestinal organoids with crypt and villous structures underwent cystic ballooning morphological changes after co-culture with pathogenic dendritic cells. Cystic changes of organoids were induced by LPLs from IL-10 KO mice, but attenuated with LPLs from nicotine-treated IL-10 KO mice. Nicotine administration to organoid-BMDC co-culture significantly inhibited ballooning change. To dissect the mechanisms of acetylcholine signaling, conditional a7nicotinic acetylcholine receptor knockout mice will be examined.

4. Mechanism of gastric metaplasia development

Hiroto Kinoshita, Yoku Hayakawa, Mitsuru Konishi, Masahiro Hata, Mayo Tsuboi, Yuki Hayata, Yoko Hikiba, Sozaburo Ihara, Hayato Nakagawa, Tsuneo Ikenoue[3], Tetsuo Ushiku[4], Masashi Fukayama[4], Yoshihiro Hirata, Kazuhiko Koike. [3]Division of Clinical Genome Research, IMSUT, [4]Department of Pathology, The University of Tokyo

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, on cholangitis and biliary cancer using originally developed mouse biliary disease models.

6. Pathogenesis of primary biliary cholangitis and novel therapy development targeting T cells

Ryo Nakagawa[5], Yoshimi Kaise, Ryosuke Muroyama[5], Yasuo Matsubara[6], Naoya Kato[5], Yoshihiro Hirata [5]Department of Gastroenterology, Chiba University, [6]Department of General Medicine, Research Hospital, IMSUT

Primary biliary cholangitis (PBC) is an autoimmune liver disease, but the causes are unknown. We focused on the gene expressions of T cells from PBC and found N-Ras is a specific transcript upregulated in PBC. N-Ras induced T cell activation, and differentially expressed T-cell receptor repertoires were identified in T cells from PBC.

7. 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.

8. Novel zinc finger protein in gastrointestinal tract

Yasuo Matsubara, Yoshihiro Hirata

The gastrointestinal tract has definite anatomical and functional boundaries between its contiguous segments. Some genetic markers that delimit gastrointestinal boundaries have been reported, but it is still unknown how such boundaries are established and maintained. We identified novel zinc finger protein in the gastric biopsy specimen by mass spectrometry. Its mRNA sequence was determined by RACE. Currently we are analyzing the molecular function of this novel zinc finger protein.

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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 new 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 the following 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 more than 1330 members and 560 institutions registered with our program. We con-

stantly assess our programs through questionnaires to get user feedback on 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 video program on "Clinical Research Act".

3. Large scale survey of public awareness of medical research terms

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo

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". We obtained 1002 valid responses (response rate, 12.8%).

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. From those results, we proposed that the researchers should understand that the public were unfamiliar with these medical research terms and, hence, should carefully explain to the research subjects the terms relevant to their research in the informed consent process.

4. 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.

5. 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. In order to accelerate data integration, Dr.Kamisato produced a common IC form.

Publication

・有澤和代, 神里彩子.研究倫理教育効果の評価手法に関する試行的考察 ― 倫理審査の質向上を目的とした倫理審査委員の教育・研修を題材として ―. 生命倫理通巻30号pp.112-120. 2019.

・神里彩子. 臨床研究法. 周産期医学50巻1号（1月号）東京医学社. 2020

Center for Stem Cell Biology and Regenerative Medicine

Division of Regenerative Medicine
再生医学分野

Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学)	谷　口　英　樹
Associate Professor	Keisuke Sekine, Ph.D.	准教授	博士(農学)	関　根　圭　輔
Project Assistant Professor	Yasuharu Ueno, Ph.D.	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 paucity of transplantable organs hinders the application of this treatment for most patients. Recently, regenerative medicine with transplantable organs has attracted attention. Regenerative medicine is a challenging scientific field that is going to convert 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), and we are going to realize transplantation of human liver primordia (liver buds [LBs]) generated from iPSCs for the treatment of liver diseases including metabolic disease and liver failure. Moreover, we expand the established technologies to cancer research and have reconstructed artificial refractory cancer tissue (cancer organoid) with a tumor microenvironment. Based on this unique cancer organoid, we develop a new drug-screening system for compounds to 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], Satoshi Okamoto[2], Tomomi Tadokoro[2], Soichiro Murata[2] and Hideki Taniguchi[1,2]:

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[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, there is a critical shortage of donor organs has highlighted the urgent need for generating organs from human induced pluripotent stem cells (iPSCs). To ensure therapeutic efficacy, however, a large number of human iPSC derived liver buds (hiPSC-LBs) need to be transplanted. Meanwhile, to ensure therapeutic safety, it is important to establish a method of evaluating the frequency of undifferentiated iPSCs in iPSC-LBs. Lin28A is expressed in iPSCs and decreases in the differentiated cells. However, hepatic endoderm cells differentiated from iPSCs have high expression levels and could not be applied to the detection of undifferentiated iPSCs in hepatic endoderm cells. Therefore, we searched for markers for detecting undifferentiated cells during

the differentiation of hepatic endoderm cells from iPSCs and extracted such three promising markers. This cell evaluation technique with the extracted markers is considered useful for safe clinical application of human iPSC-LBs.

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

Yasuharu Ueno[1], Keisuke Sekine[1], Yoshiki Kuse[1], Eriko Kanai[1], Syusaku Tsuzuki[1], Takashi Okumura[1], Yumi Horie[1], Toshiharu Kasai[1], Shinya Matsumoto[1], Satoshi Okamoto[2], Tomomi Tadokoro[2], Soichiro Murata[2] and Hideki Taniguchi[1,2]:

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Urea cycle disorders are inherited metabolic disorders that show ammonemia 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 abnormalities result in ornithine transcarbamylase deficiency (OTCD), that is the most common urea cycle disorder in humans. Unlike diffuse liver diseases, most urea cycle disorder patients show normal liver histology. Therefore, transplantation of iPSC-LBs with OTC activity into the liver would help to improve the urea cycle disorder and avoid transplantation of a living donor liver. To investigate the ammonia metabolism of iPSC-LBs, we developed quantitative metabolic flux analysis using a ^{15}N stable isotope-labeled substrate of the urea cycle. We demonstrated that the OTC activity in iPSC-LBs is about one-third of that in primary hepatocytes. Besides, we used genome editing to establish NOG-OTC^{spf} mice, which are an OTCD model with severe immunodeficiency. Combined with the concentration of ammonia in blood and urinary orotic acid, quantitative metabolic analysis suggested that NOG-OTC^{spf} mice exhibit a mild type of OTCD. Moreover, transplantation of Human iPSC-LB under the renal capsule of NOG-OTC^{spf} mice helped to improve the levels of hyperammonemia and urinary orotic acid, confirming that the effectiveness of human iPSC-LB transplantation in improving the pathology of urea cycle disorders.

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

Keisuke Sekine[1], Yasuharu Ueno[1], Yoshiki Kuse[1], Kenta Takeuchi[2], Eriko Kanai[1], Takashi Okumura[1], Yumi Horie[1], Syusaku Tsuzuki[1], Yugo Otake[2], Masaaki Hirai[2], Suzune Kobarai[2] and Hideki Taniguchi[1,2]:

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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 poor prognosis. On the basis of 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. We established a method of reproducing a high-treatment-resistant phenotype observed in pancreatic cancer patients with human primary pancreatic cancer organoids that show high resistance to anticancer drugs both in vitro and in vivo. We also found that this drug evaluation system can reproduce drug sensitivity between patients. In addition, we identified molecules that are involved in cancer cell–stromal cell interactions and are responsible for increased resistance to treatment in pancreatic cancer organoids with stroma. Therefore, drug screening using human primary pancreatic cancer organoids would help develop effective drugs for pancreatic cancer treatment.

Center for Stem Cell Biology and Regenerative Medicine

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

Professor	Atsushi Iwama, M.D., Ph.D.	教　授　博士(医学)	岩　間　厚　志
Assistant Professor	Motohiko Oshima, Ph.D.	助　教　博士(医学)	大　島　基　彦
Assistant Professor	Yaeko Nakajima, Ph.D.	助　教　博士(医学)	中　島　やえ子
Assistant Professor	Masayuki Yamashita, M.D., Ph.D.	助　教　博士(医学)	山　下　真　幸

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. The chromatin binding protein Phf6 restricts the self-renewal of hematopoietic stem cells

Satoru Miyagi[1,7,8], Patrycja Sroczynska[4,5], Yuko Kato[1], Yaeko Takagi-Nakajima[1], Motohiko Oshima[1], Ola Rizq[1], Naoya Takayama[3], Atsunori Saraya[1], Seiya Mizuno[6], Fumihiro Sugiyama[6], Satoru Takahashi[6], Yumi Matsuzaki[7], Jesper Christensen[4,5], Kristian Helin[4,5,8], and Atsushi Iwama[1,2] : [1]Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, Chiba, [2]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo, Tokyo, [3]Department of Regenerative Medicine, Graduate School of Medicine, Chiba University, Chiba, [4]Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Denmark, [5]The Novo Nordisk Center for Stem Cell Biology (Danstem), University of Copenhagen, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark, [6]Laboratory Animal Resource Center, University of Tsukuba, Tsukuba, [7]Department of Life Science, Faculty of Medicine, Shimane University, Izumo.

Recurrent inactivating mutations have been identified in the X-linked *PHF6* gene, encoding a chromatin-binding transcriptional regulator protein, in various hematological malignancies. However, the role of PHF6 in normal hematopoiesis and its tumor suppressor function remain largely unknown. We herein generated mice carrying a floxed *Phf6* allele and inactivated *Phf6* in hematopoietic cells at various developmental stages. The *Phf6* deletion in embryos augmented the capacity of hematopoietic stem cells (HSCs) to proliferate in cultures and reconstitute hematopoiesis in recipient mice. The *Phf6* deletion in neonates and adults revealed that cycling HSCs readily acquired an advantage in competitive repopulation upon the *Phf6* deletion, while dormant HSCs only did so after serial transplantations. *Phf6*-deficient HSCs maintained an enhanced repopulating capacity during serial transplantations; however, they did not induce any hema-

tological malignancies. Mechanistically, Phf6 directly and indirectly activated downstream effectors in TNFα signaling. The *Phf6* deletion repressed the expression of a set of genes associated with TNFα signaling, thereby conferring resistance against the TNFα-mediated growth inhibition on HSCs. Collectively, these results define Phf6 as a novel negative regulator of HSC self-renewal, implicating inactivating *PHF6* mutations in the pathogenesis of hematological malignancies, but also indicate that a *Phf6* deficiency alone is not sufficient to induce hematopoietic transformation.

2. KDM2B in polycomb repressive complex 1.1 functions as a tumor suppressor in the initiation of T-cell leukemogenesis

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KDM2B together with RING1B, PCGF1, and BCOR or BCORL1 comprise polycomb repressive complex 1.1 (PRC1.1), a non-canonical PRC1 that catalyzes H2AK119ub1. It binds to non-methylated CpG islands through its zinc finger-CxxC (ZF-CxxC) DNA binding domain and recruits the complex to target gene loci. Recent studies identified the loss of function mutations in the PRC1.1 gene, *BCOR* and *BCORL1* in human T-cell acute lymphoblastic leukemia (T-ALL). We previously reported that *Bcor* insufficiency induces T-ALL in mice, supporting a tumor suppressor role for BCOR. However, the function of BCOR responsible for tumor suppression, either its co-repressor function for BCL6 or that as a component of PRC1.1, remains unclear. We herein examined mice specifically lacking the ZF-CxxC domain of KDM2B in hematopoietic cells. Similar to *Bcor*-deficient mice, *Kdm2b*-deficient mice developed lethal T-ALL mostly in a NOTCH1-dependent manner. A ChIP sequence analysis of thymocytes revealed the binding of KDM2B at promoter regions, at which BCOR and EZH2 co-localized. KDM2B target genes markedly overlapped with those of NOTCH1 in human T-ALL cells, suggesting that non-canonical PRC1.1 antagonizes NOTCH1-mediated gene activation. KDM2B target genes were expressed at higher levels than the others and were marked with high levels of H2AK119ub1 and H3K4me3, but low levels of H3K27me3, suggesting that KDM2B target genes are transcriptionally active or primed for activation. These results indicate that PRC1.1 plays a key role in restricting excessive transcriptional activation by active NOTCH1, thereby acting as a tumor suppressor in the initiation of T-cell leukemogenesis.

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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. Early fluid overload predicts higher non-relapse and overall mortality in adults after single-unit cord blood transplantation.

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Early fluid overload has been associated with poor transplant outcomes after allogeneic hematopoietic cell transplantation. However, its effects on the outcomes after cord blood transplantation (CBT) are unclear. We retrospectively analyzed the data of 227 adult patients who received single-unit CBT in our institute. The cumulative incidence of grade ≥2 fluid overload was 4% at day 30 after CBT with a median onset at 16 days (range, 9-30 days) after CBT. In the multivariate analysis, grade ≥2 fluid overload was significantly associated with higher non-relapse mortality (hazard ratio [HR], 5.73; P = 0.011) and overall mortality (HR, 3.81; P = 0.006). Among the entire cohort, 133 patients were treated with low-dose dopamine (0.5-2 μg/kg/min) with a median time of initiation of low-dose dopamine therapy at 10.5 days after CBT. Use of low-dose dopamine significantly increased daily urine output and decreased body weight. These data suggested that early fluid overload was significantly associated with non-relapse and overall mortality after single CBT. The early intervention of low-dose dopamine to prevent early fluid overload is a matter of future investigation for patients undergoing allogeneic hematopoietic cell transplantations (HCT), particularly for CBT.

2. Efficacy and safety of micafungin in unrelated cord blood transplant patients.

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Micafungin (MCFG) is an echinocandin antifungal drug used for prophylaxis and treatment of fungal infections after allogeneic hematopoietic cell transplantation (HCT). However, its efficacy and safety in patients undergoing cord blood transplantation (CBT) has not been clarified. We retrospectively analyzed the efficacy and safety of MCFG in 92 adult patients undergoing CBT in our institute. Of the entire

cohort, 83 patients (90%) received MCFG for empirical or preemptive therapy. Documented breakthrough fungal infection occurred in 2 patients during MCFG treatment. Among the 49 patients who received MCFG as empirical therapy for febrile neutropenia, 41 (84%) patients had resolution of fever during neutropenia. Elevation of serum levels of hepatobiliary parameters during MCFG treatment was commonly observed, but grade 3 or higher elevation was rare. We also compared the efficacy and safety of 2 different initial daily doses of MCFG (150 mg vs. 300 mg). There were no significant differences of efficacy and safety between the two groups. These data suggest that MCFG was effective and safe for adult patients undergoing CBT. The optimal daily dose of MCFG treatment is a matter of future investigation for adult patients undergoing CBT.

3. Red blood cell transfusion burden by day 30 predicts mortality in adults after single-unit cord blood transplantation.

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Increased red blood cell (RBC) transfusion requirements are associated with morbidity and mortality after allogeneic hematopoietic cell transplantation. However, its impact on the outcomes after cord blood transplantation (CBT) is unclear. We retrospectively analyzed the data of 278 adult patients who received single-unit CBT in our institute. The median number of RBC transfusions for each patient was 12 units (range, 4-66) by day 30 and 14 units (range, 4-70) by RBC engraftment. Sex, cord blood $CD34^+$ cell dose, cytomegalovirus serostatus, total body irradiation dose in the conditioning regimen, ABO blood group incompatibility, and pre-CBT RBC transfusion requirements were significantly associated with the number of RBC transfusion units in the linear regression analysis. In the multivariate analysis, RBC transfusion ≥18 units by day 30 was significantly associated with higher overall mortality (hazard ratio, 1.86; P = 0.018). These data suggested that early RBC transfusion burden was significantly associated with overall mortality in adult patients undergoing single CBT. Early RBC transfusion burden might be a surrogate marker for poor outcomes after single CBT.

4. Fungemia due to Fusarium solani under low-dose liposomal amphotericin B in a patient after cord blood transplantation.

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The introduction of the prophylactic use of antifungal drugs caused the increased occurrence of invasive fungal infections due to previously rare molds, such as fusariosis, after allogeneic hematopoietic stem cell transplantation. We herein report the case of a patient with diffuse large B-cell lymphoma who developed fungemia due to Fusarium solani during liposormal amphotericin B on day 25 after cord blood transplantation (CBT). Because Fusarium species might differ in virulence and drug susceptibility, the sequencing of the internal transcribed spacer region of the ribosomal RNA gene accurately identified Fusarium solani to be the cause of fungemia at the species level. This case highlights Fusarium solani as the cause of fungemia in a patient under liposormal amphotericin B treatment after CBT.

5. Development of pre-engraftment syndrome, but not acute graft-versus-host disease, reduces relapse rate of acute myelogenous leukemia after single cord blood transplantation.

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The different effects of pre-engraftment syndrome (PES) and acute graft-versus-host disease (aGVHD) on outcomes after cord blood transplantation (CBT) are unclear. We retrospectively evaluated the impact of PES and aGVHD on relapse and survival after single-unit CBT in 138 adult patients with hematologic malignancies at our institution between 2004 and 2016. Multivariate analysis demonstrated that development of grade III-IV aGVHD, particularly with gut or liver involvement, significantly contributed to higher nonrelapse mortality (P < .001), but PES and grade II-IV aGVHD did not. In subgroup analyses of underlying disease type, the development of PES had a significant effect on decreased relapse (P = .032) and better disease-free survival (DFS) (P = .046) in patients with acute myelogenous leukemia (AML). These data

suggest that PES is associated with a reduced relapse rate and better DFS in AML, indicating that the early immune reaction before neutrophil engraftment may provide a unique graft-versus-leukemia effect after single-unit CBT.

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Center for Stem Cell Biology and Regenerative Medicine

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

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

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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.

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

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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 clearly showd 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 $[Ca^{2+}]_c$.

CML-LSCs (Chronic myelogenous 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 for 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 for 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 with 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 concentrated 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 for imatinib. Moreover, we found that the CML-LSCs expressed PD-L1 at higher level than the CML progenitor cells. Combination therapy of imatinib and PD-L1 blocking antibody was more efficient for eliminating CML-LSCs than mono therapy of imatinib. These data exhibited that PD-L1 expression was linked to G0 status of CML-LSCs and their resistance for TKIs.

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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 are a valuable cell source in the regenerative medicine field, hematopoietic stem cells are a valuable cell source for transplantation medicine, and pluripotent stem cells are newly emerging types of stem cells that are used from basic research to the development of curative strategies for diseases. We have focused especially on the use of human induced pluripotent stem cells (iPSCs) as a research platform to model and determine the pathophysiology of intractable diseases. We aimed to establish safe and efficacious treatment for patients suffering from various types of diseases with no curative treatment currently available.

1. Robust and highly efficient human iPSC generation from patient nonmobilized peripheral blood–derived CD34+ cells using the autoerasable Sendai virus vector

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Disease modeling using patient-derived induced pluripotent stem cells (iPSCs) is a powerful tool for determining the mechanisms underlying disease pathogenesis and for developing safe and effective treatments. Patient peripheral blood (PB) cells can be collected with minimum invasiveness and so are used to generate iPSCs. Hematopoietic stem and progenitor cells (HSPCs) are often targeted as the reprogramming source to derive iPSCs that lack immunoreceptor gene rearrangements. However, current protocols generally require HSPC mobilization and/or ex vivo expansion because of their sparsity in the steady state and low reprogramming efficiencies, making the overall procedure costly, laborious, and time consuming. We established a highly efficient method of generating iPSCs from nonmobilized PB-derived $CD34^+$ HSPCs. To reprogram $CD34^+$-rich cells to pluripotency, we used the Sendai virus vector SeVdp-302L to transfer the following four transcription factors: KLF-4, OCT-4, SOX2, and c-MYC. We established 223 iPSC lines with high reprogramming efficiencies from 15 patients with 8 different disease types. We found that our method allows the rapid appearance (within ~8 days) of primary colonies, all of which are expandable under feeder-free conditions, enabling robust establishment steps with less workload. After thawing, established iPSC lines were verified to be pluripotency marker positive and of non-T-cell origin. The majority of iPSC lines were confirmed to be transgene free, with normal karyotypes. A defined in vitro assay also verified their trilineage differentiation capability. This robust, highly efficient method enables the rapid and cost-effective establishment of transgene-free iPSC lines from a small volume of PB, facilitating the biobanking of patient-derived iPSCs and their use for disease modeling.

2. Generation of three iPSC lines from postmortem tissue derived after the sudden death of a young patient with *STXBP1* mutation

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Recreating the conditions present in life during autopsy is a regular task in the field of forensic science. Although determining the mechanisms underlying sudden, unexpected death requires analysis of living cells, because of postmortem changes, acquiring viable postmortem samples is generally impractical at autopsy. The aim is to obtain living cells at autopsy and to enable in vitro revival of the subject's lifetime conditions by generating induced pluripotent stem cells (iPSCs). Here, we report an autopsy case following the sudden, unexpected death of an 8-year-old girl with Lennox–Gastaut syndrome, who had the *R551H*-mutant *STXBP1* gene. We generated iPSCs from fibroblasts cultured postmortem. The established iPSC clones showed pluripotent characteristics with a heterozygous variant genotype shared with the original fibroblasts. The iPSC clones also demonstrated trilineage differentiation capability, indicating their utility in disease-modeling studies. This is the first study to report the establishment of iPSCs from a child who died suddenly. Using iPSC technology in forensic science is expected to be an epoch-making approach that will pave the way for precise identification of the cause of sudden death.

Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Pathology
幹細胞病理学分野

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

1. *De novo* DNA methylation at imprinted loci during reprogramming into naïve and primed pluripotency

Masaki Yagi[1], Mio Kabata[1], Tomoyo Ukai[1], Sho Ohta, Akito Tanaka[1], Yui Shimada[1], Michihiko Sugimoto[2], Kimi Araki[2], Keisuke Okita[1], Knut Woltjen[1,3], Konrad Hochedlinger[4,5,6,7], Takuya Yamamoto[1, 9, 10], Yasuhiro Yamada[1,9] : [1] Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University [2] Institute of Resource Development and Analysis, Kumamoto University [3] Hakubi Center for Advanced Research, Kyoto University [4] Department of Molecular Biology, Massachusetts General Hospital [5] Center for Regenerative Medicine, Massachusetts General Hospital [6] Cancer Center, Massachusetts General Hospital, [7] Department of Stem Cell and Regenerative Biology, Harvard University [8] Harvard Stem Cell Institute [9] AMED-CREST [10] Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University

CpG islands (CGIs) including those at imprinting control regions (ICRs) are protected from *de novo* methylation in somatic cells. However, many cancers often exhibit CGI hypermethylation, implying that the machinery is impaired in cancer cells. Here, we conducted a comprehensive analysis of CGI methylation during the somatic cell reprogramming. Although most CGIs remain hypomethylated, a small subset of CGIs, particularly at several ICRs, were often *de novo* methylated in reprogrammed pluripotent stem cells (PSCs). Such *de novo* ICR methylation was linked with the silencing of reprogramming factors, which occurs at a late stage of reprogramming. The ICR-preferred CGI hypermethylation was similarly observed in human PSCs. Mechanistically, ablation of *Dnmt3a* prevented PSCs from *de novo* ICR methylation. Notably, the ICR-preferred CGI hypermethylation was observed in pediatric cancers, while adult cancers exhibit genome-wide CGI hypermethylation. These results may have important implications in the pathogenesis of pediatric cancers and the application of PSCs.

2. Smarcb1 maintains the cellular identity and the chromatin landscapes of mouse embryonic stem cells

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ES cell (ESC) identity is stably maintained through the coordinated regulation of transcription factors and chromatin structure. SMARCB1, also known as INI1, SNF5, BAF47, is one of the subunits of SWI/SNF (BAF) complexes that play a crucial role in regulating gene expression by controlling chromatin dynamics. Genetic ablation of Smarcb1 in mice leads to embryonic lethality at the peri-implantation stage, indicating that Smarcb1 is important for the early developmental stages. However, the role of SMARCB1 in the maintenance of the ESC identity remains unknown. Here we established mouse ESCs lacking Smarcb1 and investigated the effect of Smarcb1 ablation on the differentiation propensity of ESCs. We found an increased expression of trophectoderm-related genes including Cdx2 in Smarcb1-deficient ESCs. Consistently, they exhibited an extended differentiation propensity into the trophectoderm lineage cells in teratomas. However, although Smarcb1-deficient cells were infrequently incorporated into the trophectoderm cell layer of blastocysts, they failed to contribute to mature placental tissues in vivo. Furthermore, Smarcb1-deficient cells exhibited a premature differentiation in the neural tissue of E14.5 chimeric embryos. Notably, we found that binding motifs for CTCF, which is involved in the maintenance of genomic DNA architecture was significantly enriched in chromatin regions whose accessibility was augmented in Smarcb1-deficient cells, while those for pluripotency factors were overrepresented in regions which have more closed structure in those cells. Collectively, we propose that SMARCB1-mediated remodeling of chromatin landscapes is important for the maintenance and differentiation of ESCs.

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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

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

1. 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. Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation.

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Multipotent self-renewing haematopoietic stem cells (HSCs) regenerate the adult blood system after transplantation[1], which is a curative therapy for numerous diseases including immunodeficiencies and leukaemias[2]. Although substantial effort has been applied to identifying HSC maintenance factors through the characterization of the in vivo bone-marrow HSC microenvironment or niche[3-5], stable ex vivo HSC expansion has previously been unattainable[6,7]. Here we describe the development of a defined, albumin-free culture system that supports the long-term ex vivo expansion of functional mouse HSCs. We used a systematic optimization approach, and found that high levels of thrombopoietin synergize with low levels of stem-cell factor and fibronectin to sustain HSC self-renewal. Serum albumin has long been recognized as a major source of biological contaminants in HSC cultures[8]; we identify polyvinyl alcohol as a functionally superior replacement for serum albumin that is compatible with good manufacturing practice. These conditions afford between 236- and 899-fold expansions of functional HSCs over 1 month, although analysis of clonally derived cultures suggests that there is considerable heterogeneity in the self-renewal capacity of HSCs ex vivo. Using this system, HSC cultures that are derived from only 50 cells robustly engraft in recipient mice without the normal requirement for toxic pre-conditioning (for example, radiation), which may be relevant for HSC transplantation in humans. These findings therefore have important implications for both basic HSC research and clinical haematology.

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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 two benchtop analyzers.

FCM usage performance in 2019

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 2019.

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 Ebola and influenza viruses as models.

Genetic and antigenic characterisation of influenza A(H3N2) viruses isolated in Yokohama during the 2016/17 and 2017/18 influenza seasons

Kawakami C[1], Yamayoshi S, Akimoto M[2], Nakamura K[2], Miura H[2], Fujisaki S[2], Pattinson DJ[3], Shimizu K[1], Ozawa H[1], Momoki T[1], Saikusa M[1], Yasuhara A, Usuku S[1], Okubo I[1], Toyozawa T[4], Sugita S[5], Smith DJ[3], Watanabe S[2], Kawaoka Y.

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Influenza A(H3N2) virus rapidly evolves to evade human immune responses, resulting in changes in the antigenicity of haemagglutinin (HA). Therefore, continuous genetic and antigenic analyses of A(H3N2) virus are necessary to detect antigenic mutants as quickly as possible. We attempted to phylogenetically and antigenically capture the epidemic trend of A(H3N2) virus infection in Yokohama, Japan during the 2016/17 and 2017/18 influenza seasons. We determined the HA sequences of A(H3N2) viruses detected in Yokohama, Japan during the 2016/17 and 2017/18 influenza seasons to identify amino acid substitutions and the loss or gain of potential N-glycosylation sites in HA, both of which potentially affect the antigenicity of HA. We also examined the antigenicity of isolates using ferret antisera obtained from experimentally infected ferrets. Influenza A(H3N2) viruses belonging to six clades (clades 3C.2A1, 3C.2A1a, 3C.2A1b, 3C.2A2, 3C.2A3 and 3C.2A4) were detected during the 2016/17 influenza season, whereas viruses belonging to two clades (clades 3C.2A1b and 3C.2A2) dominated during the 2017/18 influenza season. The isolates in clades 3C.2A1a and 3C.2A3 lost one N-linked glycosylation site in HA relative to other clades. Antigenic analysis revealed antigenic differences among clades, especially clade 3C.2A2 and 3C.2A4 viruses, which showed distinct antigenic differences from each other and from other clades in the antigenic map. Multiple clades, some of which differed antigenically from others, co-circulated in Yokohama, Japan during the 2016/17 and 2017/18 influenza seasons.

Serological analysis of Ebola virus survivors and close contacts in Sierra Leone: A cross-sectional study

Halfmann PJ[1], Eisfeld AJ[1], Watanabe T, Maemura T[1], Yamashita M, Fukuyama S, Armbrust T[1], Rozich I[1], N'jai A[1,3], Neumann G[1], Kawaoka Y, Sahr F[3]

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Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, USA; [2]Department of Biological Sciences, Fourah Bay College, University of Sierra Leone, Freetown, Sierra Leone; [3]34th Regimental Military Hospital at Wilberforce, Freetown, Sierra Leone

The 2013–2016 Ebola virus outbreak in West Africa was the largest and deadliest outbreak to date. Here we conducted a serological study to examine the antibody levels in survivors and the seroconversion in close contacts who took care of Ebola-infected individuals, but did not develop symptoms of Ebola virus disease. In March 2017, we collected blood samples from 481 individuals in Makeni, Sierra Leone: 214 survivors and 267 close contacts. Using commercial, quantitative ELISAs, we tested the plasma for IgG-specific antibodies against three major viral antigens: GP, the only viral glycoprotein expressed on the virus surface; NP, the most abundant viral protein; and VP40, a major structural protein of Zaire ebolavirus. We also determined neutralizing antibody titers. In the cohort of Ebola survivors, 97.7% of samples (209/214) had measurable antibody levels against GP, NP, and/or VP40. Of these positive samples, all but one had measurable neutralizing antibody titers against Ebola virus. For the close contacts, up to 12.7% (34/267) may have experienced a subclinical virus infection as indicated by detectable antibodies against GP. Further investigation is warranted to determine whether these close contacts truly experienced subclinical infections and whether these asymptomatic infections played a role in the dynamics of transmission.

Food additives as novel Influenza vaccine adjuvants

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Influenza is a major threat to public health. Vaccination is an effective strategy to control influenza; however, the current inactivated influenza vaccine has mild immunogenicity and exhibits suboptimal efficacy in clinical use. Vaccine efficacy can be improved by the addition of adjuvants, but few adjuvants have been approved for human use. To explore novel and effective adjuvants for influenza vaccines, here we screened 145 compounds from food additives approved in Japan. Of these 145 candidates, we identified 41 compounds that enhanced the efficacy of the split influenza hemagglutinin (HA) vaccine against lethal virus challenge in a mouse model. These 41 compounds included 18 novel adjuvant candidates and 15 compounds with previously reported adjuvant effects for other antigens but not for the influenza vaccine. Our results are of value to the development of novel and effective adjuvanted influenza or other vaccines for human use.

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.

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International Research Center for Infectious Diseases

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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. Roles of the Interhexamer Contact Site for Hexagonal Lattice Formation of the Herpes Simplex Virus 1 Nuclear Egress Complex in Viral Primary Envelopment and Replication

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

The scaffolding proteins of several envelope viruses required for virion assembly form high-order lattice structures. However, information on the significance of their lattice formation in infected cells is limited. Herpesviruses acquire envelopes twice during their viral replication. The first envelop acquisition (primary envelopment) is one of the steps in the vesicle-mediated nucleocytoplasmic transport of nascent nucleocapsids, which is unique in biology. HSV-1 NEC, thought to be conserved in all members of the Herpesviridae family, is critical for primary envelopment and was shown to form a hexagonal lattice structure. Here, we investigated the significance of the interhexamer contact site for hexagonal lattice formation of the NEC in HSV-1-infected cells and present evidence suggesting that the lattice formation of the NEC hexamer has an important role in HSV-1 replication by regulating primary envelopment. Our results provide insights into the mechanisms of the envelopment of herpesviruses and other envelope viruses.

During the nuclear export of nascent nucleocapsids of herpes simplex virus 1 (HSV-1), the nucleocapsids acquire a primary envelope by budding through the inner nuclear membrane into the perinuclear space between the inner and outer nuclear membranes. This unique budding process, termed primary envelopment, is initiated by the nuclear egress complex (NEC), composed of the HSV-1 UL31 and UL34 proteins. Earlier biochemical approaches have shown that the NEC has an intrinsic ability to vesiculate membranes through the formation of a hexagonal lattice structure. The significance of intrahexamer interactions of the NEC in the primary envelopment of HSV-1-infected cells has been reported. In contrast, the contribution of lattice formation of the NEC hexamer to primary envelopment in HSV-1-infected cells remains to be elucidated. Therefore, we constructed and characterized a recombinant HSV-1 strain carrying an amino acid substitution in a UL31 residue that is an interhexamer contact site for the lattice formation of the NEC hexamer. This mutation was reported to destabilize the interhexamer interactions of the HSV-1 NEC. Here, we demonstrate that the mutation

causes the aberrant accumulation of nucleocapsids in the nucleus and reduces viral replication in Vero and HeLa cells. Thus, the ability of HSV-1 to form the hexagonal lattice structure of the NEC was linked to an increase in primary envelopment and viral replication. Our results suggest that the lattice formation of the NEC hexamer has an important role in HSV-1 replication by regulating primary envelopment.

2. Identification of the Capsid Binding Site in the Herpes Simplex Virus 1 Nuclear Egress Complex and Its Role in Viral Primary Envelopment and Replication

Kosuke Takeshima, Jun Arii, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato and Yasusi Kawaguchi

Binding of HSV-1 NEC to nucleocapsids has been thought to promote nucleocapsid budding at the inner nuclear membrane and subsequent incorporation of nucleocapsids into vesicles during nuclear egress of nucleocapsids. However, data to directly support this hypothesis have not been reported thus far. In this study, we have present data showing that two amino acids in the membrane-distal face of the HSV-1 NEC, which contains the putative capsid binding site based on the solved NEC structure, were in fact required for efficient NEC binding to nucleocapsids and for efficient incorporation of nucleocapsids into vesicles during primary envelopment. This is the first report showing direct linkage between NEC binding to nucleocapsids and an increase in nucleocapsid incorporation into vesicles during herpesvirus primary envelopment.

During nuclear egress of nascent progeny herpesvirus nucleocapsids, the nucleocapsids acquire a primary envelope by budding through the inner nuclear membrane of infected cells into the perinuclear space between the inner and outer nuclear membranes. Herpes simplex virus 1 (HSV-1) UL34 and UL31 proteins form a nuclear egress complex (NEC) and play critical roles in this budding process, designated primary envelopment. To clarify the role of NEC binding to progeny nucleocapsids in HSV-1 primary envelopment, we established an assay system for HSV-1 NEC binding to nucleocapsids and capsid proteins in vitro Using this assay system, we showed that HSV-1 NEC bound to nucleocapsids and to capsid protein UL25 but not to the other capsid proteins tested (i.e., VP5, VP23, and UL17) and that HSV-1 NEC binding of nucleocapsids was mediated by the interaction of NEC with UL25. UL31 residues arginine-281 (R281) and aspartic acid-282 (D282) were required for efficient NEC binding to nucleocapsids and UL25. We also showed that alanine substitution of UL31 R281 and D282 reduced HSV-1 replication, caused aberrant accumulation of capsids in the nucleus, and induced an accumulation of empty vesicles that were similar in size and morphology to primary envelopes in the perinuclear space. These results suggested that NEC binding via UL31 R281 and D282 to nucleocapsids, and probably to UL25 in the nucleocapsids, has an important role in HSV-1 replication by promoting the incorporation of nucleocapsids into vesicles during primary envelopment.

Publications

Takeshima, K., Arii, J., Maruzuru, Y., Koyanagi, N., Kato, A., and Kawaguchi, Y. Identification of the Capsid Binding Site in the Herpes Simplex Virus 1 Nuclear Egress Complex and Its Role in Viral Primary Envelopment and Replication. J. Virol. 93: e01290-19, 2019.

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International Research Center for Infectious Diseases

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感染制御系・ウイルス学分野

Associate Professor　Takeshi Ichinohe, Ph.D.

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

1. Influenza A virus M2 protein triggers mitochondrial DNA-mediated antiviral immune responses.

Moriyama M, Koshiba T, and Ichinohe T.

Cytosolic mitochondrial DNA (mtDNA) activates cGAS-mediated antiviral immune responses, but the mechanism by which RNA viruses stimulate mtDNA release remains unknown. Here we show that viroporin activity of influenza virus M2 or encephalomyocarditis virus (EMCV) 2B protein triggers translocation of mtDNA into the cytosol in a MAVS-dependent manner. Although influenza virus-induced cytosolic mtDNA stimulates cGAS- and DDX41-dependent innate immune responses, the nonstructural protein 1 (NS1) of influenza virus associates with mtDNA to evade the STING-dependent antiviral immunity. The STING-dependent antiviral signaling is amplified in neighboring cells through gap junctions. In addition, we find that STING-dependent recognition of influenza virus is essential for limiting virus replication in vivo. Our results show a mechanism by which influenza virus stimulates mtDNA release and highlight the importance of DNA sensing pathway in limiting influenza virus replication.

2. High ambient temperature dampens adaptive immune responses to influenza A virus infection.

Moriyama M, and Ichinohe T.

Although climate change may expand the geographical distribution of several vector-borne diseases, the effects of environmental temperature in host defense to viral infection in vivo are unknown. Here, we demonstrate that exposure of mice at high ambient temperature of 36˚C impaired adaptive immune responses against infection with viral pathogens, influenza, Zika, and sever fever with thrombocytopenia syndrome phlebovirus. Following influenza virus infection, the high heat-exposed mice failed to stimulate inflammasome-dependent cytokine secretion and respiratory DC migration to lymph nodes. Although commensal microbiota composition remained intact, the high heat-exposed mice decreased their food intake and increased autophagy in the lung tissue. Induction of autophagy in room temperature-exposed mice severely impaired virus-specific CD8 T cells and antibody responses following respiratory influenza virus infection. In addition, we found that administration of glucose or dietary short-chain fatty acids

(SCFAs) restored influenza virus-specific adaptive immune responses in high heat-exposed mice. These findings uncover an unexpected mechanism by which the ambient temperature and nutritional status control the virus-specific adaptive immune responses.

3. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome.

Chen IY, Moriyama M, Chang MF, and Ichinohe T.

Nod-like receptor family, pyrin domain-containing 3 (NLRP3) regulates the secretion of proinflammatory cytokines interleukin 1 beta (IL-1β) and IL-18. We previously showed that influenza virus M2 or encephalomyocarditis virus (EMCV) 2B proteins stimulate IL-1β secretion following activation of the NLRP3 inflammasome. However, the mechanism by which severe acute respiratory syndrome coronavirus (SARS-CoV) activates the NLRP3 inflammasome remains unknown. Here, we provide direct evidence that SARS-CoV 3a protein activates the NLRP3 inflammasome in lipopolysaccharide-primed macrophages. SARS-CoV 3a was sufficient to cause the NLRP3 inflammasome activation. The ion channel activity of the 3a protein was essential for 3a-mediated IL-1β secretion. While cells uninfected or infected with a lentivirus expressing a 3a protein defective in ion channel activity expressed NLRP3 uniformly throughout the cytoplasm, NLRP3 was redistributed to the perinuclear space in cells infected with a lentivirus expressing the 3a protein. K^+ efflux and mitochondrial reactive oxygen species were important for SARS-CoV 3a-induced NLRP3 inflammasome activation. These results highlight the importance of viroporins, transmembrane pore-forming viral proteins, in virus-induced NLRP3 inflammasome activation.

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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. Comparative description of the expression profile of interferon-stimulated genes in multiple cell lineages targeted by HIV-1 infection

Hirofumi Aso, Jumpei Ito, Kei Sato

Immediately after viral infections, innate immune sensors recognize viruses and lead to the production of type I interferon (IFN-I). IFN-I upregulates various genes, referred to as IFN-stimulated genes (ISGs), and some ISGs inhibit viral replication. HIV-1, the causative agent of AIDS, mainly infects $CD4^+$ T cells and macrophages and triggers the IFN-I-mediated signaling cascade. Certain ISGs are subsequently upregulated by IFN-I stimulus and potently suppress HIV-1 replication. HIV-1 cell biology has shed light on the molecular understanding of the IFN-I production triggered by HIV-1 infection and the antiviral roles of ISGs. However, the differences in the gene expression patterns following IFN-I stimulus among HIV-1 target cell types are poorly understood. In this study, we hypothesize that the expression profiles of ISGs are different among HIV-1 target cells and address this question by utilizing public transcriptome datasets and bioinformatic techniques. We focus on three cell types intrinsically targeted by HIV-1, including $CD4^+$ T cells, monocytes, and macrophages, and comprehensively compare the expression patterns of ISGs among these cell types. Furthermore, we use the datasets of the differentially expressed genes by HIV-1 infection and the evolutionarily conserved ISGs in mammals and perform comparative transcriptome analyses. We defined 104 'common ISGs' that were upregulated by IFN-I stimulus in $CD4^+$ T cells, monocytes, and macrophages. The ISG expression patterns were different among these three cell types, and intriguingly, both the numbers and the magnitudes of upregulated ISGs by IFN-I stimulus were greatest in macrophages. We also found that the upregulated genes by HIV-1 infection included most 'common ISGs'. Moreover, we determined that the 'common ISGs', particularly those with antiviral activity, were evolutionarily conserved in mammals. To our knowledge, this study is the first investigation to comprehensively describe (i) the different expression patterns of ISGs among HIV-1 target cells, (ii) the overlap in the genes modulated by IFN-I stimulus and HIV-1 infection and (iii) the evolutionary conservation in mammals of the antiviral ISGs that are expressed in HIV-1 target cells. Our results will be useful for deeply understanding the relationship of the effect of IFN-I and the modulated gene expression by HIV-1 infec-

tion.

2. Retroviruses drive the rapid evolution of mammalian *APOBEC3* genes

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APOBEC3 (*A3*) genes are members of the *AID*/*APOBEC* gene family that are found exclusively in mammals. *A3* genes encode antiviral proteins that restrict the replication of retroviruses by inducing G-to-A mutations in their genomes, and have undergone extensive amplification and diversification during mammalian evolution. Endogenous retroviruses (ERVs) are sequences derived from ancient retroviruses that are widespread mammalian genomes. In this study we characterize the *A3* repertoire and use the ERV 'fossil record' to explore the long-term history of co-evolutionary interaction between A3s and retroviruses. We examine the genomes of 160 mammalian species and identify 1,420 *AID*/*APOBEC*-related genes, including representatives of previously uncharacterized lineages. We show that *A3* genes have been amplified in mammals and that amplification is positively correlated with the extent of germline colonization by ERVs. Moreover, we demonstrate that the signatures of A3-mediated mutation can be detected in ERVs found throughout mammalian genomes, and show that in mammalian species with expanded *A3* repertoires, ERVs are significantly enriched for G-to-A mutations. Finally, we show that *A3* amplification occurred concurrently with prominent ERV invasions in primates. Our findings establish that conflict with retroviruses is a major driving force for the rapid evolution of mammalian *A3* genes.

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The goal of our research is to explore antigen uptake receptors on specialized epithelial M cells to identify potential targets for mucosal vaccine delivery. Thus, this division aims to develop novel mucosal vaccines by taking advantage of the conjugation of M-cell-receptor ligands with various vaccine antigens.

1. Sox8 is Essential for M Cell Maturation to Accelerate IgA Response at the Early Stage after Weaning in Mice.

Shunsuke Kimura[1], Nobuhide Kobayashi[2], Yutaka Nakamura[2], Takashi Kanaya[3,4], Daisuke Takahashi[2], Rhoji Fujiki[5], Mami Mutoh[6], Yuuki Obata[2], Toshihiko Iwanaga[1], Tomoo Nakagawa[7], Naoya Kato[7], Shintaro Sato[8], Tsuneyasu Kaisho[9], Hiroshi Ohno[3,4], Koji Hase[2].: [1]Laboratory of Histology and Cytology, Graduate School of Medicine, Hokkaido University. [2]Division of Biochemistry, Faculty of Pharmacy, Keio University. [3]Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences. [4]Division of Immunobiology, Department of Medical Life Science, Graduate School of Medical Life Science, Yokohama City University. [5]Department of Applied Genomics, Kazusa DNA Research Institute. [6]Department of Orthodontics, Faculty of Dental Medicine and Graduate School of Dental Medicine, Hokkaido University. [7]Department of Gastroenterology, Graduate School of Medicine, Chiba University. [8]Mucosal Vaccine Project, BIKEN Innovative Vaccine Research Alliance Laboratories, Research Institute for Microbial Diseases, Osaka University. [9]Department of Immunology, Institute of Advanced Medicine, Wakayama Medical University.

Microfold (M) cells residing in the follicle-associated epithelium (FAE) of the gut-associated lymphoid tissue are specialized for antigen uptake to initiate mucosal immune responses. The molecular machinery and biological significance of M-cell differentiation, however, remain to be fully elucidated. Here, we demonstrate that Sox8, a member of the SRY-related HMG box transcription factor family, is specifically expressed by M cells in the intestinal epithelium. The expression of Sox8 requires activation of RANKL-RelB signaling. Chromatin immunoprecipitation and luciferase assays revealed that Sox8 directly binds the promoter region of Gp2 to increase Gp2 expression, which is the hallmark of functionally mature M cells. Furthermore, genetic deletion of Sox8 causes a marked decrease in the number of mature M cells, resulting in reduced antigen uptake in Peyer's patches. Consequently, juvenile Sox8-deficient mice showed attenuated germinal center reactions and antigen-specific IgA responses. These findings indicate that Sox8 plays an essential role in the development of M cells

to establish mucosal immune responses.

2. Fasting-Refeeding Impacts Immune Cell Dynamics and Mucosal Immune Responses.

Motoyoshi Nagai[1,2], Ryotaro Noguchi[1,2], Daisuke Takahashi[1], Takayuki Morikawa[3], Kouhei Koshida[1], Seiga Komiyama[1], Narumi Ishihara[1], Takahiro Yamada[1], Yuki I. Kawamura[2], Kisara Muroi[1], Kouya Hattori[1], Nobuhide Kobayashi[1], Yumiko Fujimura[1], Masato Hirota[1], Ryohtaroh Matsumoto[1], Ryo Aoki[4,5], Miwa Tamura-Nakano[6], Machiko Sugiyama[2,7], Tomoya Katakai[8], Shintaro Sato[9,10], Keiyo Takubo[3], Taeko Dohi[1,2], and Koji Hase[1,10,11].; [1]Division of Biochemistry, Faculty of Pharmacy and Graduate School of Pharmaceutical Science, Keio University, [2]Department of Gastroenterology, Research Center for Hepatitis and Immunology, Research Institute, National Center for Global Health and Medicine, Chiba, [3]Department of Stem Cell Biology, Research Institute, National Center for Global Health and Medicine, Tokyo, [4]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, [5]Institute of Health Sciences, Ezaki Glico Co., Ltd., Osaka, [6]Communal Laboratory, Research Institute, National Center for Global Health and Medicine, Tokyo, [7]Laboratory for Immunobiology, Graduate School of Medical Life Science, Yokohama City University, [8]Department of Immunology, Graduate School of Medical and Dental Sciences, Niigata University, [9]Mucosal Vaccine Project, BIKEN Innovative Vaccine Research Alliance Laboratories, Research Institute for Microbial Diseases, Osaka University, [10]International Research and Development Center for Mucosal Vaccines, the Institute of Medical Science, the University of Tokyo (IMSUT).

Nutritional status potentially influences immune responses; however, how nutritional signals regulate cellular dynamics and functionality remains obscure. Herein, we report that temporary fasting drastically reduces the number of lymphocytes by approximately 50% in Peyer's patches (PPs), the inductive site of the gut immune response. Subsequent refeeding seemingly restored the number of lymphocytes, but whose cellular composition was conspicuously altered. A large portion of germinal center and IgA^+ B cells were lost via apoptosis during fasting. Meanwhile, naive B cells migrated from PPs to the bone marrow during fasting and then back to PPs during refeeding when stromal cells sensed nutritional signals and upregulated CXCL13 expression to recruit naive B cells. Furthermore, temporal fasting before oral immunization with ovalbumin abolished the induction of antigen-specific IgA, failed to induce oral tolerance, and eventually exacerbated food allergy. Thus, nutritional signals are critical in maintaining gut immune homeostasis.

3. Bone Loss Caused by Dopaminergic Degeneration and Levodopa Treatment in Parkinson's Disease Model Mice.

Kazuaki Handa[1,2,3,4], Shuichi Kiyohara[3,4,5], Tomoyuki Yamakawa[1,2,3,4], Koji Ishikawa[1,3,4], Masahiro Hosonuma[1,2,4,6], Nobuaki Sakai[3,4], Akiko Karakawa[3,4], Masahiro Chatani[3,4], Mayumi Tsuji[2,4], Katsunori Inagaki[1], Yuji Kiuchi[2,4], Masamichi Takami[3,4], Takako Negishi-Koga[3,4,7].; [1]Department of Orthopaedic Surgery, [2]Department of Pharmacology, School of Medicine, [3]Department of Pharmacology, School of Dentistry, [4]Pharmacology Research Center, [5]Divition of Implant Dentistry, and [6]Division of Rheumatology, Department of Medicine, Showa University, [4]Current: Division of Mucosal Barriology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science (IMSUT), the University of Tokyo.

Patients with neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, often have smell disorder and constipation before the onset of cognitive symptoms. It has become known that altered intestinal microbiota and chronic inflammation correlate with cognitive symptoms. We focused on the correlation between age-related alterations of mucosal barrier integrity/immune responses and age-related diseases such as dementia and osteoporosis. We have shown that dopaminergic degeneration in mice, which mimic the pathology of Parkinson's disease, led to bone loss with no deficit of motor activity as observed in the patients. In addition, treatment with levodopa, the current gold-standard medication for affected patients, also reduce bone in mice. Our findings show that neurotoxin-induced dopaminergic degeneration resulted in bone loss due to accelerated osteoclastogenesis and suppressed bone formation, which was associated with elevated prolactin. On the other hand, using an experimental model of postmenopausal osteoporosis, dopaminergic degeneration did not result in exacerbation of bone loss due to estrogen deficiency, but rather reduction of bone loss. Thus, this study provides evidence for the regulation of bone metabolism by the dopaminergic system through both gonadal steroid hormone-dependent and -independent functions, leading to possible early detection of osteoporosis development in individuals with PD. Our future study will show the relationship between olfaction and intestinal microbiota/immune reaction and the onset of osteoporosis in these dementia model mice.

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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

Kosuke Fujimoto[1], Satoshi Uematsu[1]
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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. We are currently conducting monkey experiments for formulation in human.

2. Analysis of resident macrophages in small intestinal LP

Kosuke Fujimoto[1], Satoshi Uematsu[1]
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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-a 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.

4. 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.

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To explore new avenues for mucosal vaccine development, investigators have begun to employ novel adjuvants and targeting mucosal tissues and immune cells for vaccine delivery. Despite recent advanced sciences, it still 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 tolerant 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 relative large amounts of Ag and adjuvant and are exposed to the proteolytic enzymes and lower pH of the stomach. Considerably, their efficacy limits to mainly gastrointestinal mucosa. In this regard, it is essential to develop 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 oral or nasal vaccine. The sequence data are currently being analyzed using SHIROKANE super computer system in order to find novel mucosal imprinting molecules.

2. Distinct roles for Peyer's Patch B Cells in the induction of antigen-specific IgA antibody responses elicited by oral recombinant *Salmonella* delivery system

Tomomi Hashizume-Takizawa[1], Naoko Shibata[2, 3], Yosuke Kurashima[2, 4-8], Hiroshi Kiyono[2, 4, 5, 9], Tomoko Kurita-Ochiai[1], and Kohtaro Fujihashi[9, 10]

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Our previous study demonstrated an indispensable role of Peyer's patches (PPs) for the induction of antigen-specific secretory (S)IgA antibody (Ab) responses after oral immunization with recombinant *Salmonella* expressing fragment C of tetanus toxin (r*Salmonella*-Tox C). In this study, we defined the PP lymphoid structures and immune cells required for the induction of mucosal SIgA Ab responses. Adoptive transfer of mononuclear cells (MNCs) from PPs into PP deficient (PP-null) mice failed to elicit tetanus toxoid (TT)-specific mucosal immunity. However, when the same PP MNCs were transferred into lethally irradiated PP-normal recipient mice, PP MNCs preferentially emigrated to recipient PPs, leading to PP lymphoid structures and TT-specific SIgA Ab responses. Significantly reduced numbers of TT-specific IgA Ab-forming cells were detected in the mesenteric lymph nodes (MLNs) and intestinal lamina propria of mice when surface expression of the sphingosine 1-phosphate receptor on lymphocytes was inhibited by its agonist FTY720. However, FTY720 treatment did not alter dendritic cell (DC) migration or *Salmonella* dissemination into these tissues. When r*Salmonella*-Tox C-stimulated $CD4^+$ T cells isolated from PPs, MLNs and the spleen were co-cultured with B cells from these tissues, significantly increased levels of TT-specific IgA Ab responses were exclusively induced in cultures containing PP B cells. Furthermore, surface IgA^+ PP B cells produced TT-specific IgA Ab responses *in vitro*. These findings suggest that PP lymphoid structures and surface IgA^+ PP B cells are essential elements for the induction of antigen-specific intestinal SIgA Ab responses to oral *Salmonella*.

3. CpG ODN G9.1 as a novel nasal adjuvant elicits protective immunity from Influenza virus infection without inflammatory responses

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This study examined the protective efficacy of and immune response to a nasal influenza vaccine combined with a novel mucosal oligodeoxynucleotide (ODN) adjuvant, CpG-ODN G9.1 (G9.1), in a model of infection limited to the upper respiratory tract (URT) and a model of infection in the lower respiratory tract (LRT). Mice were nasally primed with an A/California/7/2009 (Cal7) split vaccine (X179A) plus G9.1 and were then nasally given a booster with X179A alone. When mice were challenged with either a large (infection of the LRT) or small (infection limited to the URT) volume of live Cal7 influenza virus, mice nasally given G9.1 combined with X179A showed a markedly high rate of protection against infection limited to the URT. Further, this group of mice promptly recovered from an infection of the LRT. When mice were subcutaneously (s.c.) given X179A as a current form of vaccination, they had no protection from an infection limited to the URT but they did recover from an infection of the LRT. The patterns of protection were closely correlated with influenza virus-specific mucosal SIgA or serum IgG Ab responses. SIgA Abs play an important role for the protection in the model of an infection limited to the URT while influenza virus-specific serum IgG Ab responses contribute for the protection from an infection of the LRT. Of importance, lungs from mice nasally given G9.1 showed low levels of type I IFN-associated protein- and transcription factor-specific mRNA expression. These results suggest that nasal G9.1 can be used as an effective and safe mucosal adjuvant for influenza vaccines since this nasal vaccine system elicits both

mucosal SIgA and serum IgG Ab responses that provide complete protection without inducing potent inflammatory responses.

Stratified Layer Analysis Reveals Intrinsic Hormone Stimulates Cryptal Mesenchymal Cells for Controlling Mucosal Homeostasis

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Mesenchymal cells in the crypt play indispensable roles in the maintenance of large 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 mouse colon, and we compared cellular properties between the luminal mucosa and crypts. The gene expression pattern in the cryptal mesenchymal cells showed that receptors of a type of hormone were highly expressed in the cryptal mesenchymal cells, and we found a decrease in the expressions of crypt factor under conditions of deficiency of the hormone, which also induced a delay in cryptal epithelial proliferation. Our novel stratified layer isolation strategies thus revealed new characteristics of cryptal mesenchymal cells.

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1. 藤橋浩太郎　呼吸器感染症と経鼻ワクチン 高齢者に有効な肺炎球菌経鼻ワクチンの開発　炎症と免疫、27(2) 121-126. 2019.
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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. BLT1 mediates commensal bacteria-dependent innate immune signals to enhance antigen-specific intestinal IgA responses

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Intestinal immune system provides the first line of defense by secreting antigen-specific IgA in the lumen, which act on the prevention of pathogen infection and neutralization of luminal toxins. However, antigen-specific intestinal IgA production is not achieved by conventional systemic vaccination, but requires oral vaccine. Therefore, the development of efficient oral vaccine system is desired. Recent accumulating evidence suggest that intestinal IgA production is regulated by intestinal environment, including dietary materials and microbiota. Here, we unveiled previously unknown function of leukotriene B_4 (LTB_4), a metabolite of w6 essential fatty acid, in the control of intestinal immune system. We found that LTB_4 receptor 1 (BLT1) is required to induce the pro-

duction of antigen-specific IgA against oral vaccine through mediating innate immune signals from commensal bacteria. B cells acquired BLT1 expression during their differentiation to IgA^+ B cells and plasma cells in Peyer's patches and the small intestinal lamina propria, respectively. BLT1 KO mice exhibited impaired production of antigen-specific fecal IgA to oral vaccine. Expression of MyD88 was decreased in BLT1 KO gut B cells and consequently led to diminished proliferation of commensal bacteria-dependent plasma cells. These results demonstrate that the LTB_4-BLT1 axis enhances the proliferation of commensal bacteria-dependent IgA^+ plasma cells through the induction of MyD88 and thereby plays a key role in oral vaccine.

2. Dietary Omega-3 Fatty Acid Dampens Allergic Rhinitis via Eosinophilic Production of the Anti-Allergic Lipid Mediator 15-Hydroxyeicosapentaenoic Acid in Mice.

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Dietary fatty acid (FA) plays important roles in the control of host's homeostasis and omega-3 FA (e.g alpha-linolenic acid [ALA], eicosapentaenoic acid [EPA]) exerts beneficial effects on immunity, inflammation and allergy. Previous studies indicated that the metabolism of dietary omega-3 FA is a key event in the regulation of allergic inflammation. We previously showed that dietary linseed oil (also called flaxseed oil), which contains high amounts ALA ameliorates egg-derived ovalbumin (OVA)-induced food allergy and that one of the omega-3 FA metabolites 17,18-epoxyeicosapentaenoic acid exerts anti-allergic effect in vivo. However, it was still unclear whether dietary omega-3 FA has anti-allergic effects at the respiratory mucosal compartment and unique anti-allergic metabolite is accumulated in nose. In this study, we found that dietary linseed oil ameliorates allergic rhinitis thorough the production of a novel anti-allergic lipid mediator 15-hydroxyeicosapentaenoic acid (15-HEPE). Lipidomic analysis unveiled the specific accumulation of 15-HEPE in nasal passage (NP) tissue of linseed-oil fed mice with allergy. 15-HEPE was produced from EPA by 15-lipoxygenase activity of eosinophil which was infiltrated into NP with allergy. Nasal administration of 15-HEPE decreased allergic symptoms by inhibiting mast cell degranulation through the interaction with peroxisome proliferator-activated receptor gamma. These results demonstrate that dietary ALA exerts anti-allergic effects through the local production of 15-HEPE by eosinophils in NP. This study also provides valuable information for both dietary and pharmaceutical strategies to ameliorate allergic rhinitis.

3. Persistent colonization of colonic lamina propria macrophages by *Stenotrophomonas maltophilia* for the creation of immunological homeostatic conditions in the large intestine.

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We explored the microbial signals that regulate anti-inflammatory characteristics of gut-resident macrophages and found that *Stenotrophomonas maltophilia* is a persistent colonizer within colonic lamina propria macrophages and helps to maintain homeostatic conditions in the colon through the induction of IL-10. We further identified a symbiotic factor encoded by the *S. maltophilia smlt2713* gene which is a responsible factor for the induction of IL-10 and consequent maintenance of less inflammatory conditions in the colon. This study suggests that the symbiotic factor encoded by the *S. maltophilia smlt2713* gene might be exploited to mine other beneficial gut bacteria for improving gut-barrier integrity and alleviating inflammatory conditions at mucosa.

4. Development of cationic nanogel-based nasal vaccines for various infectious diseases

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Nasal vaccination can induce antigen-specific mucosal and systemic immune responses. Since the mucosal surfaces such as upper and lower respiratory tracts are the entrance of the foreign microorganisms into the host, the mucosal immune responses effectively block their invasion and propagation. Based on the advantages of nasal vaccination, we are developing the nasal vaccines against various infectious diseases such as Pneumonia, Tuberculosis, or Otitis media. In addition, nasal immunization can induce an antigen-specific immune response at the genital mucosa where it is distant from the administration site. Thus, we also target the Human papilloma virus infections leading to the cervical cancer. For the nasal vaccine delivery system, we have developed a cationic type of cholesteryl group-containing pullulan (cCHP) nanogel. A complex of cCHP nanogel and vaccine antigen have shown that it can be attached on the epithelial layer of the nasal cavity after nasal immunization and elicit the effective immune responses by sustained antigen release. Most recently, we have demonstrated that the cCHP nanogel induced the dendritic cell recruitment at the underneath of the epithelial layer and deliver the vaccine antigens to the dendritic cells effectively. The mechanism of the dendritic cell recruitment after the cCHP nanogel immunization is now under investigation. For the vaccine safety, we also demonstrated that the cCHP nanogel itself or the vaccine antigens introduced with the cCHP nanogel did not migrate to the brain and the olfactory bulbs after intranasal administration both in mice and non-human primates, so the cCHP nanogel vaccine system appears to be safe and could be a promising vaccine system to target the various infectious diseases.

5. 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. Preventive monoclonal IgG antibody (Ab), which is currently available only for high-risk children, is an expensive passive immunization that requires repeated injection to maintain effectiveness. 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 recently 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. Our data showed that this vaccine induced both RSV (ec-

todomain protein)-specific serum IgG Abs and mucosal secretory IgA Abs. And we also have results showing that nasal immunization with this vaccine reduced RSV replication more in the lungs of adult mice than in those of non-immunized naive mice. We focus on the neutralizing effect of secretory IgA as one of the defensive mechanisms of this vaccine. We have preliminary results showing that nasal wash collected from mice immunized with this vaccine reduced RSV replication in HEp-2 cells more than in those of non-immunized naive mice. Moreover, this vaccine didn't induce VED after RSV infection. Our histological and flowcytometric results showing nasal immunization with this vaccine didn't induce airway eosinophil infiltration in the lungs of adult mice after natural RSV infection compared with those of formalin-inactivated (FI)-RSV immunization. These results provide promising evidence for the protective efficacy and safety of this novel nasal vaccine against RSV.

6. Development of antibody-producing MucoRice against norovirus gastroenteritis

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Human norovirus (HuNoV) usually infect by transmitted via the fecal-oral route, through contaminated food or water as aerosolized vomitus. Norovirus gastroenteritis with vomiting and diarrhea can occur in all age, but it may become severe if it infects elderly people, infants and immunocompromised humans. But there is no vaccine for norovirus gastroenteritis, since Takeda Phama prepares HuNoV GII.4 Virus-like particle (VLP) vaccine for phase III study in development. We have developed a novel therapy against norovirus gastroenteritis with transgenic rice-producing neutralizing antibody, MucoRice. Recently we could selected several antibodies to HuNoV VLP, which inhibited Norovirus proliferation using human induced pluripotent stem cells(iPSCs) established in our lab. (Sato et al., CMGH 2018, DOI: 10.1016/j.jcmgh.2018.11.001)

To introduce antibody gene in rice gene, we advanced the MucoRice system by using RNA interference (RNAi) technology to suppress the production of the two major endogenous storage proteins, prolamin and glutelin, to increase vaccine protein or antibody expression (Tokuhara et al., JCI 2013, 123:3829-3838). In present, the DNA sequence of the antibody was introduced into the rice by the *Agrobacterium*-mediated method. Then, the first generation of MucoRice introduced transgene of the antibody was harvested and the accumulation of the antibody was confirmed by SDS-PAGE and immunoblot analysis. The transgene introduced into the chromosome of the MucoRice is heterozygous due to the characteristics of Agrobacterium-mediated method, therefore the first generation needs to be self-pollinated in order to make the transgene homozygous. We will confirm the ability of the antibody derived from the transgenic rice seed in neutralization of human norovirus of antibody in transgenic rice seed of the second or later generations which become homozygous.

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International Research and Development Center for Mucosal Vaccines

Division of Mucosal Symbiosis
粘膜共生学分野

Project Associate Professor	Yoshiyuki Goto, Ph.D.		特任准教授	博士(医学)	後藤義幸
Invited Professor	Tetsuro Matano, M.D., D.M.Sc.		教授(委嘱)	博士(医学)	俣野哲朗
Project Assistant Professor	Miho Uematsu, Ph.D.		特任助教	博士(医学)	植松未帆

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 a1, 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 a1, 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 a1, 2-fucose is one of the symbiotic factors that mediate host–microbiota interaction. For example, epithelial a1, 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 a1, 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 a1, 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, Yamaguchi K, Yokoyama K, Komura M, Saito A, Kobayashi M, Yuji K, Takane K, Shibuya T, Hasegawa T, Miyagi Y, Muto K, Tojo A, Furukawa Y, Miyano S, Yamaguchi R, Imoto S

From April 2015, Medical Genomics Research Initiative The University of Tokyo is launched. For implementing clinical sequence in the Institute of Medical Science, we formed a team 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 space, 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 installed to record all logs of wet experiments and computational analyses. Together with genome analysis in clinical sequence, we now intensively focus on a method for interpreting personal genome information. In July 2015, we started to use IBM Watson for cancer research to interpret the results of genome analyses. The results of genome sequence analysis including the interpretation of IBM Watson are evaluated and discussed in biweekly sequence board meeting. In 2016, we analyzed around sequence data of 100 cancer patients (more than 250 sequencing samples) with whole genome, exome, target deep sequencings. Also, multi-omics data including genome, transcriptome and epigenome were measured 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) project "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 en-

vironmental 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.

3. Computational Methods in Systems Biology and Immunology

a. Adaptive NetworkProfiler for identifying cancer characteristic-specific gene regulatory networks.

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There is currently much discussion about sample (patient)-specific gene regulatory network identification, since the efficiently constructed sample-specific gene networks lead to effective personalized cancer therapy. Although statistical approaches have been proposed for inferring gene regulatory networks, the methods cannot reveal sample-specific characteristics because the existing methods, such as an L1-type regularization, provide averaged results for all samples. Thus, we cannot reveal sample-specific characteristics in transcriptional regulatory networks. To settle on this issue, the NetworkProfiler was proposed based on the kernel-based L1-type regularization. The NetworkProfiler imposes a weight on each sample based on the Gaussian kernel function for controlling effect of samples on modeling a target sample, where the amount of weight depends on similarity of cancer characteristics between samples. The method, however, cannot perform gene regulatory network identification well for a target sample in a sparse region (i.e., for a target sample, there are only a few samples having a similar characteristic of the target sample, where the characteristic is considered as a modulator in sample-specific gene network construction), since a constant bandwidth in the Gaussian kernel function cannot effectively group samples for modeling a target sample in sparse region. The cancer characteristics, such as an anti-cancer drug sensitivity, are usually nonuniformly distributed, and thus modeling for samples in a sparse region is also a crucial issue. We propose a novel kernel-based L1-type regularization method based on a modified k-nearest neighbor (KNN)-Gaussian kernel function, called an adaptive NetworkProfiler. By using the modified KNN-Gaussian kernel function, our method provides robust results against the distribution of modulators, and properly groups samples according to a cancer characteristic for sample-specific analysis. Furthermore, we propose a sample-specific generalized cross-validation for choosing the sample-specific tuning parameters in the kernel-based L1-type regularization method. Numerical studies demonstrate that the proposed adaptive NetworkProfiler effectively performs sample-specific gene network construction. We apply the proposed statistical strategy to the publicly available Sanger Genomic data analysis, and extract anti-cancer drug sensitivity-specific gene regulatory networks.

b. Bayesian model for analyzing human leukocyte antigen regions

Hayashi S, Yamaguchi R, Mizuno S[3], Komura M, Miyano S, Nakagawa H[4], Imoto S: [3]Center for Advanced Medical Innovation, Kyushu University, [4]RIKEN Center for Integrative Medical Sciences

Although human leukocyte antigen (HLA) genotyping based on amplicon, whole exome sequence (WES), and RNA sequence data has been achieved in recent years, accurate genotyping from whole genome sequence (WGS) data remains a challenge due to the low depth. Furthermore, there is no method to identify the sequences of unknown HLA types not registered in HLA databases. We developed a Bayesian model, called ALPHLARD, that collects reads potentially generated from HLA genes and accurately determines a pair of HLA types for each of HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, and -DRB1 genes at 3rd field resolution. Furthermore, ALPHLARD can detect rare germline variants not stored in HLA databases and call somatic mutations from paired normal and tumor sequence data. We illustrate the capability of ALPHLARD using 253 WES data and 25 WGS data from Illumina platforms. By comparing the results of HLA genotyping from SBT and amplicon sequencing methods, ALPHLARD achieved 98.8% for WES data and 98.5% for WGS data at 2nd field resolution. We also detected three somatic point mutations and one case of loss of heterozygosity in the HLA genes from the WGS data. ALPHLARD

showed good performance for HLA genotyping even from low-coverage data. It also has a potential to detect rare germline variants and somatic mutations in HLA genes. It would help to fill in the current gaps in HLA reference databases and unveil the immunological significance of somatic mutations identified in HLA genes.

d. An *in silico* automated pipeline to identify tumor specific neoantigens from next generation sequencing data

Hasegawa T, Hayashi S, Shimizu E, Mizuno S, Yamaguchi R, Miyano S, Nakagawa S, Imoto S:

Recent progress of massive parallel sequencing technology enables us to detect somatic mutations in each of cancer patients. It is known that some mutated peptides produced from missense mutations binds to the major histocompatibility complex (MHC). Since MHC presents mutated peptides to anti-tumor T cells, understanding this process is important in cancer immunotherapy. In this paper, we introduce a computational pipeline to predict binding affinity between mutated peptides and MHC molecules to detect neoantigens. We have implemented this pipeline on our supercomputer system. With nonsynonymous substitutions, frameshift insertions and deletions detected and intron retentions from whole-genome or exome sequencing data, we utilize RNA sequencing data and annotation data to make neoantigen detection pipeline more accurate.

4. Metagenome Analysis of Intestinal Microbiome

a. Analysis of intestinal microbiome.

Ozato N[5], Saito S[5], Yamaguchi T[6], Katashima M[5], Tokuda I[6], Sawada K[6], Katsuragi Y[5], Ihara K[6], Nakaji S[6], Imoto S: [5]Health Care Food Research Laboratories, Kao Corporation, [6]Graduate School of Medicine, Hirosaki University,

The gut microbiota is reported to be related to obesity, and visceral fat is reported to be strongly associated with cardiovascular disease and overall mortality. However, the association between the gut microbiota and obesity has mainly been studied using body mass index (BMI) as a proxy for obesity. We investigated the relationship of both visceral fat and BMI with the gut microbiota stratified by sex in a population-based cross-sectional study of Japanese men and women 20–76 years of age (n = 1001). Women with a higher visceral fat area (VFA) harboured a higher relative abundance of the Firmicutes phylum (P for trend <0.001) and a lower relative abundance of the Bacteroidetes phylum (P for trend 0.030), whereas men with higher VFA harboured a lower relative abundance of the Firmicutes phylum (P for trend 0.076) and a higher relative abundance of the Bacteroidetes phylum (P for trend 0.013). Similar results were obtained using BMI as an index, but the differences were not significant in men. At the genus level, Blautia was the only gut microbe significantly and inversely associated with VFA regardless of sex. In conclusion, at the genus level we found that Blautia was the only gut microbe significantly and inversely associated with VFA, regardless of sex.

b. Metagenome data about intestinal phage–bacteria association is a foundation for phage therapy development against pathobionts.

Fujimoto K, Kimura Y, Shimohigoshi M, Satoh M, Sato S, Tremmel G, Uematsu M, Kawaguchi Y, Usui Y, Nakano Y, Hayashi T, Kashima K, Yuki Y, Yamaguchi K, Furukawa Y, Kakuta M, Akiyama Y[7], Yamaguchi R, Crowe SE[8], Ernst PB[9], Miyano S, Kiyono H, Imoto S, Uematsu S.: [7]Department of Computer Science, Tokyo Institute of Technology, [8]Department of Medicine, University of California, San Diego, [9]CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California San Diego

Bacteriophages (phages) potentially regulate their host bacteria and contribute to the establishment of bacterial microbiomes (bacteriomes). The majority of intestinal commensal viruses are phages but the infectious associations between phages and bacteria in the human intestine have yet to be fully elucidated. Here, we quantitatively analysed intestinal viral microbiomes (viromes), together with bacteriomes, in 101 healthy individuals and the commensal viromes varied across individuals. Based on the genomic sequences of bacteriomes and viromes from the same fecal samples, the host–parasite associations were illustrated for both temperate and virulent phages. By using the metagenome sequence-based information, we newly identified endolysins associated with Clostridioides difficile, which could lyse a high-level toxin-producing C. difficile strain and also ameliorate acute C. difficile infection in mice effectively. Thus, these comprehensive metagenome analyses revealed not only phage–host bacteria dynamics but also constructed a foundation for the development of new phage therapies against intestinal pathobionts.

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Health Intelligence Center

Division of Health Medical Computational Science

健康医療計算科学分野

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The mission of this division is to develop computational science for transforming biomedical data to knowledge. By making full use of supercomputers, we are now focusing on annotation, translation and interpretation of genomic data including RNA sequences for supporting cancer research and clinical sequence.

1. Computational Science for Cancer Research

a. Sensitivity analysis of agent-based simulation utilizing massively parallel computation and interactive data visualization

Niida A, Hasegawa T, Miyano S

An essential step in the analysis of agent-based simulation is sensitivity analysis, which namely examines the dependency of parameter values on simulation results. Although a number of approaches have been proposed for sensitivity analysis, they still have limitations in exhaustivity and interpretability. In this study, we propose a novel methodology for sensitivity analysis of agent-based simulation, MASSIVE (Massively parallel Agent-based Simulations and Subsequent Interactive Visualization-based Exploration). MASSIVE takes a unique paradigm, which is completely different from those of sensitivity analysis methods developed so far, By combining massively parallel computation and interactive data visualization, MASSIVE enables us to inspect a broad parameter space intuitively. We demonstrated the utility of MASSIVE by its application to cancer evolution simulation, which successfully identified conditions that generate heterogeneous tumors. We believe that our approach would be a de facto standard for sensitivity analysis of agent-based simulation in an era of ever-growing computational technology. All the results form our MASSIVE analysis are available at https://www.hgc.jp/~niiyan/massive.

b. A clinical trial of somatic and germline analyses for healthy longevity in a postoperative cancer patient

Hayashi N[1,2], Kuroda Y[1], Saito T[3], Tsuruda Y[1,2], Niida A, Otsu H[1], Eguchi H[1], Masuda T[1], Suzuki Y[4], Natsugoe S[2], Mimori K[1]; [1]Kyushu University Beppu Hospital, [2]Kagoshima University Graduate School of Medical and Dental Science, [3]Oita University Hospital, [4]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences

Recent developments in molecular-targeted therapies have improved the clinical outcome of cancer patients; however, the issue of adverse effects due to treatments has often gone unconsidered. We herein report the results of a clinical trial of dual genomic

analyses for healthy longevity in a postoperative cancer patient.

We performed dual genomic analyses for a representative 79-year-old rectal cancer patient who relapsed with liver metastasis. First, we determined single-nucleotide polymorphisms according to the constitution and disease risk in the genomic DNA from the patient's saliva by referring to the data of 10,000 Japanese patients obtained from Yahoo Japan Corporation. Second, we conducted whole-exome sequencing to detect druggable mutations in the primary tumour. Forty of 59 determinable characters related to the constitution were consistent with the clinical phenotype. Several diseases classified as 'high risk' diseases actually occurred during the patient's clinical course. Of the 129 significant mutations, we identified somatic mutations in *BRAF*, *PIK3CA*, and *SMAD4* as targets. The dual genomic examination will improve the follow-up observation system to support primary care doctors in the social community for taking care of postoperative cancer patients.

c. Novel oncogene 5MP1 reprograms c-Myc translation initiation to drive malignant phenotypes in colorectal cancer

Sato K[1], Masuda T[1], Hu Q[1], Tobo T[1], Gillaspie S[5], Niida A, Thornton M[5], Kuroda Y[1], Eguchi H[1], Nakagawa T[6], Asano K[5], Mimori K[1], [5]Kansas State University, [6]Graduate School of Medical Sciences, Kyushu University

Translational reprogramming through controlled initiation from non-AUG start codons is considered a crucial driving force in tumorigenesis and tumor progression. However, its clinical impact and underlying mechanism are not fully understood.

Using a bioinformatics approach, we identified translation initiation regulator *5MP1*/*BZW2* on chromosome 7p as a potential oncogenic driver gene in colorectal cancer (CRC), and explored the biological effect of 5MP1 in CRC in vitro or in vivo. Pathway analysis was performed to identify the downstream target of 5MP1, which was verified with transcriptomic and biochemical analyses. Finally, we assessed the clinical significance of *5MP1* expression in CRC patients. 5MP1 was ubiquitously amplified and overexpressed in CRC. 5MP1 promoted tumor growth and induced cell cycle progression of CRC. c-Myc was identified as its potential downstream effector. *c-Myc* has two in-frame start codons, AUG and CUG (non-AUG) located upstream of the AUG. 5MP1 expression increased the AUG-initiated c-Myc isoform relative to the CUG-initiated isoform. The AUG-initiated c-Myc isoform displayed higher protein stability and a stronger transactivation activity for oncogenic pathways than the CUG-initiated isoform, accounting for *5MP1*-driven cell cycle progression and tumor growth. Clinically, high *5MP1* expression predicts poor survival of CRC patients. *5MP1* is a novel oncogene that reprograms *c-Myc* translation in CRC. 5MP1 could be a potential therapeutic target to overcome therapeutic resistance conferred by tumor heterogeneity of CRC.

d. Multiregion genomic analysis of serially transplanted patient-derived xenograft tumors

Sato K[1,6], Niida A, Masuda T[1], Shimizu D[1], Tobo T[1], Kuroda Y[1], Eguchi H[1], Nakagawa T[6], Suzuki Y[4], Mimori K[1]

Intratumoral heterogeneity (ITH) is a major cause underlying therapeutic difficulty of cancer. Although an understanding of ITH is critically important in order to develop novel therapeutic strategies, experimental models that enable the examination of ITH in a time series are lacking.

We developed an experimental approach based on patient-derived xenograft (PDX) mice and a multiregional sequencing approach (MRA). The multiple regions of primary colorectal cancer (CRC) and serially transplanted PDX tumors were analyzed via whole-exome sequencing and bioinformatic analyses. Our PDX-MRA of CRC indicated the spatiotemporal genetic transition of ITH. It was found that the subclonal architecture of CRC dynamically changes during serial transplantation. Furthermore, our data suggest that environmental selective pressures drive the development of minor pre-existing subclones in PDX-MRA. PDX-MRA is a useful tool for understanding the spatiotemporal dynamics of ITH.

2. Implementation of Cancer Clinical Sequence

a. An unusually short latent period of therapy-related myeloid neoplasm harboring a rare MLL-EP300 rearrangement: case report and literature review

Takeda R[7,8], Yokoyama K[7], Kobayashi S[9], Kawamata T[7,9], Nakamura S[9], Fukuyama T[7,8], Ito M[9], Yusa N[10], Shimizu E[11], Ohno N[7,9], Yamaguchi R[11], Imoto S[12], Miyano S, Uchimaru K[7,13], Tojo A[7,9]; [7]Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, [8]Division of Cellular Therapy, The Institute of Medical Science, [9]Division of Molecular Therapy, The Institute of Medical Science, [10]Department of Applied Genomics, Research Hospital, Institute of Medical Science, [11]Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, [12]Division of Health Medical Data Science, Health Intelligence Center, Institute of Medical Science, [13]Laboratory of Tumor Cell Biology, Department of Computational Biology and Medical Science, Graduate School of Frontier Sciences

Therapy-related myeloid neoplasm (t-MN) is a

late and lethal complication induced by chemotherapy and/or radiation therapy. Hematological malignancy is one of the most common primary diseases in patients with t-MN. However, the occurrence of t-MN in adult T-cell leukemia/lymphoma (ATL) patients is rarely reported, possibly due to the dismal prognosis of ATL per se. Here, we report a 62-year-old female who developed t-MN only three months after the completion of conventional chemotherapy and anti-CCR4 antibody for ATL acute type. The patient presented with persistent fever and monocytosis without any evidence of infectious diseases. Bone marrow examinations revealed chronic myelomonocytic leukemia-like disease with a chromosomal translocation of t (11;22)(q23;q13) as a solo cytogenetic abnormality, resulting in the diagnosis of t-MN. Next-generation sequencing analysis identified a rare chimeric transcript, MLL-EP300, without any additional somatic mutations. Although the patient underwent allogenic hematopoietic stem cell transplantation, she died of viral encephalomyelitis at 7 months after diagnosis of t-MN. Since recent therapeutic advances have extended the survival of patients with ATL, further evaluation of the long-term risks of developing t-MN in these patients is warranted.

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Center for Gene & Cell Therapy
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IMSUT hospital has been playing a crucial role in clinical gene therapy and stem cell transplantation in Japan. In order to reinforce this clinical development even further, IMSUT established the Center for Gene & Cell Therapy (CGCT) in 2014. The CGCT particularly focuses on the development of gene therapy and cell therapy for intractable cancer and chronic diseases, such as oncolytic virus therapy, engineered T cell therapy, gene therapy for neurological disorders using AAV vectors, T cell therapy for post-transplant viral infections, and cell therapy using mesenchymal stem cells.

1. Innovation in AAV vector toward gene and cell-based therapies to treat neuromuscular diseases

The Division of Molecular and Medical Genetics has been continuously carrying out research into the development of fundamental gene therapy techniques with the adeno-associated virus vector and cell therapeutic modalities. The main research theme is the development and clinical application of gene and cell therapies using viral vectors and MSCs for the treatment of diseases where fundamental therapies are still difficult, such as various neuromuscular diseases.

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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 the utilization of CRISPR-Cas3 gene editing technology

Tomoaki Fujii, Jinxi Wang, Kazuto Yoshimi, Tomoji Mashimo

The genome editing system could be a powerful genetic tools for development of effective cancer treatment. CRISPR-Cas9 system has been utilized as a tool for genome editing around the world. However, CRISPR-Cas9 system sometimes induce off-target,

mosaic mutations and small indels which cause unexpected phenotypes. They are often limitations for medical applications of CRISPR-Cas9 system. Recently, we have reported that genome editing using Class 1 CRISPR-Cas3 system is possible in human cells as a novel independent genome editing technology from Class 2 CRISPR-Cas9 system. The novel genomic editing system rarely induce small indels, mosaic mutations and cause loss of function mutations due to carrying a large deletion in the target genomic region and also rarely induce off-target mutations because the system has high targeting specificity. We aim for the development of safe and effective therapy for human cancers with a novel genome editing technology using CRISPR-Cas3 system.

We focus on chimeric antigen receptor T (CAR-T) cell therapy, which is an effective cancer immunotherapy and based on using genetically modified T cells to precisely target and kill cancer cells. We investigated whether CRISPR-Cas3 system induce a genetically modification on the EMX1 gene in Jurkat cells, a human acute T-cell Leukemia cell line. As a result, it causes a large deletion of hundreds to thousands of bases in its cells. This result indicates that CRISPR-Cas3 system could be genetic tools for generating safe and effective 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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動物センター

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The Laboratory Animal Research Center (LARC) was founded in 1965 as the first modern animal facility in Japan. Currently, about 30,000 mice are housed for the researches of IMSUT, and strictly maintained under the SPF condition. The building of LARC was improved in 1998 for housing larger laboratory animals, such as rats and rabbits, and performing the experiments of genetic engineering and infection (P2A, P3A). Techniques of mouse embryo manipulation and generating genetically modified mice have been introduced into the LARC.

Animal Husbandry and Housing

The building of Animal Center is a centralized facility, which is designed, constructed and maintained to meet regulatory standards that required for the operation of research animal facilities. We provide rooms to support the production and use of genetically-engineered mice, biohazardous experiments area and equipment room with X-ray Irradiator, MRI and IVIS imaging system. In 2019, 386 researchers from 37 laboratories worked in this facility with about 30,000 mice.

Techniques of mouse embryo manipulation (Microbiological cleaning and cryopreservation)

Rooms are strictly maintained under the SPF condition, therefore, we are able to 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 embryos to reduce number of using animals and laboratory space, which is also important for making back up of the strains. In 2019, embryos from103 strains and sperms from 37 strains were stored, and 119 tubes of frozen embryo were recovered.

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

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
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The Amami Laboratory of Injurious Animals was established in 1965 at Setouchi-cho in Amami-oshima Island in order to study on endemic diseases involving parasite, arthropods, and venomous snakes in the tropics or subtropics. The Amami-oshima Island belongs to the Nansei (Southwest) Islands and the fauna is quite different from that in other islands of Japan. Since the establishment of the laboratory, we have collaborated in activities to preserve the ecological status of species in this area through the trials have been carried out to utilize small mammals found unique in the Amami islands as experimental animals in addition to studies on prevention of Habu bites. The successful eradication of filariasis from this island is one of the monumental works of the laboratory. In our laboratory, it also maintains a colony of owl monkeys and squirrel monkeys, also known as the current best malaria primate models, that have adapted to the subtropical climate of Amami-oshima. Our present works are as follows:

Reproduction of squirrel monkeys and owl monkeys

The squirrel monkey (*Saimiri boliviensis*) and the owl monkey (*Aotus lemurinus griseimenbra*) were 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 closed each other, and both are very famous as the best of malaria model in primate. 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, the owl monkey is annual breeding animals. They are monogamy. Their puberty is 3 years old for both sex. Their gestation period is about 130 days. Six newborns were given in reproductive groups of squirrel monkeys in 2019, and two of them were nursed by laboratory staffs because of neglect of their mothers. On the other hand, owl monkeys have become male-only colonies, and breeding has stopped at present.

Research using non-human primates

In our facility, BSL-2 and BSL-3 grade laboratories (i.e. animal rooms, laboratories, and culture rooms) have been equipped as the primate research center of the International Research Center for Infectious Diseases from 2005. Our laboratory is the unique International Joint Usage and Research Center capable of infection experiment using squirrel monkeys, owl monkeys, and cynomolgus monkeys. We have conducted medical research in non-human primates to assess the pathogenicity of various pathogens, including highly pathogenic avian influenza virus, measles virus, equine herpes virus, mycobacteria, and cryptosporidium. Currently, we are evaluating anti-malarial activity of a novel compound in experimental infection models of squirrel monkeys and owl monkeys with collaborate researchers from some institutions. In addition, research other than infectious diseases, such as regenerative medicine, reproductive engineering, etc., in which our monkeys become suitable experimental models are also conducting at our laboratory.

Medical Proteomics Laboratory
疾患プロテオミクスラボラトリー

Professor	Jun-ichiro Inoue, Ph.D.	教　授　薬学博士	井　上　純一郎
Professor	Kouhei Tsumoto, Ph.D.	教　授　博士(工学)	津　本　浩　平
Associate Professor	Masaaki Oyama, Ph.D.	准教授　博士(医学)	尾　山　大　明
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Assistant Professor	Makoto Nakakido, Ph.D.	助　教　博士(生命科学)(大学院工学系研究科)	中木戸　　誠
Project Assistant Professor	Hiroshi Sagara, Ph.D.	特任助教　博士(医学)	相　良　　洋

The mission of our laboratory is to develop advanced technologies for integrative proteomic analyses from a physicochemical, structural and systems biology point of view. Currently, we mainly focus on functional protein-protein interaction networks related to a variety of diseases including cancer and infection. We are also engaged in collaborative researches regarding mass spectrometry and electron microscopy, which have made a substantial contribution to many scientific achievements.

<Group I>

1. 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.

2. 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 aggres-

sive 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.

3. Integrative Network Analysis Combined with Quantitative Phosphoproteomics Reveals Transforming Growth Factor-beta Receptor type-2 (TGFBR2) as a Novel Regulator of Glioblastoma Stem Cell Properties

Yuta Narushima, Hiroko Kozuka-Hata, Ryo Koyama-Nasu[1], Kouhei Tsumoto, Jun-ichiro Inoue, Tetsu Akiyama[1] and Masaaki Oyama.

Glioblastoma is one of the most malignant brain tumors with poor prognosis and their development and progression are known to be driven by glioblastoma stem cells. Although glioblastoma stem cells lose their cancer stem cell properties during cultivation in serum-containing medium, little is known about the molecular mechanisms regulating signaling alteration in relation to reduction of stem cell-like characteristics. To elucidate the global phosphorylation-related signaling events, we performed a SILAC-based quantitative phosphoproteome analysis of serum-induced dynamics in glioblastoma stem cells established from the tumor tissues of the patient. Among a total of 2876 phosphorylation sites on 1584 proteins identified in our analysis, 732 phosphorylation sites on 419 proteins were regulated through the alteration of stem cell-like characteristics. The integrative computational analyses based on the quantified phosphoproteome data revealed the relevant changes of phosphorylation levels regarding the proteins associated with cytoskeleton reorganization such as Rho family GTPase and Intermediate filament signaling, in addition to transforming growth factor-β receptor type-2 (TGFBR2) as a prominent upstream regulator involved in the serum-induced phosphoproteome regulation. The functional association of transforming growth factor-β receptor type-2 with stem cell-like properties was experimentally validated through signaling perturbation using the corresponding inhibitors, which indicated that transforming growth factor-β receptor type-2 could play an important role as a novel cell fate determinant in glioblastoma stem cell regulation.

4. Quantitative phosphoproteomics-based molecular network description for high-resolution kinase-substrate interactome analysis

Yuta Narushima, Hiroko Kozuka-Hata, Kouhei Tsumoto, Jun-ichiro Inoue and Masaaki Oyama.

Phosphorylation-dependent cellular signaling is known to play a diverse role in regulating multiple cellular processes such as proliferation, differentiation and apoptosis. Recent technological advances in mass spectrometry-based phosphoproteomics have enabled us to measure network-wide signaling dynamics in a comprehensive and quantitative manner. As conventional protein-protein interaction (PPI) information-based network analysis is insufficient to systematically analyze phosphorylation site-dependent complex interaction dynamics, here we develop and evaluate a platform to provide a high-resolution molecular network description for kinase-substrate interactome analysis. In this study, we developed a Cytoscape-based bioinformatical platform named "Post Translational Modification mapper (PTMapper)" to integrate PPI data with publicly available kinase-substrate relations at the resolution of phosphorylated amino acid residues. The previous phosphoproteome data on EGF-induced cellular signaling in glioblastoma stem cells was applied to evaluate our platform, leading to discovery of phosphorylation-dependent crucial signaling modulation in the p70S6K1-related pathway. Our study revealed that high-resolution cellular network description of phosphorylation-site dependent kinase-substrate signaling regulation should accelerate phosphoproteomics-based exploration of novel drug targets in the context of each disease-related signaling.

5. 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.

6. 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).

7. 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, such as antibody-antigen and protein-ligand complexes, to understand quantitatively how these coordinated non-covalent interactions contribute to the specific recognition in biological and artificial systems. We seek to elucidate the molecular mechanisms by which biological molecules generate high specificity and affinity from multiple angles using advanced instruments. We aim to produce functional molecules with higher performance and better properties and to build solid foundations to develop drugs that modulate specific interactions between biomolecules, helping to understand the principles of molecular interactions in our lives.

1. Effects of a remote mutation from the contact paratope on the structure of CDR-H3 in the anti-HIV neutralizing antibody PG16

Kondo HX, Kiribayashi R, Kuroda D, Kohda J, Kugimiya A, Nakano Y, Tsumoto K, and Takano Y.

PG16 is a broadly neutralizing antibody to the human immunodeficiency virus (HIV). A crystal structure of PG16 revealed that the unusually long 28-residue complementarity determining region (CDR) H3 forms a unique subdomain, referred to as a "hammerhead", that directly contacts the antigen. The hammerhead apparently governs the function of PG16 while a previous experimental assay showed that the mutation of Tyr^{H100Q} to Ala, which does not directly contact the antigen, decreased the neutralization ability of PG16. However, the molecular mechanism by which a remote mutation from the hammerhead or contact paratope affects the neutralization potency has remained unclear. Here, we performed molecular dynamics simulations of the wild-type and variants (Tyr^{H100Q} to Ala, and Tyr^{H100Q} to Phe) of PG16, to clarify the effects of these mutations on the dynamics of CDR-H3. Our simulations revealed that the structural rigidity of the CDR-H3 in PG16 is attributable to the hydrogen bond interaction between Tyr^{H100Q} and Pro^{H99}, as well as the steric support by Tyr^{H100Q}. The loss of both interactions increases the intrinsic fluctuations of the CDR-H3 in PG16, leading to a conformational transition of CDR-H3 toward an inactive state.

2. 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 (PO2−3) 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 PO2−3 and PO3 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.

3. 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.

4. Structural and thermodynamic basis for the recognition of the substrate-binding cleft on hen egg lysozyme by a single-domain antibody

Akiba H, Tamura H, Kiyoshi M, Yanaka S, Sugase K, Caaveiro JMM, and Tsumoto K.

Single-domain antibodies (VHHs or nanobodies), developed from heavy chain-only antibodies of camelids, are gaining attention as next-generation therapeutic agents. Despite their small size, the high affinity and specificity displayed by VHHs for antigen molecules rival those of IgGs. How such small antibodies achieve that level of performance? Structural studies have revealed that VHHs tend to recognize concave surfaces of their antigens with high shape-complementarity. However, the energetic contribution of individual residues located at the binding interface has not been addressed in detail, obscuring the actual mechanism by which VHHs target the concave surfaces of proteins. Herein, we show that a VHH specific for hen egg lysozyme, D3-L11, not only displayed the characteristic binding of VHHs to a concave region of the surface of the antigen, but also exhibited a distribution of energetic hot-spots like those of IgGs and conventional protein-protein complexes. The highly preorganized and energetically compact interface of D3-L11 recognizes the concave epitope with high shape complementarity by the classical lock-and-key mechanism. Our results shed light on the fundamental basis by which a particular VHH accommodate to the concave surface of an antigens with high affinity in a specific manner, enriching the mechanistic landscape of VHHs.

5. ^{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. 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. CDH17 expression in gastric cancer specimens was also analyzed.

Surface plasmon resonance analysis revealed that D2101 specifically recognizes human CDH17, but not murine CDH17. The affinity of D2101 slightly decreased as a result of the radiolabeling procedures. The biodistribution study revealed high uptake of 111In-D2101 in tumors (maximum, 39.2 ± 9.5% ID/g at 96 h postinjection), but low uptake in normal organs, including the stomach. Temporal SPECT/CT imaging with 111In-D2101 visualized tumors with a high degree of tumor-to-nontumor contrast. Immunohistochemical analysis revealed that, compared with HER2, which is a potential marker of N-stage, CDH17 had a higher frequency of positivity in specimens of primary and metastatic gastric cancer. 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.

6. Biophysical characterization of the breast cancer-related BIG3-PHB2 interaction: Effect of non-conserved loop region of BIG3 on the structure and the interaction

Chigira T, Nagatoishi S, Takeda H, Yoshimaru T, Katagiri T, and Tsumoto K.

Brefeldin A-inhibited guanine nucleotide-exchange protein 3 (BIG3) interacts with and inhibits the tumor suppressor function of prohibitin-2 (PHB2), and recent in vivo studies have demonstrated that the BIG3-PHB2 interaction is a promising target for breast cancer therapy. However, little biophysical characterization on BIG3 and its interaction with PHB2 has been reported. Here we compared the calculated 8-class secondary structure of the N-terminal domains of BIG family proteins and identified a loop region unique to BIG3. Our biophysical characterization demonstrated that this loop region significantly affects the colloidal and thermodynamic stability of BIG3 and the thermodynamic and kinetic profile of its interaction with PHB2. These results establish a model for the BIG3-PHB2 interaction and an entry for drug discovery for breast cancer.

7. A Peptoid with Extended Shape in Water

Morimoto J, Fukuda Y, Kuroda D, Watanabe T, Yoshida F, Asada M, Nakamura T, Senoo A, Nagatoishi S, Tsumoto K, and Sando S.

The term "peptoids" was introduced decades ago to describe peptide analogues that exhibit better physicochemical and pharmacokinetic properties than peptides. Oligo(N-substituted glycine) (oligo-NSG) was previously proposed as a peptoid due to its high proteolytic resistance and membrane permeability. However, oligo-NSG is conformationally flexible, and ensuring a defined shape in water is difficult. This conformational flexibility severely limits the biological application of oligo-NSG. Here, we propose oligo(N-substituted alanine) (oligo-NSA) as a peptoid that forms a defined shape in water. The synthetic method established in this study enabled the first isolation and conformational study of optically pure oligo-NSA. Computational simulations, crystallographic studies, and spectroscopic analysis demonstrated the well-defined extended shape of oligo-NSA realized by backbone steric effects. This new class of peptoid achieves the constrained conformation without any assistance of N-substituents and serves as a scaffold for displaying functional groups in well-defined three-dimensional space in water, which leads to effective biomolecular recognition.

8. Highly sensitive biomolecular interaction detection method using optical bound/free separation with grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS)

Kaya T, Nagatoishi S, Nagae K, Nakamura Y, and Tsumoto K.

Grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS) with optical bound/free (B/F) separation technique was developed by employing a highly directional fluorescence with polarization of surface plasmon-coupled emission (SPCE) to realize highly sensitive immunoassay regardless of the ligand affinity. A highly sensitive immunoassay system with GC-SPFS was constructed using a plastic sensor chip reproducibly fabricated in-house by nanoimprinting and applied to the quantitative detection of an anti-lysozyme single-domain antibody (sdAb), to compare conventional washing B/F separation with optical B/F separation. Differences in the affinity of the anti-lysozyme sdAb, induced by artificial mutation of only one amino acid residue in the variable domain were attributed to higher sensitivity than that of the conventional Biacore surface plasmon resonance (SPR) system. The detection limit (LOD; means of six replicates of the zero standard plus three standard deviations) of the GC-SPFS immunoassay with optical B/F separation, was estimated to be 1.2 ng/ml with the low-affinity ligand (mutant sdAb Y52A: KD level was of the order of 10-7 ~ 10-6 M) and was clearly improved as compared to that (LOD: 9.4 ng/ml) obtained with the conventional washing B/F separation. These results indicate that GC-SPFS with the optical B/F separation technique offers opportunities to re-evaluate low-affinity biomaterials that are neither fully utilized nor widespread, and could facilitate the creation of novel and innovative methods in drug and diagnostic development.

9. The Isolation of New Pore-Forming Toxins from the Sea Anemone Actinia fragacea Provides Insights into the Mechanisms of Actinoporin Evolution

Morante K, Bellomio A, Viguera AR, González-Mañas JM, Tsumoto K, and Caaveiro JMM.

Random mutations and selective pressure drive protein adaptation to the changing demands of the environment. As a consequence, nature favors the evolution of protein diversity. A group of proteins subject to exceptional environmental stress and known for their widespread diversity are the pore-forming hemolytic proteins from sea anemones, known as actinoporins. In this study, we identified and isolated new isoforms of actinoporins from the sea anemone Actinia fragacea (fragaceatoxins). We characterized their hemolytic activity, examined their stability and structure, and performed a comparative analysis of their primary sequence. Sequence alignment reveals that most of the variability among actinoporins is associated with non-functional residues. The differences in the thermal behavior among fragaceatoxins suggest that these variability sites contribute to changes in protein stability. In addition, the protein-protein interaction region showed a very high degree of identity (92%) within fragaceatoxins, but only 25% among all actinoporins examined, suggesting some degree of specificity at the species level. Our findings support the mechanism of evolutionary adaptation in actinoporins and reflect common pathways conducive to protein variability.

10. Structural features of methionine aminopeptidase2-active core peptide essential for binding with S100A4

Katagiri N, Nagatoishi S, Tsumoto K, and Endo H.

Methionine aminopeptidase 2 (MetAP2) is one of the effector proteins of S100A4, a metastasis-associated calcium-binding protein. This interaction is involved in angiogenesis. The region of MetAP2 that interacts with S100A4 includes amino acids 170 to 208. A peptide corresponding to this region, named as NBD, has potent anti-angiogenic activity and suppresses tumor growth in a xenograft cancer model. However, the binding mode of NBD to S100A4 was totally unknown. Here we describe our analysis of the relationship between the inhibitory activity and the structure of NBD, which adopts a characteristic helix-turn-helix structure as shown by X-ray crystallo-

graphic analysis, and peptide fragments of NBD. We conducted physicochemical analyses of the interaction between S100A4 and the peptides, including surface plasmon resonance, microscale thermophoresis, and circular dichroism, and performed docking/molecular dynamics simulations. Active peptides had stable secondary structures, whereas inactive peptides had a little secondary structure. A computational analysis of the interaction mechanism led to the design of a peptide smaller than NBD, NBD-ΔN10, that possessed inhibitory activity. Our study provides a strategy for design for a specific peptide inhibitor against S100A4 that can be applied to the discovery of inhibitors of other protein-protein interactions.

11. Identification of key modules and hub genes for small-cell lung carcinoma and large-cell neuroendocrine lung carcinoma by weighted gene co-expression network analysis of clinical tissue-proteomes

Nakamura H, Fujii K, Gupta V, Hata H, Koizumu H, Hoshikawa M, Naruki S, Miyata Y, Takahashi I, Miyazawa T, Sakai H, Tsumoto K, Takagi M, Saji H, and Nishimura T.

Small-cell lung carcinoma (SCLC) and large-cell neuroendocrine lung carcinoma (LCNEC) are high-grade lung neuroendocrine tumors (NET). However, comparative protein expression within SCLC and LCNEC remains unclear. Here, protein expression profiles were obtained via mass spectrometry-based proteomic analysis. Weighted gene co-expression network analysis (WGCNA) identified co-expressed modules and hub genes. Of 34 identified modules, six were significant and selected for protein-protein interaction (PPI) network analysis and pathway enrichment. Within the six modules, the activation of cellular processes and complexes, such as alternative mRNA splicing, translation initiation, nucleosome remodeling and deacetylase (NuRD) complex, SWItch/Sucrose Non-Fermentable (SWI/SNF) superfamily-type complex, chromatin remodeling pathway, and mRNA metabolic processes, were significant to SCLC. Modules enriched in processes, including signal recognition particle (SRP)-dependent co-translational protein targeting to membrane, nuclear-transcribed mRNA catabolic process of nonsense-mediated decay (NMD), and cellular macromolecule catabolic process, were characteristically activated in LCNEC. Novel high-degree hub genes were identified for each module. Master and upstream regulators were predicted via causal network analysis. This study provides an understanding of the molecular differences in tumorigenesis and malignancy between SCLC and LCNEC and may help identify potential therapeutic targets.

12. An epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout

Eguchi A, Nakakido M, Nagatoishi S, Kuroda D, Tsumoto K, Nagamune T, and Kawahara M.

Upon developing therapeutically potent antibodies, there are significant requirements, such as increasing their affinity, regulating their epitope, and using native target antigens. Many antibody selection systems, such as a phage display method, have been developed, but it is still difficult to fulfill these requirements at the same time. Here, we propose a novel epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout. This system is based on the competition of antibody binding, and can target membrane proteins expressed in a native form on a mammalian cell surface. Using this system, we successfully selected an affinity-matured anti-ErbB2 single-chain variable fragment variant, which had the same epitope as the original one. In addition, the affinity was increased mainly due to the decrease in the dissociation rate. This novel cell-based antibody affinity maturation system could contribute to directly obtaining therapeutically potent antibodies that are functional on the cell surface.

13. Exploring designability of electrostatic complementarity at an antigen-antibody interface directed by mutagenesis, biophysical analysis, and molecular dynamics simulations

Yoshida K, Kuroda D, Kiyoshi M, Nakakido M, Nagatoishi S, Soga S, Shirai H, and Tsumoto K.

Antibodies protect organisms from a huge variety of foreign antigens. Antibody diversity originates from both genetic and structural levels. Antigen recognition relies on complementarity between antigen-antibody interfaces. Recent methodological advances in structural biology and the accompanying rapid increase of the number of crystal structures of proteins have enabled atomic-level manipulation of protein structures to effect alterations in function. In this study, we explored the designability of electrostatic complementarity at an antigen-antibody interface on the basis of a crystal structure of the complex. We designed several variants with altered charged residues at the interface and characterized the designed variants by surface plasmon resonance, circular dichroism, differential scanning calorimetry, and molecular dynamics simulations. Both successes and failures of the structure-based design are discussed. The variants that compensate electrostatic interactions can restore the interface complementarity, enabling the cognate antigen-antibody binding. Retrospectively, we also show that these mutational effects could be predicted by the simulations. Our study

demonstrates the importance of charged residues on the physical properties of this antigen-antibody interaction and suggests that computational approaches can facilitate design of antibodies that recognize a weakly immunogenic antigen.

14. Design strategy for serine hydroxymethyltransferase probes based on retro-aldol-type reaction

Nonaka H, Nakanishi Y, Kuno S, Ota T, Mochidome K, Saito Y, Sugihara F, Takakusagi Y, Aoki I, Nagatoishi S, Tsumoto K, and Sando S.

Serine hydroxymethyltransferase (SHMT) is an enzyme that catalyzes the reaction that converts serine to glycine. It plays an important role in one-carbon metabolism. Recently, SHMT has been shown to be associated with various diseases. Therefore, SHMT has attracted attention as a biomarker and drug target. However, the development of molecular probes responsive to SHMT has not yet been realized. This is because SHMT catalyzes an essential yet simple reaction; thus, the substrates that can be accepted into the active site of SHMT are limited. Here, we focus on the SHMT-catalyzed retro-aldol reaction rather than the canonical serine-glycine conversion and succeed in developing fluorescent and 19F NMR molecular probes. Taking advantage of the facile and direct detection of SHMT, the developed fluorescent probe is used in the high-throughput screening for human SHMT inhibitors, and two hit compounds are obtained.

15. Control of Protein Adsorption to Cyclo Olefin Polymer by the Hofmeister Effect

Fujita R, Nagatoishi S, Adachi S, Nishioka H, Ninomiya H, Kaya T, Takai M, Arakawa T, and Tsumoto K.

Cyclo olefin polymer (COP) is an attractive plastic because it has low protein adsorption despite its hydrophobic chemical structure. Here, the adsorption of model proteins to the COP was evaluated in comparison with a representative plastic, polystyrene (PSt), using reflectometry interference spectroscopy (RIfS) technology. The effects of different salts on adsorption were then examined. The adsorption of bovine serum albumin onto COP increased in the presence of kosmotropic salts, whereas adsorption of IgG increased in the presence of chaotropic salts. By contrast, the adsorption of these 2 proteins to PSt was unaffected by these Hofmeister salts. Langmuir-Freundlich model of COP adsorption suggested that the COP surface is more homogeneous for protein binding than the PSt surface. Furthermore, RIfS and sum frequency generation analyses indicated that water molecules bind more weakly to COP than to PSt. Our data propose a novel viewpoint of the way protein binds to COP surface that is different from the way it binds to PSt.

16. Phospholipid Membrane Fluidity Alters Ligand Binding Activity of a G Protein-Coupled Receptor by Shifting the Conformational Equilibrium

Yoshida K, Nagatoishi S, Kuroda D, Suzuki N, Murata T, and Tsumoto K.

The affinity of a ligand for a receptor on the cell surface will be influenced by the membrane composition. Herein, we evaluated the effects of differences in membrane fluidity, controlled by phospholipid composition, on the ligand binding activity of the G protein-coupled receptor human serotonin 2B. Using Nanodisc technology to control membrane properties, we performed biophysical analysis and employed molecular dynamics simulations to demonstrate that increased membrane fluidity shifted the equilibrium toward an active form of the receptor. Our quantitative study will enable development of more realistic in vitro drug discovery assays involving membrane-bound proteins such as G protein-coupled receptors.

17. Generation and characterization of antagonistic anti-human interleukin (IL)-18 monoclonal antibodies with high affinity: Two types of monoclonal antibodies against full-length IL-18 and the neoepitope of inflammatory caspase-cleaved active IL-18

Nariai Y, Kamino H, Obayashi E, Kato H, Sakashita G, Sugiura T, Migita K, Koga T, Kawakami A, Sakamoto K, Kadomatsu K, Nakakido M, Tsumoto K, and Urano T.

Interleukin-18 (IL-18) is a pro-inflammatory cytokine that evokes both innate and acquired immune responses. IL-18 is initially synthesized as an inactive precursor and the cleavage for processing into a mature, active molecule is mediated by pro-inflammatory caspases following the activation of inflammasomes. Two types of monoclonal antibodies were raised: anti-IL-1863-68 antibodies which recognize full-length1-193 and cleaved IL-18; and anti-IL-18 neoepitope antibodies which specifically recognize the new N-terminal 37YFGKLESK44 of IL-18 cleaved by pro-inflammatory caspase-1/4. These mAbs were suitable for Western blotting, capillary Western immunoassay (WES), immunofluorescence, immunoprecipitation, and function-blocking assays. WES analysis of these mAbs allowed visualization of the IL-18 bands and provided a molecular weight corresponding to the pro-inflammatory caspase-1/4 cleaved, active form IL-1837-193, and not to the inac-

tive precursor IL-18, in the serum of patients with adult-onset Still's disease (6/14, 42%) and hemophagocytic activation syndrome (2/6, 33%). These monoclonal antibodies will be very useful in IL-18 and inflammasome biology and for diagnostic and therapeutic strategies for inflammatory diseases.

18. Affinity Improvement of a Cancer-Targeted Antibody through Alanine-Induced Adjustment of Antigen-Antibody Interface

Yamashita T, Mizohata E, Nagatoishi S, Watanabe T, Nakakido M, Iwanari H, Mochizuki Y, Nakayama T, Kado Y, Yokota Y, Matsumura H, Kawamura T, Kodama T, Hamakubo T, Inoue T, Fujitani H, and Tsumoto K.

To investigate favorable single amino acid substitutions that improve antigen-antibody interactions, alanine (Ala) mutagenesis scanning of the interfacial residues of a cancer-targeted antibody, B5209B, was performed based on X-ray crystallography analysis. Two substitutions were shown to significantly enhance the binding affinity for the antigen, by up to 30-fold. One substitution improved the affinity by a gain of binding enthalpy, whereas the other substitution improved the affinity by a gain of binding entropy. Molecular dynamics simulations showed that the enthalpic improvement could be attributed to the stabilization of distant salt bridges located at the periphery of the antigen-antibody interface. The entropic improvement was due to the release of water molecules that were stably trapped in the antigen-antibody interface of the wild-type antibody. Importantly, these effects of the Ala substitutions were caused by subtle adjustments of the binding interface. These results will be helpful to design high-affinity antibodies with avoiding entropy-enthalpy compensation.

<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. 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 30nm 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, it became possible to analyze much wider areas by using SEM to observe thin TEM sections than by using TEM. These methods are also applicable for analyzing other cells and tissues and 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, 12 projects in 8 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 70nm 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 sequentially reacted 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]Divi-

sion 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 LPCAT1 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 role of LPCAT1 (Lysophosphatidylcholine acyltransferase 1) in retinal development. With the analysis of the retina by electron microscopy, we revealed that organized photoreceptor outer-segments are formed normally in *LPCAT1*-KO mouse retinas and then degrade, suggesting that LPCAT1 have roles in maintenance of photoreceptor outer-segments. Some other projects are 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, concerning function of intestinal Paneth cells in mucosal immunity, Dr. Eguchi[5], in [5]Division of Genetics, Dr. Nakahara[6] 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. Shibata[8] in [8]Biomolecular Engineering Laboratory, Waseda University.

c. Conventional scanning electron microscopy

Conventional scanning electron microscopy is a technique used to examine the surface structure of the cells, tissues or other non-biological materials. The collaborative works using scanning electron microscopy were done with Dr. Chou[9], in [9]Department of Research and Development, Life Innovation Center, LIFEBANK Japan, Inc., about the morphology of multipotent stem cells.

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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 has established three project joint laboratories (one in Tokyo; two joint labs 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 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 new 5-year J-GRID program of Japan Agency for Medical Research and Development (AMED).

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 Zene Matsuda and Takaomi Ishida to IBP and IM, respectively, as principal investigators (PIs). Along with their Japanese and Chinese staffs, these PIs are conducting basic and translational studies of HIV, MERS coronavirus, dengue virus and norovirus. In 2015 IMSUT has set up another project laboratory in Tokyo, whose studies complement those in Beijing. The activities of the three laboratories are under Jun-ichiro

Inoue's direction. 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

J. Inoue and his group at IMSUT are trying to find small molecular weight compounds that inhibit the membrane fusion caused by emerging viruses such as Dengue virus (DENV) and Zika virus (ZIKV), in close collaboration with Z. Matsuda's group at IBPCAS (see below). For DENV and ZIKV, they developed a cell-based fusion assay for prME protein in a low pH-dependent manner, using Aedes albopictus cell line C6/36 cells expressing Renilla luciferase (RL)-based split reporter proteins, and optimized it for a 384-well format.

Using these assays, they have previously identified one candidate compound by screening 1,017 FDA-approved drugs for a possible ZIKV inhibitor. They demonstrated this year that the compound inhibited the ZIKV infection of cultured cells. They also screened this year 40,000 compounds from Drug Discovery Initiative (DDI), The University of Tokyo, in addition to 130,000 compounds already screened last year. Thus, they have completed the screening of 172,000 compounds supplied by DDI. Based on the subsequent analyses on efficacy, toxicity and structure, three compounds were selected as a possible inhibitor for ZIKV infection. Furthermore, they showed that the three compounds blocked the ZIKV infection of cultured cells.

b. Joint Laboratory at IBPCAS

Z. Matsuda and his group at IBPCAS are conducting research on structure-function relationship of the viral envelope proteins derived from HIV-1 and dengue virus (DENV). They continued their analysis of the C- terminal heptad repeat (CHR) of HIV-1 gp41. CHR forms a structure called six-helix bundle (6HB) and 6HB formation is essential for membrane fusion. They found that insertion of Q but A was tolerable at position 644 in the JRFL strain. They have developed a prokaryotic system to screen the peptide-peptide interaction and applied it to screening of the peptides that may bind to CHR and inhibit membrane fusion. In collaboration with J. Inoue's group, they established a new quantitative HIV-1 virus-cell membrane fusion assay based on a split Nanoluc reporter. The two groups are also collaborating in the study of DENV envelope protein and screening of the potential inhibitors for DENV.

c. Joint Laboratory at IMCAS

The use of combination anti-retroviral therapy (cART) has contributed to extension of the patients' life span by preventing the development of AIDS in patients infected with human immunodeficiency virus type 1 (HIV-1). However, HIV-1 eradication from the patients has not been achieved yet. HIV-1 latent reservoirs harboring silenced but replication-competent provirus are a major obstacle against viral eradication in the infected patients. The "kick and kill" strategy, which is one of promising approaches to a cure of HIV-1 infection, is aimed to reactivate the latent provirus by treatment with latency reversing agents (LRAs) in the presence of antiretroviral drugs.

T. Ishida and his group tried to find small-molecule compounds that function as an LRA. As a result the dual polo-like kinase (PLK) / bromodomain inhibitors BI-2536 and BI-6727 (volasertib) were discovered as an LRA candidate by screening 378 kinase inhibitors. BI-2536 and BI-6727 significantly reactivated silenced HIV-1 provirus at both mRNA and protein levels in two latently infected model cell lines ACH2 and U1. Importantly, BI-2536 dramatically reactivated transcription of latent HIV-1 provirus in peripheral blood mononuclear cells (PBMCs) derived from the infected patients. They are currently trying to find more LRA candidate compounds by high-throughput screening of chemical compound libraries.

d. Collaborative research program with HVRI

Since 2013, avian influenza A viruses of the H7N9 subtype (H7N9) have caused sporadic infections in humans in China. In addition, in 2016, highly pathogenic avian influenza (HPAI) H7N9 viruses emerged raising concerns of a pandemic. In 2009, the novel influenza "pandemic (H1N1) 2009" virus emerged and spread rapidly throughout the world. In addition, since 2003, HPAI H5N1 viruses have continued to

cause unprecedented global outbreaks with high case fatality rates in humans. For these reasons, HVRI (Director, Zhigao Bu) has been conducting collaborative research on influenza virus isolates 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 from wild birds, waterfowl, poultry, and swine, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral surveillance.

Their major findings this year include: (1) Characterization of H7N9 viruses isolated from duck meat products. In Japan, both HPAI and low pathogenic avian influenza (LPAI) H7N9 viruses were isolated from duck meat products carried illegally and relinquished voluntarily at the border by passengers on flights from China to Japan between 2016 and 2017. In this study, Kawaoka's group assessed the biological features of two HPAI H7N9 virus isolates (isolated from duck meat at the border) and an LPAI H7N9 virus isolate in mice and ferrets. In mice, one of the HPAI H7N9 virus isolate was more pathogenic than the other HPAI isolate and the LPAI H7N9 isolate. In ferrets, the two HPAI virus isolates replicated more efficiently in the lower respiratory tract of the animals than did the LPAI virus isolate. These results demonstrate that HPAI H7N9 viruses with the potential to cause severe disease in mammals have been illegally introduced to Japan. (2) Treatment of HPAI H7N9 virus-infected mice with baloxavir marboxil. Kawaoka's group examined the efficacy of baloxavir marboxil in mice infected with an HPAI H7N9 virus. Treatment of infected mice with a single 1.5 mg/kg dose of baloxavir marboxil protected mice from highly pathogenic human H7N9 virus infection as effectively as oseltamivir treatment at 50 mg/kg twice a day for 5 days. Daily treatment for 5 days at 15 or 50 mg/kg of baloxavir marboxil showed superior therapeutic efficacy, largely preventing virus replication in respiratory organs. These results indicate that baloxavir marboxil is a valuable treatment option for humans suffering from highly pathogenic H7N9 virus infection.

IMSUT PROJECT OFFICE

The office (M. Hayashi) supports the activities of the two joint laboratories in Beijing and one joint research program in Harbin. It serves as Secretariat for Steering Committee Meeting and files MOU and Minutes. It helps scientists visiting the joint laboratories and 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 (Group 1: Tatebayashi Group)

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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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.

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 specificities 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 are believed to phosphorylate the MAP2K Pbs2 at Ser-514 and Thr-518 (S514 and T518). 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.

In this study, we found that the MAP3K Ste11 phosphorylates only one activating phosphorylation site (Thr-518) in Pbs2, whereas the MAP3Ks Ssk2/Ssk22 can phosphorylate both Ser-514 and Thr-518 under optimal osmostress conditions. 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. Interaction between the transmembrane domains of Sho1 and Opy2 enhances the signaling efficiency of the Hog1 MAP kinase cascade in *Saccharomyces cerevisiae*

Tomomi Takayama[1], Katsuyoshi Yamamoto[1], Haruo Saito[1] and Kazuo Tatebayashi: [1]Division of Molecular Cell Signaling, IMSUT

The HOG pathway employs multiple and redundant upstream osmosensing mechanisms that all lead to Hog1 activation. Specifically, upstream osmosensing signaling of the HOG pathway consists of the SLN1 branch and the SHO1 branch. The Ste11 MAPKKK, an upstream activator of the Hog1 MAPK in the SHO1 branch, is activated by phosphorylation by the Ste20/Cla4 kinases when osmostress is applied. Overexpression of constitutively-active Ste11 mutants, such as Ste11-Q301P or Ste11-DDD, induces Hog1 activation even in the absence of osmostress. However, expressing these constitutively-active Ste11 mutant proteins at the endogenous level, *i.e.*, by using a single-copy plasmid that carries the *STE11* promoter, does not activate Hog1 in the absence of osmostress. One possible interpretation for this observation is that osmostress is still needed to activate Hog1 even when Ste11 is activated by a non-osmotic mechanism. It is possible, for example, that osmostress somehow enhances the signaling efficiency of the Ste11-Pbs2-Hog1 MAPK cascade.

In the SHO1 branch of the HOG pathway, a number of non-kinase proteins (Hkr1, Msb2, Sho1, Opy2, Ahk1, Bem1, and Ste50) are involved in activation and/or regulation of the Hog1 MAPK. The PAK-like kinase Ste20 is recruited to the membrane by the small G protein Cdc42 as well as by Hkr1 (probably through a hypothesized adaptor protein) or Msb2 through a Bem1 adaptor protein. Similarly, the MAPKK kinase (MAPKKK) Ste11 is recruited to the membrane by the Opy2-Ste50 complex. Ste50 is a cytoplasmic adaptor protein that binds both to Ste11 and to the single-path membrane anchor protein Opy2. Finally, Pbs2 is also recruited to the membrane by Sho1. Thus, both the Ste20-Ste11 reaction and the Ste11-Pbs2 reaction take place on the membrane. One or both of these activation reactions are likely regulated by osmostress; however, no such mechanisms were known.

In this study, we isolated and analyzed hyperactive mutants of Sho1 and Opy2 that harbor mutations within their TM domains. Several hyperactive mutations enhanced the interaction between Sho1 and Opy2, indicating the importance of the TM-mediated interaction between Sho1 and Opy2 for facilitating effective signaling. The interaction between the TM domains of Sho1 and Opy2 will place their respective cytoplasmic binding partners Pbs2 and Ste11 in close proximity. Indeed, genetic analyses of the mutants showed that the Sho1-Opy2 interaction enhances the activation of Pbs2 by Ste11, but not Hog1 by Pbs2. Some of the hyperactive mutants had mutations at the extracellular ends of either Sho1 TM4 or Opy2 TM, and defined the Sho1-Opy2 binding site 1 (BS1). Chemical crosslinking and mutational analyses revealed that the cytoplasmic ends of Sho1 TM1 and Opy2 TM also interact with each other, defining the

Sho1-Opy2 binding site 2 (BS2). A geometric consideration constrains that one Opy2 molecule must interact with two adjacent Sho1 molecules in Sho1 oligomer. These results raise a possibility that an alteration of the conformation of the Sho1-Opy2 complex might contributes to the osmotic activation of the Hog1 MAPK cascade.

Publications

1. Takayama, T., Yamamoto, K., Saito, H. and Tatebayashi, K. Interaction between the transmembrane domains of Sho1 and Opy2 enhances the signaling efficiency of the Hog1 MAP kinase cascade in *Saccharomyces cerevisiae*. PLOS ONE, 14(1):e0211380 (2019)

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Frontier Research Unit (Group 2: Yoneda Group)

Our major research interests are to elucidate molecular mechanism of pathogenicity and species specificity of negative and single strand RNA virus (*Mononegavirales*) including Nipah virus and measles virus, and to control viral diseases. For these purposes, we are studying virus replication and identifying viral and host factors important for the expression of pathogenicity using a novel reverse genetics technique. We are also developing new virus vaccines virus vectors and oncolytic virus by genetic engineering.

Nipah vaccine international project to fight against a deadly virus with global funding

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Nipah virus (NiV) first emerged in Malaysia in 1998. It has a high fatality rate ranging between 40% and 100%. Bats are the main reservoir for this virus, and human and animal outbreaks of NiV have been occurring in Asian countries such as Bangladesh and India every year. There are currently no effective therapeutics. For development of Nipah vaccine, we attempted to generate novel recombinant vaccine based on measles virus (MV) vector, which is an excellent vaccine vector to induce lifelong, strong immunity and used in the world more than 40 years. We succeeded in developing a new vaccine candidate for NiV infection, and then verified its effectiveness in rodent and primate models.

The Coalition for Epidemic Preparedness Innovations (CEPI) is an innovative partnership between public, private, philanthropic, and civil organizations launched in Davos in 2017 to develop vaccines to stop future epidemics. In February 2019, CEPI awarded a contract of up to US$ 31 million to UTokyo to develop Nipah vaccine we generated, with professor Kai as lead investigator of an international collaboration team including the European Vaccine Initiative, Stanford University, Batavia Biosciences, and icddr,b. Co-sponsored by CEPI and UTokyo, the international symposium "New Vaccine for Saving Lives: Focusing on Nipah Virus Infection" was successfully held on 30 September 2019 at Ito Hall in UTokyo Hongo Campus to introduce the Nipah vaccine project, inviting the project members and experts from across the globe as speakers to provide the latest firsthand updates on the project and the field of emerging infectious diseases. "Nipah Virus International Conference" was held under the auspices of CEPI et al on 10 December 2019 at Singapore, and we gave a lecture as an invited speaker.

This collaboration will enable us to carry out preclinical and clinical studies. The significant progress made in this project will contribute to the suppression of epidemics and give hope to people. We look forward to working together with our partners and CEPI to combat the threat posed by the Nipah virus.

Nipah virus V protein suppresses host interferon induction by stablilizing UBXN1.

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NiV possesses single stranded RNA genome, which contains six genes encoding structural proteins: nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G), and polymerase (L). The P gene also produces three accessory proteins, known as V, W, and C. The V and W proteins are generated by site-specific mRNA editing during viral transcription; a nontemplated one and two G nucleotides, respectively, are inserted at the editing site. The mRNA for the C protein is transcribed from an alternative open reading frame within the P gene. We have previously reported that the V and C proteins play key roles in the severe patho-

genicity of NiV in a hamster infection model. Other several reports have also indicated that V protein is a critical for the pathogenesis of NiV.

Virus infection typically activates host innate immunity including the interferon (IFN) signaling pathway, and IFN responses during virus infection have been well studied. Previous studies have demonstrated that NiV V protein suppresses the host antiviral response by targeting multiple host proteins. NiV V protein interacts with MDA5 to inhibit the activation of the IFN-β promoter. NiV V binds to the phosphatase PP1 to suppress the dephosphorylation of MDA5 and thereby its activation. NiV V also interacts with LGP2 to suppress the RIG-I-dependent induction of IFN. NiV V also blocks signaling through Toll-like receptors 7/9 (TLRs 7/9) and inhibitor of κB kinase ε to suppress IFN induction. These reports have revealed that V protein interacts with multiple host proteins to contribute to the severe pathogenicity of NiV

In this study, we identified UBX domain-containing protein 1 (UBXN1), a negative regulator of RIG-I-like receptor signaling, as a novel host protein that interacts with NiV V. NiV V interacted with the UBX domain of UBXN1 via its proximal zinc-finger motif in the C-terminal domain. NiV V increased the level of UBXN1 protein by suppressing its proteolysis. Furthermore, NiV V suppressed RIG-I and MDA5-dependent IFN signaling by stabilizing UBXN1 and increasing the interaction between MAVS and UBXN1 in addition to directly interrupting the activation of MDA5. Our results suggest a novel molecular mechanism by which the induction of IFN is potentially suppressed by NiV V protein via UBXN1.

Nipah virus nucleoprotein inhibits nuclear accumulation of STAT1 and STAT2 by interfering with their complex formation.

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As described above, it has been reported that NiV V protein inhibits host IFN responses through various mechanisms. Meanwhile, we recently found that the N protein of MV, which is phylogenetically related to NiV, inhibits host IFN responses by disrupting the JAK/STAT signaling pathway via its own nuclear translocation. NiV N protein is also distributed throughout the whole cells same as MV N, therefore we evaluated the IFN antagonist activity of NiV N.

Reporter assays demonstrated that the NiV N dose-dependently suppressed both type I and type II IFN responses. Additionally, NiV N prevented the nuclear transport of STAT1 and STAT2. However, NiV N did not associate with importin α5, importin β1, or Ran, which are members of the nuclear transport system for STATs. Although P protein is known as a binding partner of N protein and actively retains N protein in the cytoplasm, the IFN antagonist activity of N protein was not abolished by the coexpression of P protein. This suggests that the IFN inhibition by N protein occurs in the cytoplasm. Furthermore, we demonstrated that the complex formation of STATs was hampered in the N protein-expressing cells. As a result, STAT nuclear accumulation was reduced, causing a subsequent downregulation of interferon-stimulated genes due to low promoter occupancy by STAT complexes. This novel route for preventing host IFN responses by NiV N protein, in addition to NiV V protein, provides new insight into the pathogenesis of NiV.

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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 360 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 as many as 300 patients 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. Unusually short latent period of therapy-related myeloid neoplasm harboring a rare MLL-EP300 rearrangement: case report and literature review.

Takeda R[1], Yokoyama K[1], Kobayashi S[1, 2], Kawamata T[1], Nakamura S[2], Fukuyama T[1, 3], Ito M[2], Yusa N[4], Shimizu E[5], Ohno N[1], Yamaguchi R[5], Imoto S[6], Miyano S[5], Uchimaru K[1, 7], Tojo A[1, 2].

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Therapy-related myeloid neoplasm (t-MN) is a late and lethal complication induced by chemotherapy and/or radiation therapy. Hematological malignancy is one of the most common primary diseases in patients with t-MN. However, the occurrence of t-MN in adult T-cell leukemia/lymphoma (ATL) patients is rarely reported, possibly due to the dismal prognosis of ATL per se. Here, we report a 62-year-old female who developed t-MN only three months after the completion of conventional chemotherapy and anti-CCR4 antibody for ATL acute type. The patient presented with persistent fever and monocytosis without any evidence of infectious diseases. Bone marrow examinations revealed chronic myelomonocytic leukemia-like disease with a chromosomal translocation of t(11; 22) (q23; q13) as a solo cytogenetic abnormality, resulting in the diagnosis of t-MN. Next-generation sequencing analysis identified a rare chimeric transcript, MLL-EP300, without any additional somatic mutations. Although the patient underwent allogenic hematopoietic stem cell transplantation, she died of viral encephalomyelitis at 7 months after diagnosis of t-MN. Since recent therapeutic advances have extended the survival of patients with ATL, further evaluation of the long-term risks of developing t-MN in these patients is warranted.

3. Immunophenotypic analysis of cerebrospinal fluid reveals concurrent development of ATL in the CNS of a HAM/TSP patient.

Takeda R[1], Ishigaki T[2], Ohno N[1], Yokoyama K[1], Kawamata T[1], Fukuyama T[1, 3], Uchimaru K[1, 4], Tojo A[1,5].

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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 present the case of a 55-year-old female who developed cranial nerve symptoms after a 20-year history of HAM/TSP. Although multiple white matter lesions were observed on brain magnetic resonance imaging, no abnormalities were seen on a systemic computed tomography scan. Quantitative flow-cytometric analysis of cell populations in the cerebrospinal fluid (CSF) revealed that most of the infiltrating cells were not inflammatory cells, but HTLV-1-infected $CD4^+$ $CADM\text{-}1^+$ T-cells completely lacking CD7 expression. As stepwise downregulation of CD7 is correlated with disease progression from HTLV-1 carrier to aggressive ATL, the CSF cells were classified as aggressive ATL; these cells exhibited a more progressed phenotype than those in peripheral blood (PB). HAM/TSP disease activity was estimated to be low. From these and other examinations, we made a diagnosis of acute-type ATL, which unusually developed in the central nervous system at initial onset prior to systemic progression. In ATL cases with a challenging diagnosis, immunophenotypic characterization of CSF and PB is valuable for differential diagnosis and understanding disease status.

4. Clinical features and outcomes of adult Langerhans cell histiocytosis; a single-center experience.

Kobayashi M[1], Kawamata T[2], Makiyama J[2], Yokoyama K[2], Imai Y[2], Tojo[1, 2]
[1] Division of Molecular Therapy
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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 disease, particularly as new opportunities emerge for treatment.

5. Successful treatment of Epstein-Barr virus meningitis in relapsed classic Hodgkin lymphoma.

Andoh S[1], Makiyama J[1], Uchida S[1], Kawamata T[1], Yokoyama K[1], Yasui H[1, 2], Imai Y[1], Tojo A[1, 3].
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Epstein-Barr virus (EBV)-associated neurological disorders are quite rare. A 77-year-old man was diagnosed with EBV negative mixed cellularity classic Hodgkin lymphoma (MCCHL) one year ago. He received eight cycles of ABVD treatment and achieved complete response. Three months after the last cycle, he presented with fever, malaise, and anorexia. sIL-2R level was 10,400 U/mL and FDG-PET/CT indicated multiple nodal and extranodal lesions. After admission, he developed progressive consciousness disturbance, and was empirically given with intrathecal chemotherapy for suspected CNS involvement of MCCHL, but in fail. Cerebrospinal fluid (CSF) test showed WBC counts of 148/3 mm^3 with T lymphocyte dominance, 297.2 mg/dL protein, 27 mg/dL glucose, and 6,000 copies/mL of EBV DNA by PCR, but there was no evidence of other pathogens and lymphoma cells. Only EBV VCA IgG was positive in the EBV serological test. MRI findings showed enhancement of the brain surface in the left occipital lobe. Based on these findings, his diagnosis turned to be relapsed MCCHL complicated by EBV meningitis. He received acyclovir, methylprednisolone and immunoglobulin for meningitis as well as brentuximab vedotin for MCCHL. After the treatment, he showed neurological improvement with disappearance of EBV DNA in the CSF. He also achieved partial response for MCCHL. The present case suggests that special attention should be given to the analysis of possible microbial pathogens, particularly EBV by PCR |when we treat immunocompromised patients with neurological signs and symptoms.

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IMSUT Hospital

Department of Infectious Diseases and Applied Immunology

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Founded in 1981, Department of Infectious Diseases and Applied Immunology (DIDAI) started HIV clinic in 1986. In 2019, 11 new patients with HIV infection have visited to our hospital and 550 patients in total are currently under our clinical management. The total number of in-patients with HIV-infection during 2019 was 19, several beds in our ward have been constantly occupied by patients with not only HIV-infection but also other infectious diseases. Since the number of the staff members of DIDAI is too small to care both outpatients and in-patients, members of the Division of Infectious Diseases and the Department of Infectious Disease Control join the clinic. IMSUT hospital provides the most up-to-date medical treatment to hepatitis patients in Japan. DIDAI is also a treatment center in Japan for international infectious diseases such as malaria and dengue fever.

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

Tomohiko Koibuchi, Michiko Koga*, Hidenori Sato, Lay Ahyoung Lim, Kazuhiko Ikeuchi, Eisuke Adachi, Tadashi Kikuchi, Takashi Odawara, Takeya Tsutsumi*,Hiroshi Yotsuyanagi*:
***Division of Infectious Diseases, The Advanced Clinical Research Center,**

11 new patients with HIV-1 infection visited to our hospital this year (from January 1 to December 31, 2019), and 550 patients in total are under medical management in our outpatient clinic. The total number of HIV-infected in-patients during 2019 was 19. The number of total patients declined in 1997, as shown in Fig.1, because a part of patients as well as medical stuffs moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again after 1998 in accordance with Japanese statistics of HIV-infected patients (Fig. 1). Anti-retroviral therapy (ART) has been introduced to 546 HIV-infected patients in 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 98.3 % 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.

2. Treatment of hepatitis in IMSUT hospital:

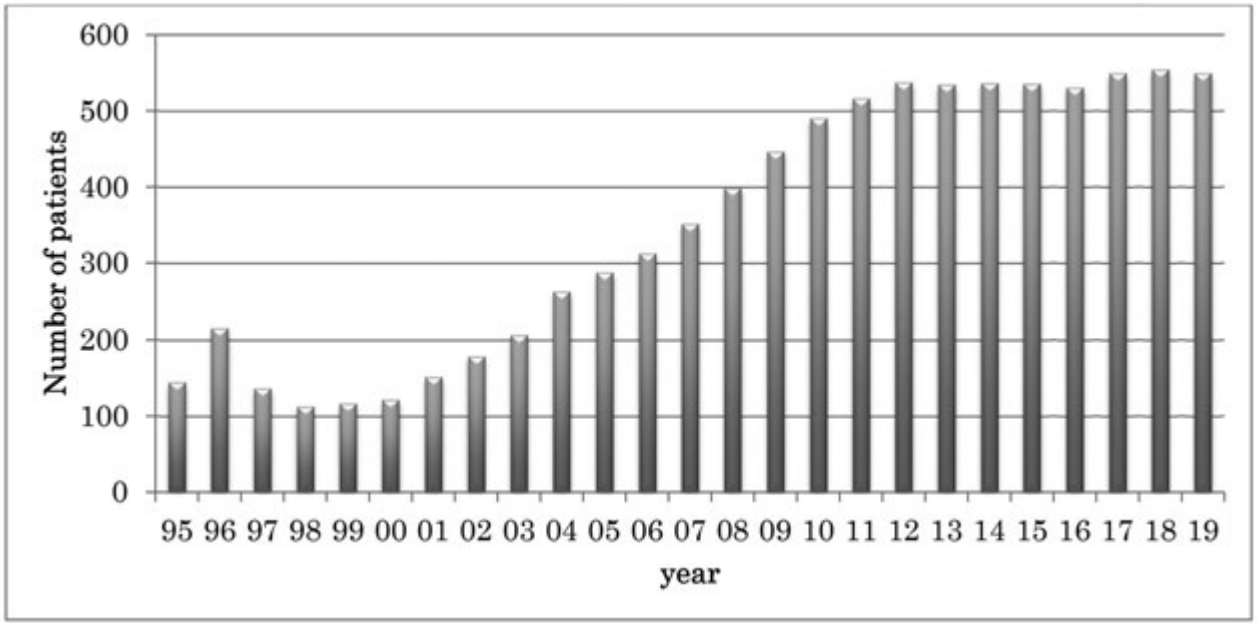

Figure 1. Number of HIV-infected outpatients in IMSUT Hospital

Takeya Tsutsumi*, Tomohiko Koibuchi, Michiko Koga*, Hidenori Sato, Lay Ahyoung Lim, Kazuhiko Ikeuchi, Eisuke Adachi, Tadashi Kikuchi, Hiroshi Yotsuyanagi*:
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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 performed abdominal ultrasonography for about 400 patients and Fibroscan® for more than 220 patients.

3. Creating Practice Guidelines for Treatment of HIV-infected Patients in Japan:

Tomohiko Koibuchi, Michiko Koga*, Hidenori Sato, Lay Ahyoung Lim, Eisuke Adachi, Tadashi Kikuchi, Takashi Odawara, Takeya Tsutsumi*, Hiroshi Yotsuyanagi*:
***Division of Infectious Diseases, The Advanced Clinical Research Center**

The Japanese guidelines for treatment of HIV-infected patients have been established since 1998 with support from Ministry of Health, Labor and Welfare. The representatives from our department have played critical roles in development of the current practice guidelines in Japan. It is vital to create practice guidelines that are specific for the unique genetic and social backgrounds of the HIV-infected population in Japan. In collaboration with other Japanese HIV-experts, the physicians from our department update the practice guidelines annually, as we deem necessary. The guidelines are available at http://www.haart-support.jp/guideline.htm and used widely by Japanese clinicians. They have been viewed 132,510 times in 2019 on the website. In Japan, where the number of HIV-experts are limited compared to other countries, the practice guidelines have substantially improved the standard of care for the HIV-infected patients in our country.

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

Tomohiko Koibuchi, Eisuke Adachi, Makoto Saito*, Michiko Koga*, Hidenori Sato, Lay Ahyoung Lim, Kazuhiko Ikekuchi, Tadashi Kikuchi, Takashi Odawara, Takeya Tsutsumi*, Hiroshi Yotsuyanagi[1]:
*** Division of Infectious Diseases, The Advanced Clinical Research Center**

This year, more than one hundred overseas travelers visited our clinic. The reasons of their visit included prescription of malaria prophylaxis, hepatitis A/B vaccination, other general health consultation, or treatment of tropical diseases such as malaria, intestinal amebiasis, post-exposure prophylaxis of rabies and so on.

Dozens of important medicines essential for treatment of tropical or parasitic diseases are not licensed in Japan. For instance, artesunate and injectable quinine for falciparum malaria, pyrimethamine and sulfadiazine for toxoplasmosis, etc. are not licensed.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 institu-

tion of the research group using these orphan drugs if needed, and colleting clinical data.

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

Tomohiko Koibuchi, Michiko Koga*, Hidenori Sato, Lay Ahyoung Lim, Kazuhiko Ikeuchi, Makoto Saito*, Eisuke Adachi, Takeya Tsutsumi*, Hiroshi Yotsuyanagi*:

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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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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, Motohisa Yamamoto, Noritada Yoshikawa, Hiroki Yamazaki, Akiko Souta-Kuribara, Erika Matsubara, Masaaki Uehara, Aya Oda, Mayu Nishimura, Satoshi Fukuyama*, Yoshihiro Kawaoka*

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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.

(i) Developing a novel therapy preventing glucocorticoid-induced muscle atrophy

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.

(ii) Developing a novel therapy to improve abnormal fat distribution in patients taking glucocorticoid therapy

Cushing's syndrome or prolonged glucocorticoid treatment is responsible for accumulation of fat in selected adipose tissue depots, especially in the face, nape of the neck, and visceral compartments including liver, resulting both clinical and cosmetic problem. We created a mouse model mimicking Cushing's syndrome and revealed that selective blockade of skeletal muscle GR by muscle specific knockout of GR (GRmKO) mitigated glucocorticoid-induced Cushingoid including metabolic disorders, i.e., insulin resistance, diabetes mellitus, hyperlipidemia, and fatty liver. We are now targeting skeletal muscle GR to develop a novel therapeutic approach improving Cushingoid features resembling metabolic syndrome.

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

Hirotoshi Tanaka, Motohisa Yamamoto, Noritada Yoshikawa, Hiroki Yamazaki, Akiko Souta-Kuribara, Erika Matsubara, Aya Oda, Masaaki Uehara, Mayu Nishimura, Satoshi Fukuyama*, Yoshihiro Kawaoka*

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(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, GRmKO mitigated such metabolic unhealthy phenotype. Moreover, GRmKO blunted coordinated regulation of systemic metabolism by glucocorticoids. 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

Sexual dimorphism in human body composition has major implication for sex differences in the risk of various diseases including metabolic syndrome. Although the metabolic syndrome tends to appear more often and/or earlier in adult males than in females, the differences in incidence decrease sharply after menopause, suggesting that sex hormones and their receptors play a certain role in metabolic pathways. Recently, we investigated considerable sexual dimorphism in transcriptome and metabolome in the mouse skeletal muscle. Interestingly, in GRmKO, such gene expression and metabolic profiles dynamically altered, suggesting that GR may modulate sexual dimorphism of such transcription and metabolic apparatus in the skeletal muscle. We found that, although the most of GR target genes related to major muscle metabolism and differentiation in the mouse skeletal muscle was common in male and female, sexually dimorphic GR target genes were also existed. 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 found that an omega-3 fatty acid, eicosapentaenoic acid (EPA), protects sarcopenia. 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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IgG4-related disease working consortium, Tanaka Y, Oshima K, Akamizu T, Nakamura S, Nakamura S, Saeki T, Umehara H, Shimosegawa T, Mizuno N, Kawano M, Azumi A, Takahashi H, Mimori T, Kamatani Y, Okazaki K, Chiba T, Kawa S, Matsuda F. A genome wide study of IgG4-related disease in the Japanese people. Lancet Rheumatol. 2019: 1(1): e14.
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IMSUT Hospital

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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

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.

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

Matsubara Y., Hirata Y.

About 800 cases of upper gastrointestinal endoscopy and about 300 cases of colonic endoscopy were performed from January 1 to December 31, 2019. We have diagnosed relatively rare disease (e.g. infectious disease, malignancy, other disease) in patients with immune dysfunction. We also participated in endoscopic health check up in Minato Ward.

3. Abdominal ultrasonography in IMSUT Hospital

Tsutsumi T

About 400 cases of abdominal ultrasonography were performed from April 1 to December 21 in 2019. We detected liver tumors, or pancreas tumors, or abdominal lymphadenopathy in some cases who were subsequently diagnosed as hepatocellular carcinoma, or pancreas cancer, or malignant lymphoma. We also performed Fibroscan® for more than 220 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. Journal of Clinical Oncology Precision Oncology. 2019 in press)

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 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, animal experiments, and multicenter studies in collaboration with other facilities.

Koichi Kimura

Clinical studies in echocardiography were conducted in collaboration with echocardiography laboratory of The University of Tokyo Hospital. Several animal experiments using CRISPR/CAS9 genome-designed rats were proceeded in collaboration with department of veterinary physiology, The University of Tokyo. Other animal experiments using disease model dogs and knockout mice were proceeded in collaboration with National Center of Neurology and Psychiatry (Tokyo). Multicenter studies, drug interventional study and observational cohort study, for patients with muscular dystrophy have been conducted 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).

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IMSUT Hospital

Department of Applied Genomics
ゲノム診療科

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Associate Professor	Tsuneo Ikenoue, M.D., Ph.D.	准教授　博士(医学)	池　上　恒　雄

Our department has been working on the application of human genome information in clinics. As clinical services in IMSUT Hospital, we provide genetic counseling, genetic tests for human 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 2019, a total of 539 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 2019, we had a total of 42 counseling cases including hereditary breast and ovarian cancer, familial adenomatous polyposis, Lynch syndrome, Li-Fraumeni syndrome, tuberous sclerosis, adrenoleukodystrophy, hemophilia A, spinal and bulbar muscular atrophy, spinocerebellar degeneration, Huntington's disease, developmental disorders, and achondroplasia. In the counseling, we provided appropriate information about hereditary diseases and took psychological care of the clients in collaboration with a clinical psychologist. Genetic testing was performed in five cases with informed consent after thoughtful discussion about its merit and demerit.

Systematic surveillance programs are provided for the clients susceptible for hereditary tumors.

3. Application of liquid-based genetic diagnosis for the screening of endometrial cancer

Kiyoko Takane[1], Kiyoshi Yamaguchi[1], Tsuneo Ikenoue, Yoichi Furukawa
[1]Division of Clinical Genome Research, Advanced Clinical Research Center

We have started 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 cytology (LBC) has increased the sensitivity of cytological diagnosis of EC compared with conventional smear cytology, the sensitivity of LBC for the detection of EC is between 70% and 96% and remains unsatisfactory. Our previous study with 48 LBC subjects revealed that LBGDx using amplicon sequencing of five genes including *PTEN*, *PIK3CA*, *CTNNB1*, *KRAS*, and *TP53* improved the sensitivity for the detection of EC (85% by LBGDx vs 75% by LBC). We are trying to establish more sensitive assay of LBGDx for screening of EC by analyzing additional genes.

4. Clinical sequencing for the implementation of genomic medicine

Kiyoko Takane, Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Eigo Shimizu[1], Rika Kasajima[1], Tetsuo Shibuya[2], Satoru Miyano[1,2], Takanori Hasegawa[3], Seiya Imoto[3], Kazuaki Yokoyama[4], Arinobu Tojo[4], Koichiro Yuji[5], Rui Yamaguchi[6],: [1]Laboratory of DNA Information Analysis, [2]Laboratory of Sequence Analysis, Human Genome Center, [3]Division of Health Medical Data Science, Health Intelligence Center, [4]Division of Molecular Therapy, [5]Division of International Advanced Medical Research, Advanced Clinical Research Center, IMSUT, [6]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute

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 working on the genetic diagnosis of patients suspected of hereditary cancer, and the 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's 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-sequencers will be help the identification of pathogenic SVs in patients with hereditary diseases.

In the second project, we have been testing interpretation of genomic data using IBM Watson for Genomics (WfG). After written informed consent was obtained from the patients with colorectal, breast, uterine, gallbladder, pancreatic cancer, lymphoma, prostate cancer, liposarcoma, glioblastoma, and hepatoblastoma, they were enrolled in this study. 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.

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Department of Radiological Technology
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The Department of Radiology undertakes radiology service at IMSUT hospital. Our expertise includes general diagnostic radiology, neuroradiology, clinical nuclear medicine, and radiation therapy. Board-certified radiologists at the Department of Radiology conduct all examinations of CT, MRI, and nuclear medicine. Radiological reports are made by the radiologists. In addition, several clinical studies are being conducted in collaboration with other departments or institutions. We also investigate the technical aspects of molecular imaging with intact small animals for its application to preclinical studies using 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 examination.

Deep learning for predicting bone marrow density of the lumbar from computed tomography

Yasaka K, Akai H, Kunimatsu A, Kiryu S[1], Abe O[2]: [1]Department of Radiology, Graduate School of Medical Sciences, International University of Health and Welfare, [2]Department of Radiology, Graduate School of Medicine, The University of Tokyo

We investigated whether a deep learning model can predict the bone mineral density (BMD) of lumbar vertebrae from unenhanced abdominal computed tomography (CT) images. Patients who received both unenhanced CT examinations and dual-energy X-ray absorptiometry (DXA) of the lumbar vertebrae, in two institutions (IMSUT Hospital and The University of Tokyo Hospital) were included. Supervised deep

learning was employed to obtain a convolutional neural network (CNN) model using axial CT images, including the lumbar vertebrae as input data and BMD values obtained with DXA as reference data. For this purpose, 1665 CT images from 183 patients in the IMSUT Hospital, which were augmented to 99900 (= 1665 × 60) images (noise adding, parallel shift and rotation were performed), were used. Internal (by using data of 45 other patients in IMSUT Hospital) and external validations (by using data of 50 patients in The University of Tokyo Hospital) were performed to evaluate the performance of the trained CNN model. Correlations and diagnostic performances were evaluated with Pearson's correlation coefficient (r) and area under the receiver operating characteristic curve (AUC), respectively. The estimated BMD values, according to the CNN model (BMDCNN), were significantly correlated with the BMD values obtained with DXA ($r = 0.852$ ($p < 0.001$) and 0.840 ($p < 0.001$) for the internal and external validation datasets, respectively). Using BMDCNN, osteoporosis was diagnosed with AUCs of 0.965 and 0.970 for the internal and external validation datasets, respectively. In conclusion, using deep learning, the BMD of lumbar vertebrae could be predicted from unenhanced abdominal CT images.

Application of CT texture analysis to assess the localization of primary aldosteronism.

Hiroyuki Akai, Koichiro Yasaka, Akira Kunimatsu, Kuni Ohtomo[3], Osamu Abe, Shigeru Kiryu: [3]International University of Health and Welfare

We performed present study to investigate whether the localization of primary aldosteronism (PA) can be predicted using quantitative texture analysis on unenhanced computed tomography (CT). Plain CT data of 82 PA patients (54 unilateral (right-sided: left-sided = 24: 30) , 28 bilateral) were analyzed retrospectively. After semi-automatically setting the region of interest to include the whole adrenal gland, texture analyses were performed with or without a Laplacian of Gaussian filter with various spatial scaling factors (SSFs). Logistic regression analysis was performed using the extracted histogram-based texture features to identify parameters capable of predicting excessive aldosterone production. The result of adrenal venous sampling served as gold standard in present study. As a result, logistic regression analysis indicated that the mean gray level intensity ($p = 0.026$), the mean value of the positive pixels ($p = 0.003$) in the unfiltered image, and entropy ($p = 0.027$) in the filtered image (SSF: 2 mm) were significant parameters. Using the model constructed by logistic regression analysis and the optimum cutoff value, the localization of PA (three multiple choices of left, right or bilateral) was determined with an accuracy of 67.1% (55/82). CT texture analysis may provide a potential avenue for less invasive prediction of the localization of PA.

Publications

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IMSUT Hospital

Department of Surgery
外科

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Assistant Professor	Tomohiro Kurokawa, M.D., Ph.D.	助教	博士（医学）	黒　川　友　博

The missions of our department are to provide surgical service for patients with surgical or gastrointestinal disease, such as malignancy or benign colon anal disease, and to develop and conduct clinical research and clinical trials in early stages (mainly, Phase I and II) on patients at the Research Hospital. We have also been offering diagnostic and therapeutic endoscopy, including upper and lower gastrointestinal endoscopic examinations.

1. Treatment for gastrointestinal malignancy

We focus on treatment of gastrointestinal cancers such as colorectal or gastric cancers. As well as standard radical surgery, surgery that emphasizes not only curability but also postoperative function preservation was performed by using preoperative chemotherapy and/or radiotherapy. Regarding advanced unresectable cancer or recurrent cancer cases, chemotherapy or palliative therapy was performed. If there is scientific evidence, we will also support non-indication treatment, and will also support participation in clinical trials. Immediate or emergency hospitalization is also possible for patients with poor general status.

2. Treatment for benign colon anal disease

Especially for anal disorders such as internal hemorrhoids, inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, or functional disorders such as irritable bowel syndrome, medical treatment at specialty hospitals was performed.

3. Endoscopic examination or treatment

Under cooperation with Department of general internal medicine, we performed many upper gastrointestinal endoscopies and colonoscopies without major complications. For the patients' satisfaction, we aggressively perform endoscopic resection of colorectal neoplasms and avoid operation as much as possible. Our fellows have learned gastrointestinal endoscopic technique and have made great progress.

4. Hospital collaboration, personnel exchange

As a part of clinical education, we sent junior fellows belonging to our department to clinical city hospitals, Dr. Yoshiaki Kanemoto to Joban Hospital.

5. International research activities

Dr. Tomohiro Kurokawa, a senior fellow belonging to our department, has studied at Massachusetts General Hospital in Boston. He return to our department in July, 2019. We also performed many research presentations at international conferences and published many papers to international journals

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IMSUT Hospital

Department of Anesthesia
麻酔科

Associate Professor Ryo Orii, M.D., Ph.D.
Assistant Professor Reiko Shibata, M.D.

准教授 博士(医学) 折井 亮
助 教 医学士 柴田玲子

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.

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 con-

tinuous CO monitors that use arterial waveform analysis. 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

Shibata R, Orii R, Ako R. Anesthesia management of arthroscopic ankle arthrodesis for a hemophilia patient after living-donor liver transplantation. Intractable Rare Dis Res. 2019; 8: 56-59.

Research Hospital

Department of Joint Surgery
関節外科

Senior Assistant Professor Hideyuki Takedani, M.D., D.M.Sc.
Assistant Professor Kumiko Ono, M.D., D.M.Sc.

講　師　博士(医学)　竹　谷　英　之
助　教　博士(医学)　大　野　久美子

Department of Joint Surgery was established in 2006. Our 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.

Surgical treatment for haemophilia

From 2006 to 2019, more than 228 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

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3. Nagao, A., N. Suzuki, H. Takedani, N. Yamasaki, Y. Chikasawa, A. Sawada, T. Kanematsu, M. Nojima, S. Higasa, K. Amano, K. Fukutake, T. Fujii, T. Matsushita and T. Suzuki (2019). "Ischaemic events are rare, and the prevalence of hypertension is not high in Japanese adults with haemophilia: First multicentre study in Asia." Haemophilia **25**(4): e223-e230.
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IMSUT Hospital

Department of Surgical Neuro-Oncology
脳腫瘍外科

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Assistant Professor	Seisaku Kanayama, M.D.	助　教		金　山　政　作
Assistant Professor	Lushun Chalise, M.D., Ph.D.	助　教	医学博士	チャリセルシュン
Assistant Professor(Thoracic surgeon)	Yoshinori Sakata, M.D., Ph.D.	助　教	医学博士(呼吸器外科医)	坂　田　義　詞
Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	医学博士	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 giving rise to an immune response against tumor-associated proteins. Clinical trials using a third-generation, triple-mutated oncolytic HSV-1, G47Δ, is ongoing in patients with olfactory neuroblastoma or malignant pleural mesothelioma. Recently we have started a new investigator-initiated clinical trial using T-hIL12 for malignant melanoma.

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 a triple-mutated oncolytic HSV-1, 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 conducteda phase II clinical trial of G47Δ in patients with glioblastoma since December 2014. 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. Daiichi Sankyo Company is going to submit a Marketing Authorization Application (MAA) for G47Δ to the Japanese Pharmaceuticals and Medical Devices Agency (PMDA).

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 conducted 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 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 have conducted a phase 1/2 clinical trial of T-hIL12 in patients with advanced malignant melanoma since August 2019. T-hIL12 is a 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, whereas in phase 2 it is response rate (RECIST 1.1).

Routine activities

Patients with brain tumors are treated by five neurosurgeons. A total of 16 operation were carried out in 2019 including 7 gliomas and 9 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 Medical Informatics
医療情報部

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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 management of hospital information system, including infrastructure for the system, at the Institute of Medical Science (IMSUT) Hospital. Hospital information system enables a 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.

In addition, we make a substantial contribution to 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 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 IT service room of IMSUT, and 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 hospital information system and network
- General work concerning the operation of hospital information system and network

2. IT support to community-based healthcare provider network

Akira Kunimatsu, Hiroyuki Akai, Koiciro Yasaka

"Community-based integrated care systems" is a keyword for the Japanese healthcare system in this decade. IMSUT hospital belongs to its own community-based healthcare provider network and we continuously improve infrastructure for mutual 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

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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. 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. Peripheral Blood Stem Cell mobilization and collection:

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.

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 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.

Mukai T, Takahashi A, Tojo 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 Cord Blood Stem Cell Bank / National BioResource Project (NBRP) (IMSUT- Cell Resource Center):

Izawa M, 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. Management of 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 herapy

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. In the next year, a da Vinci surgical system, a robotic technology, will be operable that allows surgeons to perform minimally invasive procedures.

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. In the next year, a da Vinci surgical system, a robotic technology, will be operable that allows surgeons to perform minimally invasive procedures. 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 2019

Total number of operations	161
Planned operations	149
Emergency operations	12
General anesthesia	132
Spinal	1
Epidural	0

Local	28	Others	0

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
Assistant Professor	Tomohiro Ishigaki, M.D., Ph.D.	助　教	博士(医学)	石　垣　知　寛
Project Assistant Professor	Koichi Kimura, M.D., Ph.D.	特任助教	博士(医学)	木　村　公　一

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 the steps of clinical practice, 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 been contributing 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. 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.

2. Establishment and introduction of new management system of clinical laboratory examinations for translational researches (TR).

Tomohiro Ishigaki, Osamu Takahashi, and Masato Suzuki.

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.

This subject was picked up as one of the selected topics by the chairman of the session in JSLM 2019.

3. A relationship between left ventricular non-compaction (LVNC) and therapy-related cardiac dysfunction in patients with hematological diseases.

Koichi Kimura, Hisako Ishii, and Tomohiro Ishigaki.

Our division of clinical physiology performs many kinds of ultrasonographic examinations, and echocardiography is an important one of them. A retrospective analysis was performed using echocardiographic records of patients with hematological diseases. We successfully elucidated left ventricular non-compaction (LVNC) could be one of the risk factors for therapy-related cardiac dysfunction in hematological diseases.

4. CD4+ CADM1+ cell percentage predicts disease progression in HTLV-1 carriers and indolent adult T-cell leukemia/lymphoma.

Tomohiro Ishigaki and Arinobu Tojo (first: Junya Makiyama and Seiichiro Kobayashi [Department of Hematology/Oncology]).

We recently took advantage of the universal expression of cell adhesion molecule 1 (CADM1) by CD4 + cells infected with HTLV-1 and the downregulation of CD7 expression that corresponds with the oncogenic stage of HTLV-1-infected cells to develop a flow cytometric system using CADM1 versus CD7 plotting of CD4 + cells. We risk-stratified HTLV-1 asymptomatic carriers (AC) and indolent adult T-cell leukemia/lymphoma (ATL) cases to four groups (G1-G4) based on the CADM1 + percentage, in which HTLV-1-infected clones are efficiently enriched. Our results indicate that the percentage of the CD4 + CADM1 + population predicts clinical disease progression: G1 and G2 cases are stable and considered to be at low risk; G3 cases are considered to have intermediate risk; and G4 cases are unstable and at high risk of acute transformation.

5. Laboratory contribution as a central medical department.

(1) Support for many clinical investigations and trials in this hospital.

Hiroyuki Shingyochi, Hisako Ishii, Osamu Takahashi, and Tomohiro Ishigaki.

We are also 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.

(2) Establishment of HTLV-1 carrier model using common marmoset.

Etsuko Nagai, Tamami Denda, Yukihisa Tanaka, and Arinobu Tojo (first: Lisa Hirose [Project Division of ALA Advanced Medical Research]).

For the diagnosis and treatment of adult T-cell leukemia/lymphoma (ATL) caused by human T-lymphotropic virus type 1 (HTLV-1), therapeutic models are required. Non-human primate models for ATLL would provide valuable information for clinical studies. We did a pilot study to establish an ATLL non-human primate model using common marmosets (Callithrix jacchus), and finally obtained HTLV-1 asymptomatic carriers of common marmosets by inoculating MT-2 cells.

IMSUT Hospital

Center for Clinical Safety and Infection Control
医療安全・感染制御センター

Department of Clinical trial Safety Management
医療安全管理部

Head, Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Associate Professor	Yoichi Imai, M.D., D.M.Sc.	准教授	博士(医学)	今　井　陽　一
Nurse Manager	Hatsuko Narita	看護師長		成　田　初　子
Director of Pharmacy		薬剤部長		黒　田　誠一郎
Associate Professor	Ayako Kamisato, Ph.D.	准教授	博士(法学)	神　里　彩　子

Department Medical Safety Management consisting of doctors, a nurse, a pharmacist, and an administrative staff was founded in July 2001 and is responsible for carrying out medical safety in order to prevent incidents and accidents beforehand and deliver safe medical care to patients. Especially at our hospital, we mainly focus on hematological malignancies, infectious diseases, immune diseases, refractory malignant solid tumors etc, and are performing many kinds of therapies including transplantation. So we are keeping in mind that we can adequately respond to the things those will happen in these kinds of medical activities.

Department of Infection Prevention and Control
感染制御部

Head, Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	助　教	博士(医学)	安　達　英　輔
Nurse Manager	Miya Kogayu	看護師長		小　粥　美　香
Pharmacist	Mika Yamamura	薬剤師		山　村　美　桂
Clinical laboratory technician	Hiroko Shibata	臨床検査技師		柴　田　浩　子

Department of Infection Prevention and Control builds ICT (Infection Control Team) and AST (Antimicrobial Stewardship Team) to promote the practice of hospital infection control and prevent the spread of antimicrobial resistant organisms. The ICT consists of infection control doctors, an infection control nurse, a pharmacist, a clinical laboratory technicians and an administrative staff.

Monitoring of PVL-producing MRSA in HIV-infected patients and other drug-resistant bacteria found in the hospital based on gene analysis

When an outbreak in the hospital is suspected, or colonization of multi-drug-resistant bacteria is observed in patients, genetic analysis of isolated strains is performed to investigate the route of infection.

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 manage-

ment, monitoring, and statistical works in clinical trials.

[Data management]: Planning, EDC and CRF preparation, registration, allocation, database management, data cleaning, coding

[Monitoring]: Monitoring for drug management

[Statistics]: Planning and perform for statistical analyses, Sample size calculation.

3. Support for the investigator-initiated clinical trials under an Investigational New Drug Application

All members of staff

Our mission is to develop efficient approaches for conducting investigator-initiated clinical trials under Investigational New Drug application (IND) to promote translational research. In 2019, we supported four investigator-sponsored clinical trials under IND by site management as well as project management. These four clinical trials were: oncolytic virus for glioma, peptide therapy for after rejection of non-small cell lung cancer, 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

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Professor	Kohei 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
Project Associate Professor	Motohisa Yamamoto, M.D., D.M.Sc.	特任准教授	博士(医学)	山　本　元　久
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.

Daigo Group

1. Novel therapeutic target discovery for solid cancers

Yataro Daigo, Atsushi Takano, Koji Teramoto, Hi-

detoshi 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 genes in the majority of solid cancers (lung, esophagus etc.) by genome-wide screening using the expression microarray 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, 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 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 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

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. 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.

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 immuno-

therapy 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. Clinical significance of PD-L1-positive cancer-associated fibroblasts in pN0M0 non-small cell lung cancer

Yataro Daigo, Koji Teramoto, Tomoyuki Igarashi

CAFs are a dominant cell type in tumor stroma and support the generation of pro-tumorigenic microenvironment. CAFs have frequent opportunities to interact with immune cells infiltrating the tumor stroma, but the process remains to be determined. We focused on immune checkpoint mechanism and examined the induction of programmed cell death-ligand 1 (PD-L1) on CAFs by immune cell, and the clinical significance of PD-L1-expressed CAFs in non-small cell lung cancer (NSCLC). PD-L1 mRNA and protein expression on CAFs was upregulated by exogenously supplemented interferon-gamma (IFN-γ) and downregulated through the depletion of IFN-γ. PD-L1 expression on CAFs was upregulated by co-culture with activated lymphocytes releasing IFN-γ. Immunohistochemistry revealed that PD-L1-positive CAFs were observed in 31 of 125 surgically resected pN0M0 NSCLC cases (24.8%). Postoperative relapse-free survival was significantly prolonged in patients with PD-L1-positive CAFs as compared with those with PD-L1-negative CAFs, with 5-year relapse-free probabilities of 84.5% and 66.3%, respectively ($P = 0.031$). Multivariate analysis revealed that PD-L1 expression on CAFs was an independent prognostic factor of longer relapse-free survival after surgery (hazard ratio: 3.225, P = 0.027). PD-L1 expression on CAFs is reversibly regulated by environmental stimuli including IFN-γ from activated lymphocytes. In the non-metastatic NSCLC, PD-L1 expression on CAFs suggests the induction of anti-tumor immune responses, contributing to better prognosis after surgery.

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 Mendelian randomization and pathway analysis of genome-wide association study data from never-smoking Asian women with tuberculosis infection and lung adenocarcinoma. 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, 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 55,000 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.

Nagatoishi Group

Various antibodies have been approved for therapeutic use and currently examined in clinical development. Developments and improvements of technology for the discovery and optimization of high-potency antibodies, therefore, 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. 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.

2. Biophysical characterization of the breast cancer-related BIG3-PHB2 interaction: Effect of non-conserved loop region of BIG3 on the structure and the interaction.

Chigira T, Nagatoishi S, Takeda H, Yoshimaru T, Katagiri T, Tsumoto K.

Brefeldin A-inhibited guanine nucleotide-exchange protein 3 (BIG3) interacts with and inhibits the tumor suppressor function of prohibitin-2 (PHB2), and recent in vivo studies have demonstrated that the BIG3-PHB2 interaction is a promising target for breast cancer therapy. However, little biophysical characterization on BIG3 and its interaction with PHB2 has been reported. Here we compared the calculated 8-class secondary structure of the N-terminal domains of BIG family proteins and identified a loop region unique to BIG3. Our biophysical characterization demonstrated that this loop region significantly affects the colloidal and thermodynamic stability of BIG3 and the thermodynamic and kinetic profile of its interaction with PHB2. These results establish a model for the BIG3-PHB2 interaction and an entry for drug discovery for breast cancer.

3. Structural features of methionine aminopeptidase2-active core peptide essential for binding with S100A4.

Katagiri N, Nagatoishi S, Tsumoto K, Endo H.

Methionine aminopeptidase 2 (MetAP2) is one of the effector proteins of S100A4, a metastasis-associated calcium-binding protein. This interaction is involved in angiogenesis. The region of MetAP2 that interacts with S100A4 includes amino acids 170 to 208. A peptide corresponding to this region, named as NBD, has potent anti-angiogenic activity and suppresses tumor growth in a xenograft cancer model. However, the binding mode of NBD to S100A4 was totally unknown. Here we describe our analysis of the relationship between the inhibitory activity and the structure of NBD, which adopts a characteristic helix-turn-helix structure as shown by X-ray crystallographic analysis, and peptide fragments of NBD. We conducted physicochemical analyses of the interaction between S100A4 and the peptides, including surface plasmon resonance, microscale thermophoresis, and circular dichroism, and performed docking/molecular dynamics simulations. Active peptides had stable secondary structures, whereas inactive peptides had a little secondary structure. A computational analysis of the interaction mechanism led to the design of a peptide smaller than NBD, NBD-ΔN10,

that possessed inhibitory activity. Our study provides a strategy for design for a specific peptide inhibitor against S100A4 that can be applied to the discovery of inhibitors of other protein-protein interactions.

4. Highly sensitive biomolecular interaction detection method using optical bound/free separation with grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS).

Kaya T, Nagatoishi S, Nagae K, Nakamura Y, Tsumoto K.

Grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS) with optical bound/free (B/F) separation technique was developed by employing a highly directional fluorescence with polarization of surface plasmon-coupled emission (SPCE) to realize highly sensitive immunoassay regardless of the ligand affinity. A highly sensitive immunoassay system with GC-SPFS was constructed using a plastic sensor chip reproducibly fabricated in-house by nanoimprinting and applied to the quantitative detection of an anti-lysozyme single-domain antibody (sdAb), to compare conventional washing B/F separation with optical B/F separation. Differences in the affinity of the anti-lysozyme sdAb, induced by artificial mutation of only one amino acid residue in the variable domain were attributed to higher sensitivity than that of the conventional Biacore surface plasmon resonance (SPR) system. The detection limit (LOD; means of six replicates of the zero standard plus three standard deviations) of the GC-SPFS immunoassay with optical B/F separation, was estimated to be 1.2 ng/ml with the low-affinity ligand (mutant sdAb Y52A: KD level was of the order of 10-7 ~ 10-6 M) and was clearly improved as compared to that (LOD: 9.4 ng/ml) obtained with the conventional washing B/F separation. These results indicate that GC-SPFS with the optical B/F separation technique offers opportunities to re-evaluate low-affinity biomaterials that are neither fully utilized nor widespread, and could facilitate the creation of novel and innovative methods in drug and diagnostic development.

5. An epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout.

Eguchi A, Nakakido M, Nagatoishi S, Kuroda D, Tsumoto K, Nagamune T, Kawahara M.

Upon developing therapeutically potent antibodies, there are significant requirements, such as increasing their affinity, regulating their epitope, and using native target antigens. Many antibody selection systems, such as a phage display method, have been developed, but it is still difficult to fulfill these requirements at the same time. Here, we propose a novel epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout. This system is based on the competition of antibody binding, and can target membrane proteins expressed in a native form on a mammalian cell surface. Using this system, we successfully selected an affinity-matured anti-ErbB2 single-chain variable fragment variant, which had the same epitope as the original one. In addition, the affinity was increased mainly due to the decrease in the dissociation rate. This novel cell-based antibody affinity maturation system could contribute to directly obtaining therapeutically potent antibodies that are functional on the cell surface.

6. Exploring designability of electrostatic complementarity at an antigen-antibody interface directed by mutagenesis, biophysical analysis, and molecular dynamics simulations.

Yoshida K, Kuroda D, Kiyoshi M, Nakakido M, Nagatoishi S, Soga S, Shirai H, Tsumoto K.

Antibodies protect organisms from a huge variety of foreign antigens. Antibody diversity originates from both genetic and structural levels. Antigen recognition relies on complementarity between antigen-antibody interfaces. Recent methodological advances in structural biology and the accompanying rapid increase of the number of crystal structures of proteins have enabled atomic-level manipulation of protein structures to effect alterations in function. In this study, we explored the designability of electrostatic complementarity at an antigen-antibody interface on the basis of a crystal structure of the complex. We designed several variants with altered charged residues at the interface and characterized the designed variants by surface plasmon resonance, circular dichroism, differential scanning calorimetry, and molecular dynamics simulations. Both successes and failures of the structure-based design are discussed. The variants that compensate electrostatic interactions can restore the interface complementarity, enabling the cognate antigen-antibody binding. Retrospectively, we also show that these mutational effects could be predicted by the simulations. Our study demonstrates the importance of charged residues on the physical properties of this antigen-antibody interaction and suggests that computational approaches can facilitate design of antibodies that recognize a weakly immunogenic antigen.

7. Control of Protein Adsorption to Cyclo Olefin Polymer by the Hofmeister Effect.

Fujita R, Nagatoishi S, Adachi S, Nishioka H, Ninomiya H, Kaya T, Takai M, Arakawa T, Tsumoto K.

Cyclo olefin polymer (COP) is an attractive plastic because it has low protein adsorption despite its hydrophobic chemical structure. Here, the adsorption of model proteins to the COP was evaluated in comparison with a representative plastic, polystyrene (PSt), using reflectometry interference spectroscopy (RIfS) technology. The effects of different salts on adsorption were then examined. The adsorption of bovine serum albumin onto COP increased in the presence of kosmotropic salts, whereas adsorption of IgG increased in the presence of chaotropic salts. By contrast, the adsorption of these 2 proteins to PSt was unaffected by these Hofmeister salts. Langmuir-Freundlich model of COP adsorption suggested that the COP surface is more homogeneous for protein binding than the PSt surface. Furthermore, RIfS and sum frequency generation analyses indicated that water molecules bind more weakly to COP than to PSt. Our data propose a novel viewpoint of the way protein binds to COP surface that is different from the way it binds to PSt.

8. Affinity Improvement of a Cancer-Targeted Antibody through Alanine-Induced Adjustment of Antigen-Antibody Interface.

Yamashita T, Mizohata E, Nagatoishi S, Watanabe T, Nakakido M, Iwanari H, Mochizuki Y, Nakayama T, Kado Y, Yokota Y, Matsumura H, Kawamura T, Kodama T, Hamakubo T, Inoue T, Fujitani H, Tsumoto K.

To investigate favorable single amino acid substitutions that improve antigen-antibody interactions, alanine (Ala) mutagenesis scanning of the interfacial residues of a cancer-targeted antibody, B5209B, was performed based on X-ray crystallography analysis. Two substitutions were shown to significantly enhance the binding affinity for the antigen, by up to 30-fold. One substitution improved the affinity by a gain of binding enthalpy, whereas the other substitution improved the affinity by a gain of binding entropy. Molecular dynamics simulations showed that the enthalpic improvement could be attributed to the stabilization of distant salt bridges located at the periphery of the antigen-antibody interface. The entropic improvement was due to the release of water molecules that were stably trapped in the antigen-antibody interface of the wild-type antibody. Importantly, these effects of the Ala substitutions were caused by subtle adjustments of the binding interface. These results will be helpful to design high-affinity antibodies with avoiding entropy-enthalpy compensation.

9. Phospholipid Membrane Fluidity Alters Ligand Binding Activity of a G Protein-Coupled Receptor by Shifting the Conformational Equilibrium.

Yoshida K, Nagatoishi S, Kuroda D, Suzuki N, Murata T, Tsumoto K.

The affinity of a ligand for a receptor on the cell surface will be influenced by the membrane composition. Herein, we evaluated the effects of differences in membrane fluidity, controlled by phospholipid composition, on the ligand binding activity of the G protein-coupled receptor human serotonin 2B. Using Nanodisc technology to control membrane properties, we performed biophysical analysis and employed molecular dynamics simulations to demonstrate that increased membrane fluidity shifted the equilibrium toward an active form of the receptor. Our quantitative study will enable development of more realistic in vitro drug discovery assays involving membrane-bound proteins such as G protein-coupled receptors.

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長門石曉，中木戸誠，津本浩平
材料創製を指向したタンパク質相互作用解析
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津本浩平，長門石曉
第19章抗体医薬の基礎物性評価
医薬品原薬の結晶化と物性評価：その最先端技術と評価の実際（シーエムシー出版）(2019)

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 production of recombinant viral vectors, genetic modification and/or ex vivo manipulation of patient-derived tissue 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 and documents that cover all the elements of laboratory works, including both tangible and intangible factors including equipment, facility design personnel, etc.

ISO certification

The management system of TVDC has been regularly assessed by an independent organization to meet the requirement for ISO9001 certification.

Validation of TVDC

The TVDC consists of two units; 1) Vector Unit, the primary suite for viral vector production and *ex vivo* transduction; and 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 at a Class 10,000 clean level. The facility and equipment are regularly validated 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

Associate Professor　Tokiko Nagamura-Inoue, M.D., PhD.　准教授　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).

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 supply frozen cord blood and UC-MSCs for research purposes based on joint research and material transfer agreements to for-profit companies and researchers in non-profit academic facilities in Japan and internationally.

For clinical use, we collect, process, culture, and cryopreserve the UC-MSCs with serum-free process for banking.

We began to release IMSUT-CORD for an investigator-initiated clinical trial begun in 2018 on the administration of umbilical cord-derived MSCs for treatment-resistant severe acute graft-versus-host disease (supported by Japan Agency for Medical Research and Development (AMED).

We are now preparing the IMSUT-CORD for the treatment of neonatal encephalopathy as the next clinical trial.

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, Tojo A

The objectives of establishment of stable supply system for perinatal appendage as the resource of cell therapies are two-fold. First is to establish a stable system for supplying umbilical cord blood and umbilical cords or umbilical cord-derived MSCs in the public interest from collection facilities (obstetrics and gynecology clinics) via IMSUT CORD serving for for-profit companies that conduct regenerative medicine, immunotherapies, and other forms of cell therapy. Second is to study issues regarding the social acceptability of MSCs, including ethical considerations and placenta disposal laws issues, in supplying umbilical cord-derived MSCs. This project is also supported by AMED.

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

IMSUT Hospital

Department of Nursing
看護部

Director	Eiko Yoshii, RN, CNA.
Deputy Director	Minayo Hisahara, RN.
Deputy Director	Fumiko Kasuya, RN, CNA.
Nurse Manager	Mayumi Tanii, RN, MSN, CNA.
Nurse Manager	Hatsuko Narita, RN.
Nurse Manager	Mika Kogayu, RN. MSN.
Nurse Manager	Tomoko Sato, RN.
Nurse Manager	Masako Ozawa, RN.
Nurse Manager	Hiromi Isshiki, RN.
Nurse Manager	Nozomi Linzbichler, RN.
Nurse Manager	Yukari Turu, RN.

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副看護部長		久原みな代
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看護師長		一色裕美
看護師長		リンツビヒラ希
看護師長		都留由香里

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.

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 their high quality of life.

In 2011, we introduced the Career Ladder System to support active learning and development of nurses, it keeps nurses motivated to continue learning and fulfill their career development as a nurse. Nursing skills based on good knowledge and evidence is also very important in patient care. The online training tool "Nursing Skills Japan" was also launched in 2011 to enhance nurses' learning and to brush up their skills.

In 2012, we promote that nurses can get nursing specialty training and the certification of their field. And we empowered them for role expansion of nurses. Furthermore, we are actively engaged in a discharge nursing and ethical conference.

In 2013, we introduced the Pair System as nursing delivery system to improve the quality of nursing, the effect of OJT (on the job training), and the efficiency of nursing service.

In 2014, we organized some working groups to develop clinical nurse leaders for quality assurance, cancer nursing, clinical research/ translational research nursing.

From 2015, we accelerate utilizing competency model for developing nurse manager. Nurse Managers cooperate with the competency training courses held at various places in Japan many times as facilitator.

Since 2018, we have been working with the Department of Nursing at the University of Tokyo Hospital to review the Career Ladder System and conduct

tours and training in other departments to train nurses and improve knowledge and skills in medical care and nursing.

Publication

Yamagishi Y, Konuma T, Miwa Y, Oiwa-Monna M, Tanoue S, Isobe M, Jimbo K, Mizusawa M, Narita H, Kobayashi K, Kato S Takahashi S, Tojo A. Risk factors and survival impact of readmission after single-unit cord blood transplantation for adults. International Journal of Hematology. 109(1) 115-124. 2019.

Miyashita E, Konuma T, Kataoka J, Oiwa-Monna M, Mizusawa M, Isobe M, Kato S, Sato T, Takahashi S, Tojo A. The Prognostic Impact of Pretransplantation Inflammatory and Nutritional Status in Adult Patients after Myeloablative Single Cord Blood Transplantation. Biology of Blood and Marrow Transplantation. https://doi.org/10.1016/j.bbmt.2019.01.006. [Available online 11 January 2019.]

Conference Presentation

小野谷厚子，小粥美香，亀田史絵，白井みゆき，新井保美，鯉渕智彦．患者環境の感染防御を目指し環境整備にバンドル表を用いた取り組み．第34回日本環境感染学会総会・学術集会．神戸．2019. 2. 22–23.

熊澤芽葉恵．外国人患者へ外国語表記シートを用いて衛生指導・院内感染予防を実践した看護師のケアに対する負担感の変化．第34回日本環境感染学会総会・学術集会．神戸．2019. 2. 22–23.

都留由香里，小林康司，小粥美香．A病院のがん看護に携わる看護師におけるIASM症状マネジメントツールを使用した事例検討の教育効果．第33回日本がん看護学会学術集会．福岡．2019. 2. 23–24.

宮下英太，小沼貴晶，片岡惇，大岩真希，水澤舞，磯部優理，加藤せい子，佐藤朋子，高橋聡，東條有伸．移植前栄養状態が成人臍帯血移植成績に与える影響．第41回日本造血細胞移植学会総会．大阪．2019. 3. 7–9.

中野和志，小粥美香，田村友紀，佐藤円，小林路代，小林康司．病棟看護師の血友病に関する知識向上に向けた取り組み．第41回日本血栓止血学会学術集会．愛知．2019. 6. 20–22.

小粥美香，谷井真弓，小澤昌子，小林康司，野地有子．成人血友病患者が血友病を自己管理するための支援ツールの開発・評価と支援する看護師の育成―ライフイベントを視野に入れて―．第23回日本看護管理学会学術集会．新潟．2019. 8. 23–24.

Takasaka K, Tanii M, Umemoto S, Sato M, Yuuki M, Fujiwar N. Clinical Research Nursing: The Standard of Practice for Clinical Nurses in Japanese Research Hospital. IACRN 11th Annual Conference. Hilton Philadelphia - City Avenue, Philadelphia, PA USA.2019.10.21-23

IMSUT Hospital

Department of Pharmacy
薬剤部

Director	Seiichiro Kuroda
Pharmacist	Yohei Iimura
Pharmacist	Mika Yamamura
Pharmacist	Mai Yokota

薬剤部長	黒田誠一郎
薬剤師	飯村洋平
薬剤師	山村実佳
薬剤師	横田舞

The Department of Pharmacy seeks to provide high-quality pharmaceutical care services.We contribute to the team approach to patient-oriented medical care and provides a drug distribution services. We are also trying to contribute to propel the right use of medicines for patients.

1) Prevalence of drug-drug interaction in atrial fibrillation patients based on a large claims data.

Momo K, Kobayashi H, Sugiura Y, Yasu T, Koinuma M, Kuroda SI.

PLoS One. 2019 Dec 9; 14(12): e0225297. doi: 10.1371/journal.pone.0225297. eCollection 2019. PMID: 31815956

2) Determination of factors affecting medication adherence in type 2 diabetes mellitus patients using a nationwide claim-based database in Japan.

Horii T, Momo K, Yasu T, Kabeya Y, Atsuda K.

PLoS One. 2019 Oct 8; 14(10): e0223431. doi: 10.1371/journal.pone.0223431. eCollection 2019. PMID: 31593574

3) Thiamine deficiency as a possible cofactor causing cognitive dysfunction in a patient with end-stage gastric cancer .

Iimura Y, Yasu T, Momo K, Kuroda S, Kanemoto Y, Yazawa K, Tsurita G.

Int J Clin Pharmacol Ther. 2019 Aug; 57(8): 416-419. doi: 10.5414/CP203384. PMID: 31232276

4) AKI in patients with hematological malignancies treated with various vancomycin-antibiotic combinations.

Kobayashi S, Yasu T, Momo K.

Clin Nephrol. 2019 Aug; 92(2): 108-110. doi: 10.5414/CN109790. Noabstract available. PMID: 31198173

5) Polypharmacy in >75-year-Old Patients on Admission to Tokyo-Area Hospitals.

Momo K, Yasu T, Hayashi D, Saitoh M, Ito Y, Yashiro Y, Nagumo J, SekiR, Furuya J, Okuno Y, Horii T,

Abe K, Shirota M.

Am J Ther. 2019 Nov/Dec; 26(6): e736-e738. doi: 10.1097/MJT.0000000000000889. No abstract available. PMID: 31135386

6) Effects of Formulation Changes for Deferasirox From Dispersible Tablets to Granules in Patients With Red Blood Cell Transfusion-Induced Iron Overload.

Higashino S, Yasu T, Momo K, Kuroda S.

Am J Ther. 2019 Nov/Dec; 26(6): e728-e730. doi: 10.1097/MJT.0000000000000882. No abstract available. PMID: 31135385

7) Assessment of "look-alike" packaging designs related to medication errors using information technology.

Momo K, Shimano M, Kanezaki Y, Minagawa A, Takagi A, Seino T, Koinuma M.

Pharmazie. 2019 May 1; 74(5): 310-312. doi: 10.1691/ph.2019.8924.PMID: 31109403

8) Risk factors affecting the failed low-density lipoprotein level achievement rate in working-age male population at high cardiovascularrisk.

Momo K, Yasu T, Yasui H, Kuroda SI.

J Clin Pharm Ther. 2019 Oct; 44(5): 715-719. doi: 10.1111/jcpt.12847.Epub 2019 May 6.PMID: 31062402

9) A case of under-dosing after raltegravir formulation change in an elderly patient treated for HIV.

Kobayashi S, Momo K, Yasu T, Higashino S, Kuroda S.

Pharmazie. 2019 Jan 1; 74(1): 62-63. doi: 10.1691/ph.2019.8788.PMID: 30782252

10) Simple determination of plasma ibrutinib concentration using high-performance liquid chromatography.

Yasu T, Momo K, Yasui H, Kuroda S.

Biomed Chromatogr. 2019 Mar; 33(3): e4435. doi: 10.1002/bmc.4435. Epub2018 Dec 11.PMID: 30421802

11) Thiamine deficiency as a possible cofactor causing cognitive dysfunction in a patient with end-stage gastric cancer .

Iimura Y, Yasu T, Momo K, Kuroda S, Kanemoto Y, Yazawa K, Tsurita G.

Int J Clin Pharmacol Ther. 2019 Aug; 57(8): 416-419. doi: 10.5414/CP203384. PMID: 31232276

12) Potential thiamine deficiency and neurological symptoms in patients receiving chemotherapy for gastrointestinal cancer.

Iimura Y, Kurokawa T, Nojima M, Kanemoto Y, Yazawa K, Tsurita G, Kuroda S.

Int J Clin Pharmacol Ther. 2019 Oct 28. doi: 10.5414/CP203602. [Epub ahead of print] PMID: 31657715

IMSUT Hospital

Department of AIDS Vaccine Development
エイズワクチン開発担当

Invited Professor	Tetsuro Matano, M.D., D.M.Sc.	教授（委嘱）	博士（医学） 俣 野 哲 朗
Visiting Associate Professor	Ai Kawana-Tachikawa, D.M.Sc.	客員准教授	博士（医学） 立川（川名） 愛

We are working on Microbiology and Immunology to elucidate the immune mechanism for retroviral control in vivo. We have been 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. We are developing vaccines using Sendai virus vectors eliciting antibody and/or cytotoxic T lymphocyte responses toward global control of HIV and HTLV infection.

1. CD8$^+$ T cell-based strong selective pressure on multiple SIV targets in macaques possessing a protective MHC class I haplotype.

Trang Thi Thu Hau[1], Midori Nakamura-Hoshi, Yoshiaki Kanno, Takushi Nomura[2], Masako Nishizawa[2], Sayuri Seki[2], Hiroshi Ishii[2], Ai Kawana-Tachikawa, William W. Hall[3], Lan Anh Nguyen Thi[4], Tetsuro Matano, Hiroyuki Yamamoto[2]: [1]Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan; [2]AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; [3]Centre for Research in Infectious Diseases, School of Medicine & Medical Science, University College Dublin, Dublin, Ireland; [4]Center of BioMedical Research, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

In HIV and SIV infections, MHC-I genotypes have a great impact on viral replication and MHC-I-associated viral genome mutations are selected under CD8$^+$ T-cell pressure. Association of MHC-I genotypes with HIV/SIV control has been investigated at MHC-I allele levels but not fully at haplotype levels. We previously established groups of rhesus macaques sharing individual MHC-I haplotypes. In this study, we compared viral genome diversification after SIV infection in macaques possessing a protective MHC-I haplotype, *90-010-Id*, with those possessing a non-protective MHC-I haplotype, *90-010-Ie*. These two MHC-I haplotypes are associated with immunodominant CD8$^+$ T-cell responses targeting similar regions of viral Nef antigen. Analyses of viral genome sequences and antigen-specific T-cell responses showed several candidates of viral CD8$^+$ T-cell targets associated with *90-010-Id* and *90-010-Ie*, respectively, in addition to the Nef targets. In these CD8$^+$ T-cell target regions, higher numbers of mutations were detected at the set-point after SIV infection in macaques possessing *90-010-Id* than those possessing *90-010-Ie*. These results indicate higher selective pressure on overall CD8$^+$ T-cell targets associated with the protective MHC-I haplotype, suggesting a pattern of HIV/SIV control by multiple target-specific CD8$^+$ T-cell responses.

2. HIV-1 matrix mutations that alter Gag membrane binding modulate mature core formation and post-entry events.

Yuta Hikichi, Eri Takeda[5], Masayuki Fujino[2], Emi Nakayama[5], Tetsuro Matano, Tsutomu Murakami[2]: [5]Department of Viral Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

The matrix (MA) domain of HIV-1 Gag directs

membrane binding of the Gag precursor polyprotein during the late events of virus replication. However, the effects of alteration in Gag membrane binding early post-infection are not well understood. To investigate impacts of MA mutations that alter Gag membrane binding on the phenotypes of newly produced virus particles, we extensively characterized two MA mutants by virological, biochemical, and morphological approaches. Our data suggest that HIV-1 MA plays roles in functional core formation and the following post-entry steps of the virus replication cycle.

3. HLA-associated HIV-1 CRF02_AG *gag* and *vif* polymorphisms in Ghana.

Mildred A. Adusei-Poku[1], Saori Matsuoka[2], Evelyn Y. Bonney[6], Christopher Z. Abana[6], Ewurabena O. Duker[6], Nicholas I. Nii-Trebi[7], Sampson B. Ofori[8], Taketoshi Mizutani, Aya Ishizaka, Teiichiro Shiino[2], Ai Kawana-Tachikawa, Koichi Ishikawa[2], William K. Ampofo[6], Tetsuro Matano: [6]Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Accra, Ghana; [7]School of Biomedical and Allied Health, College of Health Sciences, University of Ghana, Accra, Ghana; [8]Koforidua Regional Hospital, Koforidua, Ghana

In HIV-1 infections, cytotoxic T-lymphocyte (CTL) responses targeting HLA-restricted viral epitopes exert strong suppressive pressure on viral replication and frequently select for mutations resulting in viral escape from CTL recognition. Numerous data on these HLA-associated mutations in HIV-1 subtypes B and C have been amassed with few reports described in other subtypes. In this study, we investigated HLA-associated mutations in HIV-1 subtype CRF02_AG prevailing in Ghana, Western Africa. We determined viral *gag* sequences in 246 out of 324 HIV-1-infected Ghanaians. Phylogeny analysis revealed that 200 were infected with HIV-1 CRF02_AG. Full *gag* and *vif* sequences were obtained from 199 and 138, respectively, out of the 200 and subjected to determination of HLA-associated mutations. The analysis found HLA-associated HIV-1 CRF02_AG nonsynonymous polymorphisms at nineteen sites, thirteen in *gag* and six in *vif*, including those newly determined. Generation of this data is an important contribution to our understanding of HIV-1 CRF02_AG and host T cell interaction.

4. Estimating HIV-1 incidence in Japan from the proportion of recent infections.

Saori Matsuoka[2], Mami Nagashima[9], Kenji Sadamasu[9], Haruyo Mori[10], Takuya Kawahata[10], Shuichi Zaitsu[11], Asako Nakamura[12], Mark S. de Souza[2], Tetsuro Matano: [9]Division of Microbiology, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan; [10]Division of Microbiology, Osaka Institute of Public Health, Osaka, Japan; [11]Health Science Section, Fukuoka City Institute of Health and Environment, Fukuoka, Japan; [12]Division of Virology, Fukuoka Institute of Health and Environmental Sciences, Fukuoka, Japan

The first step of the UNAIDS/WHO 90-90-90 targets to encourage early diagnosis with treatment for the control of HIV-1 epidemic is to achieve 90% HIV-1 diagnosis in infected individuals. In Japan, approximately 30% of newly reported cases have been annually identified by AIDS onset, implying that substantial numbers of HIV-1-infected individuals remain undiagnosed. However, the proportion of undiagnosed cases has not yet been determined. In this study, the proportion of recent HIV-1 infections to newly-diagnosed cases was determined from 2006 to 2015 using a recent infection assay for three metropolitan areas in Japan: Tokyo, Osaka, and Fukuoka. Estimated median periods between infection and diagnosis were 1.0, 1.8, and 2.9 years for Tokyo, Osaka, and Fukuoka, respectively. Estimation of annual HIV-1 incidence by a back-calculation method using these data as well as HIV/AIDS national surveillance data indicated proportions of undiagnosed to new HIV-1 infections from 2006 to 2015 to be 18% in Tokyo, 22 % in Osaka, 38% in Fukuoka, and 28% in Japan. This is the first report estimating HIV-1 incidence in Japan using a serological biomarker in combination with national HIV/AIDS surveillance data.

5. Determination of a T cell receptor of potent $CD8^+$ T cells against SIV infection in Burmese rhesus macaques.

Hiroshi Ishii[2], Saori Matsuoka[2], Noriko Ikeda, Kyoko Kurihara, Takamasa Ueno[1], Masafumi Takiguchi[1], Taeko K. Naruse[13], Akinori Kimura[13], Masaru Yokoyama[14], Hironori Sato[14], Tetsuro Matano: [13]Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [14]Pathogen Genomics Center, National Institute of Infectious Diseases, Tokyo, Japan

Cumulative studies have suggested that HIV replication can be controlled by potent $CD8^+$ T-cell responses. We have previously established an AIDS model of SIV infection in Burmese rhesus macaques and found a potent $CD8^+$ T cell targeting the Mamu-A1*065: 01 restricted $Gag_{241-249}$ epitope, which is located in a region corresponding to the HIV $Gag_{240-249}$ TW10 epitope restricted by a protective MHC-I allele, HLA-B*57. In this study, we determined a T cell receptor (TCR) of this $Gag_{241-249}$ epitope specific $CD8^+$ T cell. cDNA clones encoding TCR-α and TCR-β chains were obtained from a $Gag_{241-249}$ specific $CD8^+$ T-cell clone. Coexpression of these TCR-α and TCR-β cDNAs resulted in reconstitution of a functional TCR specifically detected by $Gag_{241-249}$ epitope-

Mamu-A1*065: 01 tetramer. Two of three previously-reported $CD8^+$ T-cell escape mutations reduced binding affinity of $Gag_{241-249}$ peptide to Mamu-A1*065: 01 but the remaining one not. This is consistent with the data obtained by molecular modeling of the epitope-MHC-I complex and TCR. These results would contribute to understanding how viral $CD8^+$ T-cell escape mutations are selected under structural constraint of viral proteins.

Publications

1. Takahashi, N., Matsuoka, S., Minh, T.T.T., Ba, H.P., Naruse, T.K., Kimura, A., Shiino, T., Kawana-Tachikawa, A., Ishikawa, K., Matano, T. and Thi, L.A.N. Human leukocyte antigen-associated gag and nef polymorphisms in HIV-1 subtype A/E-infected individuals in Vietnam. Microbes Infect. 21: 113-118, 2019.
2. Tsukamoto, T., Yamamoto, H. and Matano, T. $CD8^+$ cytotoxic T lymphocyte breadth could facilitate early immune detection of immunodeficiency virus-derived epitopes with limited expression levels. mSphere 4: e00381-18, 2019.
3. Hau, T.T.T., Nakamura-Hoshi, M., Kanno, Y., Nomura, T., Nishizawa, M., Seki, S., Ishii, H., Kawana-Tachikawa, A., Hall, W.W., Nguyen, L.A.T., Matano, T. and Yamamoto, H. $CD8^+$ T cell-based strong selective pressure on multiple simian immunodeficiency virus targets in macaques possessing a protective MHC class I haplotype. Biochem. Biophys. Res. Commun. 512: 213-217, 2019.
4. Hikichi, Y., Takeda, E., Fujino, M., Nakayama, E., Matano, T. and Murakami, T. HIV-1 matrix mutations that alter Gag membrane binding modulate mature core formation and post-entry events. Virology 532: 97-107, 2019.
5. Adusei-Poku, M.A., Matsuoka, S., Bonney, E.Y., Abana, C.Z., Duker, E.O., Nii-Trebi, N.I., Ofori, S.B., Mizutani, T., Ishizaka, A., Shiino, T., Kawana-Tachikawa, A., Ishikawa, K., Ampofo, W.K. and Matano, T. Human leukocyte antigen-associated HIV-1 CRF02_AG *gag* and *vif* polymorphisms in Ghana. Jpn. J. Infect. Dis. 72: 374-380, 2019.
6. Matsuoka, S., Nagashima, M., Sadamasu, K., Mori, H., Kawahata, T., Zaitsu, S., Nakamura, A., de Souza, M.S. and Matano, T. Estimating HIV-1 incidence in Japan from the proportion of recent infections. Prev. Med. Rep. 16: 100994, 2019.
7. Ishii, H., Matsuoka, S., Ikeda, N., Kurihara, K., Ueno, T., Takiguchi, M., Naruse, T.K., Kimura, A., Yokoyama, M., Sato, H. and Matano, T. Determination of a T cell receptor of potent $CD8^+$ T cells against simian immunodeficiency virus infection in Burmese rhesus macaques. Biochem. Biophys. Res. Commun., in press.
8. 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., in press.
9. Iwamoto, M., Saso, W., Nishioka, K., Ohashi, H., Sugiyama, R., Ryo, A., Ohki, M., Yun, J.-H., Park, S.-Y., Ohshima, T., Suzuki, R., Aizaki, H., Muramatsu, M., Matano, T., Iwami, S., Sureau, C., Wakita, T. and Watashi, K. The machinery for endocytosis of epidermal growth factor receptor coordinates the transport of incoming hepatitis B virus to the endosomal network. J. Biol. Chem., in press.

IMSUT Distinguished Professor Unit

Division of Stem Cell Therapy
幹細胞治療部門

Project Professor	Hiromitsu Nakauchi, M.D., Ph.D.	特任教授	医学博士	中内啓光
Project Associate Professor	Tomoyuki Yamaguchi, Ph.D.	特任准教授	博士(医学)	山口智之
Project Associate Professor	Eiji Mizutani, Ph.D.	特任准教授	博士(農学)	水谷英二
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. Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation.

Adam C. Wilkinson,[1] Reiko Ishida,[2] Misako Kikuchi,[2] Kazuhiro Sudo,[3] Maiko Morita,[2] Ralph Valentine Crisostomo,[1] Ryo Yamamoto,[1] Kyle M. Loh,[1] Yukio Nakamura,[3] Motoo Watanabe,[2] Hiromitsu Nakauchi,[1,2] Satoshi Yamazaki[2]

[1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine

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Multipotent self-renewing haematopoietic stem cells (HSCs) regenerate the adult blood system after transplantation[1], which is a curative therapy for numerous diseases including immunodeficiencies and leukaemias. Although substantial effort has been applied to identifying HSC maintenance factors through the characterization of the in vivo bone-marrow HSC microenvironment or niche, stable ex vivo HSC expansion has previously been unattainable. Here we describe the development of a defined, albumin-free culture system that supports the long-term ex vivo expansion of functional mouse HSCs. We used a systematic optimization approach, and found that high levels of thrombopoietin synergize with low levels of stem-cell factor and fibronectin to sustain HSC self-renewal. Serum albumin has long been recognized as a major source of biological contaminants in HSC cultures; we identify polyvinyl alcohol as a functionally superior replacement for serum albumin that is compatible with good manufacturing practice. These conditions afford between 236- and 899-fold expansions of functional HSCs over 1 month, although analysis of clonally derived cultures suggests that there is considerable heterogeneity in the self-renewal capacity of HSCs ex vivo. Using this system, HSC cultures that are derived from only 50 cells robustly engraft in recipient mice without the normal requirement for toxic pre-conditioning (for example, radiation), which may be relevant for HSC transplantation in humans. These findings therefore have important implications for both basic HSC research and clinical haematology.

2. Generation of pluripotent stem cell-derived mouse kidney in Sall1-targeted anephric rats

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Regeneration of human kidneys in animal models would help combat the severe shortage of donors in transplantation therapy. Previously, we demonstrated by interspecific blastocyst complementation between mouse and rats, generation of pluripotent stem cell (PSC)-derived functional pancreas, in apancreatic *Pdx1* mutant mice. We, however, were unable to obtain rat PSC-derived kidneys in anephric *Sall1* mutant mice, likely due to the poor contribution of rat PSCs to the mouse metanephric mesenchyme, a nephron progenitor. Here, conversely, we show that mouse PSCs can efficiently differentiate into the metanephric mesenchyme in rat, allowing the generation of mouse PSC-derived kidney in anephric *Sall1* mutant rat. Glomerular epithelium and renal tubules in the kidneys are entirely composed of mouse PSC-derived cells expressing key functional markers. Importantly, the ureter-bladder junction is normally formed. These data provide proof-of-principle for interspecific blastocyst complementation as a viable approach for kidney generation.

3. CRISPR/Cas9 + AAV-mediated Intra-embryonic Gene Knocking in Mice

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Intra-embryo genome editing by CRISPR/Cas9 has enabled rapid generation of gene knockout animals. However, large fragment knock-in directly into embryos' genome is still difficult, especially without microinjection of donor DNA. Viral vectors are good transporters of knock-in donor DNA for cell lines, but seemed unsuitable for pre-implantation embryos with zona pellucida, glycoprotein membrane surrounding early embryos. We found adeno-associated virus (AAV) can infect zygotes of various mammals through intact zona pellucida. AAV-mediated donor DNA delivery following Cas9 ribonucleoprotein electroporation enables large fragment knock-in without micromanipulation.

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The mucosal immune system not only senses pathogenic antigens such as pathogens and allergens, but also establishes tolerance that does not react excessively to beneficial antigens such as food-derived proteins and commensal microorganisms. Our laboratory's mission is to elucidate and understand the uniqueness of the mucosal immune system which controls the immunological balancing act between the elimination of and commensalism with harmful and beneficial antigens, respectively, and aim to develop the basic platform for creating the novel strategies of prevention and treatment of various infectious and immunological diseases by the fusion science with agriculture, engineering and plant biology.

1. Functional analysis of desensitized intestinal mast cells in the treatment of food allergy

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Oral immunotherapy (OIT) is shown to be an effective approach to controlling food allergy; however, the detailed molecular and cellular mechanisms of OIT treatment are still unknown and understanding of the mechanisms are required for the future fundamental treatment of allergic diseases. To elucidate the mechanism of OIT especially in the phase of immunological transition from desensitization to allergy regulation, we generated a clinical OIT murine model. Mice showing gastrointestinal allergic symptoms received OIT, and desensitized intestinal MCs were generated and analyzed. Mice in which these desensitized MCs were depleted during OIT showed reduction of regulatory type cell populations. Collectively, we found the desensitization process controls MC activation and this process required for the fundamental treatment of allergic diseases.

2. Critical role of the CCR5–CCL5 interaction for preferential migration of HSV-2-specific effector cells to the vaginal mucosa upon nasal immunization.

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The mucosal immune system is a compartmentalized and interconnected system and studies of different mucosal immunization routes have shown that Ag-specific immune response is strongest at the mucosa exposed directly to the vaccine, followed by adjacent mucosal tissues. Notably, nasal immunization is exceptional in that it elicits substantial Ag-specific mucosal immune responses in the genital tract as well as the respiratory tract. This phenomenon is of special interest for possible vaccination strategy against sexually transmitted diseases (STDs), for which a nasal vaccine has not yet been approved. Therefore, studies about underlying mechanisms by which nasal vaccination induces genital immune responses are needed to provide immunological insights into successful nasal vaccine design for STDs, including herpes simplex virus 2 (HSV-2) infection. In this study, we used a murine HSV-2 TK– nasal vaccination model to investigate the involvement of interaction between chemokines and chemokine receptors in the homing of nasally primed Ag-specific effector cells to the vaginal mucosa. A nasal immunization with live attenuated HSV-2 TK– induced the upregulation of CCR5 expression in effector immune cells, including CD4 + T cells, in Ag-priming sites and vaginal tissue. The CCR5 ligands CCL3, CCL4, and CCL5 all showed upregulated expression in vaginal tissue; in particular, CCL5 expression was highly enhanced in the stromal cells of vaginal tissue after nasal immunization. Intra-vaginal blockade of CCL5 by using neutralizing antibody diminished the number of HSV-2-specific effector cells in the vagina. Furthermore, loss of CCR5, a receptor of CCL5, impaired the migration of nasally primed Ag-specific effector cells from the airway to vagina. Effector cells adoptively transferred from CCR5-deficient mice failed to migrate into vaginal tissue, consequently increasing recipient mice's susceptibility to HSV-2 vaginal infection. These results indicate that the CCR5–CCL5 chemokine pathway is required for the migration and retention of nasally primed Ag-specific effector cells in vagina for providing protective immunity against HSV-2 infection.

3. 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.

Intra-tissue opportunistic bacteria, *Alcaligenes*, induces IgA production without excessive inflammatory responses and has morphological plasticity.

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The intestinal tract is continuously exposed to a wide variety of antigens, so it develops mucosal immunity, for the elimination of pathogenic bacteria and tolerance to dietary antigens and indigenous bacteria. On the other hand, intestinal commensal bacteria affect the development and function of the host immune system, including the production of secretory IgA (SIgA) and the development of intraepithelial T lymphocytes. *Alcaligenes* are opportunistic commensal bacteria that reside in gut-associated lymphoid tissues such as Peyer's patches; however, how they create and maintain their homeostatic environment, without inducing an excessive inflammatory response remained unclear. We found that *Alcaligenes*-derived lipopolysaccharide (*Alcaligenes* LPS) acts as a weak agonist of toll-like receptor 4 and promotes IL-6 production from dendritic cells, which consequently enhances IgA production. The inflammatory activity of *Alcaligenes* LPS was weaker than that of E. coli-derived LPS and therefore no excessive inflammation was induced by *Alcaligenes* LPS in vitro or in vivo. As an application of *Alcaligenes* LPS, we found that *Alcaligenes* LPS has an adjuvanticity. *Alcaligenes* LPS induced antigen-specific humoral immune responses as well as Th1, Th2, and Th17 cells without excessive inflammation. These findings reveal the presence of commensal bacteria mediated homeostatic inflammatory conditions within Peyer's

patches that produce optimal IgA induction without causing pathogenic inflammation. In addition, we also found that *Alcaligenes* changed the morphology from rod- to filament-shape, which was determined by bacterial density and was in quorum-sensing-dependent manner. We will clarify the functional changes of *Alcaligenes* associated with morphological changes.

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再生基礎医科学国際研究拠点寄付研究部門

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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 sytem 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 2019 are as follows.

Foxr2 promotes formation of CNS-embryonal tumors in a Trp53-deficient background.

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Embryonal tumors in the central nervous system (CNS) are primary, aggressive, and poorly differentiated pediatric brain tumors. We identified forkhead box R2 (*Foxr2*) as an oncogene for medulloblastoma through a transposon-based insertional mutagenesis screen. *Foxr2* translocation has been identified in a subset of human embryonal tumors of the CNS, designated as CNS neuroblastoma with Foxr2 activation (CNS NB-*Foxr2*); however, the in vivo functions of Foxr2 remain elusive. We analyzed the effect of Foxr2 overexpression in the mouse brain by generating a transgenic strain that expresses Foxr2 in the entire brain under a transformation related protein 53

(Trp53)–deficient background. We performed histological analysis of tumors and characterized tumor-derived sphere-forming cells. We investigated gene expression profiles of tumor-derived cells. Foxr2 and Trp53 loss promoted tumor formation in the olfactory bulb (OB) and brainstem (BS). The tumors showed the common morphological features of small round blue cell tumors, exhibiting divergent, mainly neuronal and glial, patterns of differentiation, which corresponds to the definition of CNS-embryonal tumors. Importantly, all mice developed CNS-embryonal tumors. In the OB, early proliferative lesions consisting of oligodendrocyte transcription factor 2 (Olig2 +) cells were observed, indicating that Foxr2 expression expanded Olig2 + cells in the OB. Tumor-derived cells formed spheres in vitro and induced tumors that recapitulated the parental tumor upon transplantation, indicating the presence of tumor-initiating cells. Gene expression profiling revealed that OB and BS tumor cells were enriched for the expression of the genes specific to CNS NB-*Foxr2*. Our data demonstrate that Foxr2 plays a causative role in the formation of CNS-embryonal tumors.

Sall1 plays pivotal roles for lens fiber cell differentiation in mouse.

Yukihiro Baba, Yui Watabe, Hiroshi Sagara4, Sumiko Watanabe: 4 Medical proteomics laboratory, IMSUT

Mammals possess four Sall transcription factors that play various roles in organogenesis. Previously, we found that Sall1 is expressed in microglia in the central nervous system, and it plays pivotal roles in microglia maturation. In the eye, Sall1 was also expressed in the developing lens, and we examined its role in lens development. A knock-in mouse harboring the EGFP gene in the Sall1 locus (Sall1-gfp) was used to analyze the Sall1 expression pattern. In Sall1-gfp/wild, EGFP was expressed throughout the presumptive lens at E11.5, and subsequently the expression in the lens epithelium became weaker. After birth, signals were observed in the equator region. The effects of Sall1 knockout on lens development were examined in Sall1-gfp/gfp. Lens sections revealed small vacuole-like holes and gaps in the center of the lens fibers at E14.5. Subsequently, the vacuoles appeared in most regions of the fiber cells. Electron microscopic analysis indicated that the vacuoles were between the fiber cells, leading to huge gaps. In addition, contact between the lens epithelium and apical end of the fiber cell was disrupted, and there were gaps between the adjoining lens epithelial cells. However, gap junction structure was observed by electron microscopic analysis, and immunostaining of Zo1 showed rather appropriate expression pattern. Immunohistochemistry indicated that the major lens transcription factors Prox1 and Pax6 were expressed in relatively normal patterns. However, although the expression of Prox1 and Pax6 decreased in nuclei in the control lens, it remained in Sall1-gfp/gfp. In addition, lower expression level of c-Maf protein was observed. Therefore, Sall1 is strongly expressed in the lens from the early developmental stage and plays an essential role in the maintenance of fiber cell and lens epithelium adhesion.

Cd9 protects photoreceptors from injury and potentiates Edn2 expression

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Cd9 is a tetraspanin membrane protein that plays various roles in tissue development and disease pathogenesis, especially in cancer, but the expression patterns and function of Cd9 in retinal development and disease are not well understood. We asked its roles during retinal photoreceptor degeneration by using CD9-knockout mice. Cd9 knockout mice and rd1 mice were used to examine roles of Cd9 for progression of photoreceptor degeneration. RT-qPCR and immunohistochemistry were mainly used as analytical methods. Cd9 transcripts were only weakly expressed in retina at E14, but its expression level subsequently increased and peaked at around P12. In 6-week female mice derived retina, mRNA expression decreased slightly but was maintained at a significant level. RNA-sequencing and immunohistochemistry indicated that Cd9 was expressed abundantly in Müller glia and weakly in other retinal neurons. Notably, when photoreceptors were damaged, Cd9 expression was increased in rod photoreceptors and decreased in Müller glia. Cd9 knockout mice retinas developed normally; however, once the retina suffered damage, degeneration of photoreceptors was more severe in Cd9 knockout retinas than control retinas. Induction of *Edn2*, which is known to protect against photoreceptor damage, was severely hampered. In addition, induction of *Socs3*, which is downstream of gp130 (Il6st), was weaker in Cd9 knockout retinas. Taken together, these findings indicate that, although Cd9 was dispensable for normal gross morphological development, it protected rod photoreceptors by enhancing Edn2 expression, at least partially through modulation of gp130 signaling.

Apigenin regulates activation of microglia and counteracts retinal degeneration

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Photoreceptor degeneration is a major cause of blindness. Microglia are known to play key roles in the pathogenesis and progression of neural degeneration. We examined the possible use of apigenin, which is a naturally-occurring flavonoid, for the treatment of photoreceptor degeneration via regulation of microglial activities. As in vitro analyses, BV2 and MG5 mouse microglia cell lines were stimulated in the presence or absence of apigenin, and their activation profile was examined. In vivo study was done using rd1 photoreceptor degeneration model, and apigenin was administrated by intravitreal injection, and pathological feature was examined.

Cell survival was not affected by apigenin in either BV2 and MG5. Apigenin suppressed lipopolysaccharide (LPS)-induced chemokine production in both BV2 and MG5 cells, but phagocytosis was suppressed in MG5 cells but not in BV2 cells. Apigenin inhibited LPS-induced M1 activation but could not drive microglia toward the M2 phenotype. Apigenin suppressed the expression of miR-155 in a dose-dependent manner. Furthermore, the Ets protein level was also suppressed by treatment of BV2 cells with apigenin. When rd1 mice were treated with apigenin by intravitreal injection, the expression of inflammatory chemokines in the retina were reduced, and activation of microglia and Müller glia were suppressed. Furthermore, the thickness of the outer nuclear layer of the retina of rd1 mice was thicker in apigenin-treated retinas. Taken together, local administration of apigenin to the retina is a potential therapeutic treatment for photoreceptor degeneration, which involves downregulation of microglia in the retina when photoreceptors are damaged.

Characterization of human induced pluripotent stem cells carrying homozygous RB1 gene deletion

Xiaoyue Deng, Toshiro Iwagawa, Masaya Fukushima, Sumiko Watanabe

Retinoblastoma is an infant cancer that results from loss of *RB1* expression in both alleles. The *RB1* gene was the first reported cancer suppressor gene; however, the mechanism by which *RB1* loss causes cancer in the retina has not yet been clarified. Human induced pluripotent stem cells (iPSCs) provide an ideal tool for mechanistic research regarding retinoblastoma. However, because RB1 is a tumor suppressor, loss of both alleles of *RB1* in human iPS cells may affect the phenotype of the cells. To examine this possibility, we established human iPSCs with deletions in both alleles of *RB1* by CRISPR/Cas9 technique to characterize the associated phenotype. We first examined the expression of RB1 transcripts by RT-qPCR, and *RB1* transcripts were expressed in immature hiPSCs and then the expression levels of *RB1* transcripts consistently increased during retinal organoid differentiation in human iPSCs. Expression of immature markers of human iPSCs and proliferation activity were examined by RT-qPCR, immunohistochemistry, and the 5-ethynyl-2'-deoxyuridine (EdU) incorporation assay. Expression levels of immature markers including SSEA4, OCT3/4, and NANOG were indistinguishable between control iPSCs and *RB1* knockout iPSCs. Proliferative activity was also unaffected by homozygous *RB1 deletion. Taken together, we showed that homozygous deletion of RB1* did not affect the maturation and proliferation statuses of human iPSCs.

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Social Cooperation Research Program

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RNA no longer stands behind DNA or protein but stands in front of DNA and protein. Recent achievements and discovery in biological science clearly emphasize the importance of RNA in life: the discovery of RNA interference, molecular mimicry between protein and RNA, ribosome structure at atomic resolution, and RNA quality control triggered by aberrant mRNAs. Moreover, the completed human genome project revealed, to our great surprise, the existence of a large amount of protein-noncoding RNAs (ncRNAs). These ncRNAs can be classified into two types: one, like antisense and microRNA, those function with sequence complementarity to the target mRNA or DNA, while the other, like aptamer, those function independent of sequence complementarity. In our laboratory, we aim to create artificial aptamers to target proteins of therapeutic interest.

The concept of using single-stranded nucleic acids (aptamers) as affinity molecules for protein or compound binding was initially described in 1990. The concept is based on the ability of short oligonucleotides to fold, in the presence of a target, into unique three-dimensional (3D) structures that bind the target with high affinity and specificity. Aptamers are generated by a process known as systematic evolution of ligands by exponential enrichment (SELEX), which merges combinatorial chemistry with in vitro evolution from a complex library of randomized $10^{14\text{-}15}$ different sequences. Importantly, aptamer targets can be small (e.g., chemical compounds) or large (e.g., proteins), and simple (e.g., purified proteins) or complex (e.g., protein complexes or cell surface receptors). Therefore, aptamers can be used as therapeutic compounds or reagents for affinity purification or as biosensor elements.

1. A chymase inhibitory RNA aptamer improves cardiac function and survival after myocardial infarction.

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It is known that mast cell chymase, an angiotensin II-generating enzyme, is important in cardiovascular tissues. Recently, we developed a new chymase-specific inhibitory RNA aptamer, HA28, and we evaluated the effects of HA28 on cardiac function and the mortality rate after myocardial infarction. Echocardiographic parameters, such as the left ventricular ejection fraction, fractional shortening, and the ratio of early to late ventricular filling velocities, were significantly improved by treatment with HA28 after myocardial infarction. The mortality rate was significantly reduced in the HA28-treated group. Cardiac chymase

activity and chymase gene expression were significantly higher in the vehicle-treated myocardial infarction group, and these were markedly suppressed in the HA28-treated myocardial infarction group. The present study provides the first evidence that a single-stranded RNA aptamer that is a chymase-specific inhibitor is very effective in the treatment of acute heart failure caused by myocardial infarction. Chymase may be a new therapeutic target in post-myocardial infarction pathophysiology.

2. Anti-angiogenic and anti-scarring dual action of an anti-fibroblast growth factor 2 aptamer in animal models of retinal disease.

Yusaku Matsuda[1], Yosuke Nonaka[1], Satoshi Futakawa[1], Hirotaka Imai[1], Kazumasa Akita[1], Toshiaki Nishihata[1], Masatoshi Fujiwara[1], Yusuf Ali[1], Robert B. Bhisitkul[2], Yoshikazu Nakamura: [1]RIBOMIC Inc., Tokyo, Japan, [2]Department of Ophthalmology, University of California, CA, USA

Currently approved therapies for age-related macular degeneration (AMD) are inhibitors against vascular endothelial growth factor (VEGF), which is a major contributor to the pathogenesis of neovascular AMD (nAMD). Intravitreal injections of anti-VEGF drugs have shown dramatic visual benefits for AMD patients. However, a significant portion of AMD patients exhibit an incomplete response to therapy and, over the extended management course, can lose vision, with the formation of submacular fibrosis as one risk factor. We investigated a novel target for AMD treatments, fibroblast growth factor 2 (FGF2), which has been implicated in the pathophysiology of both angiogenesis and fibrosis in a variety of tissue and organ systems.

RBM-007 is anti-FGF2 aptamer composed of 36 nucleotides, whose ribose 2' positions are heavily modified to resist ribonucleases and 5'- and 3'-termini are conjugated with 40-kDa PEG and an inverted dT, respectively, to achieve sufficient pharmacokinetic profiles. It has been shown that RBM-007 binds stably and specifically to FGF2 and no other FGF family proteins or heparin-binding proteins. The Kd values ranged between 2 pM and 27 pM, indicating a high affinity of RBM-007 to different FGF2s beyond the species difference.

The anti-FGF2 aptamer, RBM-007, was examined for treatment of nAMD in animal models. In in vivo studies conducted in mice and rats, RBM-007 was able to inhibit FGF2-induced angiogenesis, laser-induced choroidal neovascularization (CNV), and CNV with fibrosis. Pharmacokinetic studies of RBM-007 in the rabbit vitreous revealed high and relatively long-lasting profiles that are superior to other approved anti-VEGF drugs. The anti-angiogenic and anti-scarring dual action of RBM-007 holds promise as an additive or alternative therapy to anti-VEGF treatments for nAMD.

3. Effect of FGF/FGFR pathway blocking on lung adenocarcinoma and its cancer-associated fibroblasts.

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Cancer-associated fibroblasts (CAFs) are known to promote tumourigenesis through various mechanisms. Fibroblast growth factor (FGF)/FGF receptor (FGFR)-dependent lung cancers have been described. We have developed a mouse model of lung adenocarcinoma that was constructed through the induction of Fgf9 overexpression in type 2 alveolar cells. The expression of Fgf9 in adult lungs resulted in the rapid development of multiple adenocarcinoma-like tumour nodules. Here, we have characterised the contribution of CAFs and the Fgf/Fgfr signalling pathway in maintaining the lung tumours initiated by Fgf9 overexpression. We found that CAF-secreted Fgf2 contributes to tumour cell growth. CAFs overexpressed Tgfb, Mmp7, Fgf9, and Fgf2; synthesised more collagen, and secreted inflammatory cell-recruiting cytokines. CAFs also enhanced the conversion of tumour-associated macrophages (TAMs) to the tumour-supportive M2 phenotype but did not influence angiogenesis. In vivo inhibition of Fgfrs during early lung tumour development resulted in significantly smaller and fewer tumour nodules, whereas inhibition in established lung tumours caused a significant reduction in tumour size and number. Fgfr inhibition also influenced tumour stromal cells, as it significantly abolished TAM recruitment and reduced tumour vascularity. However, the withdrawal of the inhibitor caused a significant recurrence/regrowth of Fgf/Fgfr-independent lung tumours. These recurrent tumours did not possess a higher proliferative or propagative potential. Our results provide evidence that fibroblasts associated with the Fgf9-induced lung adenocarcinoma provide multiple means of support to the tumour. Although the Fgfr blocker significantly suppressed the tumour and its stromal cells, it was not sufficient to completely eliminate the tumour, probably due to the emergence of alternative (resistance/maintenance) mechanism(s). This model repre-

sents an excellent tool to further study the complex interactions between CAFs, their related chemokines, and the progression of lung adenocarcinoma; it also provides further evidence to support the need for a combinatorial strategy to treat lung cancer.

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Social Cooperation Research Program

Project Division of International Advanced Medical Research

国際先端医療社会連携研究部門

Project Associate Professor　Koichiro Yuji, M.D., Ph.D.　特任准教授　博士（医学）　湯　地　晃一郎

The mission of the Project Division is to apply changes in advanced medical research at the Institute of Medical Science at the University of Tokyo (IMSUT). Our activities include field research in which innovative medicine will be implemented; cross-disciplinary education of physicians, researchers, and professionals; collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development; and establishing projections of the future healthcare system of Japan, which will be the first fully fledged aged society.

Implementing advanced medical research at IMSUT

Yuji, K.

The Project Division was established in November 2014. Our mission is to contribute to the progress of advanced medical research at IMSUT; to perform field research in which innovative medicine will be implemented; and to further the cross-disciplinary education of physicians, researchers, and professionals. Our future plans include collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development.

Projections on the future healthcare system in Japan, the first fully fledged aged society

Yuji, K.

Japan is rapidly becoming a fully fledged aged society, and the increasing dependence of the elderly population is a significant concern. We have simulated both the supply and demand features of Japan's future healthcare system.

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Social Cooperation Research Program

Project Division of ALA Advanced Medical Research

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The overall mission of our lab is to contribute to develop new science, technology, and medical treatment based on or related with the comprehensive utilization of 5-Aminolevulinic Acid (5-ALA). To achieve this goal, we especially focus on the field of basic/clinical research on gene therapy and cell therapy for malignant tumors, and basic research on hematology and regenerative medicine for the treatment of intractable diseases.

A. Basic research of 5-ALA for development of regenerative medicine-related techniques and treatment of intractable diseases.

Regenerative medicine is expected to be an essential therapeutic strategy for intractable diseases. We have been actively investigating some novel strategies to yield and evaluating the efficiency and safety of cell sources for regenerative medicine. 5-ALA, which has many biological functions in heme synthesis, mitochondria activity and cell metabolism, is expected to modify pluripotent stem cell functions because their differentiation capacity is associated with metabolic pathway. In addition, hemogrobinopathies may be benefitted from 5-ALA treatment because 5-ALA would restore its hemoglobin functions and suppress hemolysis-inducing stress.

a. Inhibition of sickling by 5-ALA in sickle cell disease mouse models

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Sickle cell disease (SCD) is a hemoglobinopathy caused by V6G mutation of haemoglobin subunit β gene. Until recently, there are only two FDA-approved drugs, hydroxyurea and L-glutamine, but neither has enough therapeutic effect for many patients. Among new reagents under clinical investigation, both of HbS polymerization inhibitor voxelotor (GBT440), and anti-P-selectin antibody crizanlizumab especially have garnered attention as next-generation SCD therapeutic agents. In November 2019, crizanlizumab became the third FDA-approved drug. The pathogenesis of SCD particularly in vaso-occlusive crisis, however, involves complex interaction of multiple factors due to hypoxia-induced HbS polymeri-

zation followed by hemolysis, endothelial dysfunction, sterile inflammation, oxidative stress, hemostatic activation, blood hyperviscosity, and cellular hyperadhesion. Therefore, in order to treat SCD more effectively, multi-agent therapy or new drugs that can simultaneously act on multiple pathological events are needed. 5-aminolevulinic acid (5-ALA) is a natural amino acid produced in mitochondria and is a precursor of heme. 5-ALA is known to increase oxygen utilization efficiency by activating the electron transport system in mitochondria, and to have multiple functions such as anti-inflammatory and anti-oxidative effects by inducing HO-1 expression. This background information encouraged us to search the possibility of 5-ALA as a new drug for SCD.

We first preliminarily examined the *in vivo* effect of 5-ALA using SCD mouse models (B6;129-Hbb$^{tm2(HBG1,HBB*)Tow}$/Hbb$^{tm3(HBG1,HBB)Tow}$ Hba$^{tm1(HBA)Tow}$/J). 200 mg/kg of 5-ALA solution or saline was administered to SCD homozygous mice daily for 15 days (n = 3 for each group). One hour after the final administration, the mice were exposed to 6% oxygen for 8 hours, and sickling rate was evaluated before and after the hypoxia exposure. Two out of 3 mice in the saline-administered (saline) group showed pulmonary infarction and acute enteric hemorrhage, respectively. On the other hand, such severe symptoms did not occur in the 5-ALA-administered (5-ALA) group. Peripheral blood smear showed sickling rate was remarkably higher in the saline group than in the 5-ALA group. These results suggested that 5-ALA could suppress hypoxia-induced vaso-occlusion. To demonstrate the mode of action of 5-ALA in SCD mice, we examined SCD mice red blood cells (RBCs) *in vitro*. To induce RBC sickling, RBCs were pre-treated with 0-500 μM 5-ALA for 4 hours under normoxia, then RBCs of SCD homozygous mice were incubated under 4% oxygen for 1.5 hours. The hypoxic stimulation significantly induced RBC sickling, and surprisingly, it was inhibited by 5-ALA treatment. Although further analysis is required to elucidate the anti-sickling mechanism of 5-ALA, our preliminary results suggested the possible application of 5-ALA for SCD treatment.

b. Non-transmissible measles virus vector with segmented RNA genome establishes different types of iPSCs from hematopoietic cells

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Recent advances in gene therapy technologies have enabled the treatment of congenital disorders and cancers and facilitated the development of innovative methods, including induced pluripotent stem cell (iPSC) production and genome editing. We recently developed a novel non-transmissible and non-integrating measles virus (MV) vector capable of transferring multiple genes simultaneously into a wide range of cells through the CD46 and CD150 receptors. The MV vector expresses four genes for iPSC generation and the GFP gene for a period of time sufficient to establish iPSCs from human fibroblasts as well as peripheral blood T cells. The transgenes were expressed differentially depending on their gene order in the vector. Human hematopoietic stem/progenitor cells were directly and efficiently reprogrammed to naive-like cells that could proliferate and differentiate into primed iPSCs by the same method used to establish primed iPSCs from other cell types. The novel MV vector has several advantages for establishing iPSCs and potential future applications in gene therapy.

c. Measles virus vector is a promising tool for T-cell engineering and establishing iPSCs

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Although great successes of chimeric antigen receptor T-cell (CAR-T) therapy highlighted the importance of anti-cancer immunity for cancer treatment, long preparation time is a problem. Therefore, rapid and safe CAR-T production systems should be developed to take the place of current systems using retrovirus vectors, which needs two to three weeks for CAR-T production cannot transduce genes into unstimulated T cells (UTs), and may induce insertional mutagenesis. To resolve these problems, non-integrating virus vectors such as Sendai virus vectors (SVs) may be useful, but there is a problem in poor transduction efficiency for UTs. We have recently developed a new non-replicating and non-integrating measles virus vector (MV) with strong infectivity to various hematopoietic cells including UTs. Compared with SVs, our MVs actually allowed more efficient gene transfer to various hematopoietic cells including UTs. Importantly, MVs induced less apoptosis compared with SVs due to their slower viral RNA amplification in transduced cells. Moreover, MVs harboring reprogramming genes could establish iPSCs from UTs 50 times more efficiently than SVs harboring the same reprogramming genes. Considering the safe history of MV vaccine, carrying capabilities of multiple genes and high transduction efficiency for hematopoietic cells including UTs, our MV vector would be a promising tool for reprogramming hematopoietic cells and developing new T-cell immunotherapies.

B. Development of new modalities including 5-ALA for gene therapy, immune cell therapy and early diagnostic methods of malignant tumors

The most commonly used therapies for malignant tumors include surgery, radiation therapy, chemotherapy, and some combination of these therapies. However, they have been not sufficiently effective for some types of tumors and the recurrent ones. In our lab, several approaches of gene therapy and immune therapy, which is expected to be an effective therapy for cancers refractory to conventional treatment, are under investigation. We have also been developing a new diagnostic method for circulating tumor cells by using 5-ALA.

a. Pilot study to detect circulating tumor cells in human peripheral blood using 5-aminolevulinic acid

Lisa Hirose[1], Miyako Sagara[1], Yoshie Miura[1], Yasuki Hijikata[1], Yasushi Soda[1], Shohei Miyamoto[1], Satoshi Takahashi[2], Masaru Shinozaki[3], Tamami Denda[4], Yukihisa Tanaka[4], Yasunori Ota[4], Eri Watanabe[5], Toru Tanaka[6], Motowo Nakajima[6], Sachiko Kiniwa[7], Ryuhei Okuyama[7], Hideo Fukuhara[8], Keiji Inoue[8], Tsutomu Namikawa[8], Kazuhiro Hanazaki[8], Kenzaburo Tani[1]
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Circulating tumor cells (CTCs) have been detected in peripheral blood (PB) of patients with a variety of cancers and expected to be of its potential diagnostic and prognostic value. Although the United States Food and Drug Administration-approved CellSearch system has been commonly used for counting CTCs in PB, this system has limitation in terms of its clinical sensitivity. Namely, this system detects only EpCAM-expressing CTCs which remain the characteristic of the epithelial tumor cells. Recent studies revealed that a proportion of CTCs does not have epithelial characteristics and such non-epithelial CTCs could be excluded in the EpCAM-based detection. Therefore, it is crucial to develop a novel method to capture and detect all tumor subpopulations of CTCs to understand accurate heterogeneity of CTCs. 5-Aminolevulinic acid (5-ALA) has been shown to be a useful fluorescent sensitizing agent for photodynamic therapy and photodynamic detection in cancer therapeutics and diagnosis. ProtoporphyrinIX (PpIX), a metabolite of 5-ALA, accumulates in tumor cells. It is expected this new method can detect both EpCAM-positive and -negative CTCs. After staining with 5-ALA, we analyzed the CTCs in PB of cancer patients by flowcytometry. Until now, we examined PB samples of twelve patients and nine healthy volunteers. In the patient samples, there were 16.2 PpIX-positive cells/mL on average while only 1.6/mL PpIX-positive cells were detected in the healthy donor samples. Moreover, the PpIX-positive cells were often lacking EpCAM expression. These results suggested that 5-ALA staining of PB samples would be a better CTC detection system than the EpCAM-dependent system. We will examine the reliability of this method in CTC detection by single cell sequencing.

b. Development of novel recombinant oncolytic Coxsackievirus B3 therapy

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Oncolytic virotherapy using enteroviruses emerges as a promising anticancer strategy. As therapeutic advantages, enteroviruses immediately induce robust oncolytic activity and do not have oncogenes that may lead to tumorigenesis. We recently showed coxsackievirus B3 wild type (CVB3-WT) infection elicited remarkably oncolytic activity against human non-small cell lung cancer cells (NSCLC). However, CVB3-WT infection caused adverse events of weight loss, pancreatitis, and myocarditis in mice. To overcome these pathogenicity, we engineered CVB3-WT genome for the development of microRNA (miRNA)-regulated oncolytic coxsackievirus. We here overcome viral specific toxicity by inserting organ specific microRNA (miR-1 or miR-217) target sequences into the CVB3 genome (CVB3-HP). We demonstrated both *in vitro* and *in vivo* that the existence of miRNA target sequences in the CVB3 genome dramatically inhibited viral replication in the cognate miRNA holding cells, decreased the level of serum biochemistry and mitigated pancreatitis with a significant tumor regression. However, non-clinical acute toxicity testing of recombinant CVB3 in mice and monkeys showed mild hematological and histopathological abnormal findings in the highest dose group. To improve its safety profile, microRNA target sequence complementary to miR-34a (enriched in normal organs) were inserted into the 5'and 3' UTR of the CVB3 genome (CVB3-BHP). The novel microRNA-targeted coxsackievirus B3 retained high cytotoxicity for cancer cells without causing unwanted side effects in mice with high dose administration. Here, we attempted to explore the oncolysis to triple-negative breast cancer (TNBC) because TNBC are highly aggressive and intractable tumors with dismal prognosis. We performed *in vitro* crystal violet staining to examine the effect of CVB3-BHP on TNBC. These results showed that CVB3-BHP had potent oncolytic activity against TNBC cell lines in a MOI-dependent manner. Furthermore, consecutive administrations of CVB3-BHP into subcutaneous xenografts of human TNBC pre-established in athymic nude mice significantly suppressed the tumor growth with a prolonged survival rate. The intratumoral CVB3-BHP administrations into human TNBC xenograft tumor mice model displayed dramatically decreased side effects of CVB3-WT-induced pathogenicity. Collectively, we showed that CVB3-BHP infection indicated marked oncolytic activity against human NSCLC and TNBC cells *in vitro* and *in vivo* as well as CVB3-WT. This approach could be a promising new therapeutic modality to improve survival in patients suffering from NSCLC and TNBC in advanced stage.

c. Oncolytic CVB3 therapy is an antitumor immunity inducer

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Oncolytic virotherapy emerges as a novel anticancer therapeutic modality because of its distinctive cytotoxic mechanism of conventional therapies such as chemotherapy and radiotherapy. Oncolytic viruses are self-replicating, tumor-selective viruses, with an ability to directly induce cancer cell death, and have emerged as a promising treatment platform for cancer therapy. Enteroviruses have recently been used as an oncolytic virus for cancer virotherapy. We carried out the screening of 38 enteroviral strains and found that coxsackievirus B3 (CVB3) possessed specific oncolytic activity against cell lines of human lung cancer, malignant pleural mesothelioma and breast cancer. In addition, we previously demonstrated that CVB3 had potent oncolytic activity with immunostimulatory properties, abundant cell surface calreticulin expression and secreted ATP as well as translocated extranuclear high-mobility group box 1 (HMGB1) in CVB3-infected lung cancer cell lines, which are required for immunogenic cell death (ICD). Moreover, intratumoral CVB3 injection markedly recruited natural killer (NK) cells and granulocytes, both of which contributed to the antitumor effects as demonstrated by depletion assays, macrophages, and mature dendritic cells (DCs) into tumor tissues. To further confirm the ICD induction by CVB3 treatment, we injected CVB3-infected fibrosarcoma MCA205 cells subcutaneously. We found marked antitumor immune response by this treatment while injection of CDDP (non-ICD inducer)-treated MCA205 cells failed to induce antitumor immunity. In conclusion, because CVB3 is a strong ICD inducer, long-lasting therapeutic effect after CVB3 treatment in cancer patients will be expected.

d. Successful clinical sequencing by molecular tumor board in an elderly patient with refractory Sézary syndrome.

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Sézary syndrome (SS) a rare, aggressive, leukemic variant of cutaneous T-cell lymphoma (CTCL), comprises the malignant clonal proliferation of T lymphocytes and is prone to skin involvement. Most patients with SS are elderly, with erythroderma present on more than 80% of the body. SS is rarely curable and has a poor prognosis, for which the median survival of patients with Stage IV SS is approximately 2 years. Recent SS genomic profiling with next-generation sequencing (NGS) demonstrated impaired normal signaling, suggesting the possibility of new treatment targets.

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) at The Institute of Medical Science, The University of Tokyo. Clinical sequencing is associated with several potential challenges in analysis, interpretation, and drug development for rare cancers such as SS. Here, we report the use of clinical sequencing to achieve a complete response in an elderly SS patient refractory to standard treatment.

e. Safe use of nivolumab in a patient with epipharyngeal carcinoma and preexisting ulcerative colitis: a histologically proven case report.

Yasuki Hijikata[1,2], Yasuo Matsubara[2], Yasunori Ota[3], Lay Ahyoung Lim[2], Kenzaburo Tani[1,2], Yoshihiro Hirata[2], Hiroshi Yotsuyanagi[2]
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Nivolumab, an antibody against human programmed cell death 1 (PD-1), enhances pre-existing immune responses and has antitumor activity. However, it may also cause undesirable immune-related adverse events (irAEs), such as anti-PD-1-related colitis. In addition, Nivolumab can worsen pre-existing autoimmune diseases. Ulcerative colitis (UC) is a chronic inflammatory disease of the colon. Its exact cause is unknown, but may involve dysregulation of the mucosal immune response. Therefore, it is of great interest whether nivolumab can affect UC activity. This is the first report of a patient with epipharyngeal carcinoma and ulcerative colitis safely treated with nivolumab as determined by autopsy findings.

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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. 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.

2. 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.

3. Program for supporting biospecimen analysis for the diagnosis and treatment of hematological malignancies

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To support cancer scientists in promoting translational research and genome medicine, we have established a platform for supporting cohort studies and biospecimen analysis. Under this program, we are collecting and managing clinical materials, including tumor cells, serum, and peripheral blood mononuclear cells from patients at high risk of hematologic malignancies as well as patients with blood cancer. We provide support for obtaining and/or analyzing biomaterials, as requested by researchers, and contribute to their clinical studies and publications.

4. Investigator-initiated clinical trials under an Investigational New Drug application for the development of novel cancer therapeutics and biomarkers

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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.

5. Development of novel immunodiagnostics and cancer immunotherapy

Hiroshi Yasui, Asako Kobayashi, Reika Li, 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 started to develop novel immunodiagnostics to evaluate activities of immune cells in patients with allogenic hematopoietic stem cell transplantation. It is also expected to contribute the development of the novel cancer immunotherapy in hematologic malignancies.

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 at 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

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Various antibodies have been approved for therapeutic use and currently examined in clinical development. Developments and improvements of technology for the discovery and optimization of high-potency antibodies, therefore, 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. 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.

2. Biophysical characterization of the breast cancer-related BIG3-PHB2 interaction: Effect of non-conserved loop region of BIG3 on the structure and the interaction.

Chigira T, Nagatoishi S, Takeda H, Yoshimaru T, Katagiri T, Tsumoto K.

Brefeldin A-inhibited guanine nucleotide-exchange protein 3 (BIG3) interacts with and inhibits the tumor suppressor function of prohibitin-2 (PHB2), and recent in vivo studies have demonstrated that the BIG3-PHB2 interaction is a promising target for breast cancer therapy. However, little biophysical characterization on BIG3 and its interaction with PHB2 has been reported. Here we compared the calculated 8-class secondary structure of the N-terminal domains of BIG family proteins and identified a loop region unique to BIG3. Our biophysical characterization demonstrated that this loop region significantly affects the colloidal and thermodynamic stability of BIG3 and the thermodynamic and kinetic profile of its interaction with PHB2. These results establish a model for the BIG3-PHB2 interaction and an entry for drug discovery for breast cancer.

3. Structural features of methionine aminopeptidase2-active core peptide essential for binding with S100A4.

Katagiri N, Nagatoishi S, Tsumoto K, Endo H.

Methionine aminopeptidase 2 (MetAP2) is one of the effector proteins of S100A4, a metastasis-associated calcium-binding protein. This interaction is involved in angiogenesis. The region of MetAP2 that interacts with S100A4 includes amino acids 170 to 208. A peptide corresponding to this region, named as NBD, has potent anti-angiogenic activity and suppresses tumor growth in a xenograft cancer model. However, the binding mode of NBD to S100A4 was totally unknown. Here we describe our analysis of the relationship between the inhibitory activity and the structure of NBD, which adopts a characteristic helix-turn-helix structure as shown by X-ray crystallographic analysis, and peptide fragments of NBD. We conducted physicochemical analyses of the interaction between S100A4 and the peptides, including surface plasmon resonance, microscale thermophoresis, and circular dichroism, and performed docking/molecular dynamics simulations. Active peptides had stable secondary structures, whereas inactive peptides had a little secondary structure. A computational analysis of the interaction mechanism led to the design of a peptide smaller than NBD, NBD-ΔN10, that possessed inhibitory activity. Our study provides a strategy for design for a specific peptide inhibitor against S100A4 that can be applied to the discovery of inhibitors of other protein-protein interactions.

4. Highly sensitive biomolecular interaction detection method using optical bound/free separation with grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS).

Kaya T, Nagatoishi S, Nagae K, Nakamura Y, Tsumoto K.

Grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS) with optical bound/free (B/F) separation technique was developed by employing a highly directional fluorescence with polarization of surface plasmon-coupled emission (SPCE) to realize highly sensitive immunoassay regardless of the ligand affinity. A highly sensitive immunoassay system with GC-SPFS was constructed using a plastic sensor chip reproducibly fabricated in-house by nanoimprinting and applied to the quantitative detection of an anti-lysozyme single-domain antibody (sdAb), to compare conventional washing B/F separation with optical B/F separation. Differences in the affinity of the anti-lysozyme sdAb, induced by artificial mutation of only one amino acid residue in the variable domain were attributed to higher sensitivity than that of the conventional Biacore surface plasmon resonance (SPR) system. The detection limit (LOD; means of six replicates of the zero standard plus three standard deviations) of the GC-SPFS immunoassay with optical B/F separation, was estimated to be 1.2 ng/ml with the low-affinity ligand (mutant sdAb Y52A: KD level was of the order of 10-7 ~ 10-6 M) and was clearly improved as compared to that (LOD: 9.4 ng/ml) obtained with the conventional washing B/F separation. These results indicate that GC-SPFS with the optical B/F separation technique offers opportunities to re-evaluate low-affinity biomaterials that are neither fully utilized nor widespread, and could facilitate the creation of novel and innovative methods in drug and diagnostic development.

5. An epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout.

Eguchi A, Nakakido M, Nagatoishi S, Kuroda D, Tsumoto K, Nagamune T, Kawahara M.

Upon developing therapeutically potent antibodies, there are significant requirements, such as increasing their affinity, regulating their epitope, and using native target antigens. Many antibody selection systems, such as a phage display method, have been developed, but it is still difficult to fulfill these requirements at the same time. Here, we propose a novel epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout. This system is based on the competition of antibody binding, and can target membrane proteins expressed

in a native form on a mammalian cell surface. Using this system, we successfully selected an affinity-matured anti-ErbB2 single-chain variable fragment variant, which had the same epitope as the original one. In addition, the affinity was increased mainly due to the decrease in the dissociation rate. This novel cell-based antibody affinity maturation system could contribute to directly obtaining therapeutically potent antibodies that are functional on the cell surface.

6. Exploring designability of electrostatic complementarity at an antigen-antibody interface directed by mutagenesis, biophysical analysis, and molecular dynamics simulations.

Yoshida K, Kuroda D, Kiyoshi M, Nakakido M, Nagatoishi S, Soga S, Shirai H, Tsumoto K.

Antibodies protect organisms from a huge variety of foreign antigens. Antibody diversity originates from both genetic and structural levels. Antigen recognition relies on complementarity between antigen-antibody interfaces. Recent methodological advances in structural biology and the accompanying rapid increase of the number of crystal structures of proteins have enabled atomic-level manipulation of protein structures to effect alterations in function. In this study, we explored the designability of electrostatic complementarity at an antigen-antibody interface on the basis of a crystal structure of the complex. We designed several variants with altered charged residues at the interface and characterized the designed variants by surface plasmon resonance, circular dichroism, differential scanning calorimetry, and molecular dynamics simulations. Both successes and failures of the structure-based design are discussed. The variants that compensate electrostatic interactions can restore the interface complementarity, enabling the cognate antigen-antibody binding. Retrospectively, we also show that these mutational effects could be predicted by the simulations. Our study demonstrates the importance of charged residues on the physical properties of this antigen-antibody interaction and suggests that computational approaches can facilitate design of antibodies that recognize a weakly immunogenic antigen.

7. Control of Protein Adsorption to Cyclo Olefin Polymer by the Hofmeister Effect.

Fujita R, Nagatoishi S, Adachi S, Nishioka H, Ninomiya H, Kaya T, Takai M, Arakawa T, Tsumoto K.

Cyclo olefin polymer (COP) is an attractive plastic because it has low protein adsorption despite its hydrophobic chemical structure. Here, the adsorption of model proteins to the COP was evaluated in comparison with a representative plastic, polystyrene (PSt), using reflectometry interference spectroscopy (RIfS) technology. The effects of different salts on adsorption were then examined. The adsorption of bovine serum albumin onto COP increased in the presence of kosmotropic salts, whereas adsorption of IgG increased in the presence of chaotropic salts. By contrast, the adsorption of these 2 proteins to PSt was unaffected by these Hofmeister salts. Langmuir-Freundlich model of COP adsorption suggested that the COP surface is more homogeneous for protein binding than the PSt surface. Furthermore, RIfS and sum frequency generation analyses indicated that water molecules bind more weakly to COP than to PSt. Our data propose a novel viewpoint of the way protein binds to COP surface that is different from the way it binds to PSt.

8. Affinity Improvement of a Cancer-Targeted Antibody through Alanine-Induced Adjustment of Antigen-Antibody Interface.

Yamashita T, Mizohata E, Nagatoishi S, Watanabe T, Nakakido M, Iwanari H, Mochizuki Y, Nakayama T, Kado Y, Yokota Y, Matsumura H, Kawamura T, Kodama T, Hamakubo T, Inoue T, Fujitani H, Tsumoto K.

To investigate favorable single amino acid substitutions that improve antigen-antibody interactions, alanine (Ala) mutagenesis scanning of the interfacial residues of a cancer-targeted antibody, B5209B, was performed based on X-ray crystallography analysis. Two substitutions were shown to significantly enhance the binding affinity for the antigen, by up to 30-fold. One substitution improved the affinity by a gain of binding enthalpy, whereas the other substitution improved the affinity by a gain of binding entropy. Molecular dynamics simulations showed that the enthalpic improvement could be attributed to the stabilization of distant salt bridges located at the periphery of the antigen-antibody interface. The entropic improvement was due to the release of water molecules that were stably trapped in the antigen-antibody interface of the wild-type antibody. Importantly, these effects of the Ala substitutions were caused by subtle adjustments of the binding interface. These results will be helpful to design high-affinity antibodies with avoiding entropy-enthalpy compensation.

9. Phospholipid Membrane Fluidity Alters Ligand Binding Activity of a G Protein-Coupled Receptor by Shifting the Conformational Equilibrium.

Yoshida K, Nagatoishi S, Kuroda D, Suzuki N, Murata T, Tsumoto K.

The affinity of a ligand for a receptor on the cell surface will be influenced by the membrane composition. Herein, we evaluated the effects of differences in

membrane fluidity, controlled by phospholipid composition, on the ligand binding activity of the G protein-coupled receptor human serotonin 2B. Using Nanodisc technology to control membrane properties, we performed biophysical analysis and employed molecular dynamics simulations to demonstrate that increased membrane fluidity shifted the equilibrium toward an active form of the receptor. Our quantitative study will enable development of more realistic in vitro drug discovery assays involving membrane-bound proteins such as G protein-coupled receptors.

Publications

Hirano A, Nagatoishi S, Wada M, Tsumoto K, Maluf KN, 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. 2020 Jan; 109(1): 524-531

Chigira T, Nagatoishi S, Takeda H, Yoshimaru T, Katagiri T, Tsumoto K. Biophysical characterization of the breast cancer-related BIG3-PHB2 interaction: Effect of non-conserved loop region of BIG3 on the structure and the interaction. Biochem Biophys Res Commun. 2019 518(1): 183-189.

Katagiri N, Nagatoishi S, Tsumoto K, Endo H. Structural features of methionine aminopeptidase2-active core peptide essential for binding with S100A4. Biochem Biophys Res Commun. 2019 516(4): 1123-1129.

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PLoS One. 2019 14(8): e0220578.

Eguchi A, Nakakido M, Nagatoishi S, Kuroda D, Tsumoto K, Nagamune T, Kawahara M. An epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout. Biotechnol Bioeng. 2019 116(7): 1742-1751.

Yoshida K, Kuroda D, Kiyoshi M, Nakakido M, Nagatoishi S, Soga S, Shirai H, Tsumoto KExploring designability of electrostatic complementarity at an antigen-antibody interface directed by mutagenesis, biophysical analysis, and molecular dynamics simulations. Sci Rep. 2019 9(1): 4482.

Fujita R, Nagatoishi S, Adachi S, Nishioka H, Ninomiya H, Kaya T, Takai M, Arakawa T, Tsumoto K. Control of Protein Adsorption to Cyclo Olefin Polymer by the Hofmeister Effect. J Pharm Sci. 2019 108(5): 1686-1691.

Yamashita T, Mizohata E, Nagatoishi S, Watanabe T, Nakakido M, Iwanari H, Mochizuki Y, Nakayama T, Kado Y, Yokota Y, Matsumura H, Kawamura T, Kodama T, Hamakubo T, Inoue T, Fujitani H, Tsumoto K. Affinity Improvement of a Cancer-Targeted Antibody through Alanine-Induced Adjustment of Antigen-Antibody Interface. Structure. 2019 27(3): 519-527.

Yoshida K, Nagatoishi S, Kuroda D, Suzuki N, Murata T, Tsumoto K. Phospholipid Membrane Fluidity Alters Ligand Binding Activity of a G Protein-Coupled Receptor by Shifting the Conformational Equilibrium. Biochemistry. 2019 58(6): 504-508.

長門石曉，中木戸誠，津本浩平
材料創製を指向したタンパク質相互作用解析
高分子，68巻３月号，126−127（高分子学会）(2019)

津本浩平，長門石曉
第19章　抗体医薬の基礎物性評価
医薬品原薬の結晶化と物性評価：その最先端技術と評価の実（(シーエムシー出版）(2019)

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.
Project Associate Professor Hiroaki Uchida, M.D., Ph.D.

特任教授 医学博士 田 原 秀 晃
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

Yu Mizote, Mika Uematsu-Hamada, Miho Kudo, Hiroaki Uchida, Hideaki Tahara

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 focus include the investigation on the significance of tumor-derived MFG-E8, which has been implicated in our histological examination on human samples. 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, Yukinari Kato[1], Hideaki Tahara ([1]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), carcinoembryon-

ic 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 are now testing whether syncytium formation in tumors would be associated with more potent antitumor effects *in vivo*. We are also investigating whether our retargeted oncolytic HSV vectors would 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[2], Hideaki Tahara ([2]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. We expect that this novel Ab-screening system may lead to a new generation of RR-oHSV vectors.

Publications

1. Hayato, Okamoto., Yasuhiro, Yoshimatsu., Taishi, Tomizawa., Akiko, Kunita., Rina, Takayama., Teppei, Morikawa., Daisuke, Komura., Kazuki, Takahashi., Tsukasa, Oshima., Moegi, Sato., Mao, Komai., Katarzyna, Inoue., Hiroaki, Uchida., Hirofumi, Hamada., Katsuhito, Fujiu., Shumpei, Ishikawa., Masashi, Fukayama., Takeshi, Fukuhara. and Tetsuro, Watabe. Interleukin-13 receptor α2 is a novel marker and potential therapeutic target for human melanoma. Sci Rep. 9: 1281, 2019.

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], Momoko Horikoshi[2], Yoichiro Kamatani[3], 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.

Genome wide association studies (GWAS) have identified a large number of single nucleotide polymorphisms (SNPs) that are associated with susceptibility to various human diseases. However, the contribution of most of single SNPs in disease susceptibility is quite small and their clinical significance in disease development as well as its prevention is largely limited. In order to overcome this problem, a limited number of unique SNPs showing clinically significant functions is extracted, including SNPs involved in metabolic activities of enzymes for nutrients or drugs through literature search for numerous related papers published. For example, functional variants in *ADH1B* and *ALDH2* alter enzymatic activity involved in alcohol metabolism and make the population less tolerant to alcohol consumption. The A allele of rs1229984:A＞G in *ADH1B* causes the rapid oxidation of ethanol to acetaldehyde by ADH1B, which increases an aversive reaction to alcohol, while the A allele of rs671:G＞A in *ALDH2* gene causes the functional deficiency of ALDH2, which slows the metabolism of acetaldehyde. In addition, these variants are known to be highly pleiotropic and associated with many complex human traits (Ref. Matoba N,

Kamatani Y, Murakami Y, Okada Y *et al*, *Nature Human Behavior, in press*). The utilization of the clinically significant genomic information in possible life style improvement for individuals and its validity from the view point of ethical, legal and social issues (ELSI) are being investigated in collaboration with medical doctors in several hospitals. 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 on the basis of a large number of SNPs information through poly genic risk score (PRS) analysis is being investigated (Ref. Sakaue S, Kamatani Y, Murakami Y, Okada Y *et al.*, *Nature Medicine, in press*). 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 and enhance the mutual cooperation and alliance among teaching and research staff, administration staff, and technical staff, in order to execute the activities in our institute effectively. For these purposes, we carry out several tasks such as planning for new institutional research programs or 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, Noriko Endo

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:
- "China-Japan Research Collaboration on Defense against Emerging and Reemerging Infections" supported by the program of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) 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 was 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, or 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 manages these activities, edits documents pertaining to various investigations and submits evaluation reports requested by MEXT in collaboration with the Research Promotion Team, Research Support Division, Administration Office.

In November 2018, IMSUT was 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 collect and stock data every year using an original format to construct a data system available any time for evaluation, submission of various reports, public relation activities, basic data for application of external funding.

4. Others

Kiyomi Nakagawa, Ayako Miyake, Yoko Udagawa, Noriko Endo

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

Dean's Office

Office of Support for Platforms for Advanced Technologies and Research Resources

学術研究基盤支援室

Chair and Professor Jun-ichiro Inoue, Ph.D.
Mutsuhiro Takekawa, M.D., Ph.D.

教授・室長　薬学博士　井　上　純一郎
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. In order to achieve the goal, the General Management Group was organized to facilitate a close cooperation between four platforms comprising 52 universities and 24 research institutions nationwide which provide 77 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 16 members participated: four platform representatives and 12 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, Mutsuhiro Takekawa and Jun-ichiro Inoue

The following activities have been performed under the management of this office in FY 2019.

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 exhibitions and luncheon seminars in various scientific meetings.
7. Facilitating cooperative networks between our platforms and other domestic or international groups that support life science researches.

SCIENTIFIC MEETINGS & SEMINARS

46th IMSUT Founding Commemorative Symposium Recent Advances in Stem Cell Science and Therapies

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「幹細胞サイエンスにおける最近の進歩：基礎から臨床応用まで」というテーマで講演をお願いした。

日　時：令和元年 5 月 31 日（金） 13：00 ～ 17：00
会　場：医科学研究所　1 号館講堂

Atsushi Iwama (Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, IMSUT)
Epigenetic alterations in aged hematopoietic stem cells and age-associated malignancies

Tohru Masui (Guest professor, Keio University, School of Medicine / Project researcher, National Center of Neurology and Psychiatry)
In the process of implementation of stem cell science: a philosophical change in the practice of sciences from the basic research into application

Hideki Taniguchi (Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, IMSUT)
Generation of human organoids using iPS cells for regenerative therapies

Hirohide Saito (Department of Life Science Frontiers, Kyoto University, CiRA)
Synthetic RNA technologies for stem cell science

Nobuyuki Takakura (Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University)
Endothelial stem cell population in health and diseases

学友会セミナー

（平成 31 年 1 月～令和 1 年 12 月）

1 月 7 日　演題：　IgG4-related disease (IgG4-RD) の疾患概念確立と病態解明・治療法開発に向けて
　　演者：　山本　元久

1 月 9 日　演題：　Targeting Spliceosomal Dysfunction in Leukemias
　　演者：　吉見　昭秀

1 月 10 日　演題：　Microbiome-gut-brain axis and its therapeutic manipulation after stroke
　　演者：　Juneyoung Lee

1 月 16 日　演題：　インフルエンザウイルス感染肺の生体イメージング
　　演者：　植木　紘史

1 月 23 日　演題：　Functional Annotation of Mammalian Genomes in the FANTOM projects
　　演者：　DE HOON Michiel Jan Laurens

1 月 29 日　演題：　Combination and inducible adjuvants targeting nucleic acid sensors.
　　演者：　Burcu Temizoz

1 月 29 日　演題：　Dok-7/MuSK シグナルによる NMJ 形成・維持機構
　　演者：　江口　貴大

2 月 4 日　演題：　疾患病態の解明に向けた網羅的エピゲノム解析
　　演者：　永江　玄太

2 月 4 日　演題：　大腸がんの進化と腫瘍内不均一性
　　演者：　新井田厚司

2 月 4 日　演題：　Regulation of hematopoietic stem cells by programmed cell death（プログラム細胞死による造血幹細胞の制御）
　　演者：　山下　真幸

2 月 14 日　演題：　TAT-MYC Recombinant Fusion Protein Enhances Hematopoietic Stem Cell Graft Performance and Immune Cell Reconstitution after Transplantation
　　演者：　Yosef Refaeli

2 月 14 日　演題：　Big Data, Smart Data, and Actionable Data: Shaping the Future of Precision Medicine and Healthcare
　　演者：　Yu Shyr

2 月 20 日　演題：　マウス生体内における ATP 動態の可視化と応用
　　演者：　山本　正道

2 月 22 日　演題：　動物における感染症に注目！
　　演者：　前田　　健

3 月 6 日　演題：　新規アジュバント開発と非感染性疾患ワクチン開発の試み
　　演者：　小檜山康司

3月6日　演題：　消化器癌を標的としたオンコリティック・アデノウィルスの開発と今後の展望
　　演者：　山本　正人
3月8日　演題：　Potential almighty anti-cancer therapeutic strategy through CROX (Cluster regulation of RUNX family).
　　演者：　上久保靖彦
3月11日　演題：　免疫応答時における臓器間対話 個体を対象としたシステム生物学的アプローチ
　　演者：　角木　基彦
3月13日　演題：　インフルエンザウイルスのゲノム適応進化
　　演者：　鈴木　由紀
3月13日　演題：　脳腫瘍マウスモデルに対する遺伝子組換え単純ヘルペスウイルス治療におけるモニタリング解析
　　演者：　伊藤　博崇
3月14日　演題：　自己免疫疾患に関わる内在性 RNA の同定とその応答を制御する方法の開発研究
　　演者：　根岸　英雄
3月14日　演題：　新規ペア型免疫受容体 LMIR6/CD300e の機能解析
　　演者：　磯部　優理
3月18日　演題：　鼻咽腔における肺炎球菌保菌機序と母乳栄養による感染予防
　　演者：　保富　宗城
3月26日　演題：　Stomach ILC2s are regulated by commensal bacteria and can be activated in response to the pathobiont Helicobacter pylori.
　　演者：　佐藤　尚子
4月8日　演題：　Exploring phenotypic mosaicism in a mouse model of glioma　to understand intratumoral heterogeneity and cellular states in GBM.
　　演者：　原　　敏朗
4月11日　演題：　薬剤耐性とインフルエンザ
　　演者：　山下　　誠
4月26日　演題：　Regulation of RNA-protein interactions
　　演者：　Miguel A. Esteban
5月20日　演題：　B7-H3 をターゲットとした膵癌に対する CAR-T 療法の開発
　　演者：　黒川　友博
5月22日　演題：　骨と免疫系の総括的な理解による骨疾患の病態解明
　　演者：　古賀　貴子
6月10日　演題：　腸管炎症と小胞体ストレス　～ 小胞体 Distress と Eustress ～
　　演者：　細見　周平
6月21日　演題：　ウイルス学×公衆衛生×分子進化
　　演者：　古瀬　祐気
6月21日　演題：　大規模ゲノム改変に向けた新規ゲノム編集法および新規ツールの開発
　　演者：　吉見　一人

6 月 27 日　演題：　私の、医科研における、抗ガン剤開発への歩み　S100A4-targeted peptide inhibitor
　　演者：　遠藤　英也
7 月 23 日　演題：　Science Publishing: Myths and Legends
　　演者：　Zoltan Fehervari
7 月 25 日　演題：　Inhibition of plasmin regulates cytokine storm and effector cell trafficking by suppressing MMP-9
　　演者：　佐藤　亜紀
7 月 30 日　演題：　Targeting the general cancer metabolic defect of methionine addiction with recombinant methioninase in patient-derived orthotopic xenograft (PDOX) mouse models
　　演者：　Robert M. Hoffman
8 月 5 日　演題：　RS ウイルス感染症とワクチン開発
　　演者：　柴田　岳彦
8 月 6 日　演題：　妊婦における熱帯熱マラリアの治療
　　演者：　齋藤　　真
8 月 9 日　演題：　Formation, function, and regeneration of corticospinal circuits underlying skilled movements skilled movements「運動系の神経回路の形成、機能、再生」
　　演者：　吉田　　富
8 月 9 日　演題：　NOD2 の機能獲得変異によって肉芽腫を形成する自己炎症
　　演者：　神戸　直智
8 月 23 日　演題：　Restoration of chromatin based information behind replication forks
　　演者：　Constance Alabert
9 月 5 日　演題：　研究の一スタイルとしてのフーダニット（who done it）
　　演者：　片山　義雄
9 月 5 日　演題：　肺癌免疫微小環境の理解～トランスジェニックマウスモデルから臨床検体へ～
　　演者：　小山　正平
9 月 6 日　演題：　疾患特異的マクロファージの機能的多様性
　　演者：　佐藤　　荘
9 月 20 日　演題：　Neutralizing monoclonal antibodies to prevent and treat Zika, dengue and yellow fever virus infection.
　　演者：　David I Watkins
9 月 25 日　演題：　DeepCleave: a deep learning-based approach and tool for more accurate prediction of protease-specific cleavage sites
　　演者：　Jiangning Song
10 月 1 日　演題：　マウス非アルコール性脂肪肝モデルにおける時間栄養学的観点からの病態解明
　　演者：　横田　伸一
10 月 8 日　演題：　AI からゲノミクスを学ぶ
　　演者：　小井土　大

10 月 10 日演題：　ポスドクを始めて現在に至るまでの軌跡；アメリカの研究大学の仕組み
演者：　泉屋　吉宏
10 月 10 日演題：　3D chromatin organizational principles involved　in B-cell lymphoma gene expression
演者：　Nagai Luis Augusto Eijy
10 月 28 日演題：　RNA プロセシング異常に起因する疾患「RNA 病」―その原因解明と治療への挑戦―
演者：　片岡　直行
10 月 30 日演題：　Intestinal permeability and IgA provoke immune vasculitis linked to cardiovascular inflammation
演者：　Moshe Arditi
11 月 1 日　演題：　菌類・植物・動物ウイルスの生き様を数理モデルの助けを借りて比較する
演者：　宮下　脩平
11 月 11 日演題：　A trip in “The New Microbiology” with the bacterial pathogen Listeria monocytogenes.
演者：　Pascale COSSART
11 月 15 日演題：　Building and rebuilding the retina: one cell at a time.
演者：　Seth Blackshaw
11 月 19 日演題：　ゲノム編集治療のための多重ガイド RNA 発現アデノベクターの開発と特許
演者：　中西　友子
12 月 2 日　演題：　Immune Regulation and Immunopathology of Malaria
演者：　Michelle S.J. Lee
12 月 3 日　演題：　HIV-1 潜伏感染モデル細胞を用いた抗 HIV-1 治療薬の探索と潜伏感染分子機構解析への応用
演者：　合田　　仁
12 月 4 日　演題：　Regulation of microbiota-derived metabolites on gut homeostasis
演者：　Mi-Na Kweon
12 月 4 日　演題：　For the development of vaccines against infectious diseases
演者：　吉岡　靖雄
12 月 6 日　演題：　Transcriptional regulation mechanism of Plasmodium spp. parasites.
演者：　岩永　史朗
12 月 20 日演題：　A Roadmap for Fast and Efficient Genome Analysis
演者：　Mohammed H. K. Alser
12 月 20 日演題：　人工知能（AI）入門と医療分野への応用
演者：　門脇　嗣郎

国際共同利用・共同研究拠点セミナー

（令和元年 11 月～令和元年 12 月）

11 月 25 日演題： Understanding and Targeting Cancer Microenvironment

演者： Zhihai Qin

12 月 2 日 演題： Recent evolution of a TET controlled and DPPA3/STELLA driven pathway of passive demethylation in mammals

演者： Heinrich Leonhardt

12 月 2 日 演題： Genetic and structural investigations of DNA methylation maintenance mechanisms

演者： Pierre-Antoine Defossez

12 月 17 日演題： Comprehensive 3D Imaging by Tissue Clearing Technique CUBIC

演者： 田井中一貴

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。2019 年には、「ゲノム編集の基礎と応用」というテーマの下で次のようなセミナーが行われた。

ゲノム編集の基礎と応用

	月　日	講師名		演　　題
1	4 月 8 日	高橋　　智	筑波大学医学医療系 教授	ゲノム編集を用いたヒト疾患モデルマウスの作製
2	4 月 15 日	伊川　正人	大阪大学・微生物病研究所 教授	ゲノム編集マウス作製の基礎と受精研究への応用
3	4 月 22 日	山本　　卓	広島大学大学院理学研究科 教授	ゲノム編集の開発の歴史と基本原理
4	5 月 13 日	畑田　出穂	群馬大学生体調節研究所 教授	ゲノム編集の基礎と応用
5	5 月 20 日	Woltjen Knut	京都大学 iPS 細胞研究所 准教授	Precision Engineering of the Human Genome in iPS Cells
6	5 月 27 日	西田　敬二	神戸大学 先端バイオ工学研究センター 教授	塩基編集 Base editing の開発と応用
7	6 月 3 日	石井　哲也	北海道大学安全衛生本部 教授	ゲノム編集を伴う生殖医療の可能性と課題
8	9 月 30 日	堀田　秋津	京都大学 iPS 細胞研究所 特定拠点講師	ゲノム編集の医療応用
9	10 月 7 日	足立　典隆	横浜市立大学 教授	外来 DNA 組込みと二本鎖切断修復機構
10	10 月 21 日	山田　泰広	東京大学医科学研究所 先進病態モデル研究分野 教授	iPS 細胞技術によるがん細胞の理解と制御
11	10 月 28 日	濡木　　理	東京大学大学院理学系研究科 生物科学専攻 教授	CRISPR-Cas の分子機構とゲノム編集ツールの開発
12	11 月 11 日	三宅　健介	東京大学医科学研究所 感染遺伝学分野 教授	自然免疫研究におけるゲノム編集の応用
13	11 月 18 日	三谷　幸之介	埼玉医科大学ゲノム医学研究センター 遺伝子治療部門 教授	ゲノム編集技術の臨床応用に向けて

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：令和元年 12 月 14 日（土）　9：15 ～ 16：40
　　令和元年 12 月 15 日（日）　9：30 ～ 16：40
場　所：医科学研究所 1 号館 1 階講堂

教員および題目

12 月 14 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
中西　　真	癌・細胞増殖部門 癌防御シグナル分野	老化を理解する
井元　清哉	ヘルスインテリジェンスセンター 健康医療データサイエンス分野	ゲノムビッグデータ解析
COBAN CEVAYIR	感染・免疫部門 マラリア免疫学分野	Host-Plasmodium parasites interactions
関根　圭輔	幹細胞治療研究センター 再生医学分野	オルガノイドを用いたヒトバイオロジーへのアプローチ

12 月 15 日（日）

講　師　名		題　　目
四柳　　宏	先端医療研究センター 感染症分野	難治性ウイルス感染症の克服を目指して
古川　洋一	先端医療研究センター 臨床ゲノム腫瘍学分野	ゲノムで読み解くがんと体質
石井　　健	感染・免疫部門 ワクチン科学分野	近未来のワクチン研究
山田　泰広	システム疾患モデル研究センター 先進病態モデル研究分野	マウス発生工学を駆使した医科学研究

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