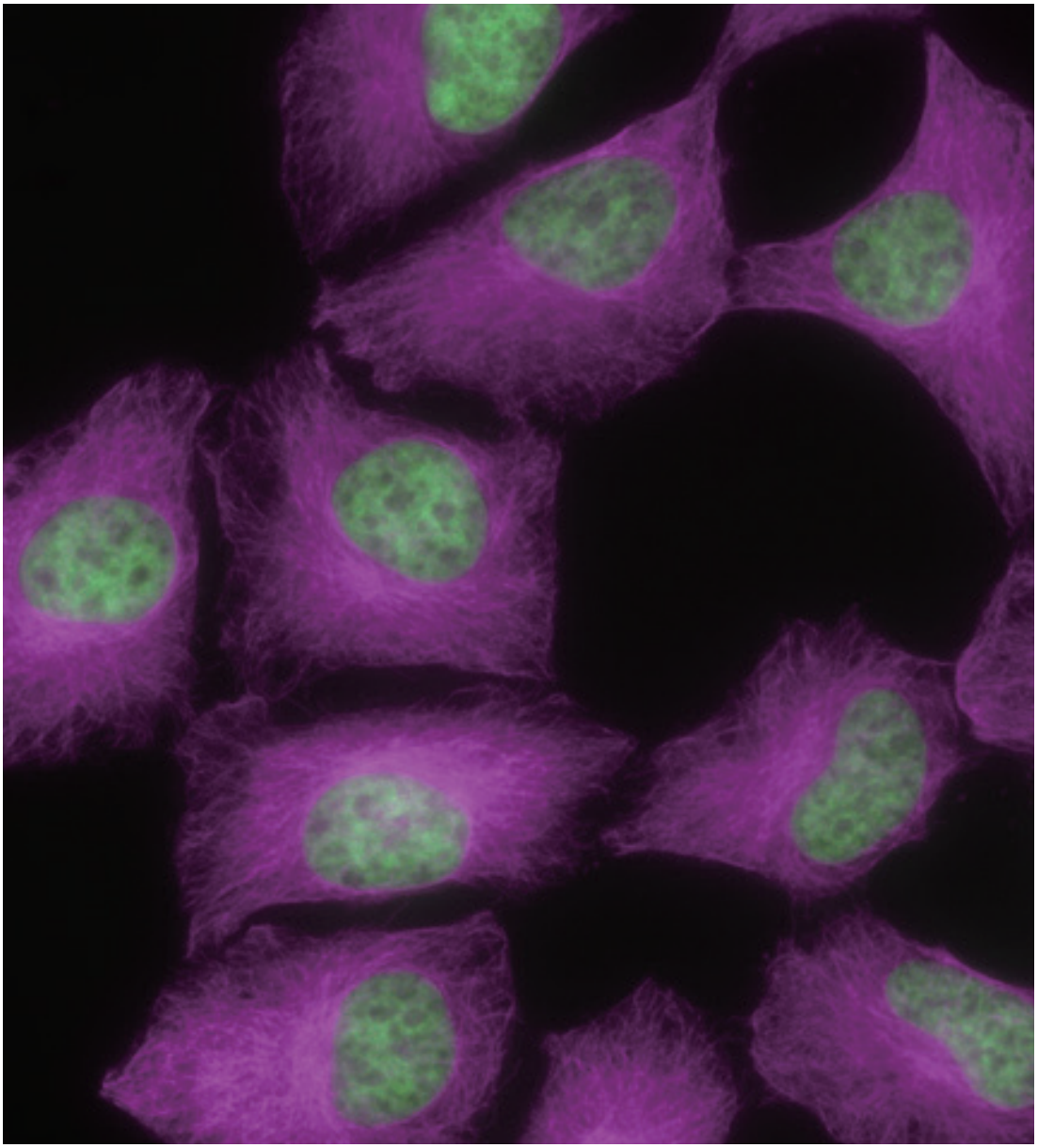
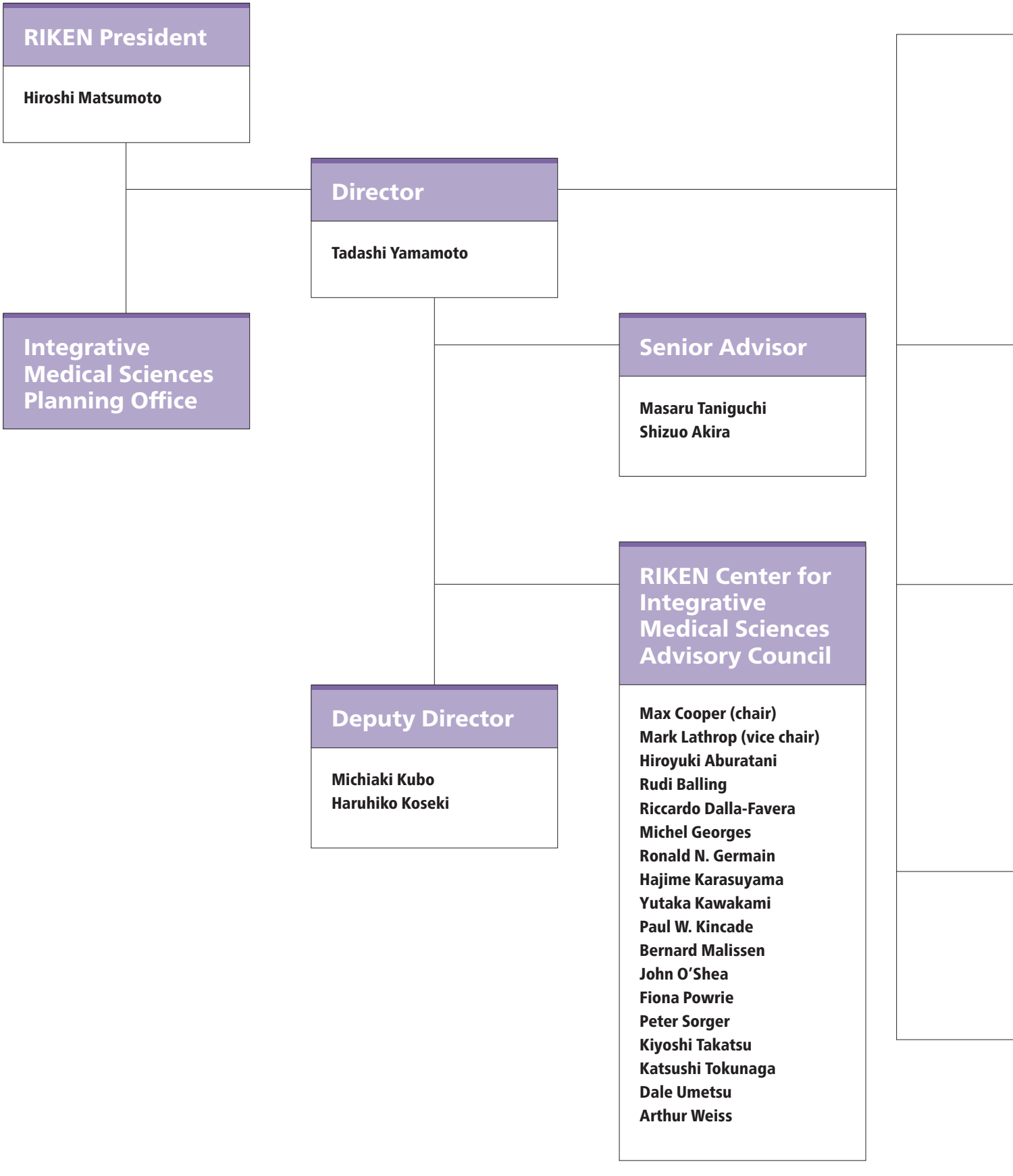


RIKEN IMS Annual Report 2016

RIKEN Center for Integrative Medical Sciences

RIKEN Center for Integrative Medical Sciences Organization Chart



Core for Homeostatic Regulation

- Lab. for Cell Signaling: **Takashi Saito**
- Lab. for Lymphocyte Differentiation: **Tomohiro Kurosaki**
- Lab. for Transcriptional Regulation: **Ichiro Taniuchi**
- Lab. for Immune Cell Systems: **Shigeo Koyasu**
- Lab. for Human Disease Models: **Fumihiko Ishikawa**
- Lab. for Intestinal Ecosystem: **Hiroshi Ohno**
- Lab. for Mucosal Immunity: **Sidonia Fagarasan**
- Lab. for Gut Homeostasis: **Kenya Honda**
- Lab. for Immune Homeostasis: **Shohei Hori**
- Lab. for Skin Homeostasis: **Masayuki Amagai**
- Lab. for Metabolic Homeostasis: **Naoto Kubota**
- Lab. for Immune Crosstalk: **Hilde Cheroutre**
- Lab. for Inflammatory Regulation: **Takashi Tanaka**
- Lab. for Cytokine Regulation: **Masato Kubo**
- Lab. for Innate Immune Systems: **Kazuyo Moro**

Core for Precise Measuring and Modeling

- Lab. for Developmental Genetics: **Haruhiko Koseki**
- Lab. for Integrative Genomics: **Osamu Ohara**
- Lab. for Disease Systems Modeling: **Hiroaki Kitano**
- Lab. for Medical Science Mathematics: **Tatsuhiko Tsunoda**
- Lab. for Immunogenetics: **Tadashi Yamamoto**
- Lab. for Integrated Bioinformatics: **Todd Duane Taylor**
- Lab. for Tissue Dynamics: **Takaharu Okada**
- Lab. for Integrated Cellular Systems: **Mariko Okada**
- Lab. for Metabolomics: **Makoto Arita**
- Lab. for Microbiome Sciences: **Masahira Hattori**

Core for Genomic Medicine

- Lab. for Genotyping Development: **Yukihide Momozawa**
- Lab. for Genome Sequencing Analysis: **Hidewaki Nakagawa**
- Lab. for Statistical Analysis: **Yoichiro Kamatani**
- Lab. for Pharmacogenomics: **Taisei Mushiroda**
- Lab. for International Alliance on Genomic Research: **Taisei Mushiroda**
- Lab. for Cardiovascular Diseases: **Kaoru Ito**
- Lab. for Autoimmune Diseases: **Kazuhiko Yamamoto**
- Lab. for Digestive Diseases: **Kazuaki Chayama**
- Lab. for Bone and Joint Diseases: **Shiro Ikegawa**
- Lab. for Endocrinology, Metabolism and Kidney Diseases: **Momoko Horikoshi**
- Lab. for Respiratory and Allergic Diseases: **Mayumi Tamari**

Program for Medical Innovations

- Lab. for Immune Regulation: **Masaru Taniguchi**
- Lab. for Immunotherapy: **Shin-ichiro Fujii**
- Drug Discovery Antibody Platform Unit: **Toshitada Takemori**

Young Chief Investigator Program

- YCI Laboratory for Immune Regeneration: **Tomokatsu Ikawa**
- YCI Laboratory for Cellular Bioenergetic Network: **Toshimori Kitami**
- YCI Laboratory for Trans-omics: **Katsuyuki Yugi**
- YCI Laboratory for Immunological Transcriptomics: **Hideyuki Yoshida**

Contents

Part 1
Research Highlights

Part 2
Lab Activities

Part 3
Research Projects

Part 4
Events

Part 5
Data and Statistics

Director's Report

This is the fourth annual report of the RIKEN Center for Integrative Medical Sciences (IMS), and one and half years have been passed since I became IMS Director.

IMS held its second Advisory Council Meeting (IMAC) in September, 2016, and RIKEN's Advisory Council (RAC) met in December. It was my first experience to attend these meetings, but I was very much encouraged because in both meetings, IMS was characterized as one of the key pillars of RIKEN's medical and life science research.

IMS has been making tremendous contributions to RIKEN's multidisciplinary projects: for example, Aging Project, Epigenetics Project, Medical Innovation Hub Program, and Single Cell Project. In addition, IMS has started a discussion with the Division of Genomic Technologies, led by Dr. Piero Carninci, about our future collaborations and common visions.

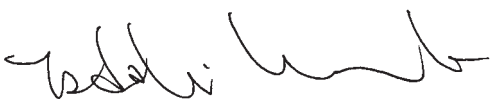
Our body constantly attempts to maintain its internal stability, but environmental and genetic factors can disrupt this homeostasis. When the body responds to environmental changes, the result is always changes in gene expression. Thus, we hope to understand how genetic and environmental factors regulate our body homeostasis and diseases, and thereby we will contribute to human health. To succeed in this aim, it is necessary to collect data in multiple layers and then to integrate them to understand and simulate the whole system. I believe that IMS's expertise in immunology and genomics, together with DGT's expertise in translational regulation, will facilitate elucidation of these mechanisms and ultimately result in translation of this knowledge into practical applications to promote our health.

The IMAC, chaired by Dr. Max Cooper of Emory University USA, encouraged IMS researchers to integrate genomic and biological science at IMS, although their academic cultures are quite different. The former thrives on team science to answer large scale questions, but on the other hand, the latter thrives on individual/small group science to investigate basic issues that may have clinical applications. The IMAC felt that the two groups could synergize in a very powerful fashion. The comments from the IMAC stimulated IMS researchers very much, regardless of their field of specialization.

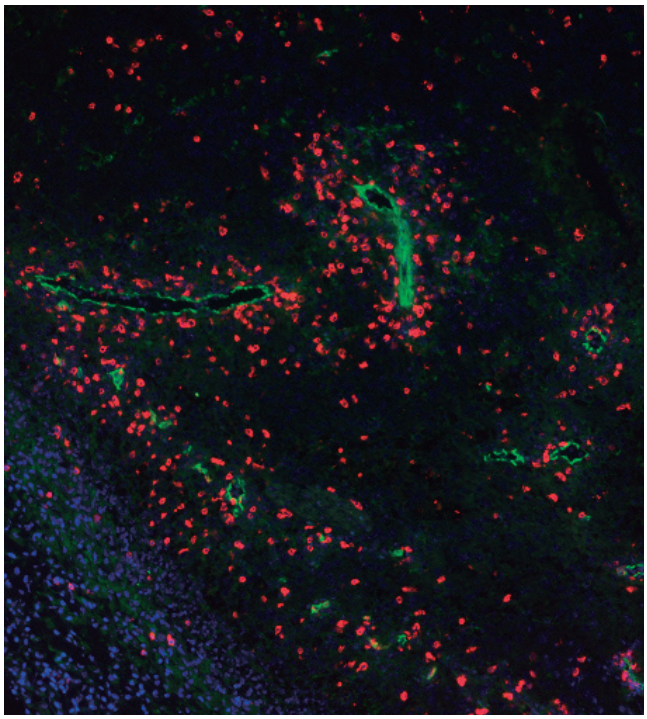
In 2016 after the AC, IMS newly started an internal discussion group and a seminar series, PI Club and Researcher Seminar, to facilitate the synergy between IMS laboratories. At the PI Club, timely research topics at IMS are introduced and PIs discuss the directions of the research or possibilities for internal collaboration. At the Researcher Seminar, two selected IMS researchers give presentations about their projects. Also, personally, I started to have lunch with several young researchers, which I enjoy very much. I feel that gradually people are starting to recognize the Center's direction and are becoming enthusiastic about contributing to it.

IMS continued to publish papers in significant journals in 2016. Hidewaki Nakagawa reported whole genome sequencing of 300 liver cancers in Japan and discovered 25 genes repeatedly mutated in different patients (*Nature Genetics* 2016). Masato Kubo discovered a novel mechanism in which anti-viral antibody is generated in the absence of T_{FH} cells (*Nature Immunology* 2016). Hisahiro Yoshida published a paper (*Journal of Clinical Investigation* 201) culminating his 15 years of work. He used a pioneering integrative approach to collect data from multiple layers, resulting from a collaboration between the former RIKEN GSC and RCAI, and university hospitals. There were 289 papers published from IMS in 2016.

I believe that our unique approach will pioneer a new area of life sciences, and hope that our challenges will be strongly supported by RIKEN and the government.



Tadashi Yamamoto
Director,
RIKEN Center for Integrative Medical Sciences



Part 1

Research Highlights

Liver cancer's genomic landscape

A massive DNA sequencing effort has exposed the mutations behind liver cancer in Japanese patients

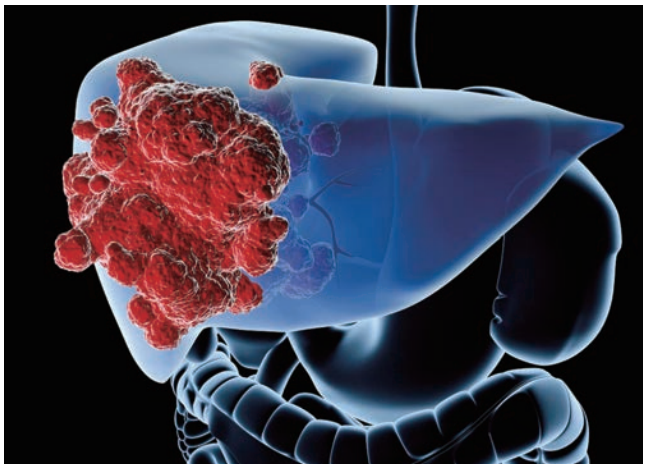


Figure: Liver tumors (like the one shown in red here) are major killers. A new study has shown that liver cancer can be classified into six different types.

In one of the largest ever genomic studies of a single-organ cancer, scientists in Japan have discovered a multitude of mutations responsible for causing liver cancer. The research, led by a team from the RIKEN Center for Integrative Medical Sciences, could help doctors develop personalized drug regimens tailored to each patient's genetic signature.

"Whole-genome sequencing revealed that about 40% of liver cancers had a mutation in genes related to therapeutic targets and that are hence expected to be actionable," says Hidewaki Nakagawa, senior author of the study.

The fifth most common cancer and the fourth leading cause of cancer-related deaths in Japan, liver cancer was responsible for an estimated 19,000 deaths in the country last year alone. Chronic infection with hepatitis B and C viruses can often lead to liver cancer, but so can alcohol abuse, metabolic diseases and certain environmental toxins, suggesting that many different genetic drivers may interact with lifestyle factors to bring about liver tumors.

In search of mutations that could explain the diversity of the disease, Nakagawa and his colleagues sequenced the entire genomes of liver cancers from 300 Japanese patients. That much sequencing yielded more than 300 terabytes of data, and the analysis required using the supercomputer SHIROKANE—the fastest in the life-science research sector in Japan.

The researchers discovered 25 genes that were repeatedly mutated in different patients' cancer samples, causing changes in tumor suppressors or other regulatory proteins. They also found a number of recurrently mutated sites in the genome that did not code for a protein, as well as structural rearrangements that popped up time and again with an effect on expression of nearby genes.

Looking across the mutational landscape, Nakagawa and his team found that liver cancer among Japanese patients could be broken down into six types—and that these types were strongly linked to survival outcomes.

"Our study revealed that the prognosis of patients with mutations in some genes is worse than in others," says Akihiro Fujimoto, who worked on the research at RIKEN before moving to the Kyoto University Graduate School of Medicine. "Although validation with an independent cohort is required, this result can contribute to predict patient prognosis in the future."

Knowing the different liver cancer types and the mutations that underlie them could also help scientists develop new, precision drug treatments. "Our analysis identified novel driver gene candidates, which can be targets for therapies," says Fujimoto.

This article was reproduced from RIKEN Research http://www.riken.jp/en/research/rikenresearch/highlights/8220/

Original Paper:
Fujimoto, A., Furuta, M., Totoki, Y., Tsunoda, T., Kato, M., Shiraishi, Y., Tanaka, H., Taniguchi, H., Kawakami, Y., Ueno, M. et al. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. ***Nat Genet*** 48, 500–509 (2016)

Mutational signatures mark cancer's smoking gun

A study of cancer genome sequences identifies telltale mutational signatures associated with smoking tobacco.

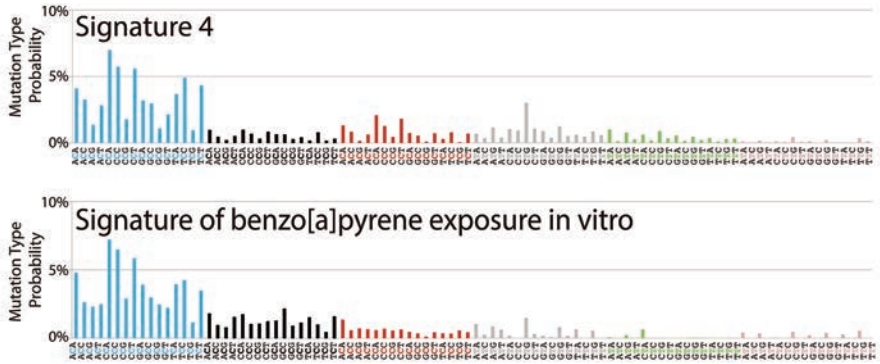


Figure: Signature 4, extracted from cancer associated with tobacco smoking, is very similar to the mutational signature induced *in vitro* by exposing cells to benzo[α]pyrene. Signature 4 was detected at significantly higher level in lung cancers, larynx cancers and (Japanese) liver cancers of smokers.

A broad computational study of cancer genome sequences identifies telltale mutational signatures associated with smoking tobacco and demonstrates, for the first time, that smoking increases cancer risk by causing somatic mutations in tissues directly and indirectly exposed to tobacco smoke. The international study conducted by researchers representing 16 institutions in the US, Europe, and Asia, including Los Alamos National Laboratory, Wellcome Trust Sanger Institute and RIKEN Center for Integrative Medicine, was published in the November 4 issue of *Science*.

Previous large-scale epidemiological studies have associated tobacco smoking with increased risk for 17 different types of cancer, including cancer in tissues not directly exposed to smoke. However, the mechanisms by which tobacco smoke causes cancer have previously remained elusive. This study demonstrates that smoking increases cancer risk by causing somatic mutations that both directly damage DNA and increase the speed of an endogenous molecular clock.

"This research brings together Big Data generated by international cancer consortia and supercomputing and machine-learning capabilities to address one of the leading public health issues of our time," said Dr. Charlie McMillan of Los Alamos. "The work leverages pattern-recognition software in genomic screening and represents a creative breakthrough in cancer research."

The study focused on identifying the mutation signatures and DNA methylation changes in 5,243 genome sequences of smoking-related cancers by comparing the cancers of smokers to those of non-smokers. All mutations–harmless or cancer-promoting–are due to the activity of endogenous or exogenous mutation processes, each one of which leaves a signature of scrambled DNA code on the base pairs of that cell's genome. The new study found more than 20 mutational signatures across the 17 cancer types associated with tobacco smoking. However, only five of these signatures were elevated in cancers from smokers. Some cancer types had only a single mutational signature elevated in smokers, while others had multiple.

One signature, called signature 4, can be traced to DNA being damaged by direct exposure to tobacco smoke and it was detected at significantly higher level in the smokers' lung cancers, larynx cancers and (Japanese) liver cancers, interestingly. Signature 4 is likely the direct mutational consequence of misreplication of DNA damage induced by tobacco carcinogens, particularly benzo[α]pyrene, according to the study. Signature 5, found by previous research to occur in all cells and to trigger mutations with clock-like regularity, also correlated with increased mutations in smokers versus non-smokers. Dr. Ludmil Alexandrov of Los Alamos explains that smoking accelerates the clock function, mostly likely by altering the molecular machinery underlying this signature.

The study modeled the cancer mutational processes as a blind-source-separation problem to distinguish coherent signals from a noisy background, a methodology used in other areas of Los Alamos research related to its nuclear-security program, taking advantage of high-performance computing resources and expertise, as well as expertise in numerical optimization problems.

This article was redacted from Los Alamos National Laboratory Press Release http://www.lanl.gov/discover/news-release-archive/2016/November/11.03-mutational-signtres-mark-cancers-smoking-gun.php

Original paper:
Alexandrov LB, Ju YS, Haase K, Van Loo P, Martincorena I, Nik-Zainal S, Totoki Y, Fujimoto A, Nakagawa H, Shibata T, Campbell PJ, Vineis P, Phillips DH, Stratton MR. Mutational signatures associated with tobacco smoking in human cancer. ***Science*** 354, 618–622 (2016)

New anti-cancer strategy mobilizes both innate and adaptive immune responses

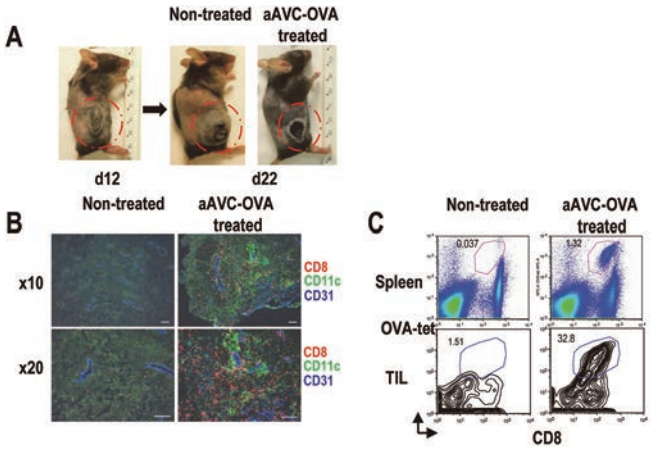


Figure: The antitumor effect and the mechanism of aAVC
A. C57BL/6 mice were injected with OVA-expressing B16 melanoma cells s.c. and then treated with or without aAVC-OVA on day 12. Images are representative of pre- (d12) and post-aAVC-OVA treatment. B. The infiltration of $CD8^+$ T cells into the tumor was analyzed by immunohistochemistry. Scale bar, 100 µm. Closely-aggregated structures composed of $CD11c^+$ DCs (green) and antigen-specific $CD8^+$ T cells (red) around the blood vessels (blue) were seen in aAVC-OVA treated tumor sites. C. The frequency of OVA antigen-specific $CD8^+$ T cells in the spleen and tumor (TIL) in the untreated or aAVC-OVA treated, tumor-bearing mice was analyzed using OVA-tetramer binding. The frequency of OVA-specific $CD8^+$ T cells in both tumor (TIL) and spleen was higher in aAVC-OVA treated mice, and in the treated mice was much higher in the tumor, the site of tumor destruction, than in the spleen (30% vs 1% respectively).

Scientists from the RIKEN Center for Integrative Medical Sciences have developed a new vaccine that involves injecting cells that have been modified so that they can stimulate both an innate immune response and the more specific adaptive response to the tumor. This approach allows the body to develop "memory" of the original cancer and then attack any new tumor cells as they form or spread. In the study published in *Cancer Research*, the RIKEN scientists found that the vaccine made it possible for killer $CD8^+$ T-cells—important players in the immune response against cancer—to enter the tumor and target cancerous cells.

According to Shin-ichiro Fujii, leader of the Laboratory for Immunotherapy, who led the study, "Cancer cells each have different sensitivities to the innate and adaptive response, so it is important to target both responses in order to eradicate them. We have developed a special type of modified cell-based vaccine, called aAVC, which we found can do this."

The aAVC are not taken from the subject's own body but are foreign cells, which are modified by adding a natural killer T cell ligand, which permits them to stimulate innate-like natural killer T cells (NKT), along with an antigen associated with the particular cancer. The group found that when NKT cells are activated, they in turn promote the maturation of dendritic cells, which act as coordinators of the innate and acquired responses. Dendritic cells are key because they allow the activation of immune memory, where the body remembers and responds to a threat even years later.

To find out whether the aAVC actually worked in the body, they conducted experiments in mice inoculated with a virulent form of melanoma that also expresses a model antigen called OVA. Tests in mice showed that aggressive tumors could be shrunken by vaccinating the animals with aAVC cells that were programmed to carry OVA antigen. Following this treatment, the tumors in the treated animals were smaller and necrotic in the interior—a sign that the tumor was being attacked by killer $CD8^+$T-cells.

Fujii continues, "We were interested in discovering a mechanism, and found that the aAVC treatment led to the development of blood vessels in the tumors that expressed a pair of important adhesion molecules, ICAM-1 and VCAM-1, which are not normally expressed in tumors. This allowed the killer $CD8^+$T cells to penetrate into the tumor."

Importantly, they also found that in animals that had undergone the treatment, cancer cells injected even a year later were eliminated. "This indicates," says Fujii, "that we have successfully created an immune memory that remembers the tumor and attacks it even much later."

Looking to the future, Fujii says, "Our therapy with aAVC is promising because typical current immunotherapies have to be tailor-made with the patient's own cells. In our case we use foreign cells, which can be produced in a uniform quality and used in any patient. Because we found that our treatment can lead to the maturation of dendritic cells, immunotherapy can move from local treatment to more systemic treatment based on immune memory."

This article was reproduced from
http://www.riken.jp/en/pr/press/2016/20160701_2/RIKEN Press Release

Original article:
Shimizu K, Yamasaki S, Shinga J, Sato Y, Watanabe T, Ohara O, Kuzushima K, Yagita H, Komuro Y, Asakura M, Fujii S. Systemic DC activation modulates the tumor microenvironment and shapes the long-lived tumor-specific memory mediated by $CD8^+$ T cells. ***Cancer Res*** 76, 3756–3766 (2016)

Hyperactivation of JAK1 tyrosine kinase induces atopic dermatitis

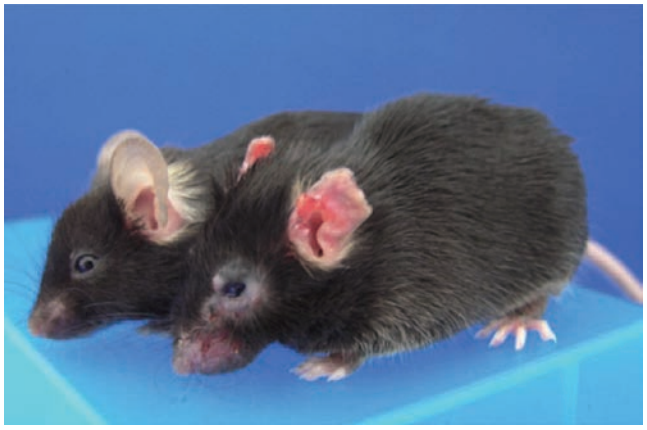


Figure: Spade mutant (right) and a wildtype littermate (left) at 20 weeks of age.

Atopic dermatitis (AD) is an inflammatory skin disease that primarily affects children. AD can be triggered by environmental factors such as stress, detergents, weather, and food allergies, but genetic factors also contribute to AD pathogenesis. Hisahiro Yoshida and his team at RIKEN IMS discovered that JAK1 hyperactivation affects the skin barrier system and induces atopic dermatitis.

Yoshida's team worked on phenotype screening of *N*-ethyl *N*-nitrosourea (ENU)-induced mutant mice to find genetically-based allergic diseases. Among 3,000 mice examined, they found an interesting line where the mice showed continuous scratching and eventually developed dermatitis. The disease onset was from 8 to 12 weeks after birth and, interestingly, serum IgE and IgG1 elevation and serum histamine elevation were not detected until about three weeks after the dermatitis onset. Because of those characteristics, they named this mutant "Stepwise progressive atopic dermatitis (Spade)."

By genetic mapping of the *Spade* mutant, they found a point mutation in the coding region of the *Jak1* gene, which encodes a tyrosine kinase used in signaling through many cytokine receptors. This amino acid change caused hyperactivation of JAK1, therefore, Yoshida decided to test whether treating the mice with a JAK inhibitor suppressed dermatitis. As they predicted, painting JAK inhibitors on the skin delayed the onset of dermatitis.

Because Jak1 is a well-known signal transduction molecule for various cytokine receptors and strongly affects immune cells, they decided to assess the role of hematopoietic cells in the dermatitis. When *Spade* mouse bone marrow was transferred into a normal mouse, the mouse did not develop dermatitis, suggesting that non-hematopoietic/immune cells contribute to the dermatitis. In fact, dermatitis onset was caused by a skin barrier dysfunction. When they applied petrolatum to the *Spade* mouse skin, dermatitis was prevented for more than a month.

"The result was surprising to us. Some immunologists said that this mutant cannot be an atopic dermatitis disease model because it develops independently of acquired immune function. However, many dermatologists encouraged us, saying that such a phenotype sounded similar to juvenile type atopic dermatitis development in human," Yoshida commented. "In particular, the human patients usually have a defect in skin barrier function, and the disease starts with itchy dermatitis independent of serum IgE or histamine elevation."

To test whether JAK1 hyperactivation is also seen in human atopic dermatitis, they examined six independent atopic dermatitis skin biopsies and detected increased JAK1 activation in four of them.

"The results indicate that signal transduction by JAK1 plays an important role in the skin barrier and this can be modified by environmental conditions. We hope that the *Spade* mouse will be a good model to investigate human atopic dermatitis and to better understand the disease onset," said Yoshida.

Original paper:
Yasuda T, Fukada T, Nishida K, Nakayama M, Matsuda M, Miura I, Dainichi T, Fukuda S, Kabashima K, Nakaoka S, Bin BH, Kubo M, Ohno H, Hasegawa T, Ohara O, Koseki H, Wakana S, Yoshida H. Hyperactivation of JAK1 tyrosine kinase induces stepwise, progressive pruritic dermatitis. ***J Clin Invest.*** 2016;126 (6):2064–76.

Immune system overreaction may trigger eczema into becoming a chronic disease

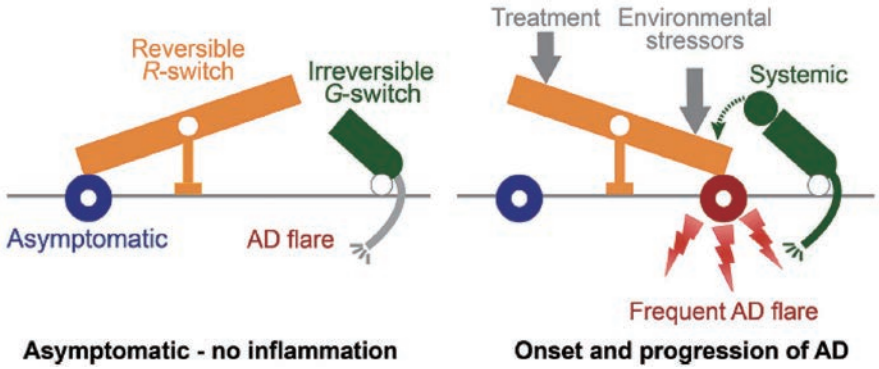


Figure: Double-switch mechanism for the onset and progression of AD

Atopic dermatitis (AD) is a medical condition primarily affecting children, where patches of skin become rough and inflamed with blisters, causing itching and bleeding. It is a chronic condition triggered by a combination of factors such as stress, detergents, weather, allergies to certain foods, and genetic factors also play a role.

Now, bioengineers from Imperial College London, RIKEN Center for Integrative Medical Sciences and Trinity College Dublin have collated data from previous studies on AD and developed a mathematical model that suggests how AD may progress to become chronic. The team's model showed that repeated flare-ups of AD trigger an immune system overreaction, which, once triggered, cannot be reversed. This creates a cycle where the threshold for triggering further AD outbreaks becomes lower, the flare-ups are more severe, and the condition progresses to becoming long-term. Severe flare-ups happen as a result of the complex interactions between the body's immune system, the skin's protective barrier, and environmental factors such as stress.

The team examined approximately 500 clinical and experimental studies, and carried out research involving mice, to develop their mathematical model, which modelled four different types of AD, ranging from no symptoms to severe flare-ups. They also looked at how genetic factors affect the skin and the immune system.

The researchers stress that theirs is a general model of AD. However, they believe it suggests that preventing an immune system overreaction from being triggered in the first place may be the key to preventing AD from becoming more severe.

Two recent clinical trials demonstrated that babies who received moisturizing treatments, known as emollients, which were applied directly to the skin to reduce water loss and cover it with a protective film, were less like to develop AD. The authors of this study also explained the possible mechanism behind these observed preventive effects. The emollients provide an effective barrier, preventing the itching and scratching cycle that can progress the condition further. They also demonstrated that these preventive effects are applicable not only for babies with genetic markers that indicate a predisposition to AD, but also for those without genetic markers.

Dr. Reiko Tanaka at Imperial College London, said: "Our mathematical modeling is helping us to make a clearer connection between a systemic reaction in AD and its progression. More work needs to be done to verify our results in patients. However, we think a crucial preventative measure may be the use of ointments on all babies early on. It doesn't matter which moisturizing treatment is used as long as a barrier on the skin is created, which may stop the AD cycle from triggering a systemic reaction."

This approach of using mathematical models to understand the underlying causes of diseases or conditions is called systems medicine. Dr. Tanaka and her colleagues now hope to develop a model that can be tailored to each patient based on their individual clinical data. This could enable doctors to tailor each treatment to the specific needs of the patients, optimizing its effectiveness and thus lessening the severity and impact of the condition.

This article was redacted from Imperial College London Press Release
http://www3.imperial.ac.uk/newsandeventspggrp/imperialcollege/newssummary/news_24-11-2016-15-14-54

Original paper:
Domínguez-Hüttinger E, Okada-Hatakeyama M, Kubo M, Tanaka RJ et al. Mathematical modeling of atopic dermatitis reveals "double switch" mechanisms underlying four common disease phenotypes. ***J Allergy Clin Immunol*** S0091–6749(16)31433–6 (2016)

New mechanism of antiviral protection

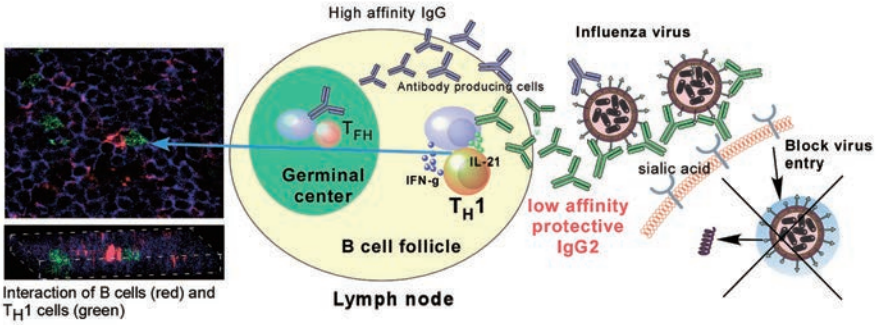


Figure: Antiviral protection by IgG2 antibody.

Influenza pandemics have become a serious public health concern in recent history and vaccination is critical for preventing the spread of the influenza virus in the population. However, there is no vaccine that offers adequate protection against emerging flu viruses. Thus, a better understanding of the mechanism of neutralizing antibody induction during influenza infection is essential to develop alternative vaccines against emerging pandemic influenza.

It has been long believed that generation of high-affinity neutralizing antibody is critical for antiviral humoral immunity. For the production of high-affinity antibodies, formation of germinal centers (GC) in lymph nodes and the presence of a subset of T cells called follicular helper T (TFH) cells in the GC are essential. Because of this, efficient activation of TFH cells in GC was thought to be an essential strategy for the development of antiviral vaccines.

However, a research team led by Masato Kubo of the RIKEN Center for Integrative Medical Sciences discovered a novel pathway that regulates induction of neutralizing antibodies independently of TFH cells and GC.

"Generation of not only TFH but also helper T1 (TH1) cells has been reported in the mouse model of acute influenza infection. So we decided to investigate the role of TH1 cells in protection against influenza virus," says Kubo. They used genetically modified mice lacking TFH cells or GC. To their surprise, after vaccination these mutant mice were still protected from a lethal dose of pandemic H1N1 or highly pathogenic H5N1 influenza A virus. The production of high-affinity IgG1 antibody was massively reduced as a consequence of the loss of TFH cells and GC, however, the function of TH1 cells remained normal, and there was no effect on the production of IgG2 antibody. Although the avidity of the IgG2 antibody was reduced in the TFH/GC-deficient mice, its neutralizing ability was not affected.

To confirm the role of TH1 cells in the production of IgG2 antibody, Kubo and colleagues isolated TH1 cells from the vaccinated mice and transferred them into genetically engineered mice that have a defect in IgG antibody production due to the lack of helper T cells. Mice with transferred TH1 cells generated protective anti-viral IgG2 antibodies after influenza virus infection.

"Activation of TH1 cells resulted in production of cytokines such as IFN-γ and IL-21, and we found that these cytokines were essential for production of IgG2 antibodies. The IgG2 antibodies induced by TH1 cells have high neutralizing activity although their affinity is low. This insight should contribute to new strategies to develop novel types of vaccines against newly emerging influenza viruses" commented Kubo.

Original paper:
Miyauchi K, Sugimoto-Ishige A, Harada Y, Adachi Y, Usami Y, Kaji T, Inoue K, Hasegawa H, Watanabe T, Hijikata A, Fukuyama S, Maemura T, Okada-Hatakeyama M, Ohara O, Kawaoka Y, Takahashi Y, Takemori T, Kubo M. Protective neutralizing influenza antibody response in the absence of T follicular helper cells. ***Nat Immunol*** 17, 1447–1458 (2016)

Mechanism of memory B cell differentiation

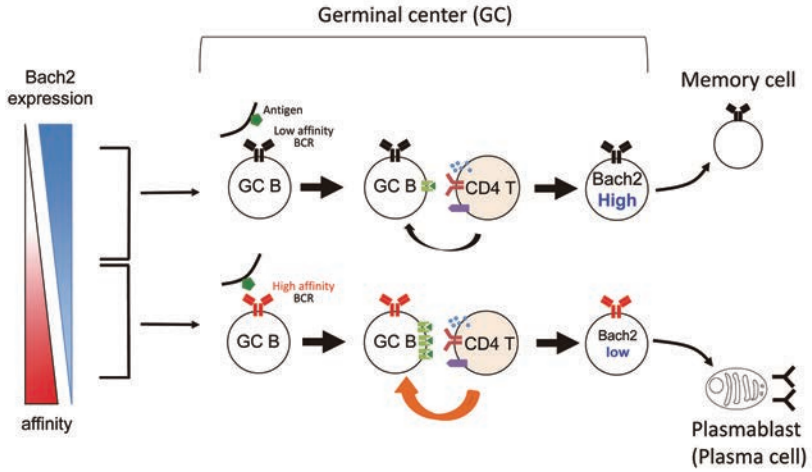


Figure: Schematic view of memory B cell and plasma cell development from GC B cells
The affinity of the B cell receptor for antigen and the expression level of Bach2 are inversely correlated in germinal center B cells. Low affinity germinal center B cells, which receive weaker stimulation from antigen and antigen-specific CD4 T cells, maintain a relatively high level of Bach2 expression and tend to be induced to form memory B cells in the germinal center. On the other hand, high affinity cells, which receive strong stimulation from antigen and antigen-specific CD4 T cells, have difficulty maintaining Bach2 expression at high levels and tend to be induced to form plasmablasts (plasma cells).

When our body encounters a pathogen during an infection, our immune system remembers it even after the infection has cleared. If we are re-exposed to the same infection, a robust antibody response is quickly induced to get rid of the pathogen. The "memory" B cell is the main player in remembering pathogens and inducing faster protection from re-infection, however, it has been unclear how memory B cells are generated and selected during the first infection.

Ryo Shinnakasu and Tomohiro Kurosaki of RIKEN Center for Integrative Medical Sciences and IFReC, Osaka University, published a paper in *Nature Immunology* showing that memory B cells are predominantly generated during the early phase of the germinal center (GC) response after immunization. This was a surprising finding since it had been thought that these cells arose later.

To understand the mechanism behind the memory B cell induction, they first generated a transgenic mouse in which they could irreversibly mark B cells in the GC, the site where the B cells are responding to antigen, by administration of a drug called tamoxifen. After immunizing the mice, they administered tamoxifen for three days starting at different time points, days 5–7, 9–11, 19–21, and 29–31. This allowed them to analyze the generation of GC B cells and memory B cells in a time-dependent manner. The results clearly indicated that memory B cells were generated mainly at the early stage, 9-11 days after immunization.

"Traditionally, it was believed that memory B cells induced late in the immune response have an advantage because they are derived from B cells with high-affinity for the antigen," said Kurosaki. "Therefore, we analyzed the affinity maturation of GC B cells and memory B cells 20 days after immunization. To our surprise, the results indicated that low-affinity GC B cells are preferentially selected to become memory B cells."

They further found that high expression of the transcription factor Bach2 in GC B cells is important for memory B cell differentiation. "Our findings have the potential to change current vaccine strategies," said Kurosaki. "Bach2 might be a novel target for the development of new vaccines that induce efficient differentiation of memory B cells from low-affinity B cells."

Original paper:
Shinnakasu R, Inoue T, Kometani K, Moriyama S, Adachi Y, Nakayama M, Takahashi Y, Fukuyama H, Okada T, Kurosaki T. Regulated selection of germinal-center cells into the memory B cell compartment. ***Nat Immunol*** 17, 861–869 (2016)

GWAS identifies seven novel loci for type 2 diabetes

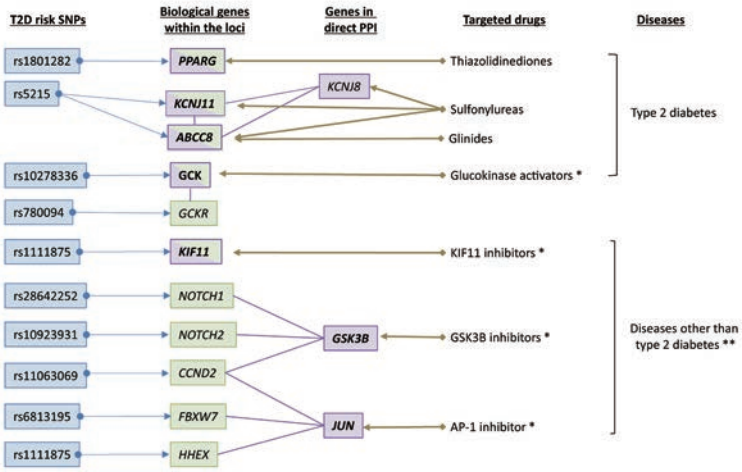


Figure: Connection of type 2 diabetes risk genes to drug targets. PPI, protein-protein interactions.

Diabetes is one of the largest global health problems. There are currently an estimated 415 million adults in the world with diabetes, and this figure will reach 642 million by 2040. The Ministry of Health, Labour and Welfare estimates that more than 9.5 million Japanese are already diabetic or pre-diabetic, and 90% of them are affected by Type 2 diabetes due to insulin resistance and/or insufficient insulin secretion.

Genome-wide association studies (GWAS) have identified more than 80 susceptibility loci for type 2 diabetes, but most of its heritability still remains to be elucidated. The relative importance of the causative mechanisms appears to differ between Eastern and Western populations. Accumulating clinical evidence suggests that defective insulin secretion contributes more to the pathogenesis of Japanese type 2 diabetes, whereas insulin resistance seems more important for European type 2 diabetes. Thus, it is important to know the genetic differences in diabetes pathogenesis in Japanese and Europeans.

Minako Imamura and Shiro Maeda at RIKEN Center for Integrative Medical Sciences, in collaboration with Takashi Kadowaki and Toshimasa Yamauchi at Tokyo University Hospital, analyzed DNA samples of 15,463 Japanese type 2 diabetes patients and 26,183 controls for 5.8 million SNPs. "This was the largest sample size ever for GWAS studies of Japanese type 2 diabetes," says Imamura. They subjected 17 candidate SNPs identified in this GWAS to a second round of analysis, using a cohort of 7,936 patients and 5,536 controls. Based on these data, they identified seven novel loci (*CCDC85A, FAM60A, DMRTA1, ASB3, ATP8B2, MIR4686,* and *INAFM2*) with a statistically significant linkage to type 2 diabetes.

They next analyzed the association of these seven loci with disease susceptibility in populations other than Japanese. When they examined the association of these loci with type 2 diabetes on 65,936 patients and 158,030 controls from East Asian, South Asian, European and Mexican populations, they found that five of the seven loci were also linked to type 2 diabetes susceptibility in these non-Japanese populations.

To search for potential drug targets for type 2 diabetes, they investigated 752 genes, 40 genes which lie on the 90 risk loci (the 7 new loci identified in this study and 83 previously identified loci) and 712 genes which have direct protein-protein interaction with the products of the 40 genes, that overlap with drug target genes corresponding to approved, clinical trial stage or experimental drugs for various human diseases. Among the overlapping genes, indeed, three genes that are targets for already-approved drugs widely used for the treatment of type 2 diabetes. In addition, they found drug targets that are under clinical trials: *KIF11* for treatment of cancer, *GSK3B* for leukemia and *JUN* for rheumatoid arthritis; these drugs may be useful for the treatment of type 2 diabetes. "We could propose several new potential drug targets for type 2 diabetes treatment using a systematic bioinformatics approach. GWAS is still useful to identify novel susceptibility loci and, by integrating the findings with other genetic, biological and pharmacological studies, we hope to develop new treatments for type 2 diabetes," says Imamura.

Original paper:
Imamura M, Takahashi A, Yamauchi T, Hara K, Yasuda K, Grarup N, Zhao W, Wang X, Huerta-Chagoya A, Hu C, Moon S, Long J, Kwak SH, Rasheed A, Saxena R, Ma RC, Okada Y, Iwata M, Hosoe J, Shojima N, Iwasaki M, Fujita H, Suzuki K, Danesh J, Jørgensen T, Jørgensen ME, Witte DR, Brandslund I, Christensen C, Hansen T, Mercader JM, Flannick J, Moreno-Macías H, Burtt NP, Zhang R, Kim YJ, Zheng W, Singh JR, Tam CH, Hirose H, Maegawa H, Ito C, Kaku K, Watada H, Tanaka Y, Tobe K, Kawamori R, Kubo M, Cho YS, Chan JC, Sanghera D, Frossard P, Park KS, Shu XO, Kim BJ, Florez JC, Tusié-Luna T, Jia W, Tai ES, Pedersen O, Saleheen D, Maeda S, Kadowaki T. Genome-wide association studies in the Japanese population identify seven novel loci for type 2 diabetes. ***Nat Commun*** 7, 10531 (2016)

Sending bone growth down the spine

Genetic variant causes unwanted bone growth in a spinal disorder common among Asians

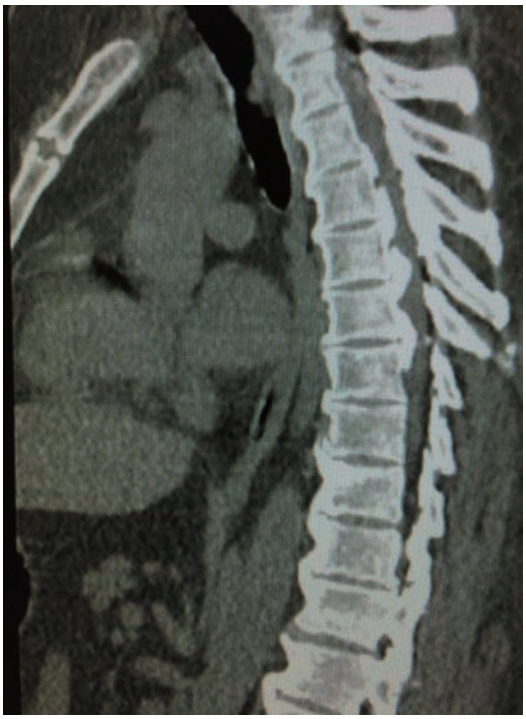


Figure: Patients with the spinal disorder ossification of the posterior longitudinal ligament (OPLL) of the spine have aberrant bone growth in the spine.

For the first time, RIKEN researchers have shown how a genetic variant increases the risk of a spinal disorder prevalent throughout Asia.

Ossification of the posterior longitudinal ligament of the spine (OPLL) is a degenerative disease that afflicts more than 2% of people in Japan. It results when bone starts developing in the soft tissue of the spinal cord, leading to nerve compression, pain and numbness—debilitating symptoms that can severely affect daily life.

Over the years, scientists have found several gene variants that occur more often in people with OPLL, but it was not known if or how any of these genes directly contributed to the patients' aberrant bone growth. Now a team led by Shiro Ikegawa at the RIKEN Center for Integrative Medical Sciences tested the role of one potential susceptibility gene, known as *RSPO2*.

The team previously conducted a comprehensive gene sleuthing study, which found six sites in the genome that seemed to confer OPLL disease risk. One of these genomic segments included the gene *RSPO2*, which carries the instructions for making a protein called R-spondin 2. The protein was known to play a critical role in skeletal development, but the researchers wanted to drill deeper into how RSPO2 works at a molecular level. They used mouse and human bone precursor cells to examine gene expression in a lab dish.

They showed that RSPO2 normally acts to put the brakes on bone formation by inhibiting the development of the early cartilage cells, which are later replaced by bone cells. However, the gene variant linked to OPLL decreases gene activity by changing the promoter region—the 'on switch'—of *RSPO2*. Specifically, the disease-associated variant alters the binding of a transcription factor that would otherwise allow *RSPO2* expression and thereby halt bone growth. Without this binding, stem cells in the spinal ligament get misdirected into bone.

"Our findings provide new insights into the etiology and pathogenesis of OPLL, as well as a new target for treatment of the disease," says Masahiro Nakajima, who conducted the study. If drug companies can find a way to block the pathway unleashed by low *RSPO2* expression, Nakajima explains, they could help improve the lives of people with the disease.

The team plans to investigate the role of other potential OPLL genes in search of additional drug targets. "We believe it is possible to find other causal variants and susceptibility genes using the same approach," Nakajima says.

This article was reproduced from RIKEN Research http://www.riken.jp/en/research/rikenresearch/highlights/8262/

Original article:
Nakajima M, Kou I, Ohashi H, Genetic Study Group of the Investigation Committee on the Ossification of Spinal Ligaments, Ikegawa S. Identification and functional characterization of *RSPO2* as a susceptibility gene for ossification of the posterior longitudinal ligament of the spine. ***Am J Hum Genet*** 99, 202–207 (2016)

GWAS identifies a genetic risk factor associated with clozapine-induced agranulocytosis/granulocytopenia

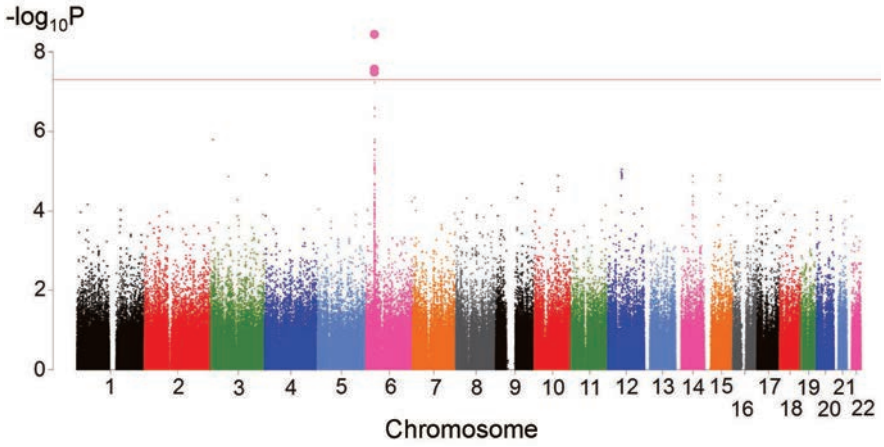


Figure: A Manhattan plot of GWAS results for CIAG in the Japanese population. The horizontal axis indicates each SNP's chromosomal location, while the vertical axis indicates the -log$_{10}$(P value) of the association. SNPs positioned higher on the plot have a more reliable association with CIAG.

Schizophrenia is a chronic and serious mental disorder that affects how a person thinks, feels and behaves. Its lifetime prevalence is 1% of the world population, and there are 700–800 thousand patients in Japan suffering from schizophrenia. The most useful therapeutic option for schizophrenia is antipsychotics; however, approximately one third of patients do not respond adequately to these medications, resulting in treatment-resistant schizophrenia.

Clozapine, a second generation "atypical" antipsychotic, is a gold standard drug for treatment-resistant schizophrenia. It has a lower incidence of movement abnormalities and its efficacy is superior to that of other atypical antipsychotics for treatment-resistant schizophrenia. Despite these advantages, clozapine may have side effects, some serious and potentially fatal, and therefore the use of clozapine is significantly restricted.

Agranulocytosis or granulocytopenia is an acute and severe leukopenia, potentially life threatening. About 1–3% of people who take clozapine develop the side effect of clozapine-induced agranulocytosis (CIA)/granulocytopenia (CIG) (CIAG). Several pharmacogenetic/pharmacogenomic studies in European populations suggested that certain genetic polymorphisms in the MHC (HLA) region may be associated with CIAG.

Taisei Mushiroda of the RIKEN Center for Integrative Medical Sciences, in collaboration with researchers at Fujita Health University and Osaka University, analyzed 900 thousand SNPs in genomic DNA samples from 50 Japanese patients with CIAG and 2,905 controls. They identified four SNPs in the HLA region on chromosome 6 associated with CIAG (Figure). To determine the associated HLA allele, they further conducted HLA typing. As a result, they found that a specific allele named HLA-B*59:01 was significantly associated with CIAG in Japanese.

In addition, they explored the risk of developing CIA in clozapine rechallenge treatment. "Our results indicated that clozapine rechallenge is a possible option for patients who respond only to clozapine," say the research group. "Our explorative estimates showed that about 60% of CIG patients without the HLA-B*59:01 allele have the potential to avoid future CIA. Of course, frequent monitoring of the white blood cells and absolute neutrophil count is essential during the rechallenge."

Original paper:
Saito T, Ikeda M, Mushiroda T, Ozeki T, Kondo K, Shimasaki A, Kawase K, Hashimoto S, Yamamori H, Yasuda Y, Fujimoto M, Ohi K, Takeda M, Kamatani Y, Numata S, Ohmori T, Ueno S, Makinodan M, Nishihata Y, Kubota M, Kimura T, Kanahara N, Hashimoto N, Fujita K, Nemoto K, Fukao T, Suwa T, Noda T, Yada Y, Takaki M, Kida N, Otsuru T, Murakami M, Takahashi A, Kubo M, Hashimoto R, Iwata N. Pharmacogenomic Study of Clozapine-Induced Agranulocytosis/Granulocytopenia in a Japanese Population. ***Biol Psychiatry*** 80, 636–642 (2016)

Keeping viral DNA at bay

Scientists find that an epigenetic interplay keeps virus-derived DNA sequences repressed in embryos but not in the surrounding placenta

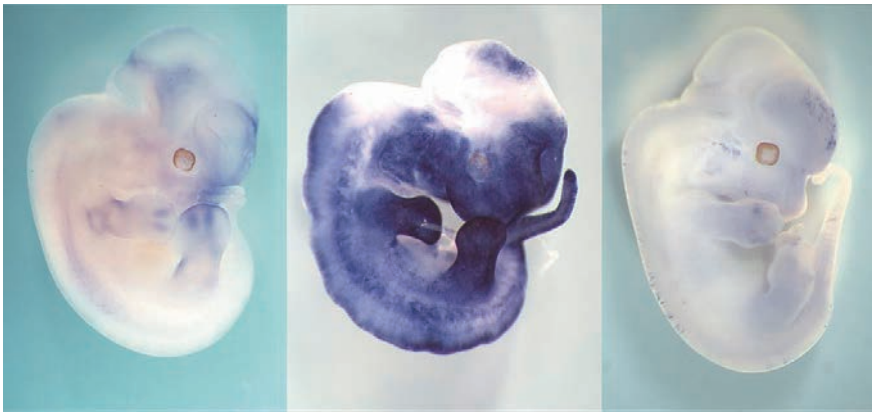


Figure: Wild-type (left), *Dnmt1*-deficient (center) and *Np95*-deficient (right) mouse embryos. Only the *Dnmt1* mutant shows activity of endogenous retroviruses, indicated by the blue stain.

The human genome is riddled with virus-derived sequences considered to be remnants of viral infections during our evolutionary past. A new study by RIKEN researchers explains how these so-called endogenous retroviruses (ERVs) are repressed in the developing embryo, but also finds that they spring into action in the cells of the placenta.

"In my field, people mostly think that ERVs are harmful and therefore should be silenced in all cells," says Jafar Sharif of the RIKEN Center for Integrative Medical Sciences, who led the study with colleagues at RIKEN and collaborators in Japan, Canada and the US. "But our work shows that ERV expression takes place even under perfectly normal conditions, such as in the placenta during development."

Sharif and his colleagues first wanted to clarify how these viral sequences are silenced in the embryonic lineage.

An enzyme called DNMT1 was known to help to inactivate these rogue genetic elements by ensuring that methyl tags are placed in the right locations on the DNA backbone to maintain proper gene expression and regulation.

When functioning DNMT1 is absent, the viral elements kick into action, which was thought to be due to a lack of DNA methylation. But that turned out to be only part of the story. The RIKEN team has shown that the partially methylated DNA wrought by depletion of DNMT1 actually attaches itself to a second protein called NP95, which triggers the regulatory cascade responsible for releasing the brakes on viral repression.

The researchers discovered that prolonged binding with NP95 disrupted the way DNA was packaged into chromatin fibers and this allowed ERVs to be expressed. They confirmed the crucial role of Np95 by removing the protein in mouse embryos, either alone or in combination with Dnmt1 removal. In both cases, the viruses remained dormant (Figure, the Dnmt1/Np95 double-deficient results are not shown).

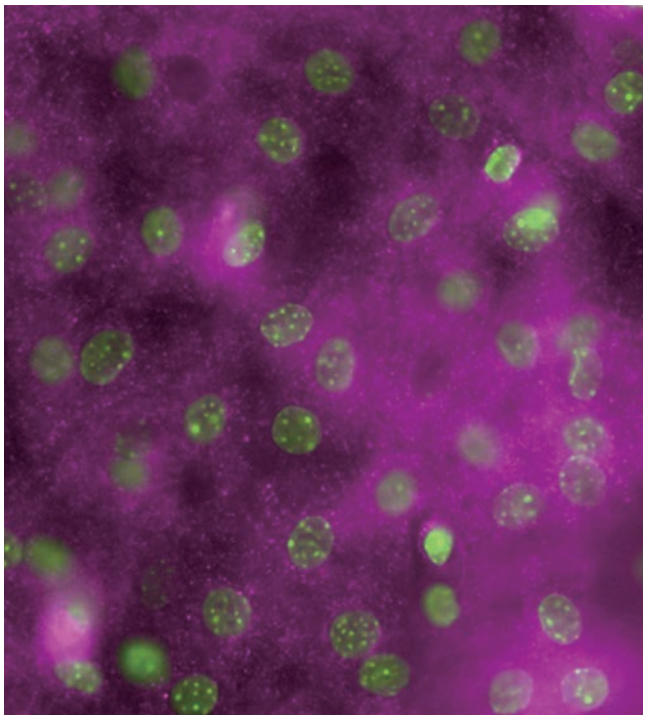
"I was very surprised," says Sharif of this unexpected finding. "In fact, at first I thought my experiments were not going well when I found deleting the *Np95* gene together with the Dnmt1 gene inexplicably extinguished the activation of ERVs."

But more surprising was that the Np95 protein and ERVs are highly expressed in placental cells.

This discrepancy between ERV activity in embryonic and extra-embryonic tissues remains a mystery. "There is still no physiological explanation for why these ERVs are expressed in the placenta," notes Sharif, who intends to find out why. "I like to think that ERV expression must have some biological meaning, or else it would not be tolerated in the placenta," Sharif says.

This article is reproduced from RIKEN Research http://www.riken.jp/en/research/rikenresearch/highlights/8230/

Original paper:
Sharif J, Endo TA, Nakayama M, Karimi MM, Shimada M, Katsuyama K, Goyal P, Brind'Amour J, Sun MA, Sun Z, Ishikura T, Mizutani-Koseki Y, Ohara O, Shinkai Y, Nakanishi M, Xie H, Lorincz MC, Koseki H.
Activation of endogenous retroviruses in *Dnmt1*$^{-/-}$ ESCs involves disruption of SETDB1-mediated repression by NP95 binding to hemimethylated DNA. ***Cell Stem Cell*** 19, 81-94 (2016)



Part 2

Lab Activities

Core for Homeostatic Regulation

The ultimate goal of the Core for Homeostatic Regulation is to elucidate the mechanisms of onset of human diseases and to create new scientific paradigms. This Core clarifies the regulation of homeostasis in individuals, focusing on their immune, metabolic and environmental response systems. In addition, the Core for Homeostatic Regulation will validate the disease models established by the Core for Precise Measuring and Modeling in a multi-tier timeframe from before to after the onset of diseases.

There are 15 laboratories in the Core for Homeostatic Regulation which are divided into four areas;

[1] Immune homeostasis

Cell signaling (T. Saito), Lymphocyte differentiation (T. Kurosaki), Immune homeostasis (S. Hori), Metabolic homeostasis (N. Kubota)

[2] Lymphocyte development

Transcriptional regulation (I. Taniuchi), Human disease models (F. Ishikawa)

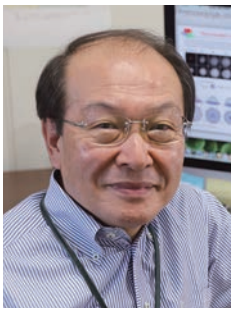
[3] Mucosal immunity

Intestinal ecosystem (H. Ohno), Mucosal immunity (S. Fagarasan), Immune cell systems (S. Koyasu), Gut homeostasis (K. Honda), Immune crosstalk (H. Cheroutre)

[4] Allergy and inflammation

Skin homeostasis (M. Amagai), Inflammatory regulation (T. Tanaka), Cytokine regulation (M. Kubo), Innate immune systems (K. Moro)

All of these areas elucidate the basic mechanisms of immune regulation at cellular tissue and systemic levels. We ultimately aim to analyze the onset of autoimmune diseases, metabolic disorders [1], primary immunodeficiency [2], inflammatory bowel disease and colitis [3], and atopic dermatitis and allergic diseases [4].



Laboratory for Cell Signaling

Group Director: Takashi Saito

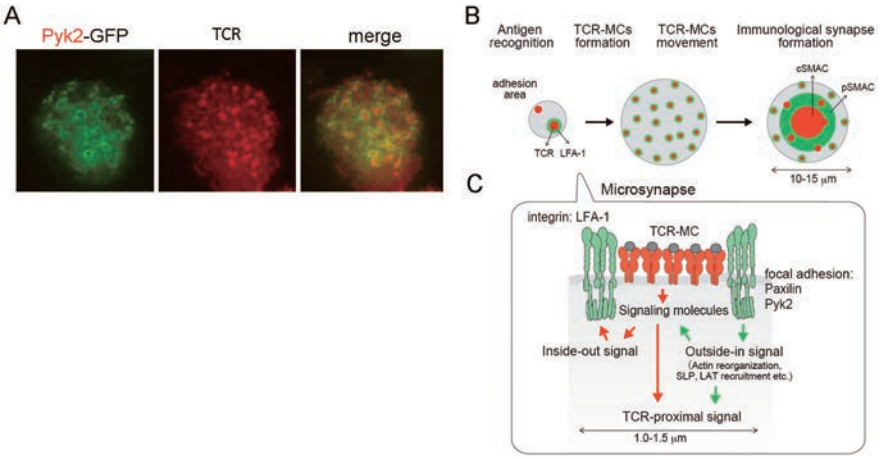


Figure: The microsynapse, composed of a micro-adhesion ring surrounding a TCR microcluster, is essential for T cell activation
[A] The Pyk2 focal adhesion molecule (green) forms a micro-adhesion ring around TCR-microclusters (red) upon T cell stimulation
[B] Schematic diagram of a microsynapse. The microsynapse, composed of a micro-adhesion ring around a TCR-microcluster, is formed during early activation and facilitates formation of a microcluster and cSMAC and in T cell activation, particularly under weak stimulation.
[C] An adhesion-ring in a microsynapse induces outside-in signals through LFA1/focal adhesion molecules, thus helping induce activation signals through TCR-microclusters.

Recent Major Publications

Ishikawa E, Kosako H, Yasuda T, Ohmuraya M, Araki K, Kurosaki T, Saito T, Yamasaki S. Protein kinase D regulates positive selection of $CD4^+$ thymocytes through phosphorylation of SHP-1. ***Nat Commun*** 7, 12756 (2016)

Hashimoto-Tane A, Sakuma M, Ike H, Yokosuka T, Kimura Y, Ohara O, Saito T. The Micro adhesion-ring surrounding each TCR microclusters forms synapse-like structure essential for T cell activation. ***J Exp Med*** 213, 1609–1625 (2016)

Takeuchi A, Badr MESG, Miyauchi K, Ishihara C, Onishi R, Guo Z, Sasaki T, Ike H, Takumi A, Tsuji NM, Murakami Y, Katakai T, Saito T. CRTAM determines the $CD4^+$ cytotoxic T lymphocyte lineage. ***J Exp Med*** 213, 123–138 (2016)

Invited Presentations

Saito T. "Single cell molecular imaging for T cell activation" The 54th Annual Meeting of the Biophysical Society of Japan (Tsukuba, Japan) November, 2016

Saito T. "Microsynapse composed of micro-adhesion ring surrounding TCR microcluster is essential for T cell activation" EMBO conference (Siena, Italy) September, 2016

Saito T, Hashimoto-Tane A. "Microsynapse composed of focal adhesion molecules surrounding TCR microcluster is essential for T cell activation" International Congress of Immunology 2016 (Melbourne, Australia) August, 2016

Saito T. "Dynamic Regulation of T cell Activation at Immune Synapse" POSTEC Symposium (Pohang, Korea) July, 2016

Saito T. "Development and function of $CD4^+$CTL in inflammation diseases" SICORP Japan-New Zealand Joint Research on Functional Foods (Wellington, New Zealand) February, 2016

T cells play central roles in immune regulation. They initiate immune responses, induce activation and generate various effector T cells, which protect against infection and oncogenesis. Aberrant T cell function results in infectious, cancer and autoimmune diseases. Our group aims to determine the molecular mechanism of T cell activation, differentiation and homeostasis, particularly from a signaling perspective.

T cell activation is induced through TCR-microclusters (MC), the signaling clusters generated by recruiting TCR and proximal signaling molecules. We found that TCR-MCs at the initial activation stage are surrounded by an adhesion-ring composed of integrin and focal adhesion molecules. This structure resembles an immune synapse in micro-scale, and is termed the microsynapse (Figure). The microsynapse supports integrin outside-in signaling, which facilitates formation of the MC and cSMAC and then T cell activation. The microsynapse is particularly important for activation under weak stimulation condition. Thus, the microsynapse and MCs seem to be the major membrane structures for inducing an activation signal in T cells.

We also investigate the regulation of T cell activation by innate signals. We have analyzed the function of TLRs and nucleic acid recognition in T cells. STING is known to be a major intracellular DNA sensor in innate cells. Since STING is highly expressed in T cells, its function in T cell activation was analyzed. Surprisingly, STING stimulation induced inhibition of T cell proliferation and anti-viral responses. Thus, STING ligands may serve as specific inhibitors of activated T cells.

The ultimate aim of our diverse approaches is to elucidate the mechanisms of inducing autoimmune diseases through aberrant T cell function in order to be able to modulate them and inhibit/prevent autoimmunity and allergic inflammation. We are analyzing the function and regulation of T cells by phosphatases (PTPN22, PTPN2), whose deficiency is associated with induction of autoimmune diseases. The onset of autoimmunity will be analyzed in gene-targeted mouse models.

Laboratory for Lymphocyte Differentiation

Group Director: Tomohiro Kurosaki

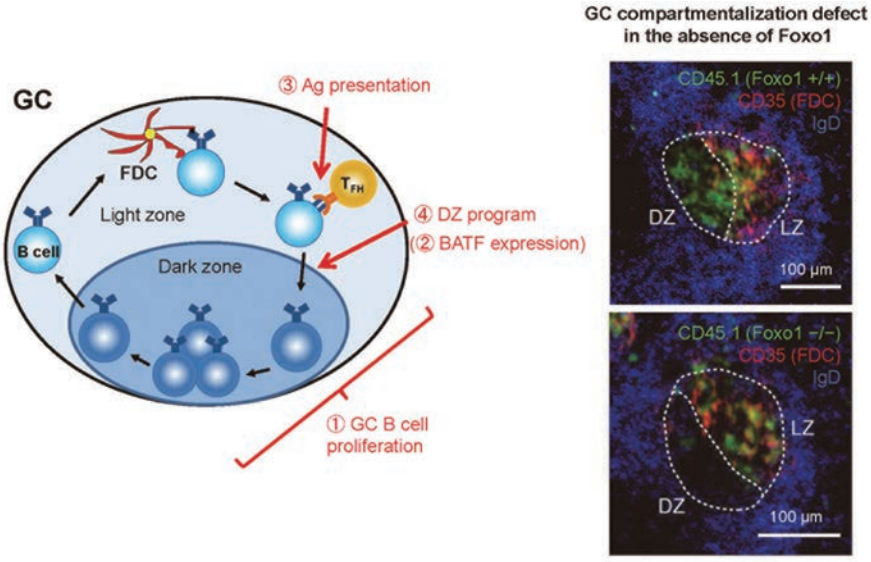


Figure: Foxo1 controls GC B cell proliferation in response to T cell help
(Left) Schematic illustration of Foxo1 function in GC B cells. Foxo1 controls GC B cell proliferation (1), partly by mediating BATF up-regulation in the LZ B cells (2). In the LZ B cells, Foxo1 is also required for antigen presentation to T_{FH} cells (3) and up-regulation of the DZ program genes in response to T cell help (4), which triggers the switch from the LZ to the DZ. (Right) Immunohistochemical analysis of spleen sections showing the GC compartmentalization defect resulting from Foxo1-ablation. Expression of CD45.1(*Foxo1*$^{+/+}$ or *Foxo1*$^{-/-}$ derived donor cells), CD35 (FDC network), and IgD (follicular B cells) are shown.

Humoral memory relies on the development of memory B cells and long-lived plasma cells, and the vast majority of these cells are derived from germinal center (GC) B cells. Therefore, our lab has been focusing on characterizing of these three cell types and clarifying how memory B and plasma cells are generated through GC reactions.

GC B cells cycle between two states, the light zone (LZ) and the dark zone (DZ) and, in the latter, they proliferate and hypemutate their immunoglobulin V genes (Igs). How this functional transition takes place is still controversial. We found that ablation of the transcription factor Foxo1 after GC development led to loss of the DZ GC B cells and disruption of the GC architecture (Figure). Mechanistically, even upon provision of adequate T cell help, Foxo1-deficient GC B cells manifested less proliferative expansion than controls. Moreover, we found that the transcription factor BATF was transiently induced in a small subset of GC B cells in a Foxo1-dependent manner and that deletion of BATF similarly led to GC disruption. Thus, the switch from the LZ to the DZ is triggered after receipt of T cell help, and Foxo1-mediated BATF up-regulation is at least partly involved in this switch.

In regard to differentiation of GC B cells into long-lived plasma cells, only high affinity GC B cells are selected into the plasma cell pool, however the underlying mechanism is still obscure. We found that the IRF4^{hi}Bcl6^{lo} LZ GC B cell subset with high affinity BCRs favored the plasma cell fate over GC cycling. Given that IRF4 acts to facilitate differentiation towards plasma cells in a dose-dependent manner, our data suggest that a strong BCR signal and T cell help modulate the expression of IRF4 and Bcl6, thereby facilitating plasma cell differentiation and antagonizing the GC program.

Recent Major Publications

Inoue T, Shinnakasu R, Ise W, Kawai C, Egawa T, Kurosaki T. The transcription factor Foxo1 controls germinal center B cell proliferation in response to T cell help. ***J Exp Med*** 214, 1181–1198 (2017)

Igarashi K, Kurosaki T, Roychoudhuri R. BACH family transcription factors in innate and adaptive immunity. ***Nat Rev Immunol*** (in press)

Shinnakasu R, Inoue T, Kometani K, Moriyama S, Adachi Y, Nakayama M, Takahashi Y, Fukuyama H, Okada T, Kurosaki T. Regulated selection of germinal-center cells into the memory B cell compartment. ***Nat Immunol*** 17, 861–869 (2016)

Invited Presentations

Kurosaki, T. "Fate decisions of germinal center B cells into the memory B cell or plasma cell compartment" 4th Antibody Symposium (Singapore, Singapore) November, 2016

Kurosaki, T. "Overview of germinal center immunity" The 13th International Workshop on Autoantibodies and Autoimmunity (IWAA2016) (Kyoto, Japan) October, 2016

Kurosaki, T. "Transcriptional regulation in memory B cell development" EMBO Conference: Lymphocyte Antigen Receptor Signaling (Siena, Italy) September, 2016

Kurosaki, T. "Instructive selection of germinal center B cells into the memory compartment" International Congress of Immunology 2016 (Melbourne, Australia) August, 2016

Kurosaki, T. "Instructive selection of germinal center B cells into the memory compartment" Antibody and Fc Receptor Biology: Bench to Bedside, A Special Symposium to Honor Jeffrey V. Ravetch on his 65th Birthday (New York, USA) May, 2016

Laboratory for Transcriptional Regulation

Group Director: Ichiro Taniuchi

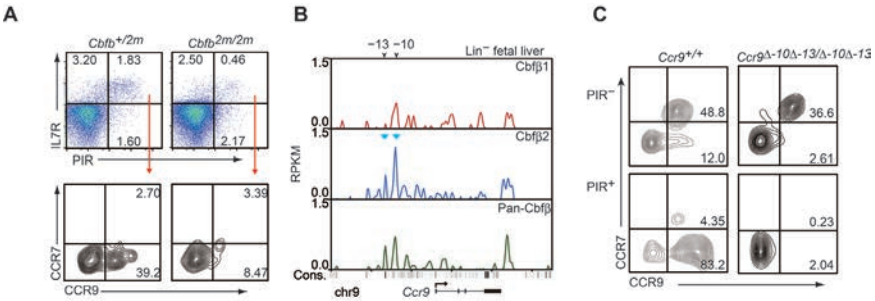


Figure: The evolutionarily conserved Cbf*β*2 variant confers thymus-homing capacity.
In mutant mice lacking Cbfβ2, which is one of two RNA splice variants encoded by the *Cbfb* gene, differentiation of IL7R$^+$PIR$^+$ fetal liver cells and induction of Ccr9 in those cells are impaired (A). Cbfβ2 binds predominantly to two upstream regions in the *Ccr9* gene (B). Removal of these two regions eliminated CCR9 expression specifically in PIR$^+$ but not PIR$^-$ cells (C).

Recent Major Publications

Seo W, Muroi S, Akiyama K, Taniuchi I. Distinct requirement of Runx complexes for TCRβ enhancer activation at distinct developmental stages. ***Sci Rep*** 7, 41351 (2017)

Kitagawa Y, Ohkura N, Kidani Y, Vandenbon A, Hirota K, Kawakami R, Yasuda K, Motooka D, Nakamura S, Kondo M, Taniuchi I, Kohwi-Shigematsu T, Sakaguchi S. Guidance of regulatory T cell development by Satb1-dependent super-enhancer establishment. ***Nat Immunol*** 18, 173–183 (2017)

Taniuchi I. Views on helper/cytotoxic lineage choice from a bottom-up approach. ***Immunol Review*** 271, 98–113 (2016)

Invited Presentations

Taniuchi I. "Regulation of Runx Complex Function by Modulating C-terminal Sequences in Its Two Components" Cancer Science Institute of Singapore. (Singapore, Singapore) November, 2016

Taniuchi I. "Regulation of T cell development in the thymus by transcription factors." CELL AND DEVELOPMENTAL BIOLOGY SEMINAR SERIES at University of Michigan. (Arbor, USA) October, 2016

Taniuchi I. "Regulation of Langerhans cell development by Runx transcription factor complexes." Immunological Diseases and Translation Research Forum 2016 (Shanghai, China) October, 2016

Taniuchi I. "Transcription factors in regulation of T cell development and function." The first RIKEN-IITU Works (Beijing, China) September, 2016

Taniuchi, I. "T cell development in the thymus" The 26th Japan Metrology Association Academic Conference y (Fukuoka, Japan) July, 2016

The vertebrate immune system consists of two components, innate and acquired. The acquired immune system is responsible for antigen-specific recall responses, is the principle for vaccination, and appeared latter during evolution, at least by acquisition of the system for generating pools of lymphocytes with a broad variety of antigen-specificities. Thus, one of the major questions in developmental biology is how the genetic program that generates a primary lymphoid organ, the thymus, for supporting T lymphocyte development has been exploited.

My laboratory has been addressing functions of Runx transcription factor complexes, which consist of a Runx protein and a non-DNA binding Cbfβ protein. Our recent work provided novel insight into how modulation of Runx complexes contributed to formation of lymphoid organs/tissues. Two functional Cbfβ variants, Cbfβ1 and Cbfβ2, are generated from the mammalian *Cbfb* gene by alternative RNA splicing. We found that the Cbfβ2 variant is essential not only for differentiation of thymic progenitors, defined as IL7R$^+$PIR$^+$ fetal liver cells, but also for endowing them with thymus-homing capacity through activating cell-type specific enhancers in the *Ccr9* gene, which encodes the primary thymus homing receptor. Our comparative genomics analyses revealed that RNA splicing, which produces Cbfβ2, was likely to have emerged at some point in the evolution toward bony fish, when the thymus appeared in its current form. Thus, acquisition of novel splice variants of the *Cbfb* gene established regulation that allows firm interaction between hematopoietic and stromal cells to generate a primary lymphoid organ, the thymus. In addition, our results suggest that this ancestral regulation was likely co-opted later to exploit development of lymphoid tissue inducer (LTi) cells to generate secondary lymphoid tissues. These results illustrate how an increase in the functional diversity of transcription factors was adapted to modulate the immune system.

Laboratory for
Immune Cell Systems

Group Director: Shigeo Koyasu

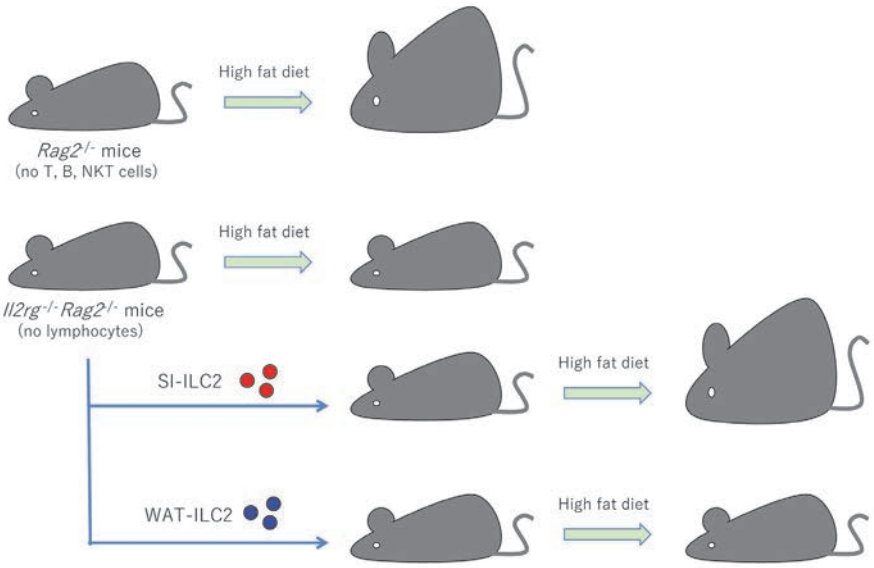


Figure: ILC2 in the small intestine are involved in the induction of diet-induced obesity.
While *Rag2*$^{-/-}$ mice lacking acquired immune cells gain weight upon high-fat diet feeding, *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice lacking all lymphocytes including innate lymphoid cells (ILCs) are resistant to diet-induced obesity. Adoptive transfer of ILC2 from small intestine but not white adipose tissue restored diet-induced obesity in *Il2rg*$^{-/-}$ *Rag2*$^{-/-}$ mice.

We have been working on the role of the natural helper (NH) cell, one of the group 2 innate lymphoid cells (ILC2). Because we originally discovered this population in the mesentery, one of largest adipose tissues in the body, we have been interested in the role of ILC2 in adipose tissue homeostasis. Obesity induces adipose tissue inflammation associated with infiltration of immune cells, such as T cells, B cells and macrophages, into adipose tissues and reduction of Tregs. Although it is believed that complex interactions of immune cells are involved in the induction of obesity, the involvement of the immune system, specifically lymphocytes, has not been fully explored before. We found that *Il2rg*$^{-/-}$ *Rag2*$^{-/-}$ mice, lacking all lymphocytes, but not *Rag2*$^{-/-}$ mice, lacking only acquired immune cells, were resistant to diet-induced obesity. Transplantation of *Rag2*$^{-/-}$ bone marrow cells into *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice abolished this resistance, indicating the involvement of ILCs. Mice lacking ILC2 or ILC3 but not NK cells were resistant to obesity, with stronger phenotypes in mice lacking only ILC2. Adoptive transfer of naïve ILC2 isolated from small intestine (SI) but not white adipose tissue (WAT) restored the induction of diet-induced obesity in *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice. These results suggest a role of small intestinal ILC2s in the induction of obesity.

Recent Major Publications

Morita H, Moro K, Koyasu S. Innate lymphoid cells in allergic and non-allergic inflammation. ***J Allergy Clin Immunol*** 138, 1253–1264 (2016)

Yamazumi Y, Sasaki O, Imamura M, Oda T, Ohno Y, Shiozaki-Sato Y, Nagai S, Suyama Kamoshita S, Funato Y, Yasui K, Kikutani T, Yamamoto K, Dohi M, Koyasu S, Akiyama T. The RNA binding protein Mex-3B is required for IL-33 induction in the development of allergic airway inflammation. ***Cell Rep*** 16, 2456–2471 (2016)

Invited Presentations

Koyasu S. "IL-7 and Notch conditionally control the commitment of ILC2 differentiation" 2nd EMBO Conference "Innate lymphoid cells" (Berlin, Germany) December, 2016

Koyasu S. "Regulatory mechanisms for ILC2 activation in allergic inflammation" 4th International Cytokine and Interferon Society (San Francisco, USA) October, 2016

Koyasu S. "Innate lymphoid cells and inflammation" Cold Spring Harbor Asia Symposium on "Frontiers of Immunology in Health and Disease" (Awaji, Hyogo) October, 2016

Koyasu S. "Innate lymphoid cells and inflammation" 5th International GK Symposium on Regulators of Adaptive Immunity (Erlangen, Germany) September, 2016

Laboratory for Human Disease Models

Group Director & Chief Scientist: **Fumihiko Ishikawa**

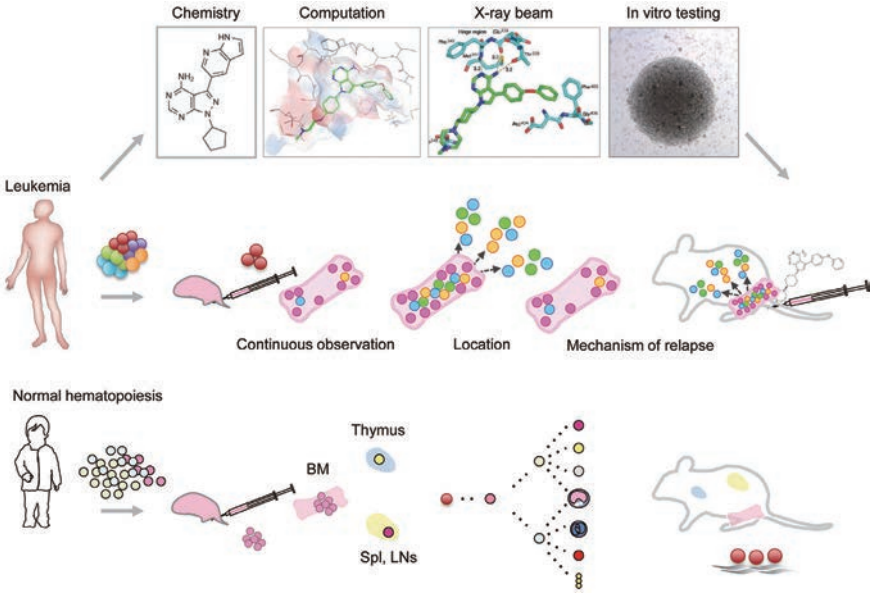


Figure: Creating new therapeutic strategies targeting poor prognosis leukemia through a multi-faceted approach
To understand the *in vivo* behavior of normal and leukemic stem cells, we have developed humanized mouse systems. Using the humanized mice, we identified where human stem cells home, how human AML develops, and how human hematopoietic stem cells generate myeloid and lymphoid progeny *in vivo*. Through this multi-faceted approach integrating medicinal chemistry, computational science, and protein structural analysis, we have been identifying new drug candidates targeting human leukemia. We hope to translate humanized mouse research into future medicine.

The specific aim of our laboratory has been to understand normal and diseased human hematopoiesis and immunity and to apply our research findings to clinical medicine. To this end, we have developed humanized mouse models by intravenously injecting human normal and disease-initiating stem cells into immune-compromised NOD/SCID/Il2rgKO (NSG) newborns. By injecting normal human hematopoietic stem cells, we have succeeded in reconstituting multiple organs of the recipient mice with human acquired and innate immunity. Furthermore, to better understand human hematopoiesis and immunity, we have created a new generation humanized mice expressing human molecules that serve essential roles in mediating environmental signaling. Using these new humanized mice, we are examining roles of human cytokines and adhesion molecules in differentiation, maturation, and function of human immune cell types.

Among various human diseases, we have been focusing on poor prognosis leukemia. Through identification of leukemia stem cells responsible for disease initiation and relapse, we have been searching for small molecules and antibodies that can eradicate human leukemia at the stem cell level. We found that RK-20449, a dual kinase inhibitor for HCK and FLT3, could effectively target FLT3-ITD mutated acute myeloid leukemia in bone marrow, spleen, and peripheral blood. We will further assess the therapeutic and adverse effects of RK-20449 and its related compounds toward establishment of a new therapeutic strategy for FLT3-ITD mutated AML.

Recent Major Publications

Najima Y, Tomizawa-Murasawa M, Saito Y, Watanabe T, Ono R, Ochi T, Suzuki N, Fujiwara H, Ohara O, Shultz LD, Yasukawa M, Ishikawa F. Induction of WT1-specific human $CD8^+$ T cells from human HSCs in HLA class I Tg NOD/SCID/IL2rgKO mice. ***Blood*** 127, 722–734 (2016)

Aoki Y, Watanabe T, Saito Y, Kuroki Y, Hijikata A, Takagi M, Tomizawa D, Eguchi M, Eguchi-Ishimae M, Kaneko A, Ono R, Sato K, Suzuki N, Fujiki S, Koh K, Ishii E, Shultz LD, Ohara O, Mizutani S, Ishikawa F. Identification of $CD34^+$ and $CD34^-$ leukemia-initiating cells in MLL-rearranged human acute lymphoblastic leukemia. ***Blood*** 125, 967–980 (2015)

Invited Presentations

Ishikawa F. "Exploring complexity and heterogeneity of human hematopoiesis *in vivo*" (Hawaii, USA) February, 2017

Ishikawa F. "Targeting acute myeloid leukemia with genetic complexity and heterogeneity" 75th Annual Meeting for Japan Cancer Association (Yokohama, Japan) October, 2016

Ishikawa F. "Toward understanding human immunity and diseases" Merck Meeting for Preclinical Models for Immunotherapy (San Francisco, USA) September, 2016

Ishikawa F. "Exploring complexity and heterogeneity of human acute myeloid leukemia" 2016 the Japanese Society of Medical Oncology Annual Meeting (Kobe, Japan) July, 2016

Laboratory for Intestinal Ecosystem

Group Director: Hiroshi Ohno

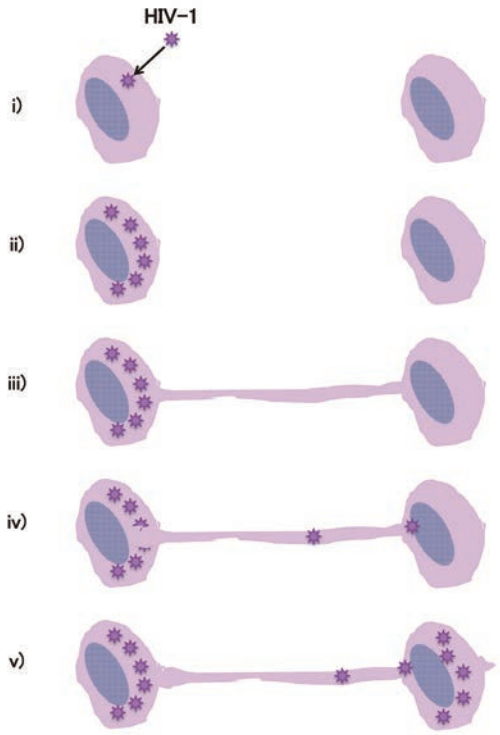


Figure: Schematic representation of the mechanism for tunneling nanotube-mediated HIV-1 spreading by direct intercellular transmission-
From top to bottom, i) HIV-1 infects a macrophage, ii) HIV-1 replication, iii) formation of a tunneling nanotube, an intercellular connection of the plasma membranes via interaction of HIV-1 Nef protein and host cell M-Sec, iv) transmission of HIV-1 from an infected to a non-infected macrophage, v) replication of HIV-1 in the newly infected macrophage.

Enormous numbers of bacteria, collectively called the gut microbiota, reside in our intestines; nevertheless, the gut does not unconditionally accept commensal microorganisms. The intestinal immune system somehow senses the type and quantity of bacteria in the gut lumen and tries to contain them. Reciprocally, the gut microbiota shape the host immune system, and the host-gut microbiota interaction deeply impacts host physiology and pathology. The aim of our laboratory is to understand the mechanisms by which the host and its gut microbiota interact, especially focusing on how gut microbes are delivered across the intestinal epithelial barrier to be recognized by the intestinal immune system, how the gut microbiota shape host defense and immune system, and how host-gut microbiota interactions affect host health and disease status.

The delivery of particulate antigens such as bacteria is thought to be mainly achieved by a unique subset of intestinal epithelial cells, M cells. These reside in a limited region of the epithelial layer called the follicle-associated epithelium (FAE), which covers the lymphoid follicles of gut-associated lymphoid tissue (GALT), such as Peyer's patches (PP). In 2016, we reported the identification of epithelium-intrinsic microRNAs important for M-cell maturation.

Regarding the host-gut microbiota interactions, we are employing an integrated multiple omics approach, combining exhaustive (meta)genomic, (meta)transcriptomic, and metabolomic analyses. In collaboration with Professor Shinkura, we have reported that high-affinity IgA regulates the gut microbiota and prevents colitis in mice. The integrated multiple omics approach is currently being applied to understand the impact of the gut microbiota on human diseases such as infantile allergic diseases and type 2 diabetes.

We have also shown that tunneling nanotubes are important for HIV-1 to spread directly through intercellular connections among macrophages (Figure).

Recent Major Publications

Okai S, Usui F, Yokota S, Hori-I Y, Hasegawa M, Nakamura T, Kurosawa M, Okada S, Yamamoto K, Nishiyama E, Mori H, Yamada T, Kurokawa K, Matsumoto S, Nanno M, Naito T, Watanabe Y, Kato T, Miyauchi E, Ohno H, Shinkura R. High-affinity monoclonal IgA regulates gut microbiota and prevents colitis in mice. ***Nat Microbiol*** 1, 16103 (2016)

Nakato G, Hase K, Sato T, Kimura S, Sakakibara S, Sugiyama M, Obata Y, Hanazato M, Iwanaga T, Ohno H. Epithelium-Intrinsic MicroRNAs Contribute to Mucosal Immune Homeostasis by Promoting M-Cell Maturation. ***PLoS One*** 11, e0150379 (2016)

Hashimoto M, Bhuyan F, Hiyoshi M, Noyori O, Nasser H, Miyazaki M, Saito T, Kondoh Y, Osada H, Kimura S, Hase K, Ohno H, Suzu S. Potential Role of the Formation of Tunneling Nanotubes in HIV-1 Spread in Macrophages. ***J Immunol*** 196, 1832–1841 (2016)

Invited Presentations

Ohno H. "Gut microbiota and human diseases" International Society of Cardiomyopathies and Heart Failure (ISCHF) Congress 2016 (Kyoto, Japan) December, 2016

Ohno H. "Regulation by the gut microbiota of the intestinal immune system: the largest immune system in the body" The 6th Chemical Festa of the Chemical Society of Japan (Tokyo, Japan) November, 2016

Ohno H. "Human gut microbiota as novel therapeutic targets-An overview-" BioJapan 2016 (Yokohama, Japan) October 2016

Ohno H. "Integrated multi-omics approach for understanding the gut ecosystem" International Conference on Beneficial Microbes (ICOBM) 2016 (Phuket, Thailand) May-June, 2016

Ohno H. "Gut Microbiota, Host Defense and Immunity" The 5th NIF Winter School on Advanced Immunology (Awaji, Japan) January, 2016

Laboratory for Mucosal Immunity

Team Leader: Sidonia Fagarasan

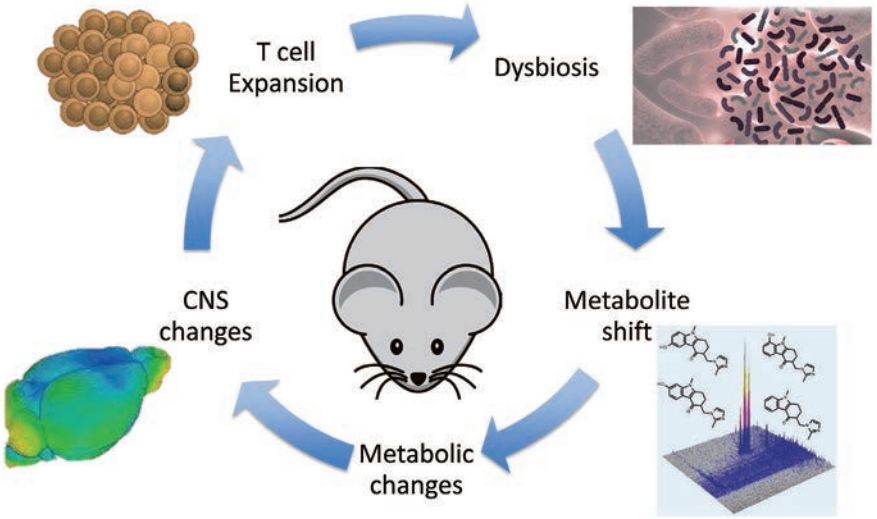


Figure: System immunology
A scheme portraying the systemic effects of T cell activation in PD-1 deficient mice. Activation of T cells in the absence of PD-1 leads not only to abnormal expansion of T cells with effector/memory phenotype but also to a series of modifications leading to microbiota, serum metabolome and metabolic changes as well as biochemical shifts in the central nervous system. We aim to uncover the mechanisms leading to such systemic effects of immune activation.

Activation of T cells is accompanied by metabolic reprogramming for energy and biosynthesis necessary for sustaining expansion, survival and effector functions. The consequences of such T cell reprogramming for immunity are widely accepted. However, it is currently unknown whether T cell activation impacts on systemic metabolic homeostasis, which would then affect the function of other major physiological systems of the body. This is the fundamental question our laboratory aims to answer.

Blockade of the inhibitory receptor PD-1 by antibodies reinvigorates T cell immunity and boosts antitumor activity, making PD-1 an important target in cancer immunotherapy. We exploit PD-1 deficiency as a mouse model of sustained immune activation and used a systemic approach to evaluate the metabolic outcome of T cell activation.

We find that persistent T cell activation in mice lacking PD-1 results in dramatic systemic metabolome changes and that such changes affect other major physiological system of the body, such as the central nervous system. Strikingly, in a tumor model we also find that activation of T cells in the presence of PD-1 blockade, even for a short period of time, reproduces the metabolic phenotype of PD-1 deficient mice.

We are interested in further exploring the mechanisms leading to the systemic metabolic shift as a basis to understand the systemic pathologies associated with chronic activation of the immune system. Such fundamental knowledge may contribute to understanding the processes associated with aging.

In addition to dissecting the immune system impact on various physiological systems we aim to understand how the microbiota impact on the immuno-metabolic phenotype in PD-1-deficient mice and the contribution of gut bacteria to the efficacy of PD1 antibody blockade.

In our quest to decipher the basic principles of systems immunology we employ approaches that combine genetic and immunologic methods with metagenomic, mass spectrometry-based proteomic and metabolomic methodologies together with extensive phenotypic and functional *in vivo* studies.

Recent Major Publications

Chamoto K, Chowdhury PS, Kumar A, Fagarasan S, Honjo T. Mitochondria activation chemicals synergize with PD-1 checkpoint blockade for T cell-dependent anti-tumor activity. ***Proc Natl Acad Sci U S A*** 114, E761–E770 (2017)

Zhang B, Chikuma S, Hori S, Fagarasan S, Honjo T. Nonoverlapping roles of PD-1 and FoxP3 in maintaining immune tolerance in a novel autoimmune pancreatitis mouse model. ***Proc Natl Acad Sci U S A*** 113, 8490–8495 (2016)

Sutherland DB, Suzuki K, Fagarasan S. Fostering of advanced mutualism with gut microbiota by Immunoglobulin A. ***Immunol Rev*** 270, 20–31 (2016)

Invited Presentations

Fagarasan S. "PD-1, a key regulator of immune-brain communication" The 2016 Kyoto Prize Workshop in Basic Sciences: "From the Molecular Immunity to the Suppression of Cancer" (Kyoto, Japan) November, 2016

Fagarasan S. "Involvement of PD-1 in antibody diversification and body homeostasis" Japan-UK Clinical Translational Research Symposium: Metabolic profiling for precision medicine (Tokyo, Japan) October, 2016

Fagarasan S. "IgA and T cells: Regulators of gut and systemic homeostasis" The 5th International GK symposium Regulators of adaptive immunity (Erlangen, Germany) September, 2016

Fagarasan S. "On germinal centers, IgA synthesis and the intestinal ecosystem", International Congress of Immunology ICI 2016: B cells (Melbourne, Australia) August, 2016

Fagarasan S. "On germinal centers, IgA synthesis and the intestinal ecosystem" Keystone Symposia: T Follicular Helper Cells and Germinal Centers (Monterey, USA) March, 2016

Laboratory for Gut Homeostasis

Team Leader: Kenya Honda

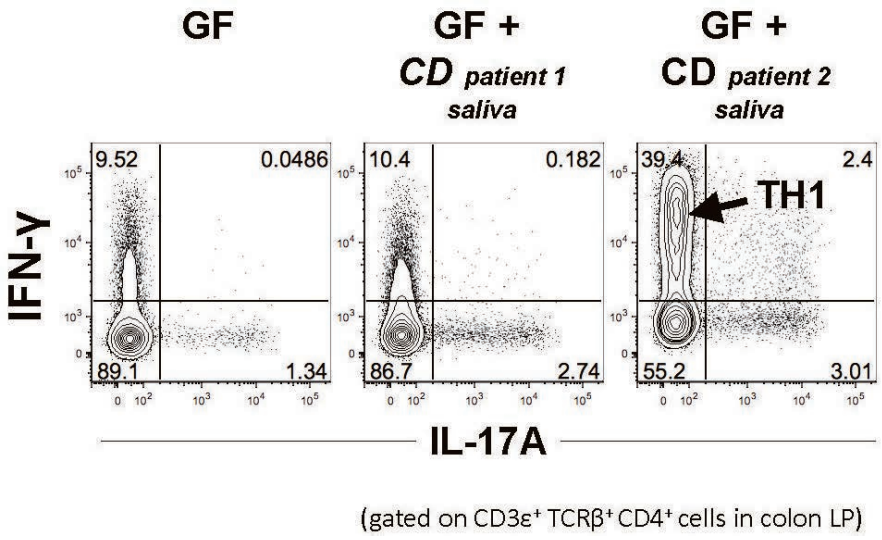


Figure: Identification and isolation of a colonic TH1cell-inducing *K. pneumoniae* strain derived from human saliva microbiota
FACS analysis of colonic LP T cells from exGF mice inoculated with saliva samples from patients with Crohn's disease (CD).

Recent Major Publications

Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. ***Nature*** 535, 75–84 (2016)

Saito T, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, Maeda Y, Hamaguchi M, Ohkura N, Sato E, Nagase H, Nishimura J, Yamamoto H, Takiguchi S, Tanoue T, Suda W, Morita H, Hattori M, Honda K, Mori M, Doki Y, Sakaguchi S. Two FOXP3$^+$CD4$^+$ T cell subpopulations distinctly control the prognosis of colorectal cancers. ***Nat Med*** 22, 679–684 (2016)

Tanoue T, Atarashi K, Honda K. Development and maintenance of intestinal regulatory T cells. ***Nat Rev Immunol*** 16, 295–309 (2016)

Invited Presentations

Honda K. "Regulation of T Cells by the Gut Microbiota" Gordon Research Conference (GRC) in Immunochemistry and Immunobiology (Lucca (Barga), Italy) June, 2016

Honda K. "T cell stimulatory bacteria colonizing in the human oral cavity" CSH ASIA: Frontiers of Immunology in Health & Disease (Awaji, Japan) October, 2016

Honda K. "Gut microbiota in immune activation" 4th Annual meeting of the International Cytokine and Interferon Society (San Francisco, USA) October, 2016

The Laboratory for Gut Homeostasis has been working on the identification of specific members of the gut microbiota that have a deep impact on the immune system. We have succeeded in isolation of 17 human gut-associated commensal bacterial strains belonging to the class Clostridia that can potently trigger accumulation of colonic CD4$^+$ Foxp3$^+$ regulatory T (Treg) cells. The induced Treg cells expressed RORγt and IL-10 and had strong suppressive activity for effector T cells. Introducing the 17 Clostridia strains reduced GVHD severity through production of short chain fatty acids.

We have also focused on Th17 cells, which are known to accumulate in response to colonization with a subgroup of intestinal microbes such as segmented filamentous bacteria (SFB). Our previous study showed that SFB bound to small intestinal epithelial cells (ECs) and induced Th17 cells strictly in a host-specific manner. Further analyses of Th17 cell induction by the intestinal pathogens *Citrobacter rodentium*, *Escherichia coli* O157:H7 and their adhesion-defective mutants revealed a strong correlation between epithelial adhesion and Th17 induction. Moreover, 20 bacterial strains isolated from feces of an Ulcerative Colitis patient were able to induce Th17 cells in the colonic lamina propria of germ-free mice, and these strains also exhibited EC-adhesive characteristics. These results support our hypothesis that the physical interaction of the microbiota with the gut epithelium is essential for Th17 cell differentiation. Currently, we are in the process of elucidating the nature of microbiota-inducing Th17 cells from healthy human donors by metagenomic 16S rRNA sequencing.

Intestinal colonization of bacteria of oral origin correlates with several negative health outcomes, such as inflammatory bowel diseases (IBD). In our recent project, we have identified a strong intestinal Th1 cell-inducing *Klebsiella pneumoniae* strain from a saliva sample of an IBD patient (Figure). The *K. pneumoniae* strain produces innate immune ligands, possibly packaged in outer membrane vesicles, to induce Th1 cells. Our work suggests that there may be a sub-type of IBD in which bacteria of oral origin contribute to the pathogenesis.

Laboratory for Immune Homeostasis

Team Leader: Shohei Hori

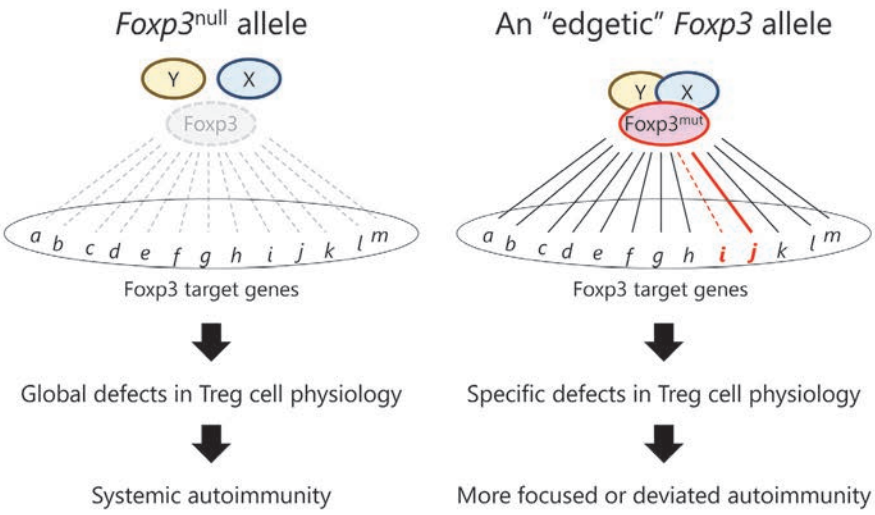


Figure: Differential impacts of null versus "edgetic" *Foxp3* alleles on Treg cells and self-tolerance
A *Foxp3*^{null} allele globally compromises Treg cell physiology by removing not only Foxp3 itself but also all the interactions between Foxp3 and its target genes as well as partner proteins, and thereby leads to severe systemic autoimmunity. On the other hand, an "edgetic" Foxp3 allele, which perturbs only certain molecular interactions with its specific target genes or partner proteins, could impair only specific facets of Treg cell physiology and thereby result in more focused or deviated autoimmunity.

Regulatory T (Treg) cells expressing the transcription factor Foxp3 play an indispensable role in the establishment and maintenance of immunological self-tolerance and tissue homeostasis, a concept well illustrated by the discovery that defective generation or function of Treg cells underlies the development of a fatal autoimmune disease in Foxp3-mutant mice and in humans suffering from the IPEX syndrome. Findings that Foxp3^{+} Treg cells exert tissue-protective or immune-suppressive functions under diverse circumstances have raised the question of what mechanisms ensure the robustness of Treg cell functions, and thus of immunological self-tolerance, in the face of unpredictable perturbations in the extracellular environment. To answer this question, we have focused on the mechanisms that control stability and adaptability of Treg cells in changing environments.

We have previously shown that, although Treg cells exhibit phenotypic plasticity, they retain epigenetic memory of, and thus remain committed to, Foxp3 expression and regulatory functions. We are now addressing the mechanisms underlying this epigenetic memory of Treg cell phenotype and function.

Another focus of our research is to understand how Foxp3 and Treg cells control immunological self-tolerance and tissue homeostasis in changing environments. To address this question, we have addressed how Foxp3 mutations found in human IPEX impinge on Treg cells *in vivo* using knock-in mutagenesis in mice. Our analysis revealed that, while many mutations are loss-of-function mutations, one particular mutation acts as an "edgetic" perturbation that specifically alters Foxp3 interactions with specific target genes by broadening its DNA-binding specificity. Furthermore, this mutation preferentially impairs the ability of Treg cells to adapt to certain non-lymphoid tissue environments and thereby leads to tissue-restricted autoimmunity. By taking advantage of this unique edgetic *Foxp3* allele, we are currently investigating how Treg cells adapt to diverse and fluctuating tissue environments.

Recent Major Publications

Zhang B, Chikuma S, Hori S, Fagarasan S, Honjo T. Nonoverlapping roles of PD-1 and FoxP3 in maintaining immune tolerance in a novel autoimmune pancreatitis mouse model. ***Proc Natl Acad Sci U S A*** 113, 8490-8495 (2016)

Ono T, Okamoto K, Nakashima T, Nitta T, Hori S, Iwakura Y, Takayanagi H. IL-17-producing gammadelta T cells enhance bone regeneration. ***Nat Commun*** 7, 10928 (2016)

Nishio J, Baba M, Atarashi K, Tanoue T, Negishi H, Yanai H, Habu S, Hori S, Honda K, Taniguchi T. Requirement of full TCR repertoire for regulatory T cells to maintain intestinal homeostasis. ***Proc Natl Acad Sci U S A*** 112, 12770-12775 (2015)

Invited Presentations

Hori S. "Functional dissection of the Foxp3-centered molecular network in regulatory T cells" The 45th Annual Meeting of the Japanese Society for Immunology (Okinawa, Japan) December, 2016

Hori S. "Dissecting Foxp3-dependent mechanisms of regulatory T cell function" The 39th Annual Meeting of the Molecular Biology Society of Japan (Yokohama, Japan) December, 2016

Laboratory for Skin Homeostasis

Team Leader: Masayuki Amagai

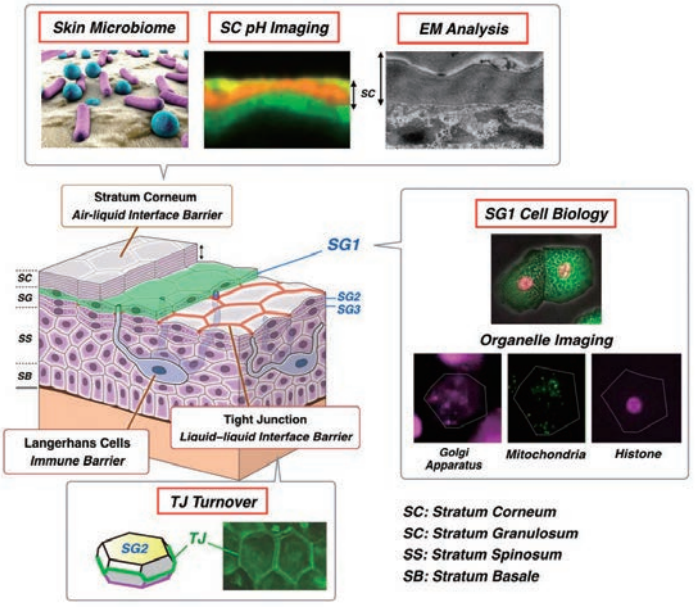


Figure: Comprehensive analysis of skin barrier homeostasis
Our team is trying to clarify the mechanisms of skin barrier homeostasis by focusing on stratum corneum (SC), tight junction (TJ), and SG1 cells.

Skin is the site where immunity meets external antigens. Cutaneous sensitization is now thought to be the initial key step for many allergic disorders, not only atopic dermatitis (AD), but also asthma, food allergy and anaphylaxis. Skin harbors several barriers to prevent easy penetration of external antigens into the body; however, the exact molecular mechanisms by which the skin barriers form and are maintained are largely unknown. The epidermis is keratinized stratified squamous epithelia and is the outermost component of the skin. From bottom to top, the epidermis is composed of the stratum basale, stratum spinosum, stratum granulosum (SG) and stratum corneum (SC). Our group has been focusing on the SC barrier as an air-liquid barrier, and on tight junctions (TJ) as a liquid-liquid barrier formed between SG2 cells, among many other skin barriers. There is a fundamental biophysical paradox regarding the function of the epidermis, namely, how it can maintain the barrier but still constantly replace and shed cells. Our group is trying to clarify how epidermal barrier homeostasis in maintained under normal conditions and how impaired barrier function occurs and affects microenvironments of the skin in various disease conditions. We use comprehensive approaches combining molecular biology, biochemistry, ultrastructural anatomy, live cell imaging, microbiology, and systems biology. For example, we have recently succeeded in isolating and characterizing SG1 cells and also in visualizing SC-pH in living mice, which enables us to perform several unique experiments to understand SC homeostasis. Another of our strengths is to be able to go back and forth between the findings in basic science in mice and those in clinical science in humans with various skin diseases. Our goal is to understand skin barrier homeostasis in health and diseases, and to provide patients suffering from severe allergic diseases with more targeted, ideal therapeutic approaches with fewer side effects.

Recent Major Publications

Amagai M. Modulating immunity to treat autoimmune disease. ***New Eng J Med*** 375, 1487–1489, 2016

Yokouchi M, Atsugi T, Logtestijn MV, Tanaka RJ, Kajimura M, Suematsu M, Furuse M, Amagai M, Kubo A. Epidermal cell turnover across tight junctions based on Kelvin's tetrakaidecahedron cell shape. ***eLife*** 5, e19593 (2016)

Kobayashi T, Glatz M, Horiuchi K, Kawasaki H, Akiyama H, Kaplan DH, Kong HH, Amagai M, Nagao K: Dysbiosis and Staphyloccus aureus Colonization Drives Inflammation in Atopic Dermatitis. ***Immunity*** 42, 756–766 (2015)

Invited Presentations

Amagai M. "Atopic diseases as a result of skin barrier failure" 41st Annual Meeting of the Japanese Society for Investigative Dermatology (Sendai, Japan) December, 2016

Amagai M. "The skin as a barrier in a dirty world" 136th Annual Meeting of American Dermatological Association (Santa Barbara, USA) October, 2016

Amagai M. "Skin barrier homeostasis and its failure in atopic dermatitis" Plenary Lectures at the 25th Congress of European Academy of Dermatology and Venereology (Vienna, Austria) September–October, 2016

Amagai M. "Skin barrier function and dysfunction in atopic diseases" 4th Taiwan Dermatology Aesthetic Conference/2016 Taiwanese Dermatological Association Spring Meeting (Kaohsiung, Taiwan) April–May, 2016

Amagai M. "Skin barrier homeostasis by stratum corneum and tight junction" Keystone Symposia Conference: Immunity in Skin Development, Homeostasis and Disease (Tahoe, USA) February–March, 2016

Laboratory for Metabolic Homeostasis

Team Leader: Naoto Kubota

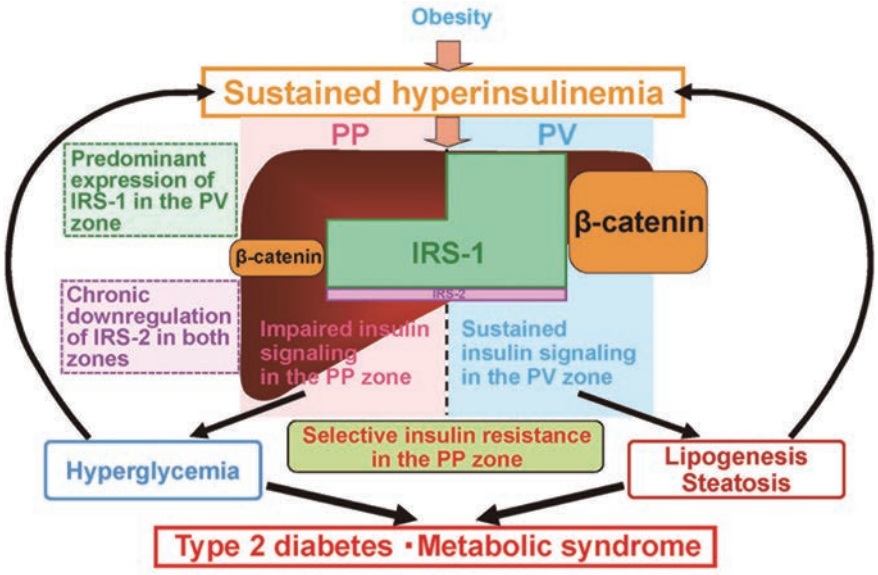


Figure: The mechanism of 'selective insulin resistance' in type 2 diabetes with obesity
In type 2 diabetes with obesity, hepatocyte IRS-1 expression levels remain unaffected by the hyperinsulinemia, but the expression of IRS-2 is downregulated in both the periportal (PP) and perivenous (PV) zones. Thus, in the PP zone, where IRS-1 is less abundantly expressed and IRS-2 expression is downregulated, insulin signaling is impaired despite the hyperinsulinemia, leading to the impaired suppression of gluconeogenesis and hyperglycaemia. By contrast, in the PV zone, where IRS-1 is abundantly expressed, insulin signaling is rather stimulated in the presence of hyperinsulinemia despite the downregulation of IRS-2, resulting in increased lipogenesis and development of steatosis.

Recent Major Publications

Sato H, Kubota N, Kubota T, Takamoto I, Iwayama K, Tokuyama K, Moroi M, Sugi K, Nakaya K, Goto M, Jomori T, Kadowaki T. Anagliptin increases insulin-induced skeletal muscle glucose uptake via an NO-dependent mechanism in mice. ***Diabetologia*** 59, 2426–2434 (2016)

Kubota N, Kubota T, Kajiwara E, Iwamura T, Kumagai H, Watanabe T, Inoue M, Takamoto I, Sasako T, Kumagai K, Kohjima M, Nakamuta M, Moroi M, Sugi K, Noda T, Terauchi Y, Ueki K, Kadowaki T. Differential hepatic distribution of insulin receptor substrates causes selective insulin resistance in diabetes and obesity. ***Nat Commun*** 7, 12977 (2016)

Kubota T, Kubota N, Sato H, Inoue M, Kumagai H, Iwamura T, Takamoto I, Kobayashi T, Moroi M, Terauchi Y, Tobe K, Ueki K, Kadowaki T. Pioglitazone Ameliorates Smooth Muscle Cell Proliferation in Cuff-Induced Neointimal Formation by Both Adiponectin-Dependent and -Independent Pathways. ***Sci Rep*** 6, 34707 (2016)

Invited Presentations

Kubota N. "Diabetes and Cardiovascular diseases" Diabetes Scientific Update Meeting in Tokyo 2016 (Tokyo, Japan) October, 2016

Kubota N. "Diabetes, obesity and gut microbiota" Bio-Japan 2016 (Yokohama, Japan) October, 2016

Kubota N. "Molecular mechanism of 'selective insulin resistance' in the liver" The 10th Diabetes Leading-edge Conference (Tsukuba, Japan) August, 2016

Kubota N. "Molecular mechanisms in the dysregulation of glucose and lipid homeostasis in the liver" Annual Scientific Meeting of Insulin Resistance and Metabolic Syndrome Study Group 2016 (Tokyo, Japan) June, 2016

Kubota N. "Elucidation of the molecular mechanism of 'selective insulin resistance' in the liver" The 59th Annual Meeting of the Japan Diabetes Society (Kyoto, Japan) May, 2016

In recent years, there has been a rapid increase in the incidence of type 2 diabetes in both Western and Asian countries; however, the precise molecular mechanisms underlying the progression of type 2 diabetes remain poorly understand. The goal of our team is to identify molecular mechanisms of insulin secretion and insulin resistance.

1) Molecular mechanism of insulin secretion

Impaired insulin secretion leads to the development of type 2 diabetes. This impaired insulin secretion is thought to be partially caused by genetic factors. Most of the common variant single-nucleotide polymorphisms (SNPs) identified by genome-wide association study (GWAS) have been reported to be associated with defective pancreatic islet function. However, the functional role of genes identified by GWAS remains unclear. To elucidate the physiology and pathophysiological role of such genes *in vivo*, we have generated genetically engineered mice (Diabetologia 2014). Specifically, we are investigating common variants in potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) and ubiquitin-conjugating enzyme E2 E2 (*UBE2E2*), which confer the largest effect on the risk of type 2 diabetes in Asians.

2) Molecular mechanism of insulin resistance

Obesity-induced insulin resistance plays a crucial role in the pathogenesis of lifestyle-related diseases, including metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes. Insulin resistance is defined as a condition in which physiological insulin signals are impaired for some reason, such as chronic inflammation. Once insulin binds to the insulin receptor, insulin receptor substrate (IRS)-1 and IRS-2 are activated and mediate intracellular insulin signaling. Until now, we have been studying the role of IRS-1 and IRS-2, which show ubiquitous expression patterns (Diabetes 2000, Circulation 2003, J Clin Invest 2004, Cell Metab 2008, Cell Metab 2011, Cell 2012, Nat Commun 2016). We will clarify the underlying mechanisms of insulin resistance by studying the role of IRS-1 and IRS-2.

Laboratory for Immune Crosstalk

Team Leader: Hilde Cheroutre

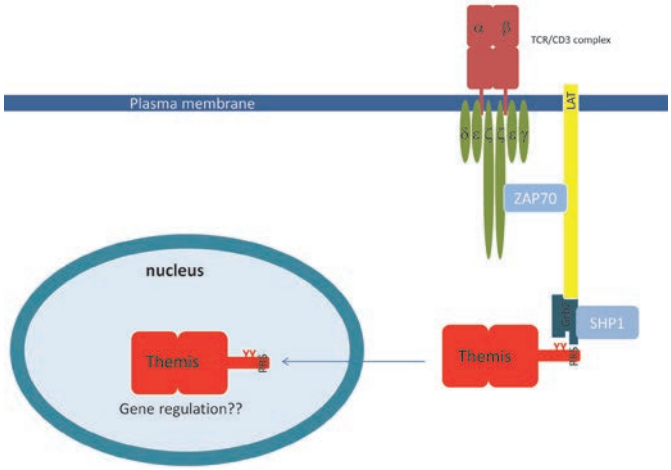


Figure: Simplified model of the interaction between Themis and T cell receptor signaling molecules
Themis is constitutively associated with Grb2 which binds both LAT and SHP1. This association requires two tandem tyrosine residues as well as a proline-rich sequence (PRS) in the C terminal region of Themis. Although Themis translocates to the nucleus in response to TCR stimulation, some amount of Themis also exists in the nucleus at steady state. The Themis mutant knock-in mouse, in which Themis cannot reside in the nucleus, has a deficiency in T cell development indicating an essential role for Themis in the nucleus.

By phenotype screening of ENU-induced mutant mice, we newly identified Themis as an essential gene for T cell development. Themis functions by interacting with Grb2 and SHP1, thus reducing TCR signals and allowing positive selection of thymocytes that further mature to naïve T cells. We found that, although Themis deletion impaired conventional selection, it did not affect positive agonist selection of self-specific T cells. By generating specific nuclear localization signal mutants we now find that the nuclear localization of Themis is critical and essential for its function. Therefore, in addition to its function in proximal TCR signaling, we speculate that Themis also plays a role in the nucleus to control gene expression. In order to elucidate its genomic function, we established a x3 FLAG tagged Themis knock-in mouse by CRISPR/Cas9 gene editing in order to perform highly precise ChIP seq analysis. Since GWAS studies identified Themis as associated with celiac disease and atopic dermatitis, and since Themis has been linked with inflammatory bowel disease, and furthermore, since Themis is essential for the pathogenesis of C. Malaria and for protection from P. Tuberculosis, it appears that Themis plays central roles in regulating peripheral T cells. To examine the genomic role of Themis in mature T cells *in vivo*, we generated mutant mice that contained one allele of the nuclear translocation mutation and one allele of floxed wild type (WT) Themis. These mice were crossed onto various Cre-transgenic mice to delete the WT Themis allele in specific cells or with specific kinetics. This allows us to study the genomic role of Themis during thymic development and selection as well as in various disease settings. This approach will allow us to understand the role of Themis and other factors in controlling and translating pre-TCR and TCR signal strength during thymic selection and fate decisions of immature and mature T cells. We are also using unique and novel loss-and gain-of-function approaches, designed in collaboration with Dr. Taniuchi's laboratory. In another study we discovered cytoplasmic retinoic acid receptor alpha (RARα) as a new member of the TCR signalosome and a ZAP70 interacting partner. We found that cytoplasmic RARα plays a critical non-nuclear role in the proximal TCR signaling complex as well as in TCR-induced NOTCH activation, c-Myc expression and activation-induced proliferation. Furthermore, we characterized cytoplasmic RARα at the molecular level and identified it as encoded by an alternative splice form of the nuclear RARα1 and -2, with a conserved C-terminus. Cytoplasmic RARα is expressed by mature T cells as well as immature thymocytes, indicating that it might also serve non-nuclear roles during thymic development and selection. We are now designing various *in vitro* and *in vivo* strategies to fully characterize cytoplasmic RARα at the molecular and cellular level and under physiological and disease settings.

Recent Major Publications

Larange A, Cheroutre H. Retinoic Acid and Retinoic Acid Receptors as Pleiotropic Modulators of the Immune System. ***Annu Rev Immunol*** 34, 369–394 (2016)

Satoh R, Kakugawa K, Yasuda T, Yoshida H, Sibilia M, Katsura Y, Levi B, Abramson J, Koseki Y, Koseki H, van Ewijk W, Hollander GA, Kawamoto H. Requirement of Stat3 Signaling in the Postnatal Development of Thymic Medullary Epithelial Cells. ***PLoS Genet*** 12, e1005776 (2016)

Krause P, Morris V, Greenbaum JA, Park Y, Bjoerheden U, Mikulski Z, Muffley T, Shui JW, Kim G, Cheroutre H, Liu YC, Peters B, Kronenberg M, Murai M. IL-10-producing intestinal macrophages prevent excessive antibacterial innate immunity by limiting IL-23 synthesis. ***Nat Commun*** 6, 7055 (2015)

Invited Presentations

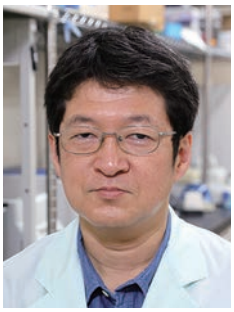
Cheroutre H. KAI-IBS Joint International Conference (Seoul, Korea) November, 2016

Cheroutre H. "Food for Thought: Mucosal Immune Protection and Regulation Controlled by the Diet" Institute for Medical Science, University of Tokyo (Tokyo, Japan) September, 2016

Cheroutre H. Yale University School of Medicine (New Haven, USA) September, 2016

Cheroutre H. Inaugural Chiba University- University of California, San Diego Symposium (San Diego, USA) February, 2016

Cheroutre H. The 55th Midwinter Conference of Immunologists (Pacific Grove, USA) January, 2016



Laboratory for Inflammatory Regulation

Team Leader: Takashi Tanaka

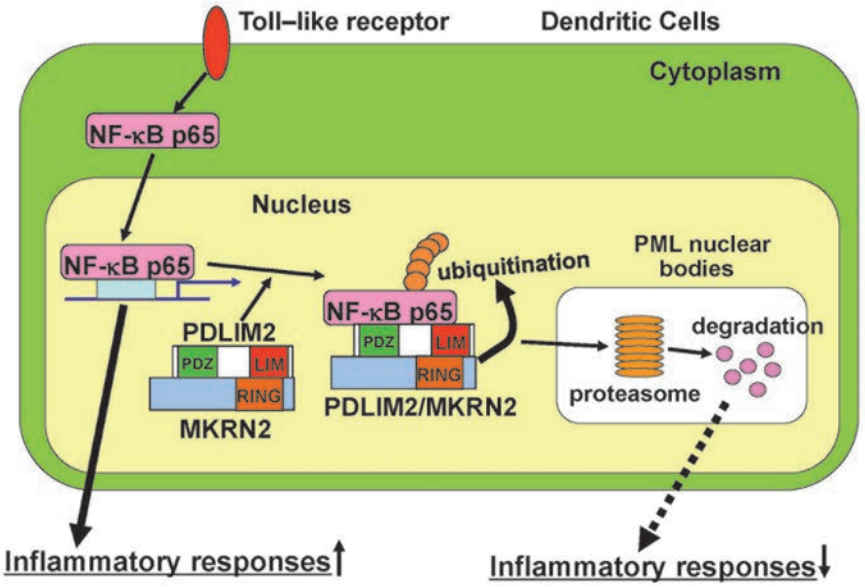


Figure: MKRN2 is a novel ubiquitin E3 ligase for the p65 subunit of NF-κB and negatively regulates inflammatory responses
MKRN2 and PDLIM2 are ubiquitin E3 ligases for the p65 subunit of NF-κB, form heterodimers and cooperatively promote polyubiquitination and degradation of p65, thereby negatively regulating NF-κB-mediated inflammatory responses.

The inflammatory response is an important host defense mechanism to sense and eliminate invading microbial pathogens. However, these inflammatory responses must be terminated at the appropriate time point, otherwise excessive responses can damage normal tissue and may lead to autoimmune diseases. Our research goal is to identify a series of key negative regulators of inflammation-related signal transduction pathways and clarify the complete picture of the molecular mechanisms for regulating inflammatory responses. We predict that dysfunction of these negative regulators might be the cause of human autoimmune diseases.

We previously identified PDLIM2 (PDZ and LIM domain-containing protein-2), a nuclear protein that belongs to a large family of LIM proteins, as one of the key factors negatively regulating inflammatory responses. PDLIM2 is a ubiquitin E3 ligase for the p65 subunit of NF-κB in dendritic cells, negatively regulating NF-κB-mediated inflammation. Since both PDZ and LIM domains are generally thought of as structures involved in protein-protein interaction, we assumed that PDLIM2 controls inflammatory responses through its interaction with other intracellular molecules that are also important for regulating inflammatory signaling. We therefore sought to isolate PDLIM2-interacting proteins that are critical for suppressing NF-κB signaling. We recently identified MKRN2, a RING finger domain-containing protein that belongs to the makorin ring finger gene family, as a novel p65 ubiquitin E3 ligase. We have demonstrated that MKRN2 binds to PDLIM2 and that the heterodimer then synergistically promotes polyubiquitination and degradation of p65, thereby negatively regulating NF-κB-mediated inflammatory responses. Moreover, to elucidate the mechanisms by which initially helpful inflammation can lead to pathogenic chronic inflammation, we also analyzed the regulation of *in vivo* inflammatory responses using PDLIM2-deficient mice. These studies should help clarify the pathogenesis of human autoimmune and inflammatory diseases and may lead to the development of new therapeutic tools for these diseases.

Recent Major Publications

Shin C, Ito Y, Ichikawa S, Tokunaga M, Sakata-Sogawa K, Tanaka T. MKRN2 is a novel ubiquitin E3 ligase for the p65 subunit of NF-κB and negatively regulates inflammatory responses. ***Sci Rep*** 7, 46079 (2017)

Tanaka T. Clarification of the molecular mechanisms that negatively regulate inflammatory responses. In: Miyasaka M, Takatsu K. (eds.), ***Chronic Inflammation: Mechanisms and Regulation, 1st edition***, Tokyo, Japan: Springer Japan KK, pp109–118 (2016)

Ono R, Kaisho T, Tanaka T. PDLIM1 inhibits NF-κB-mediated inflammatory signaling by sequestering the p65 subunit of NF-κB in the cytoplasm. ***Sci Rep*** 5, 18327 (2015)

Invited Presentations

Tanaka T. "The roles of LIM protein family in the regulation of inflammatory responses" The 45th Annual Meeting of the Japanese Society for Immunology (Okinawa, Japan) December, 2016

Tanaka T. "Negative regulation for inflammatory responses and its association with autoimmune diseases" The 2nd RIKEN-IITU Joint Symposium (Yokohama, Japan) December, 2016

Laboratory for Cytokine Regulation

Team Leader: Masato Kubo

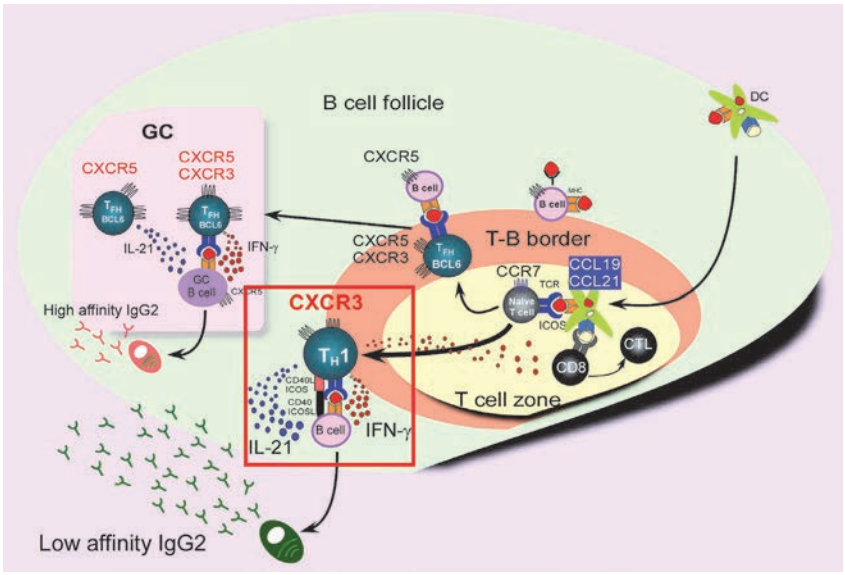


Figure: T_H1 cells control protective IgG2 antibody responses
Influenza virus preferentially induces development of two different type of IFN-γ producing effector T cells, $CXCR3^+$ $CXCR5^+$ TFH and $CXCR3^+$ T_H1 cells. T_H1 cells are the dominant subset responsible for the IFN-γ-producing CD4 T cells and the production of IgG2 protective antibodies. These T_H1 cells secreted IL-21 and IFN-γ, both of which were required for low affinity IgG2 antibody production.

T cells play a central role in the effector and regulatory functions of the immunological surveillance system, and aberrations in these functions can lead to various immunological disorders. Cytokines are critical factors in the transmission of information from the receptor to the nucleus as well as in the establishment of communication networks among cells. Our overall long-term goal is to understand how effector cytokines are controlled during cell lineage commitment.

We have focused on antibody responses in systemic vaccination against Influenza A virus (IAV). Follicular helper T (T_{FH}) cells are critical for development of the germinal center (GC) environment. Although TFH cells are important in anti-viral humoral immunity, IgG2 antibodies predominate in the response to vaccination with inactivated IAV and were responsible for protective immunity to lethal challenge with pathogenic H5N1 and pandemic H1N1 IAVs, even in B or T cell-specific *Bcl6*-deficient mice. IL-21 and IFN-γ secreted from T_H1 cells were essential for greater persistence and higher titers of IgG2 protective antibodies. These results suggest that T_H1 induction could be a promising strategy to induce effective neutralizing antibodies against emerging influenza viruses.

Recent Major Publications

Domínguez-Hüttinger E, Christodoulides P, Miyauchi K, Irvine AD, Okada-Hatakeyama M, Kubo M, Tanaka RJ. Mathematical modeling of atopic dermatitis reveals "double-switch" mechanisms underlying 4 common disease phenotypes. ***J Allergy Clin Immunol*** S0091–6749(16)31433-6 (2016)

Miyauchi K, Sugimoto-Ishige A, Harada Y, Adachi Y, Usami Y, Kaji T, Hasegawa H, Watanabe T, Hijikata A, Fukuyama S, Maemura T, Ohara O, Kawaoka Y, Takahashi Y, Takemori T, Kubo M. Protective neutralizing influenza antibody response in the absence of T follicular helper cells. ***Nat Immunol*** 17, 1447–1458 (2016)

Yasuda T, Fukada T, Nishida K, Nakayama M, Matsuda M, Miura I, Dainichi T, Fukuda S, Kabashima K, Nakaoka S, Bin BH, Kubo M, Ohno H, Hasegawa T, Ohara O, Koseki H, Wakana S, Yoshida H. Hyperactivation of JAK1 tyrosine kinase induces stepwise, progressive pruritic dermatitis. ***J Clin Invest*** 126, 2064–2076 (2016)

Invited Presentations

Kubo M. "Cytokine signaling in skin homeostasis" NEXT Lecture Meeting in Chiba (Chiba, Japan) October, 2016

Kubo M. "Role of T follicular helper (TFH) in humoral immunity" The 13th International Workshop on Autoantibodies and Autoimmunity (IWAA2016) (Kyoto, Japan) October, 2016

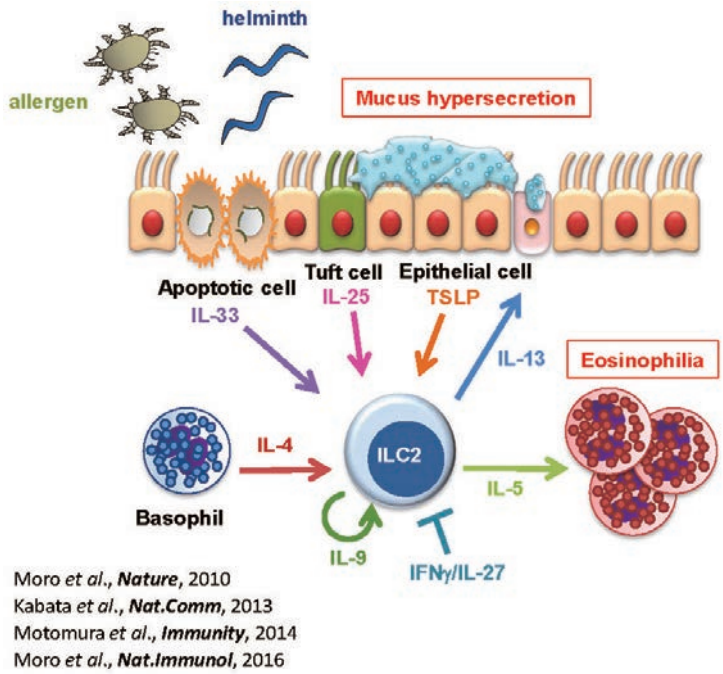
Kubo M. "T dependent antibody responses in influenza virus vaccination" Seminar series of new wave of virology (Kyoto, Japan) September, 2016

Kubo M. "Cross talk between innate aquired immunity in allergic responses" The 65th Annual Meeting of Japanese Society Allergology (Tokyo, Japan) August, 2016

Kubo M. "New therapeutic approach for atopic dermatitis (AD) using comprehensive analysis and system biology" 2016 International Biomedical Interface Symposium (Taipei, Taiwan) March, 2016

Laboratory for
Innate Immune Systems

Team Leader: Kazuyo Moro



Moro *et al.*, ***Nature***, 2010
Kabata *et al.*, ***Nat.Comm***, 2013
Motomura *et al.*, ***Immunity***, 2014
Moro *et al.*, ***Nat.Immunol***, 2016

Figure: Group 2 innate lymphoid cells and the cytokine network
Group 2 innate lymphoid cells (ILC2) are known to be activated by epithelial-related cytokines such as IL-33, IL-25 and TSLP, which are produced by apoptotic epithelial cells, tuft cells, and activated epithelial cells, respectively. Production of type-2 cytokines, including interleukin IL-4, IL-5, IL-9, and IL-13 promote alternative activation of eosinophilia, goblet-cell hyperplasia and smooth-muscle contractility that contribute to expulsion of helminth parasites but also worsen allergic inflammation. ILC2 are tissue-resident cells and are strongly suppressed by IFNγ and IL-27 in a STAT1-dependent manner.

Recent studies have revealed new types of lymphocytes functioning in innate immune responses that are collectively called innate lymphoid cells (ILC). Unlike T and B lymphocytes, ILCs lack Rag-dependent antigen-specific receptors and are activated by cytokines produced by other innate immune cells or epithelial cells. ILCs have been divided into three groups based on their cytokine production profiles; group 1 ILC including NK cells and ILC1 produce IFNγ, group 2 ILC (ILC2) including natural helper cells, nuocytes and innate helper type 2 cells produce type 2 cytokines such as IL-5, IL-6 and IL-13, and group 3 ILC including lymphoid tissue inducer (LTi) cells and ILC3s produce IL-17 and IL-22.

Our research group focuses on group 2 innate lymphoid cells (ILC2), an innate lymphocyte lineage that we identified in 2010. ILC2 localize to a variety of tissues such as fat, lung, intestine, liver and skin, and mediate immunity to helminth and fungal infections via strong type 2 cytokine production. Infection with helminths or fungi induces IL-25 and IL-33 production by epithelial cells or endothelial cells and activates ILC2, leading to eosinophilia and goblet cell hyperplasia resulting from the production of IL-5 and IL-13, respectively. These immune responses are extremely important for defense against infection, but recent studies have shown that ILC2 can also exacerbate allergic inflammation using the same mechanisms.

We think that it is particularly important to determine the ILC2 activation and suppression mechanisms during infection and inflammation in order to develop effective treatments for ILC2-related allergic diseases. Recently, we found that ILC2 are involved in obesity, which leads to a variety of metabolic syndromes. To gain a comprehensive understanding of the role of ILC2, our lab focuses on their development, cytokine signaling pathways, miRNA regulation as well as their function in adipose tissue inflammation, autoimmune disease, helminth infection, and a number of allergic diseases.

Recent Major Publications

Morita H, Moro K, Koyasu S. Innate lymphoid cells in allergic and nonallergic inflammation. ***J Allergy Clin Immunol*** 138, 1253-1264 (2016)

Moro K, Kabata H, Tanabe M, Koga S, Takeno N, Mochizuki M, Fukunaga K, Asano K, Betsuyaku T, Koyasu S. Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. ***Nat Immunol*** 17, 76-86. (2016)

Vasanthakumar A, Moro K, Xin A, Liao Y, Gloury R, Kawamoto S, Fagarasan S, Mielke LA, Afshar-Sterle S, Masters SL, Nakae S, Saito H, Wentworth JM, Li P, Liao W, Leonard WJ, Smyth GK, Shi W, Nutt SL, Koyasu S, Kallies A. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. ***Nat Immunol*** 16, 276-285 (2015)

Invited Presentations

Moro K. "The role of type 2 innate lymphoid cells in the immune systems" The 44th Annual Meeting of the Japan Society for Clinical Immunology (Tokyo, Japan) August, 2016

Moro K. "Suppression mechanism of Group 2 innate lymphoid cells" FASEB: IgE and Allergy, 50 Years & Onward (West Palm Beach, USA) July, 2016

Moro K. "Mechanism for ILC2-mediated eosinophilia in allergic inflammation" The 65th Annual Meeting of Japanese Society of Allergology (Tokyo, Japan) June, 2016

Moro K. "IFNs and IL-27 regulate type 2 innate lymphoid cells" The 89th Annual Meeting of Japanese Society for Bacteriology (Osaka, Japan) March, 2016

Moro K. "Group 2 Innate lymphoid cell, a novel target of the treatment for allergic diseases" The 34th Annual Meeting of the Japan society of Immunology & Allergology in Otolaryngology (Mie, Japan) February, 2016

Core for Precise Measuring and Modeling

Toward the ultimate goal of obtaining a comprehensive understanding of the pathogenesis of human diseases, the functions of the Core for Precise Measuring and Modeling are three pronged: production of mouse models, multiomics measurements and quantitative bioimaging, and bioinformatics/modeling of human disease processes. Through close interactions among these three branches of the core, we aim to collect a wide variety of quantitative data in order to build a computational and predictive network of the disease process. As for the production of genetically engineered mice that will be used as models of human diseases, the laboratory for Developmental Genetics has begun to apply recent advances in genome engineering technology, e.g., CRISPR/Cas9-based genome editing, and thereby considerably enhance the production capacity and power of the disease models. Regarding the precise quantitative measurements branch, we have recently enhanced the power of metabolite analysis by the Laboratory for Metabolomics. Together with mRNA/protein profiling by the Laboratory for Integrative Genomics, the enhanced multiomics measurements and bio-imaging (Laboratory for Tissue Dynamics) will greatly contribute to exploration of the etiology of human diseases. After being processed by bioinformatics (Laboratory for Integrated Bioinformatics), the datasets are used for modeling (Laboratories for Disease Systems Modeling and Integrated Cellular Systems). The laboratory for Medical Science Mathematics is working to fill the gap between humans and mice. As a leading IMS project, an atopic dermatitis model mouse, provided by the Laboratory for Immunogenetics, has been extensively analyzed from several different angles, fully exploiting the analysis powers of this core. These efforts should enable us to identify new biomarkers for early diagnosis and prevention of atopic dermatitis in the very near future.

Laboratory for Developmental Genetics

Group Director: Haruhiko Koseki

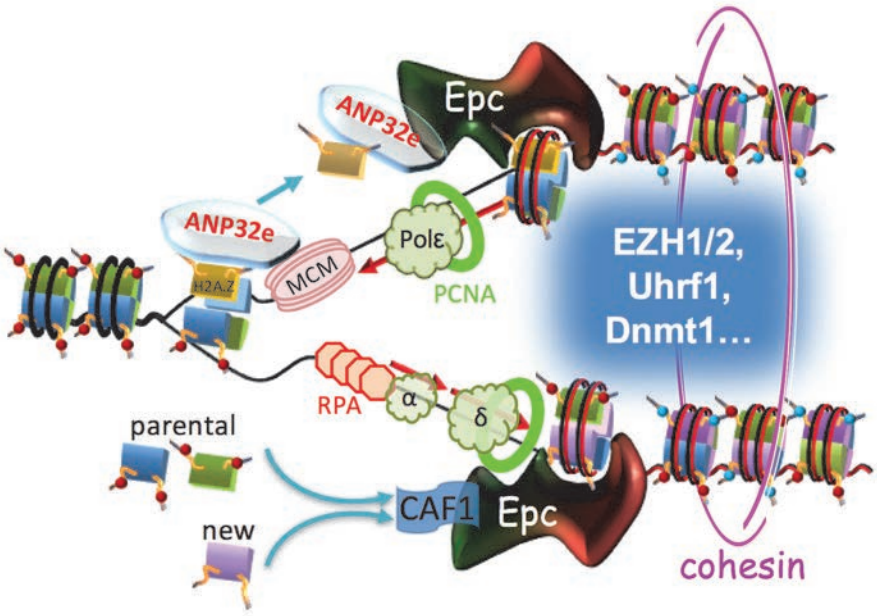


Figure: EPC1/2-mediated epigenetic inheritance that is coupled with the replication machinery
EPC1/2 are coupled with the replication machinery and are expected to contribute to chromatin replication by linking replication and epigenetic mechanisms.

The Developmental Genetics Research Group is pursuing a research program to elucidate the molecular mechanisms underlying organ development and stem cell functions. Particular emphasis has been put on epigenetic regulation mediated by the combinatorial actions of Polycomb group (PcG) gene products, DNA methylation mechanisms and cell cycle regulation.

EPC1/2 (Enhancer of Polycomb) are orphan Polycomb factors. We found that EPC1/2 are essential for proliferation of ES cells by generating conditional double knockout (*Epc*-dKO) ES cells. By using immunofluorescence staining and isolation of proteins on nascent DNA (iPOND), we observed that EPC1/2 accumulate at nascent chromatin and move along with the replication fork similar to PCNA, a processivity factor for DNA polymerase. Further, mass spectrometry analysis of the purified EPC1-Flag complex revealed its association with the NuA4 complex, Ino80 chromatin remodeling complex, and cohesin complex, supporting its functions in DNA replication. Indeed, depletion of EPC significantly impaired DNA replication, and cells became more sensitive to replication stress. To dissect how EPC depletion influences replication, we monitored the binding of replication factors to nascent chromatin. We observed a clear reduction in histone chaperones CAF-1 and ANP32e, FACT, Polδ, and PCNA in *Epc*-dKO cells. These results indicated that EPC plays a role as a platform to stabilize the replication fork.

EPC1/2 have been reported to bind to nucleosomes via their N-terminal regions, therefore we presumed that EPC1/2 would contribute to nucleosome assembly at the replication fork. We indeed found that both parental and newly generated histones were less efficiently incorporated in *Epc*-dKO. Consistently, the chromatin structure at promoter regions was not sufficiently reassembled and loosened. Further, we found alternative deposition of histone variants, an impaired incorporation of H2A.Z while H3.3 was increased, as well as defects in epigenetic features at pericentric heterochromatin (PHC), reduced trimethylation levels at H3K9 and H3K27, loss of HP1 binding and DNA methylation. Based on these results together with other data, we suggest that EPC1/2 contribute to mediate epigenetic inheritance mechanisms during replication.

Recent Major Publications

Yamada D, Iyoda T, Vizcardo R, Shimizu K, Sato Y, Endo TA, Kitahara G, Okoshi M, Kobayashi M, Sakurai M, Ohara O, Taniguchi M, Koseki H, Fujii SI. Efficient regeneration of Human Vα24^{+} invariant NKT cells and their anti-tumor activity *in vivo*. ***Stem Cells*** 34, 2852–2860 (2016)

Sharif J, Endo TA, Nakayama M, Karimi MM, Shimada M, Katsuyama K, Goyal P, Brind'Amour J, Sun M-A, Sun Z, Ishikura T, Mizutani-Koseki Y, Ohara O, Shinkai Y, Nakanishi M, Xie H, Lorincz MC, Koseki H. Activation of Endogenous Retroviruses in Dnmt1$^{-/-}$ ESCs Involves Disruption of SETDB1-Mediated Repression by NP95 Binding to Hemimethylated DNA. ***Cell Stem Cell*** 19, 81–94 (2016)

Yakushiji-Kaminatsui N, Kondo T, Endo TA, Koseki Y, Kondo K, Ohara O, Vidal M, Koseki H. RING1 proteins contribute to early proximal-distal specification of the forelimb bud by restricting Meis2 expression. ***Development*** 143, 276–285 (2016)

Invited Presentations

Koseki, H. "The role of PRC1 variants during activation of Meis2" Seminar at Max-Planck Institute of Immunobiology and Epigenetics (Freiburg, Germany) November, 2016

Koseki, H. "Regeneration of NKT cell using iPS cell technology and its adoptive immunotherapy for cancer" The 25th Annual Meeting of Japanese Society of Strategies for Cancer Research and Therapy (Chiba, Japan) June, 2016

Koseki, H. "Polycomb in transcriptional phase transition of developmental genes" Mini Symposium, Genetic Control of Mouse embryonic Development at Hubrecht Institute (Utrecht, the Netherlands) June, 2016

Koseki, H. "PCGF6-PRC1 to suppress premature activation of meiosis/germ cell related genes in ES cells" Seminar at Trinity College, Oxford University (Oxford, UK) June, 2016

Koseki, H. "Personalized Preventive Medicine: chronic dermatitis" Workshop on Personalised Medicine, Tübingen University Hospital, Center for Personalised Medicine (Tübingen, Germany) May, 2016

Laboratory for Integrative Genomics

Group Director: Osamu Ohara

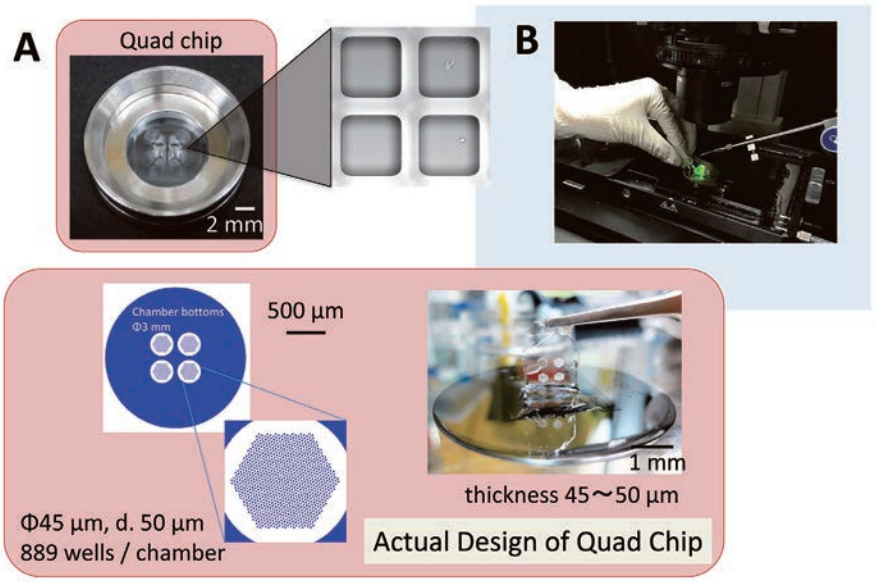


Figure: Development of A Real-Time Monitoring Platform for Single-cell Omics
In a previous study, we used a single-hole chip for measurement of protein secretion by single cells (Sci Rep, 2014, 4:4736). To improve the throughput of the measurements, we developed a four-hole chip, termed "Quad Chip", which enabled us to measure single-cell behaviors under four different conditions simultaneously (Panel A). Together with improvements in the optical system, the new platform equipped with a "Quad Chip" greatly enhances measurement performance. In addition, we also improved a device for retrieval of single cells at a time point of interest (Panel B). Consequently, the new platform allows us to monitor the behavior of intracellular and secreted proteins in real-time by fluorescent microscopy and obtain mRNA or epigenetic profiles of single cells at their endpoint.

While the original mission of the Laboratory for Integrative Genomics at the beginning of the center was to function as a "Gateway" to genomics, genomics has become the approach of choice to tackle complex problems in medical sciences. Thus, we are currently involved in many intramural and extramural collaborations and various strategic projects organized by the center, besides providing our colleagues in the center with technical support as a central support function. However, it is not our intent to be overwhelmed by these activities: Every effort has also been made to continuously update new technologies with the least delay, but only after they become mature and robust enough to be useful in a general laboratory setting. This is still the basic mission of our laboratory.

Technology development has had a big impact on the design of biological experiments and greatly deepens our understanding of biological systems. As we have experienced it first hand by the recent emergence of next-generation DNA sequencing technology, new technology drastically changes the experimental approaches in biology; even a data-driven approach can now be personalized. In this context, the most critical issue for our laboratory is to correctly decide on the direction of technology development. As one layer in the multi-scale structure of a biological system, our current focus is on the development of single-cell measurement technologies and their applications. In particular, we are most interested in the temporal dynamics of cellular responses at single-cell resolution. As a fruit of studies in this direction, through collaboration with Dr. Uemura's Laboratory at the University of Tokyo (Department of Biological Sciences, Graduate School of Science), we have recently developed a single-cell omics platform shown in the Figure. Together with snapshot omics data, either in bulk or at single-cell resolution, the datasets of temporal dynamics of cellular responses obtained by this platform greatly contribute to a comprehensive understanding of the systems behaviors of a cellular ensemble of interest.

Recent Major Publications

Kagawa R, Fujiki R, Tsumura M, Sakata S, Nishimura S, Itan Y, Kong XF, Kato Z, Ohnishi H, Hirata O, Saito S, Ikeda M, El Baghdadi J, Bousfiha A, Fujiwara K, Oleatro M, Yancoski J, Perez L, Danielian S, Ailal F, Takada H, Hara T, Puel A, Boisson-Dupuis S, Bustamante J, Casanova JL, Ohara O, Okada S, Kobayashi M. Alanine-scanning mutagenesis of human signal transducer and activator of transcription 1 to estimate loss- or gain-of-function variants. ***J Allergy Clin Immunol*** pii: S0091–6749, 31281–31287 (2016)

Miyauchi K, Sugimoto-Ishige A, Harada Y, Adachi Y, Usami Y, Kaji T, Inoue K, Hasegawa H, Watanabe T, Hijikata A, Fukuyama S, Maemura T, Okada-Hatakeyama M, Ohara O, Kawaoka Y, Takahashi Y, Takemori T, Kubo M. Protective neutralizing influenza antibody response in the absence of T follicular helper cells. ***Nat Immunol*** 17, 1447–1458 (2016)

Yasuda T, Fukada T, Nishida K, Nakayama M, Matsuda M, Miura I, Dainichi T, Fukuda S, Kabashima K, Nakaoka S, Bin BH, Kubo M, Ohno H, Hasegawa T, Ohara O, Koseki H, Wakana S, Yoshida H. Hyperactivation of JAK1 tyrosine kinase induces stepwise, progressive pruritic dermatitis. ***J Clin Invest*** 126, 2064–2076 (2016)

Invited Presentations

Ohara O. "Snapshot vs Real-Time Monitoring: Toward an Understanding of Population Dynamics of Immune Cells at the Single-Cell Resolution" The 6th International Multidisciplinary Conference on Optofluidics (Beijing, China) July, 2016

Ohara O. "From genotype to phenotype: Mind the gap in multiscale structure of the phenotype" 5th Sardinian Summer School: From genome-wide association studies (GWAS) to function (Pula, Italy) June, 2016

Laboratory for Disease Systems Modeling

Group Director: Hiroaki Kitano

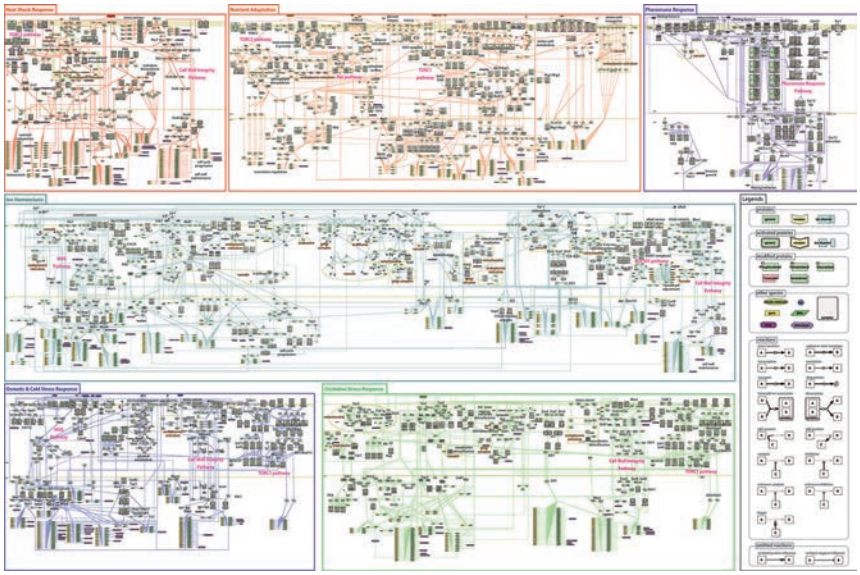


Figure: Comprehensive maps of the stress response pathways in budding yeast
This is the most comprehensive signaling map of an organism ever produced. Pathways were categorized into six groups and contain multiple layers of information, which are not included in simple interaction networks such as protein-protein interaction (PPI). Network structure analysis revealed a novel mechanism to ensure robustness of signaling networks.

The Laboratory for Disease Systems Modeling (LDSM) aims at in-depth understanding of several biological processes relevant to disease systems, and possible applications to clinical practice. There are three interrelated projects: (1) Skin Homeostasis, (2) Aging, and (3) Precision systems biology using budding yeast. Skin homeostasis is linked with the IMS center project on atopic dermatitis. We are striving to create computational models combined with a rage of cell-based experiments at LDSM together with mouse data from other IMS teams. Aging is a new topic with the long-range objectives to understand possible control of the aging process and uncovering molecular mechanisms behind it. We are focusing on the effect of SIRT1 (mammalian) and Sir2 (budding yeast), and nicotinamide mononucleotide as a chemical substance to affect these genes. We are linking skin homeostasis and aging to low-grade chronic inflammation as the common thread. Precision systems biology using budding yeast strengthens our understanding of key biological process by providing deeper insights into detailed molecular processes with our original gTOW genome-wide assay system.

In addition, the LDSM has extensive involvement with the Medical Innovation Hub project in its feasibility study stage, but that will not be reported here.

Recent Major Publications

Kawakami E, Nakaoka S, Tazro Ohta T, Kitano H. Kawakami E, Nakaoka S, Tazro Ohta T, Kitano H. Weighted enrichment method for prediction of transcription regulators from transcriptome and global chromatin immunoprecipitation data. ***Nucleic Acids Res*** 44, 5010–5021 (2016)

Kitano H. Artificial intelligence to win the Nobel Prize and beyond: Creating the engine of scientific discovery. ***AI Magazine*** 37, 39–49 (2016)

Kawakami E, Singh VK, Matsubara K, Ishii T, Matsuoka Y, Hase T, Kulkarni P, Siddiqui K, Kodilkar J, Danve N, Subramanian I, Katoh M, Shimizu-Yoshida Y, Ghosh S, Jere A, Kitano H. Network analyses based on comprehensive molecular interaction maps reveal robust control structures in yeast stress response pathways. ***NPJ Syst Biol Appl*** 2, 15018 (2016)

Invited Presentations

Kitano H. "Nobel Turing Challenge" The 39th Annual Meeting of the Molecular Biology Society of Japan (Yokohama, Japan) December, 2016

Kitano H. "AI-Driven biomedical research – Grand Challenge of AI and Systems Biology" Precision Medicine in Metabolic Disease: A new data driven approach to prevent the onset of metabolic disease (Sydney, Australia) October, 2016

Kitano H. "Healthy Aging in the context of Systems Biology, Digital Health, and Artificial Intelligence" JST(/AMED)-Leibniz Association Joint Workshop on Healthy Ageing (Tokyo, Japan) June, 2016

Kitano H. "Artificial Intelligence for Scientific Discovery in Biomedical Sciences" GET Conference (Boston, USA) April, 2016

Kitano H. "Cellular systems" The 3rd EMBO Conference on Visualizing Biological Data (VIZBI 2016) (Heidelberg, Germany) March, 2016

Laboratory for Medical Science Mathematics

Group Director: Tatsuhiko Tsunoda

Recent Major Publications

Fujimoto A+, Furuta M+, Totoki Y+, Tsunoda T+, Kato M+ (+: co-first), Shiraishi Y, Tanaka H, Taniguchi H, Kawakami Y, Ueno M, Gotoh K, Ariizumi S, Wardell CP, Hayami S, Nakamura T, Aikata H, Arihiro K, Boroevich KA, Abe T, Nakano K, Maejima K, Sasaki-Oku A, Ohsawa A, Shibuya T, Nakamura H, Hama N, Hosoda F, Arai Y, Ohashi S, Urushidate T, Nagae G, Yamamoto S, Ueda H, Tatsuno K, Ojima H, Hiraoka N, Okusaka T, Kubo M, Marubashi S, Yamada T, Hirano S, Yamamoto M, Ohdan H, Shimada K, Ishikawa O, Yamaue H, Chayama K, Miyano S, Aburatani H, Shibata T, Nakagawa H. Whole genome mutational landscape and characterization of non-coding and structural mutations in liver cancer. ***Nat Genetics*** 48, 500–509 (2016)

Sharma A, Boroevich KA, Shigemizu D, Kamatani Y, Kubo M, Tsunoda T. Hierarchical Maximum Likelihood Clustering Approach. ***IEEE Trans Biomed Eng*** 64, 112–122 (2016).

Fujimoto A, Furuta M, Shiraishi Y, Nguyen HH, Shigemizu D, Gotoh K, Kawakami Y, Nakamura T, Ueno M, Ariizumi S, Shibata T, Abe T, Boroevich KA, Nakano K, Sasaki A, Kitada R, Maejima K, Yamamoto Y, Tanaka H, Shibuya T, Ojima H, Shimada K, Hayami S, Shigekawa Y, Aikata H, Arihiro K, Ohdan H, Marubashi S, Yamada T, Ishikawa O, Kubo M, Hirano S, Yamamoto M, Yamaue H, Chayama K, Miyano S, Tsunoda T*, Nakagawa H* (*: co-last). Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. ***Nat Commun*** 6, 6120 (2015)

Invited Presentations

Lysenko A and Kamola P. "Strategies for discovering disease-associated modules in integrated biological networks" RECOMB/ISCB Conference on Regulatory and Systems Genomics (Phoenix, USA) November, 2016

Shigemizu D. "Current status of exome analysis for identifying disease gene mutation" Grant-in-Aid for Scientific Research A Symposium (I) Large scale data analysis and modeling to understand complicated biological phenomena (Kurume, Japan) November, 2016

Tsunoda T. "Medical big data analysis drives precision and preemptive medicine" Symposium 'Basics and Medical Application of Statistical Genetics', 23rd Annual Meeting of the Japanese Society for Gene Diagnosis and Therapy (Tokyo, Japan) October, 2016

Tsunoda T. "Disease multi-omics analysis utilizing medical big data" CREST Symposium 'Elucidation of the life system by trans-omics' (Tokyo, Japan) March, 2016

Tsunoda T. "Big data analysis frontiers personalized medicine" Software Japan 2016 (Tokyo, Japan) February, 2016

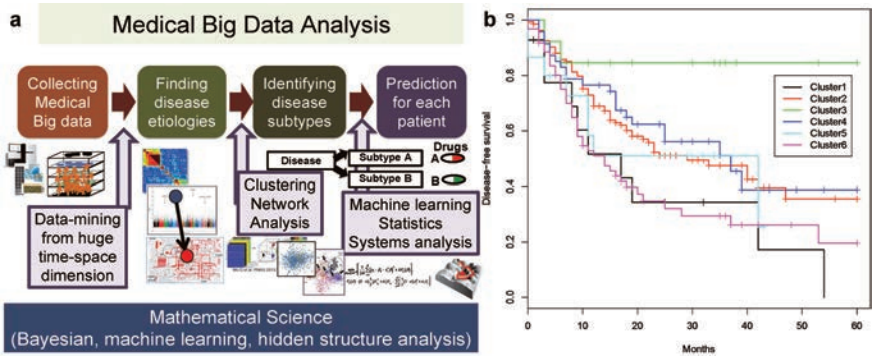
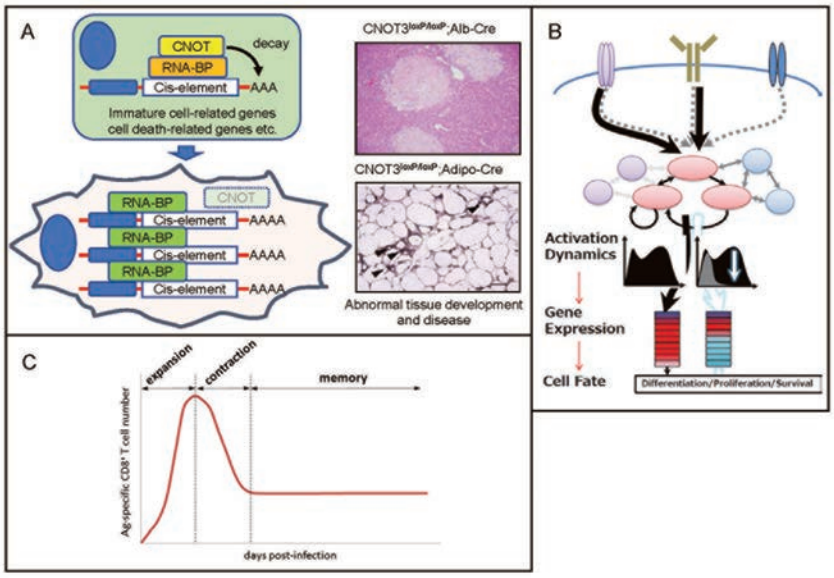


Figure: Medical Big Data Analysis for Precision Medicine.
(a) Common analysis steps and methodologies. (b) Application to omics data from 300 hepatocellular carcinoma cases revealed six clusters, which showed significantly different disease-free survival rates (***Nat Genet*** 48, 500–509 (2016)).

The application of rapidly progressing omic profiling technologies and, in particular, the promotion of personalized/precision/preventive medicine have recently become major goals of medical research. Traditional therapies do not adequately take into account the individuality of each patient. Our laboratory develops strategies to overcome such medical science issues through a combination of mathematics and computational sciences. Nowadays, biomedical big data, consisting of both clinical and omic profiles, are collected from hospitals and medical institutions. First, driven by need for integrative analysis of clinical and omic data, we explore etiologies of intractable diseases, e.g., cancer, common diseases, and neurodegenerative diseases. Next, we classify each disease into finer categories through molecular profiles, and clarify disease causing mechanisms through a systems approach. Last, we apply mathematical methods, e.g., machine learning techniques, to optimize therapy prediction for each patient when she/he visits a hospital or medical institute. We can also apply these methods to disease prevention based on an individual's medical history. Our laboratory does biomedical research and genomic medicine research driven by prediction through complete utilization of advanced mathematics and computational sciences with the goal of developing personalized/precision/preventive medicine strategies and methodologies. Recently, we developed statistical and computational methodologies for analyzing up-to-date omics data, as well as for understanding and predicting cancer progression based on genomic data, with a final aim of establishing precision medicine and applying it to real data [***Nat Genet*** 48, 500–509 (2016); ***IEEE Trans Biomed Eng*** 64, 112–122 (2016); ***J Theor Biol*** 393, 67–74 (2016); ***BMC Bioinformatics*** 17, 319 (2016); ***BMC Med Genomics*** 9(Suppl 3), 74, 2016; ***IEEE Trans Nanobioscience*** 14, 915–926 (2015)]. On the basis of these methodologies, we succeeded in observing a significant correlation between the molecular clustering of omic data and clinical information, e.g. survival time, from liver cancer omic analysis, and have recently published these results (***Nat Genet*** 48, 500–509 (2016)).

Laboratory for Immunogenetics

Team Leader: Tadashi Yamamoto



Figures: Research projects in the laboratory

A. Defects in the mRNA decay system result in abnormal tissue development
The CNOT complex-mediated mRNA decay pathway contributes to tissue development by regulating gene expression in the transition from undifferentiated to differentiated cells. When mRNA decay is deregulated by inhibition of the CNOT complex or by an ectopic increase in mRNA stabilizing molecules (green), abnormal tissues and various diseases develop. Photos: necrosis in liver (upper) and reduction of adipocyte size and macrophage infiltration in white adipose tissue (lower) in mice lacking the CNOT3 component of the CNOT complex.

B. Activation dynamics lead to specific gene expression
Signaling networks generate the distinct activation dynamics induced by different stimuli. The differences in activation dynamics may determine specific gene expression profiles and, hence, biological output.

C. The kinetics of Ag-specific CD8 T cell numbers following infection

Recent Major Publications

Li X, Morita M, Kikuguchi C, Takahashi A, Suzuki T* and Yamamoto T*. (*corresponding author) Adipocyte-specific disruption of mouse Cnot3 causes lipodystrophy. ***FEBS Lett*** 591, 358–368 (2017).

Shinohara H*, Inoue K, Yumoto N, Nagashima T, Okada-Hatakeyama M. (*corresponding author) Stimulus-Dependent Inhibitor of Apoptosis Protein Expression Prolongs the Duration of B Cell Signaling. ***Sci Rep*** 6, 27706 (2016)

Setoguchi R. IL-15 boosts the function and migration of human terminally differentiated CD8+ T cells by inducing a unique gene signature. ***Int Immunol*** 28, 293–305 (2016)

Invited Presentations

Yamamoto T. "The CCR4-NOT dedenylase: its roles in controlling cells' survival and differentiation in various systems" The 39th Annual Meeting of the Molecular Biology Society of Japan (Yokohama, Japan) December, 2016

Yamamoto T. "The CCR4-NOT deadenylase: a key player of novel paradigm of gene regulation" The 89th Annual Meeting of the Japanese Biochemical Society (Sendai, Japan) September, 2016

Three projects are ongoing in our team to understand the molecular basis of tissue development and the dynamics of signal transduction.

1) Tissue development involves dramatic changes in gene expression. Control of mRNA stability as well as transcriptional regulation contribute to these gene expression changes. We have found that suppression of the deadenylase activity of the CNOT complex, which mediates mRNA degradation, leads to abnormal development of liver and adipose tissues concomitant with cell death and inflammation. Augmentation of mRNA binding proteins that stabilize target mRNAs are also observed in various diseases. We are investigating how those molecules regulate tissue development and function in a developmental stage-specific manner, and how they are deregulated in diseases.

2) By analyzing protein modification that controls signal dynamics as a drug target, it is possible to develop therapeutic agents without side effects. Therefore, the importance of understanding the dynamics of signal transduction has become increasingly clear. In B cells, cell differentiation is determined by signals from the antigen receptor and cooperative receptors such as CD40. The mechanisms that produce different cellular responses despite the qualitatively identical molecules activated by various stimuli will be mediated by changes in active kinetics controlled by temporal information. We have quantitatively analyzed the induced protein complexes and dynamics of signaling activity and cellular responses by time-controlled ubiquitin modification. This has helped to clarify the signaling networks and control mechanisms of induced cell differentiation.

3) Memory CD8 T cells are long-lived antigen (Ag)-specific cells that can respond quickly, proliferate robustly, and exert effector functions faster than naïve CD8 T cells upon reencounter with their cognate Ags. Memory CD8 T cells are maintained as a stable population over a long period, but the mechanisms by which their population size is maintained remain elusive. One of objectives of our study is to reveal how memory CD8 T cells are maintained *in vivo*. This research can provide new vaccination strategies for induction of protective immunity against tumor cells and in chronic infections.

Laboratory for Integrated Bioinformatics

Team Leader: Todd D. Taylor

Figure: iCLiKVAL Overview
iCLiKVAL is a web-based tool that uses the power of crowdsourcing to accumulate annotation information for all scientific media found online. Annotations in the form of key-relationship-value tuples are added by users through a variety of methods. Users can create or join common interest groups to work as part of a community. Controlled vocabulary lists can be created, edited and shared. Media can be bookmarked, followed, reviewed and searched. Annotations can be sorted, filtered and edited. The database is completely searchable, without registration, and all of the data are freely available to registered users via our application programming interface.

We are developing an integrated database and sample-tracking system to handle both small- and large-scale datasets of various types of experimental outputs. The system brings together an array of wet-lab experimental types being generated by various labs. Our goal is to develop a flexible system that makes it easy for users to manage, access, analyze, integrate, and visualize their own data as per their requirements.

We also develop general-purpose bioinformatic tools capable of efficiently processing and analyzing data from a variety of sources, with an emphasis on metagenomic data (e.g., taxonomic classification, phylogenic tree visualization, 16S rRNA curation), host-microbiome interactions, and scientific discovery through big data curation. We have constructed a highly-curated genomic-based 16S ribosomal RNA gene database, which is continually being updated as new sequences appear in the public databases. Because metagenomic samples can contain hundreds or thousands of different species and are not easy to visualize or quantify, we developed an alternative visualization method for displaying phylogenic trees that allows users to, at a glance, comprehend the distribution of the species within their samples. Big data in the form of scientific media comes in many languages and formats: journal articles, books, images, videos, etc. While there are many resources for browsing, searching and annotating some of this media, there is no single place to search them all at once, and generalized search engines do not allow for the comprehensive and precise searches researchers require. To address these issues, we have developed a web-based tool that uses the power of crowdsourcing to accumulate annotation information for all scientific media found online. This will allow for richer data searches and discovery of novel connections by integrating all forms of scientific knowledge through common terminology.

Recent Major Publications

Lau N-S, Makita Y, Kawashima M, Taylor TD, Kondo S, Othman AS, Shu-Chien AC, Matsui M. The rubber tree genome shows expansion of gene family associated with rubber biosynthesis. ***Sci Rep*** 6, 28594 (2016)

Hebrard M, Taylor TD. MetaTreeMap: an alternative visualization method for displaying metagenomic phylogenic trees. ***PLOS ONE*** 11, e0158261 (2016)

Jinda W, Poungvarin N, Taylor TD, Suzuki Y, Thongnoppakhun W, Limwongse C, Lertrit P, Suriyaphol P, Atchaneeyasakul L. A novel start codon mutation of the MERTK gene in a patient with retinitis pigmentosa. ***Mol Vis*** 22, 342-351 (2016)

Invited Presentations

Taylor TD. "Improving the connectivity, searchability and discoverability of scientific media through annotations with iCLiKVAL" Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand) June, 2016

Taylor TD. "Turning 'big data' into 'small data' through crowdsourced curation: integrating all types of medical and scientific knowledge" 3rd AIST-RIKEN Joint Bioinformatics Seminar, RIKEN (Yokohama, Japan) June, 2016

Laboratory for Tissue Dynamics

Team Leader: Takaharu Okada

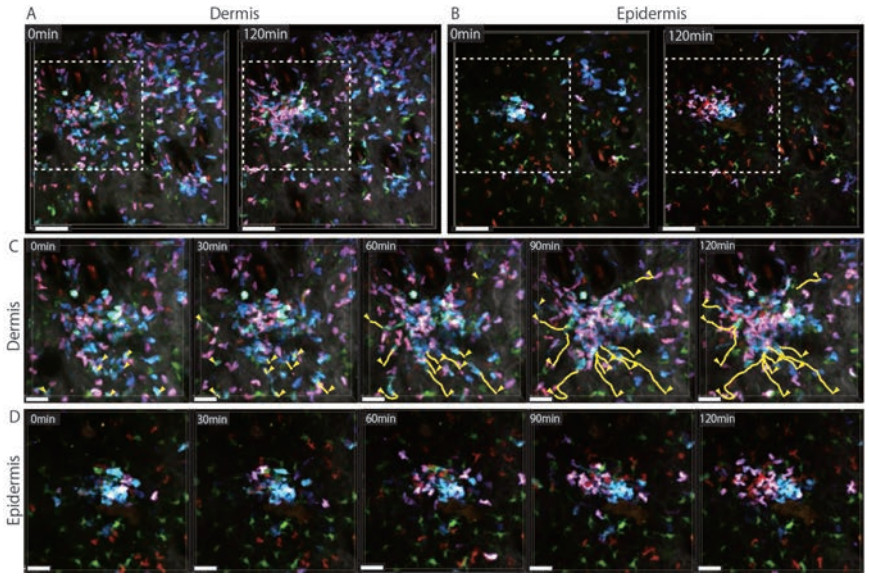


Figure: Multicolor imaging of dendritic cells and T cells in the mouse model of contact dermatitis by intravital multiphoton microscopy. (Modified from Okada et al. *Pflugers Arch* 468, 1793–1801, 2016).
Chicken ovalbumin-reactive T cell receptor transgenic CD4$^+$ T cells expressing tdTomato plus EGFP (pink to purple) and CD8$^+$ T cells expressing tdTomato (red) were transferred to an Xcr1$^{gfp/+}$ CD11c-YFP mouse for visualization of their interactions with XCR1$^+$ dendritic cells (light blue) and other dendritic cells (green). The mouse was subcutaneously immunized in the flank with ovalbumin plus poly (I:C). Four days after immunization, the mice were then intradermally injected in the dorsum of the foot with ovalbumin alone. Seven days later, the mouse was anesthetized, and the skin of the dorsum of the foot was imaged on an inverted multiphoton microscope with four external detectors. Excitation wavelength was 910 nm. **(A)** Projection images of ten z-slices of the dermis (33–60-μm depth from the skin surface) at the beginning and end of the 2-h recording. Scale bar: 80 μm. Collagen fibers (white) were also visualized by second harmonic generation. **(B)** Projection images of four z-slices of the epidermis (12–21-μm depth from the skin surface) at the same x-y position as (A). The scattered epidermal dendritic cells in green are mostly Langerhans cells. **(C and D)** Time-lapse images of the region indicated by dotted lines in (A) and (B). Scale bar: 40 μm. Yellow lines in (C) are paths of dendritic cell migration tracked every minute. Yellow arrowheads indicate starting positions of the tracks.

The goal of the laboratory is to mechanistically understand the *in vivo* cellular dynamics that shape immune responses and inflammation. To this end, we conduct multi-dimensional fluorescent imaging analysis by two-photon microscopy. This microscopy method has been revealing the striking dynamics of immune cells in various organs, underlining the importance of this approach to resolve the complexity of the immune system. By applying the imaging strategy to relevant mouse models, we aim to reveal immune cell dynamics that are critical for generation of immunological memory and tolerance. We have continued to study various mouse strains, in which (1) dynamics of helper T cells specialized for B cell immune responses are perturbed, (2) cross-presenting dendritic cells (DCs) are fluorescently labeled for *in vivo* imaging, and (3) dynamics and function of regulatory T (Treg) cell subsets are perturbed to break peripheral tolerance of autoreactive B cells and CD8$^+$ T cells.

In addition, we are studying the roles for the peripheral nervous system in modulation of inflammation. Particularly, we are interested in structural and functional changes in sensory neurons in dermatitis. In this study as well, the imaging approaches are very powerful for deciphering the diversity and dynamics of cellular activities. Through this study, we aim to find strategies to suppress chronic inflammation based on the modulation of neuronal activities.

Recent Major Publications

Okada T, Takahashi S, Ishida A, Ishigame H. *In vivo* multiphoton imaging of immune cell dynamics. ***Pflugers Arch*** 468, 1793–1801 (2016)

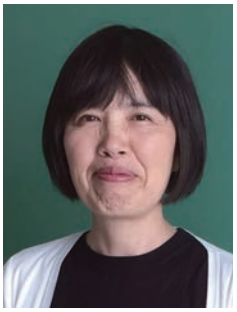
Shinnakasu R, Inoue T, Kometani K, Moriyama S, Adachi Y, Nakayama M, Takahashi Y, Fukuyama H, Okada T, Kurosaki T. Regulated selection of germinal-center cells into the memory B cell compartment. ***Nat Immunol*** 17, 861–869 (2016)

Kitano M, Yamazaki C, Takumi A, Ikeno T, Hemmi H, Takahashi N, Shimizu K, Fraser SE, Hoshino K, Kaisho T, Okada T. Imaging of the cross-presenting dendritic cell subsets in the skin-draining lymph node. ***Proc Natl Acad Sci U S A*** 113, 1044–1049 (2016)

Invited Presentations

Okada T. "Cellular dynamics shaping adaptive immune responses in the lymph node" The 54th Annual Meeting of The Biophysical Society of Japan (Tsukuba, Japan) November, 2016

Okada T. "Imaging of cellular dynamics shaping the adaptive immune system" The 68th Annual Meeting of The Japan Society for Cell Biology (Kyoto, Japan) June, 2016



Laboratory for Integrated Cellular Systems

Team Leader: Mariko Okada

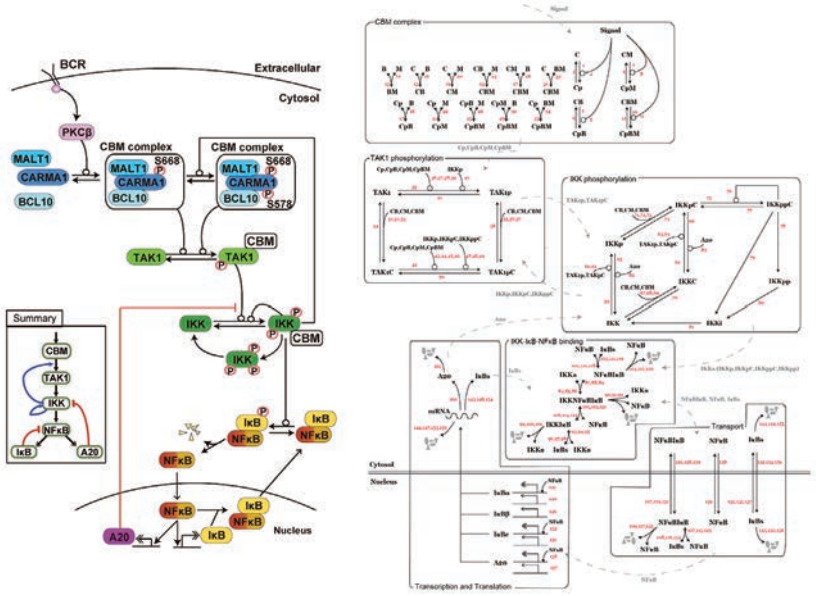


Figure: A mathematical model of the BCR- NF-κB network
Left: A model overview. Right: Detailed diagram of the model. Black arrows indicate functional modifications or association/dissociation of proteins. Gray dashed arrows indicate links of inter-modules (CBM complex, TAK1 phosphorylation, IKK phosphorylation, IKK-IκB-NF-κB binding, transport, transcription and translation).

The aims of the laboratory are to define the general regulatory rules in signal transduction-transcriptional networks in cell determination processes and to apply this knowledge of regulatory principles to the understanding and treatment of human diseases. Using systems biology approaches, we uncovered several unique properties in signal-transcription networks in immune cell development and cancer. For example, the potency and duration of signaling responses are controlled by self-regulatory mechanisms. One of such examples is negative feedback regulation in signaling cascades. We recently found that transcriptionally-induced PHLDA1 suppresses activation of ErbB receptors and downstream kinases after growth factor stimulation of breast cancer cells and thereby acts as a negative feedback regulator. Our LC-MS analysis showed that PHLDA1 binds to ErbB3. Mathematical modeling followed by single molecule analysis of fluorescent-labeled ligand binding to the receptors indicated that PHLDA1 inhibits high-order oligomerization of ErbB receptors, which suggested a novel inhibitory mechanism of ErbB receptor signaling (Manuscript in revision). On the other hand, intracellular signaling pathways often act as amplifiers to activate transcription factors for thresholding setting during cellular commitment. Our earlier study indicated that IKK activity is regulated by positive feedback from IKK to TAK1, mediated by the scaffolding protein CARMA1 at Ser-578, and that this feedback regulation could induce switch-like activation of NF-κB in B cells (Shinohara et al. *Science*, 2014). Our recent mathematical analysis further indicated that this switch activation of NF-κB is important for induction of target genes, which are co-regulated by NF-κB oscillation dynamics (Inoue, et al. *npj Syst. Biol. Appl.* 2016). Thus, our studies showed that this signaling-transcription network is controlled in the short term by post-transcriptional regulation and in the long term by transcription-mediated regulation. Moreover, the resulting dynamics itself contains important messages for cell regulatory mechanisms. Our lab also contributed to research society in terms of large-scale data analysis and mathematical modeling.

Recent Major Publications

Inoue K, Shinohara H, Behar M, Yumoto N, Tanaka G, Hoffmann A, Aihara K, Okada-Hatakeyama M. Oscillation dynamics underlies functional switching of NF-κB for B cell activation. ***NPJ Syst Biol Appl*** 2, 16024 (2016)

Yoshida K, Maekawa T, Zhu Y, Renard-Guillet C, Chatton B, Inoue K, Uchiyama T, Ishibashi K, Yamada T, Ohno N, Shirahige K, Okada-Hatakeyama M, Ishii S. The transcription factor ATF7 mediates lipopolysaccharide-induced epigenetic changes in macrophages involved in innate immunological memory. ***Nat Immunol*** 16, 1034–1043 (2015)

Mina M, Magi S, Jurman G, Itoh M, Kawaji H, Lassmann T, Arner E, Forrest ARR, Carninci P, Hayashizaki Y, Daub CO, the FANTOM Consortium, Okada-Hatakeyama M, Furlanello C. Promoter-level expression clustering identifies time development of transcriptional regulatory cascades initiated by ErbB receptors in breast cancer cells. ***Sci Rep*** 5, 11999 (2015)

Invited Presentations

Okada M. "Multiple-scale cooperativity in signal-transcription network for cellular commitment" Trans-Omics: New Approaches in Biology and Medicine 2016 (Fukuoka Japan) November, 2016

Okada-Hatakeyama M." Experimental and modeling analysis of signal transduction network in mammalian cells" 2016 A3 Workshop on Interdisciplinary Research Connecting Mathematics and Biology (Beijing, China) April, 2016

Okada-Hatakeyama M. "Experimental and mathematical analysis of signaling network". National Taiwan University (Taipei, Taiwan) April, 216

Okada-Hatakeyama M. "Switch-like activation of transcription factors in cell decision program". Academia Sinica, Taiwan (Taipei, Taiwan) April, 2016

Okada-Hatakeyama M. "Modeling Cellular Signaling Functions in Mammalian Cells" The 4th Bioscience and Biotechnology International Symposium. Multifaceted Approaches to Disease Intervention (Yokohama, Japan) January, 2016

Laboratory for Metabolomics

Team Leader: Makoto Arita

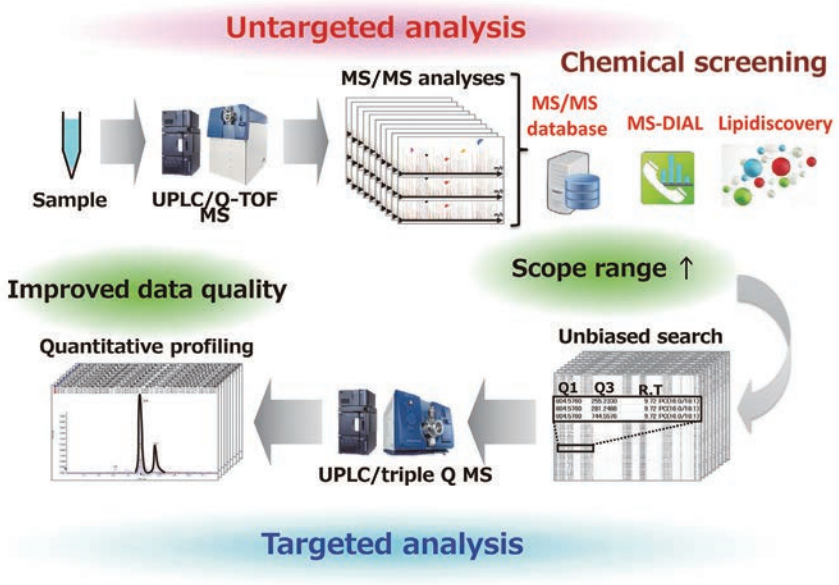


Figure: Untargeted multi-lipidomics platform

Lipids are recognized as extremely diverse molecules. Precise determination of each molecular species of lipid, namely Lipo-Quality (Quality of Lipids), becomes a prerequisite not only to understand their biological functions in physiology and disease, but also to discover novel bioactive lipids that may link lipid metabolism and biological phenotypes. A powerful method for the analysis of lipid metabolites is liquid chromatography tandem mass spectrometry (LC-MS/MS). Our research is aimed at elucidating structure and function of endogenous lipid metabolites that regulate inflammation and tissue homeostasis.

A triple quadrupole (TripleQ) mass spectrometer is capable of carrying out a targeted MS method called multiple reaction monitoring (MRM). A quadrupole Time-of Flight (Q-TOF) mass spectrometer provides the ability to perform untargeted analyses with high resolution and accurate MS/MS information. The MS/MS trigger is set at a low threshold level in order to detect as globally as possible, and lipid structures are unbiasedly annotated by lipid database screening. By taking advantage of Q-TOF (global lipid screening) and TripleQ (quantitative analyses) mass spectrometry, our new approach has a strong potential to search for lipids of interest globally, and to identify unknown lipid species in a non-biased fashion. We are developing a measured MS/MS library of diverse lipid molecular species for Q-TOF-based untargeted analyses, especially focusing on bioactive lipid mediators, oxidized phospholipids, skin barrier lipids such as acylceramides, and unique lipid metabolites produced by gut microbiota. We are also developing software that enables us to search for lipid structures more precisely and comprehensively.

Recent Major Publications

Hirabayashi T, Anjo T, Kaneko A, Senoo Y, Shibata A, Takama H, Yokoyama K, Nishito Y, Ono T, Taya C, Muramatsu K, Fukami K, Muñoz-Garcia A, Brash AR, Ikeda K, Arita M, Akiyama M, Murakami M. PNPLA1 has a crucial role in skin barrier function by directing acylceramide biosynthesis. ***Nature Commun*** (in press)

Sakayori N, Kikkawa T, Tokuda H, Kiryu E, Yoshizaki K, Kawashima H, Yamada T, Arai H, Kang JX, Katagiri H, Shibata H, Innis SM, Arita M, Osumi N. Maternal dietary balance between omega-6 and omega-3 polyunsaturated fatty acids impairs neocortical development via epoxy metabolites. ***Stem Cells*** 34, 470–482 (2016)

Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, Kanazawa M, VanderGheynst J, Fiehn O, Arita M. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. ***Nature Methods*** 12, 523–526 (2015)

Invited Presentations

Arita M. "Lipidomics & Discovery of Novel Bioactive Omega-3 Metabolites" ISOR Inaugural Symposium "Omega-3 in Health Promotion & Disease Management" (Boston, USA) January, 2017

Arita M. "Advanced lipidomics to understand the quality difference of fatty acids in biological systems" The 39th Annual Meeting of the Molecular Biology Society of Japan (Yokohama, Japan) November, 2016

Arita M. (Award Lecture) "Omega-3 fatty acid metabolism in controlling inflammation and related diseases" The 25th Annual Meeting of Japan Society for Lipid Nutrition (Akita, Japan) September, 2016

Arita M. "Emerging roles of lipid metabolism in phagocyte function" The 24th International Symposium on Molecular Cell Biology of Macrophages (Tokyo, Japan) June, 2016

Arita M. (Plenary Lecture) "Lipidomic approach to uncover anti-inflammatory properties of omega-3 polyunsaturated fatty acids" 2016 Korea-Japan Bioactive Lipid Joint Symposium (Jeju, Korea) May, 2016

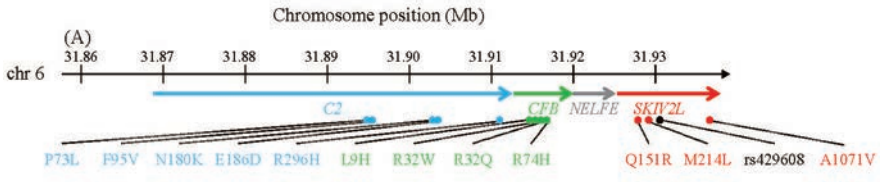
Core for Genomic Medicine

The Core for Genomic Medicine is performing genomic research on human diseases, especially the common diseases. The aims of Core for Genomic Medicine are 1) to identify genetic variations related to disease susceptibility, disease outcome and drug responses (efficacy/adverse reaction), 2) to provide useful information about possible molecular targets for drug discovery, 3) to examine the interactions between genetic and environmental factors to understand the pathogenesis and the progression of diseases, and 4) finally to construct an evidence base for the implementation of personalized medicine.

To identify genetic variations related to disease susceptibility and drug responses, the Core for Genomic Medicine first showed a proof of concept of the genome-wide association study (GWAS) in 2002. To advance this strategy, the Core for Genomic Medicine has organized laboratories to facilitate comprehensive genomic research on common diseases. To produce comprehensive genomic information, the Laboratory for Genotyping Development is mainly working on large-scale SNP genotyping and genome sequencing for various diseases. The resulting huge amount of genomic variation data was mainly analyzed at the Laboratory for Statistical Analysis to extract significant genomic variations related to disease susceptibility and drug responses. These laboratories are in close communication with the research group of pharmacogenomics (Laboratory for Pharmacogenomics and Laboratory for International Alliance on Genomic Research), laboratories for disease-causing mechanisms (Laboratory for Cardiovascular Diseases, Autoimmune Diseases, Digestive Diseases, Bone and Joint Diseases, Endocrinology, Metabolism and Kidney Diseases, and Respiratory and Allergic Diseases) and many other collaborators worldwide for further analyses. In addition to this strategy, the Laboratory for Genome Sequencing Analysis is mainly working on whole genome sequencing of cancer genomes to clarify the pathogenesis of carcinogenesis.

Laboratory for Genotyping Development

Team Leader: Yukihide Momozawa



(B)

No	C2					CFB				SKIV2L			GWAS	CaseFreq	ControlFreq	P value	OR	OR 95%CI
	73	95	180	186	296	9	32W	32Q	74	151	214	1071	rs429608	(n = 2,884)	(n = 9,309)			
1	C	T	C	G	G	T	C	G	G	A	C	C	G	0.4079	0.4208			
2	C	T	C	G	G	T	C	G	G	A	A	C	G	0.4187	0.3790	9.10E-05	1.140	(1.067-1.217)
3	C	T	C	G	G	T	T	G	G	A	C	C	G	0.0811	0.0600	5.10E-08	1.394	(1.237-1.569)
4	C	T	C	G	G	T	C	A	G	G	C	T	A	0.0279	0.0417	2.54E-05	0.691	(0.576-0.825)
5	C	T	C	G	A	T	C	A	G	G	C	T	A	0.0173	0.0256	1.08E-03	0.698	(0.554-0.872)
6	C	T	C	C	G	A	C	G	G	A	C	C	A	0.0149	0.0249	3.42E-05	0.619	(0.483-0.784)
7	T	T	C	G	G	T	C	G	A	A	C	C	G	0.0078	0.0165	1.56E-06	0.486	(0.346-0.669)
8	C	G	C	G	A	T	C	A	G	G	C	T	A	0.0061	0.0113	8.90E-04	0.555	(0.375-0.799)
9	C	T	C	G	G	T	T	G	G	A	C	T	G	0.0127	0.0104	0.105	1.259	(0.945-1.664)
10	C	T	G	G	G	T	C	A	G	G	C	T	A	0.0033	0.0063	0.010	0.541	(0.314-0.885)
11	C	T	C	G	G	T	C	G	A	A	C	C	G	0.0000	0.0012	4.62E-03	0.000	(0.000-0.580)

Figure: Diagram and association results of haplotypes in the C2-CFB-SKIV2L locus with susceptibility to age-related macular degeneration

The aims of the Laboratory for Genotyping Development are 1) to produce precise and large-scale genomic data to identify genetic variants related to disease susceptibility, outcomes, and drug responses in close collaboration with the Laboratory for Statistical Analysis and 2) to develop methods and databases useful for personalized medicine. Our laboratory has worked as a research hub of large-scale genomic analysis, collaborating with domestic and international universities, research institutes, and pharmaceutical companies.

Our laboratory published 51 papers in 2016. Among them, we newly reported a multiplex PCR based targeted sequencing method that enables us to sequence specific genomic regions in tens of thousands of samples. This method showed a high concordance rate (99.97%) of genotypes with other established methods. We applied this method to investigate the contribution of low frequency variants in 34 genes to age-related macular degeneration (AMD) susceptibility in 2,886 cases and 9,337 controls. While we showed that low frequency variants in the *CFB* gene were protective against AMD (odds ratio = 0.43), disruptive variants in the *CETP* gene increased disease risk (odds ratio = 2.48). These findings were considered East Asian-specific. In addition, since dysfunction of CETP is also known to increase high-density lipoprotein cholesterol (HDL-C), a previously unknown connection between HDL-C and AMD was proposed. These findings highlight the importance of targeted sequencing to reveal the impact of rare or low-frequency coding variants on disease susceptibility in different ethnic populations.

We will continue to work as a research hub for large-scale genomic analysis so that we can contribute to the implementation of personalized medicine.

Recent Major Publications

Momozawa Y, Akiyama M, Kamatani Y, Arakawa S, Yasuda M, Yoshida S, Oshima Y, Mori R, Tanaka K, Mori K, Inoue S, Terasaki H, Yasuma T, Honda S, Miki A, Inoue M, Fujisawa K, Takahashi K, Yasukawa T, Yanagi Y, Kadonosono K, Sonoda KH, Ishibashi T, Takahashi A, Kubo M. Low-frequency coding variants in CETP and CFB are associated with susceptibility of exudative age-related macular degeneration in the Japanese population. ***Hum Mol Genet*** 25, 5027–5034 (2016).

Zhou K, Yee SW, Seiser EL, van Leeuwen N, Tavendale R, Bennett AJ, Groves CJ, Coleman RL, van der Heijden AA, Beulens JW, de Keyser CE, Zaharenko L, Rotroff DM, Out M, Jablonski KA, Chen L, Javorský M, Židzik J, Levin AM, Williams LK, Dujic T, Semiz S, Kubo M, Chien HC, Maeda S, Witte JS, Wu L, Tkáč I, Kooy A, van Schaik RH, Stehouwer CD, Logie L; MetGen Investigators.; DPP Investigators.; ACCORD Investigators., Sutherland C, Klovins J, Pirags V, Hofman A, Stricker BH, Motsinger-Reif AA, Wagner MJ, Innocenti F, Hart LM, Holman RR, McCarthy MI, Hedderson MM, Palmer CN, Florez JC, Giacomini KM, Pearson ER. Variation in the glucose transporter gene SLC2A2 is associated with glycemic response to metformin. ***Nat Genet*** 48, 1055–1059 (2016)

Sun C, Molineros JE, Looger LL, Zhou XJ, Kim K, Okada Y, Ma J, Qi YY, Kim-Howard X, Motghare P, Bhattarai K, Adler A, Bang SY, Lee HS, Kim TH, Kang YM, Suh CH, Chung WT, Park YB, Choe JY, Shim SC, Kochi Y, Suzuki A, Kubo M, Sumida T, Yamamoto K, Lee SS, Kim YJ, Han BG, Dozmorov M, Kaufman KM, Wren JD, Harley JB, Shen N, Chua KH, Zhang H, Bae SC, Nath SK. High-density genotyping of immune-related loci identifies new SLE risk variants in individuals with Asian ancestry. ***Nat Genet*** 48, 323–330 (2016)

Invited Presentations

Momozawa Y. "Direction of canine behavior genetics" Meeting for behavior genetics (Mishima, Japan) October, 2016

Laboratory for Genome Sequencing Analysis

Team Leader: Hidewaki Nakagawa

Recent Major Publications

Furuta M, Ueno M, Fujimoto A, Hayami S, Yasukawa S, Kojima F, Arihiro K, Kawakami Y, Wardell CP, Shiraishi Y, Tanaka H, Nakano K, Maejima K, Sasaki-Oku A, Tokunaga N, Boroevich KA, Abe T, Aikata H, Ohdan H, Gotoh K, Kubo M, Tsunoda T, Miyano S, Chayama K, Yamaue H, Nakagawa H. Whole genome sequencing discriminates hepatocellular carcinoma with intrahepatic metastasis from multi-centric tumors. ***J Hepatol*** 66, 363–373 (2017)

Alexandrov LB, Ju Y, Haase K, Van Loo P, Martincorena I, Nik-Zainal S, Totoki Y, Fujimoto A, Nakagawa H, Shibata T, Campbell PJ, Vineis P, Phillips DH, Stratton MR. Mutational signatures associated with tobacco smoking in human cancer. ***Science*** 354, 618–622 (2016)

Fujimoto A, Furuta M, Totoki Y, Tsunoda T, Kato M, Shiraishi Y, Tanaka H, Taniguchi H, Kawakami Y, Ueno M, Gotoh K, Ariizumi S, Wardell CP, Hayami S, Nakamura T, Aikata H, Arihiro K, Boroevich KA, Abe T, Nakano N, Maejima K, Sasaki-Oku A, Ohsawa A, Shibuya T, Nakamura H, Hama N, Hosoda F, Arai Y, Ohashi S, Urushidate T, Nagae G, Yamamoto S, Ueda H, Tatsuno K, Ojima H, Hiraoka N, Okusaka T, Kubo M, Marubashi S, Yamada T, Hirano S, Yamamoto M, Ohdan H, Shimada K, Ishikawa O, Yamaue H, Chayama K, Miyano S, Aburatani H, Shibata T, Nakagawa H. Whole genome mutational landscape and characterization of non-coding and structural mutations in liver cancer. ***Nature Genetics*** 48, 500–509 (2016)

Invited Presentations

Nakagawa H. "Sequencing analysis of plasma and pancreatic juice cell-free DNAs for cancer diagnosis" The 75th Annual Meeting of Japanese Cancer Association (Yokohama, Japan) October, 2016

Nakagawa H and Aburatani H. "Molecular analysis for liver cancer progression: step-wise progression and recurrent diagnosis" The 12th Scientific Workshop of ICGC (Boston, USA) September, 2016

Nakagawa H. "Immunogenomic Analysis in PCAWG (PanCancer Analysis for Whole Genomes) of ICGC/TCGA" The 25th Korean Genome Organization Annual Conference (Seoul, Korea) September, 2016

Nakagawa H. "HLA genotyping and immunogenomics of PanCancer" PCAWG Workshop, ICGC/TCGA (Barcelona, Spain) April, 2016

Nakagawa H. "Whole genome sequencing of liver cancer and forwarding to precision medicine" The 102nd Annual Meeting of Japanese Society of Gastroenterology (Tokyo, Japan) April, 2016

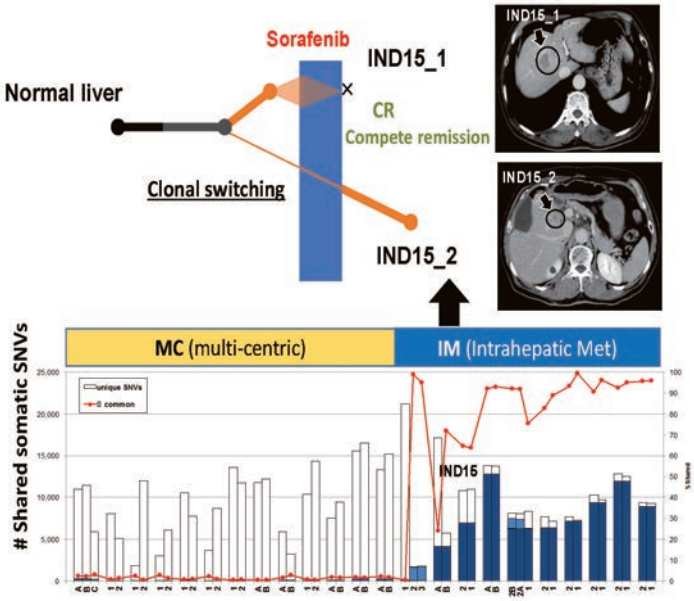


Figure: Molecular discrimination of multi-centric tumors (MC) and intrahepatic metastasis (IM) of liver cancer by the number of shared somatic SNVs detected by whole genome sequencing (WGS).
Phylogenetic tree depicts the patterns of clonal evolution of the IND15_1 tumor nodule, which showed complete remission after sorafenib treatment, and the IND15_2 nodule, which was diagnosed as a MC after complete remission by sorafenib, but WGS analysis indicated that it was an IM after clonal switching.

Cancer is essentially a "disease of the genome" that evolves in the background of germline variants with the accumulation of diverse mutations caused by environmental exposure. Germline variants are biomarkers predisposing to cancer development and genetic analysis of certain specific genes, such as *BRCA1/2*, is commonly performed for cancer risk, while somatic mutations of driver genes have been targeted for cancer treatment and genotype-based personalized cancer therapy is now a reality. Furthermore, emerging immune therapies, which modulate immune cell activity or immuno-genes in cancer cells, have been demonstrated to be effective in many types of cancers. Now we have to understand more about cancer genome-immune interactions and their diversity to develop new and more effective therapies. Recent explosive advances in next-generation sequencing (NGS) and bioinformatics enable a systematic, genome-wide identification of all somatic abnormalities and immune activity within cancer tissues by whole genome sequencing (WGS), whole exome sequencing and RNA sequencing. It is important to analyze the likely implications of the huge genome datasets from NGS and to reach a consensus about how to interpret the biological and clinical aspects of somatic and germline variants. Our research objectives are to understand cancer genome and microenvironmental activities, including immune activity, by utilizing NGS to identify novel genomic biomarkers and to develop analysis platforms that can be used in clinics for precision medicine.

Laboratory for Statistical Analysis

Team Leader: Yoichiro Kamatani

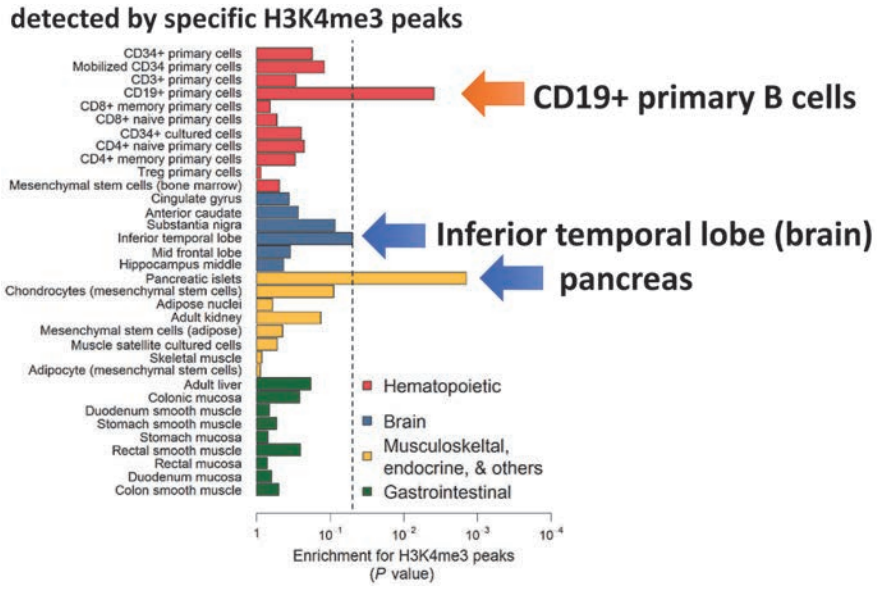


Figure: Enrichment of obesity susceptible SNPs in CD19⁺ primary B cells
We performed GWAS for obesity using 173,430 subjects and found 85 genome-wide significant loci, including 49 novel obesity variants. We used them and conducted enrichment analysis of these significant variants into cell-type specific regulatory marks obtained from Roadmap H3K4me3 epigenomics data. We successfully replicated previously reported enrichment of obesity variants in pancreatic islets and brain tissue, and found novel enrichment in primary CD19⁺ B cells.

Our laboratory aims at identifying susceptibility variants of complex traits by using genetic association study, as well as connecting the genetic association findings with biology and medicine. Until now, we have used genome-wide SNP array data and performed genome wide association studies (GWAS) for dozens of traits. In 2016, we have completed GWAS for 45 different diseases using samples from ~200,000 BioBank Japan participants with ~30,000 population controls genotyped for nearly a million SNPs. Additionally, we have performed GWAS for more than 50 quantitative traits including anthropometric traits and clinical laboratory data, and also for life-style using the BioBank Japan clinical information database. These are catalogs of the largest non-European GWAS ever for each trait.

We then moved into addressing three key issues in this field; missing heritability, functional interpretation of the identified genetic variants, and the genetic architecture of the diseases in question. Firstly, we have started whole genome sequence (WGS) analysis for thousands of patients affected by common disorders to evaluate the contribution of rare variants or structural variations which are not covered by SNP array data. Genotype imputation of the SNP results of the ~230,000 subjects described above using a WGS-based reference panel is being conducted in parallel. Secondly, we are now focusing on the interpretation of GWAS signals. We conducted integrative analysis of GWAS signals with publicly available expression and epigenomic data, and found a novel genetically-associated immune cell, the CD19⁺ B cell, for obesity, which should be one of the target cell-types to elucidate functional roles of GWAS variants. We are also using in-house eQTL data of lymphocyte subtypes generated by the Laboratory for Autoimmune Diseases and long non-coding RNA expression data from several cell types as a collaborative project with the RIKEN Center for Life Science Technologies (CLST). Lastly, we are attempting to use machine learning techniques to reveal non-additive effects of genomic variants that have not been investigated well in this field. The novel machine learning techniques will facilitate detecting such effects.

Recent Major Publications

Shiraishi K, Okada Y, Takahashi A, Kamatani Y, Momozawa Y, Ashikawa K, Kunitoh H, Matsumoto S, Takano A, Shimizu K, Goto A, Tsuta K, Watanabe S, Ohe Y, Watanabe Y, Goto Y, Nokihara H, Furuta K, Yoshida A, Goto K, Hishida T, Tsuboi M, Tsuchihara K, Miyagi Y, Nakayama H, Yokose T, Tanaka K, Nagashima T, Ohtaki Y, Maeda D, Imai K, Minamiya Y, Sakamoto H, Saito A, Shimada Y, Sunami K, Saito M, Inazawa J, Nakamura Y, Yoshida T, Yokota J, Matsuda F, Matsuo K, Daigo Y, Kubo M, Kohno T. Association of variations in HLA class II and other loci with susceptibility to EGFR-mutated lung adenocarcinoma. ***Nat Commun*** 7, 12451 (2016)

Okada Y, Suzuki A, Ikari K, Terao C, Kochi Y, Ohmura K, Higasa K, Akiyama M, Ashikawa K, Kanai M, Hirata J, Suita N, Teo YY, Xu H, Bae SC, Takahashi A, Momozawa Y, Matsuda K, Momohara S, Taniguchi A, Yamada R, Mimori T, Kubo M, Brown MA, Raychaudhuri S, Matsuda F, Yamanaka H, Kamatani Y, Yamamoto K. Contribution of a Non-classical HLA Gene, HLA-DOA, to the Risk of Rheumatoid Arthritis. ***Am J Hum Genet*** 99, 366–374 (2016)

Okada Y, Momozawa Y, Ashikawa K, Kanai M, Matsuda K, Kamatani Y, Takahashi A, Kubo M. Construction of a population-specific HLA imputation reference panel and its application to Graves' disease risk in Japanese. ***Nat Genet*** 47, 798–802 (2015)

Invited Presentations

Kamatani Y. "The Biobank Japan" International Stroke Genetics Consortium 2016 Fall (Milan, Italy) November, 2016

Kamatani Y. "Genetic analysis of non-European population" Neurepiomics (Boston, USA) September, 2016

Kamatani Y. "The largest Asian GWAS of ischemic stroke" International Stroke Genetics Consortium 2016 Spring (Boston, USA) May, 2016

Laboratory for Pharmacogenomics

Group Director: Taisei Mushiroda

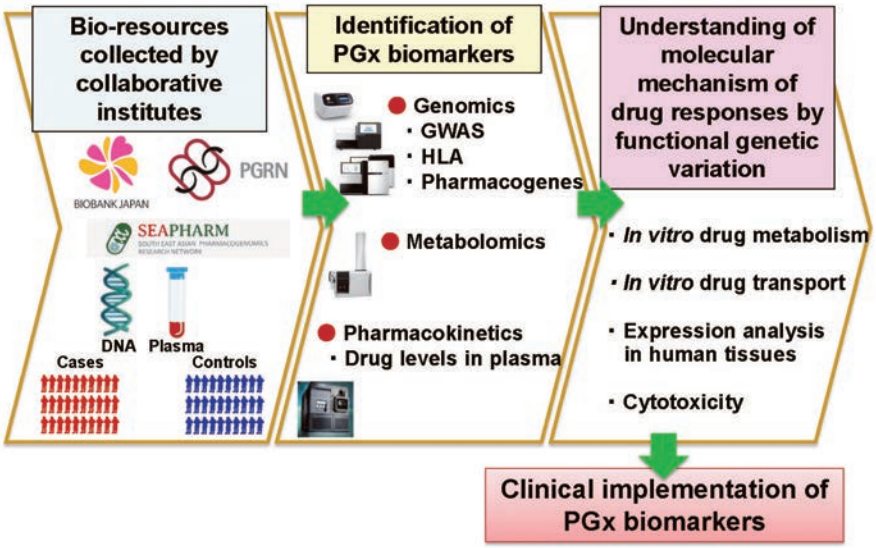


Figure: Identification of genomic biomarkers associated with drug responses and implementation into clinical practice for pharmacogenomics-based individualization of drug therapy

Genomic analyses, such as genome-wide association study (GWAS) and HLA genotyping, are effective for the identification of pharmacogenomics (PGx) biomarkers. GWAS presently stands as a standard method in IMS, but can explain only a part of the relationship between genomic variations and drug responses. Thus, we have developed a new targeted re-sequencing method that can identify all genomic variations in 100 drug-related genes (pharmacogenes) using next generation sequencing. In addition to genomic analysis, pharmacokinetics and metabolomics analyses using ultra high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) may be necessary for development of accurate predictive systems for risk of adverse drug reactions (ADRs) and for drug efficacy.

Although we have mainly used BioBank Japan, which contains DNA samples and clinical information from about 220,000 Japanese patients, it is difficult for individual countries acting alone to collect a sufficient number of samples for PGx research. We will continue two international PGx collaborative projects with the South East Asian Pharmacogenomics Research Network (SEAPharm) and the US NIH Pharmacogenomics Research Network (PGRN).

Since our mission is implementation of PGx testing, we will conduct prospective clinical trials to test the clinical utility of genetic test using the PGx biomarkers identified by our basic research. If successful, this will lead to use of the PGx biomarkers as *in-vitro* diagnostics under the Japan national health insurance system. However, from the viewpoint of a limited budget and human resources, it will not be practical to conduct clinical trials for all of the identified PGx biomarkers. Thus, we will have to create a plan to investigate whether pre-emptive PGx testing is clinically useful for patients and cost-effective without actually conducting prospective clinical trials. This strategy is similar to that of the Ubiquitous Pharmacogenomics (U-PGx) project, which has started prospective collection of pre-emptive PGx testing data and its embedment into the electronic medical record in European countries.

Recent Major Publications

Wattanapokayakit S, Mushiroda T, Yanai H, Wichukchinda N, Chuchottawon C, Nedsuwan S, Rojanawiwat A, Denjanta S, Kantima T, Wongyai J, Suwankesawong W, Rungapiromnan W, Kidkeukarun R, Bamrungram W, Chaiwong A, Suvichapanich S, Mahasirimongkol S, Tokunaga K. NAT2 slow acetylator associated with anti-tuberculosis drug-induced liver injury in Thai patients. ***Int J Tuberc Lung Dis*** 20, 1364–1369 (2016)

Fukunaga K, Nakagawa H, Ishikawa T, Kubo M, Mushiroda T. ABCB1 polymorphism is associated with atorvastatin-induced liver injury in Japanese population. ***BMC Genet*** 17, 79 (2016)

Low SK, Fukunaga K, Takahashi A, Matsuda K, Hongo F, Nakanishi H, Kitamura H, Inoue T, Kato Y, Tomita Y, Fukasawa S, Tanaka T, Nishimura K, Uemura H, Hara I, Fujisawa M, Matsuyama H, Hashine K, Tatsugami K, Enokida H, Kubo M, Miki T, Mushiroda T. Association Study of a Functional Variant on ABCG2 Gene with Sunitinib-Induced Severe Adverse Drug Reaction. ***PLoS One*** 11, e0148177 (2016).

Invited Presentations

Mushiroda T. "Current status of pharmacogenomics in Japan and abroad and germline BRCA mutation testing to determine eligibility for PARP inhibitor maintenance therapy" The 37th Annual Meeting of The Japanese Society of Clinical Pharmacology and Therapeutics (Yonago, Japan) December, 2016

Mushiroda T, "Identification of genomic biomarkers associated with cutaneous adverse drug reactions and validation of clinical utility of genetic testing" The 31st Annual Meeting of The Japanese Society for the Study of Xenobiotics (Matsumoto, Japan) October, 2015

Mushiroda T, "Identification of genomic biomarkers associated with drug responses and pharmacogenomics-based individualization of drug therapy" The 2nd Annual Meeting of Japanese Society of Drug Safety (Gifu, Japan) July, 2016

Mushiroda T, "Pharmacogenomics-based individualization of drug therapy" The 89th Annual Meeting of The Japanese Pharmacological Society (Yokohama, Japan) March, 2016

Laboratory for Cardiovascular Diseases

Team Leader: Kaoru Ito

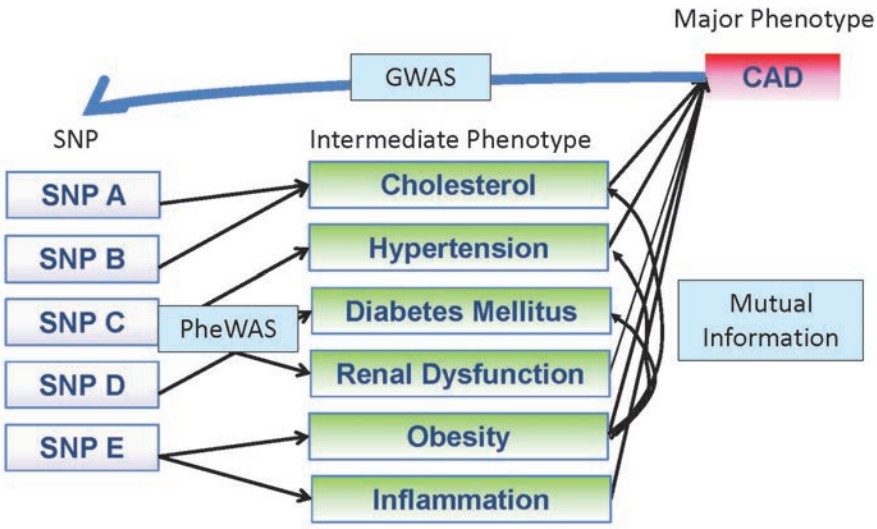


Figure: The Landscape of Coronary Artery Diseases revealed by Modified Phenome-wide Association Study
Significant SNPs are identified by a conventional GWAS approach. To understand the biological and clinical implications of those SNPs, the modified phenome-wide association study reveals intermediate phenotypes and the relationship among them.

Since cardiovascular diseases cause more than 15% of the deaths in the Japanese population and represent more than 20% of the total medical expenses in Japan, it is important for our society to understand the mechanisms underlying these disorders and to uncover new therapeutic targets for their treatment. To achieve these goals, we combine "dry" (NGS technologies, OMICS strategies and computer science) and "wet" (so-called conventional molecular biology) technologies, in an attempt to achieve a comprehensive and precise understanding of these diseases. In other words, there is no border between "dry" and "wet" research in our lab, since we postulate that both will be required in order to expand the horizon of our knowledge.

Diseases of our interest to date are coronary artery diseases (CAD), atrial fibrillation (AF), Kawasaki disease (KD), peripheral artery disease (PAD) and cardiomyopathy (CM). We are currently seeking to 1) understand the interactive effect between Japanese top SNPs (e.g. SNPs in *BRAP, ALDH2*, etc.) and other genes, 2) provide a reasoned interpretation of significant SNPs from the viewpoint of clinical phenotypes (Modified Phenome-wide Association Study, see Figure), and 3) develop a more sophisticated genetic risk scoring system by machine learning algorithms in the CAD project. Additionally, in the CM project, we are developing an *in silico* splicing variant prediction algorithm, a high-throughput cell-based splicing assay and a downstream *in silico* pipeline to uncover cryptic splicing variants, which have been overlooked in the current established pipeline.

We are conducting our research with not only a scientific mind but also a clinical eye, because our ultimate goal is to provide improved diagnostic / management / therapeutic approaches for patients suffering from those diseases.

Recent Major Publications

Konta A, Ozaki K, Sakata Y, Takahashi A, Morizono T, Suna S, Onouchi Y, Tsunoda T, Kubo M, Komuro I, Eishi Y, Tanaka T. A functional SNP in FLT1 increases risk of coronary artery disease in a Japanese population. ***J Hum Genet*** 61, 435–441 (2016)

Ozaki K, Tanaka T. Molecular genetics of coronary artery diseases. ***J Hum Genet*** 61, 71–77 (2016)

Onouchi Y, Fukazawa R, Yamamura K, Suzuki H, Kakimoto N, Suenaga T, Takeuchi T, Hamada H, Honda T, Yasukawa K, Terai M, Ebata R, Higashi K, Saji T, Kemmotsu Y, Takatsuki S, Ouchi K, Kishi F, Yoshikawa T, Nagai T, Hamamoto K, Sato Y, Honda A, Kobayashi H, Sato J, Shibuta S, Miyawaki M, Oishi K, Yamaga H, Aoyagi N, Yoshiyama M, Miyashita R, Murata Y, Fujino A, Ozaki K, Kawasaki T, Abe J, Seki M, Kobayashi T, Arakawa H, Ogawa S, Hara T, Hata A, Tanaka T. Variations in ORAI1 gene associated with Kawasaki Disease. ***PLoS One*** 11, e0145486 (2016)

Invited Presentations

Kaoru I. "High-throughput Splicing Analysis Revealed LMNA and MYBPC3 Silent Mutations Affect RNA Splicing and Cause Pathogenicity" American Heart Association Scientific Session 2016 (New Orleans, USA) November, 2016

Kaoru I. "Searching for a hidden variant for laminopathy using machine learning and NGS" The 20th Annual Scientific Meeting of the Japanese Heart Failure Society (Sapporo, Japan) October, 2016

Kaoru I. "Heart Failure from the viewpoint of Genetics" The 20th Annual Scientific Meeting of the Japanese Heart Failure Society (Sapporo, Japan) October, 2016

Kaoru I. "Heart Failure and Atherosclerosis in Genetics" Science Harmony vol.3 (Tokyo, Japan) October, 2016

Kaoru I. "High-throughput Multiplexed Splicing Analysis Pipeline Revealed that Lamin A/C Synonymous and Missense Mutations Affect RNA Splicing and Cause Pathogenicity." The 22nd Congress of TMFC (Osaka, Japan) July, 2016

Laboratory for Autoimmune Diseases

Team Leader: Kazuhiko Yamamoto

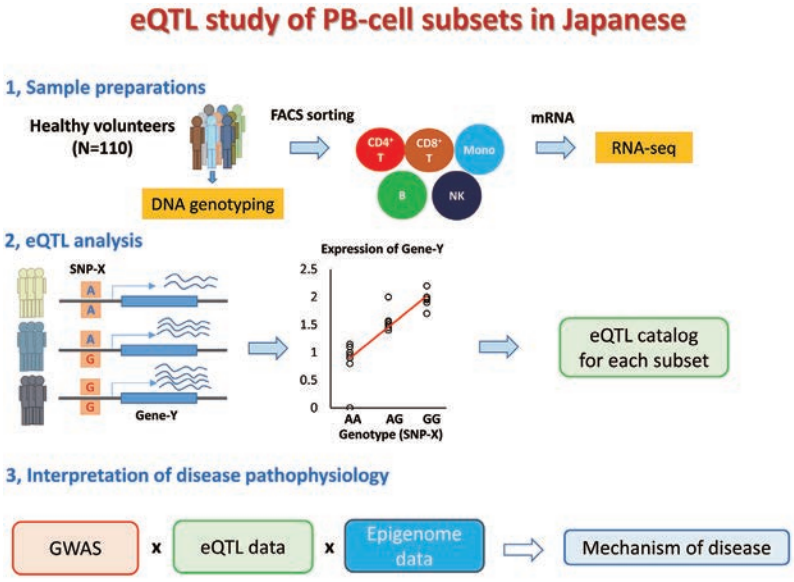


Figure: eQTL study of peripheral-blood (PB) subsets in Japanese
1. We collected peripheral blood samples from Japanese healthy volunteers and sorted the cells into five subsets. We extracted mRNAs and quantified gene expression by RNA-seq.
2. By examining the association between SNP genotype and gene expression, we identified all eQTLs to establish an eQTL catalog for each subset.
3. By combining disease GWAS data, eQTL data, and epigenome data using bioinformatics approaches, we identified disease causal genes to understand disease mechanisms.

Most autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Graves' disease (GD), are multifactorial diseases involving both genetic and environmental factors. The aim of our laboratory is to elucidate the etiology of these autoimmune diseases by dissecting their genetic contributions. Furthermore, through functional analyses of disease-associated variants and genes together with epigenetic studies, we will also clarify the mechanisms of disease pathogenesis. The majority of disease risk variants of complex traits and diseases are expression quantitative trait loci (eQTLs) and these eQTLs mostly behave functionally in a cell type-specific manner. Therefore, subpopulations of immune related cells, such as T cells, B cells, dendritic cells, macrophages, and others should be isolated and investigated. One advantage of research on immune mediated diseases is that we can relatively easily obtain such immune-related cells from human subjects, e.g., from peripheral blood (PB). Through genetic as well as informatics analyses of these samples, we are investigating the gene expression profiles and functions of each subset of immune-related cells and will dissect their roles in the global immune systems as well as in several autoimmune diseases.

Recent Major Publications

Sun C, Molineros EJ, Looger LL, Zhou X, Kim K, Okada Y, Ma J, Qi Y, Kim-H X, Motghare P, Bhattarai K, Adler A, Bang S-Y, Lee HS, Kim TH, Kang YM, Suh CH, Chung WT, Park YB, Choe JY, Shim SC, Kochi Y, Suzuki A, Kubo M, Sumida T, Yamamoto K, Lee SS, Kim YJ, Han BG, Dozmorov M, Kaufman MK, Wren DJ, Harley BJ, Shen N, Chua KH, Zhang H, Bae SC, Nath KS. High-density genotyping of immune-related loci identifies new SLE risk variants in individuals with Asian ancestry. ***Nat Genet*** 48, 323–330 (2016)

Kochi Y. Genetics of autoimmune diseases: perspectives from genome-wide association studies. ***Int Immunol*** 28, 155–161 (2016)

Yamamoto K, Okada Y, Suzuki A, Kochi Y. Genetics of rheumatoid arthritis in Asia--present and future. ***Nat Rev Rheumatol*** 11, 375–379 (2015)

Invited Presentations

Yamamoto K. "Regulation of B cell functions and cellular metabolism in autoimmunity" Cold Spring Harbor Asia (Awaji, Japan) October, 2016

Yamamoto K. "Genomics and functional genomics of rheumatoid arthritis" 20th Asia Pacific League of Associations for Rheumatology Congress (Shanghai, China) September, 2016

Yamamoto K. "TGF-β3 as a novel target of immunotherapy" 10th International Congress on Autoimmunity (Leipzig Germany) April, 2016

Yamamoto K. "Genetics of rheumatoid arthritis: From genetics to functional genetics" 18th Conference on Advances in Targeted Therapies (ATT) (Palma, Spain) March, 2016

Yamamoto K. "Genetics of rheumatic diseases: Functional genetics of rheumatoid arthritis." IRA - APLAR COURSE ON RHEUMATOLOGY (Jakarta, Indonesia), February, 2016

Laboratory for Digestive Diseases

Team Leader: Kazuaki Chayama

Recent Major Publications

Furuta M, Ueno M, Fujimoto A, Hayami S, Yasukawa S, Kojima F, Arihiro K, Kawakami Y, Wardell CP, Shiraishi Y, Tanaka H, Nakano K, Maejima K, Sasaki-Oku A, Tokunaga N, Boroevich KA, Abe T, Aikata H, Ohdan H, Gotoh K, Kubo M, Tsunoda T, Miyano S, Chayama K, Yamaue H, Nakagawa H. Whole genome sequencing discriminates hepatocellular carcinoma with intrahepatic metastasis from multi-centric tumors. ***J Hepatol*** 66, 363-373 (2017)

Fujimoto A, Furuta M, Totoki Y, Tsunoda T, Kato M, Shiraishi Y, Tanaka H, Taniguchi H, Kawakami Y, Ueno M, Gotoh K, Ariizumi S, Wardell CP, Hayami S, Nakamura T, Aikata H, Arihiro K, Boroevich KA, Abe T, Nakano K, Maejima K, Sasaki-Oku A, Ohsawa A, Shibuya T, Nakamura H, Hama N, Hosoda F, Arai Y, Ohashi S, Urushidate T, Nagae G, Yamamoto S, Ueda H, Tatsuno K, Ojima H, Hiraoka N, Okusaka T, Kubo M, Marubashi S, Yamada T, Hirano S, Yamamoto M, Ohdan H, Shimada K, Ishikawa O, Yamaue H, Chayama K, Miyano S, Aburatani H, Shibata T, Nakagawa H. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. ***Nat Genet*** 48, 500–509 (2016)

Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, Nakamura T, Ueno M, Ariizumi S, Nguyen HH, Shigemizu D, Abe T, Boroevich KA, Nakano K, Sasaki A, Kitada R, Maejima K, Yamamoto Y, Tanaka H, Shibuya T, Shibata T, Ojima H, Shimada K, Hayami S, Shigekawa Y, Aikata H, Ohdan H, Marubashi S, Yamada T, Kubo M, Hirano S, Ishikawa O, Yamamoto M, Yamaue H, Chayama K, Miyano S, Tsunoda T, Nakagawa H. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. ***Nat Commun*** 6, 6120 (2015)

Invited Presentations

Chayama K. "Best Strategy for Curing HCV Genotype 1 and 4: East vs. West" 5th The Asian Pacific Association for the Study of the Liver (APASL) Single Topic Conference on Hepatitis C (Kaohsiung, Taiwan) June, 2016

Chayama K. "HCV Treatment Update-a New Era of All-oral HCV Treatment" The 46th Annual Meeting of GEST and The 25th Annual Meeting of DEST (Taipei, Taiwan) March, 2016

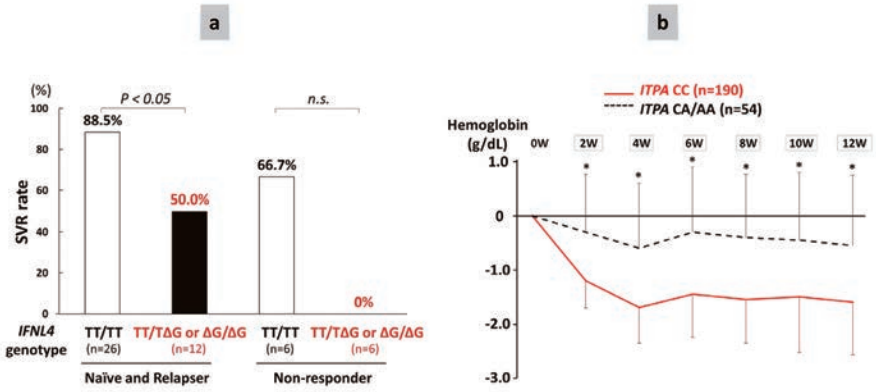


Figure: The effects of *IFNL4* and *ITPA* polymorphisms on novel regimes for the treatment of HCV.

a) Relationship between treatment response and *IFNL4* genotype in older Japanese patients with genotype 1 chronic hepatitis C. Sustained virological response rates for simeprevir triple therapy grouped by response to prior interferon treatment and *IFNL4* genotype. b) Reduction of hemoglobin levels correlated with an *ITPA* polymorphism during sofosbuvir and ribavirin combination treatment. Patients were grouped by *ITPA* rs1127354 genotype.

Using a GWAS approach, we have focused on investigating host genetic factors involved in various liver diseases, such as chronic HBV and HCV infection, HCV-induced liver cirrhosis and cancer, and responsiveness to therapy. We have now intensively investigated and verified how to apply such genetic information to clinical practice. We demonstrated that a polymorphism in the *interferon lambda-4 (IFNL4)* gene affects the outcome of simeprevir, peginterferon and ribavirin therapy for older patients with genotype 1 chronic hepatitis C (***Hepatol Res*** in press). Analysis of *IFNL4* polymorphisms is a valuable predictor in both younger and older patients. We also found that an *inosine triphosphatase* (*ITPA*) polymorphism influences hemoglobin levels and incidence of ribavirin dose reduction during simeprevir triple therapy, indicating the importance of monitoring anemia during treatment, particularly for *ITPA* genotype CC patients (***Hepatol Res*** 2016). In addition, we investigated 244 patients with genotype 2 chronic hepatitis C, who were treated with the interferon-free regimen of sofosbuvir plus ribavirin, and reported *ITPA* polymorphism effects on the decrease of hemoglobin during combination therapy (***J Gastroenterol*** in press).

We have also participated in whole-genome sequencing (WGS) analyses that have identified several recurrently mutated genes/pathways, HBV integration events, mutations in non-coding regions, and structural variations associated with liver cancer, in collaboration with Laboratory for Genome Sequencing Analysis (***Nat Genet*** 2016, ***Nat Commun*** 2015). We recently showed that WGS of multiple liver tumors enabled the accurate diagnosis of multi-centric occurrence and intrahepatic metastasis prior to selecting a therapeutic strategy for multiple tumors in the liver (***J Hepatol*** in press). By using WGS data, we analyzed circulating tumor DNA and found that it reflects tumor progression, microscopic vascular invasion of the portal vein and cancer recurrence (***Cell Mol Gastroenterol Hepatol*** 2015).

In collaboration with other IMS teams, we are currently investigating the host immune response to further elucidate the mechanism of hepatitis and carcinogenesis.

Laboratory for
Bone and Joint Diseases

Team Leader: **Shiro Ikegawa**

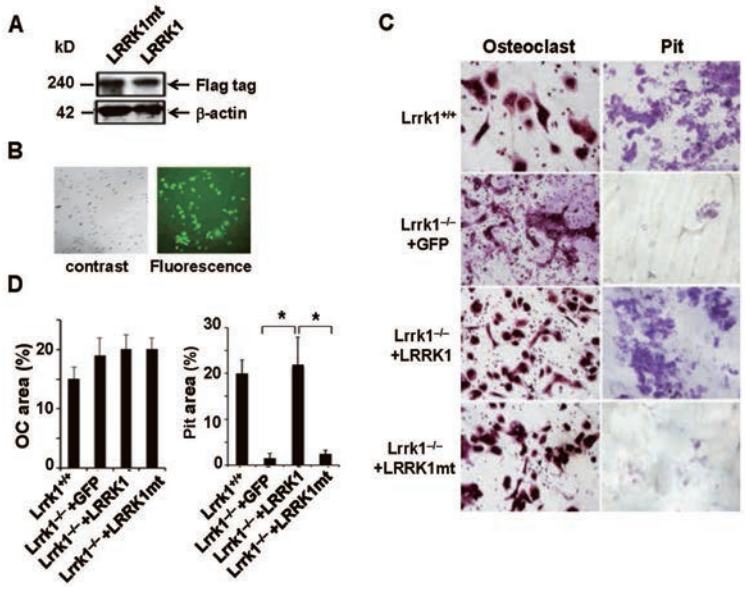


Figure: LRRK1 mutant does not rescue the bone resorption defect of *Lrrk1*-deficient osteoclasts. (A) Expression of Flag-tagged wild type and mutant (p.E1980Afs*66) human LRRK1 proteins. Western blot using anti-Flag antibody. (B) Efficient transduction of lentiviral particle in osteoclast precursors. The transduction efficiency was monitored by GFP expression. (C) TRAP positive osteoclasts and resorptive pit formation on bone slices (100x). Left panel: lentivirus transduced monocytes were differentiated on bone slices for 6 days, followed by TRAP staining. Right panel: transduced monocytes were differentiated on bone slices for 10 days. (D) Quantitative data of TRAP-positive multinuclear cells (left) and pit formation (right) (N = 9). Data are mean ± SEM. *: P< 0.01.

a) Genomic Study of Common Diseases

Common bone and joint diseases have become serious concerns for the world health and economy, as exemplified by the WHO initiative "Bone and Joint Decade" (2000–2010) and the "Locomo" campaign in Japan. We are searching for susceptibility genes for common (polygenic) bone and joint diseases including osteoarthritis (OA), lumbar disc disease (LDD)/herniation (LDH), osteoporosis, avascular necrosis of the femoral head (ANF), scoliosis, and ossification of the posterior longitudinal ligament of the spine (OPLL).

Through genome-wide association studies (GWASs) and next-generation sequencing approaches, we identify and characterize susceptibility genes, and then clarify their disease-causing mechanisms at the molecular level. Using the genome information obtained by these studies, we will realize our final goal, "precision medicine" for these diseases. GWASs for OA, LDD/LDH, adolescent idiopathic scoliosis (AIS), OPLL, and ANF are in progress, and some of them have already succeeded in identification of susceptibility genes. Functional studies of the genes *in vitro* and using animal models are underway.

b) Genomic Study of Skeletal Dysplasia

Skeletal dysplasia is a group of heritable (monogenic) disorders affecting the skeleton, with more than 450 diseases belonging to this category. Skeletal dysplasia is an intractable disease and thus many patients are waiting for treatment. We are engaging in clinical and basic studies of these difficult diseases. By large-scale mutation screening, including exome sequencing, we are identifying the disease genes. So far, we identified new genes for 24 diseases. One of them which we recently identified is *LRRK1* in osteosclerotic metaphyseal dysplasia. We found an elongation mutation of the gene that resulted in the loss of the bone-resorbing capacity of osteoclasts (Figure).

Though analysis of their phenotypes and diseases genes, we approach molecular mechanisms of bone and joint formation, pathogenesis of common bone and joint diseases, as well as the diagnosis and treatment of these crippling intractable diseases ('nanbyo'). Using the disease genes for skeletal dysplasia as candidate genes, we are performing association studies for the corresponding common bone and joint diseases.

Recent Major Publications

Guo L, Girisha KM, Iida A, Hebbar M, Shukla A, Shah H, Nishimura G, Matsumoto N, Nismath S, Miyake N, Ikegawa S. Identification of a novel LRRK1 mutation in a family with osteosclerotic metaphyseal dysplasia. ***J Hum Genet*** 62, 437–441 (2017)

Nishimura G, Nakajima M, Takikawa K, Haga N, Ikegawa S. Distinctive skeletal phenotype in high bone mass osteogenesis imperfecta due to a COL1A2 cleavage site mutation. ***Am J Med Genet A*** 170, 2212–2214 (2016)

Guo L, Yamashita H, Kou I, Takimoto A, Meguro-Horike M, Horike S, Sakuma T, Miura S, Adachi T, Yamamoto T, Ikegawa S, Hiraki Y, Shukunami C. Functional investigation of a non-coding variant associated with adolescent idiopathic scoliosis in zebrafish: elevated expression of the ladybird homeobox gene causes body axis deformation. ***PLoS Genet*** 12, e1005802 (2016)

Invited Presentations

Ikegawa S. "How to Study Genetic Diseases." Taipei Medical University Invited Seminar (Taipei, Taiwan) January 2017

Ikegawa S. "Identification of the disease gene by whole exome sequencing" 10th Annual Introductory Course on Skeletal Dysplasia (Lausanne, Switzerland) July, 2016

Ikegawa S. "Why study scoliosis genetics? A genetical perspective" (Singapore, Singapore) May, 2016.

Ikegawa S. "Genomic study of common spinal diseases." 6th Jishuitan Orthopedics Forum (Beijing, China) April, 2016

Ikegawa S. "How to identify disease genes in skeletal dysplasia" Third Scandinavian Skeletal Dysplasia Workshop (Oslo, Norway) March, 2016

Laboratory for Diabetes, Metabolism and Renal diseases

Team Leader: Momoko Horikoshi

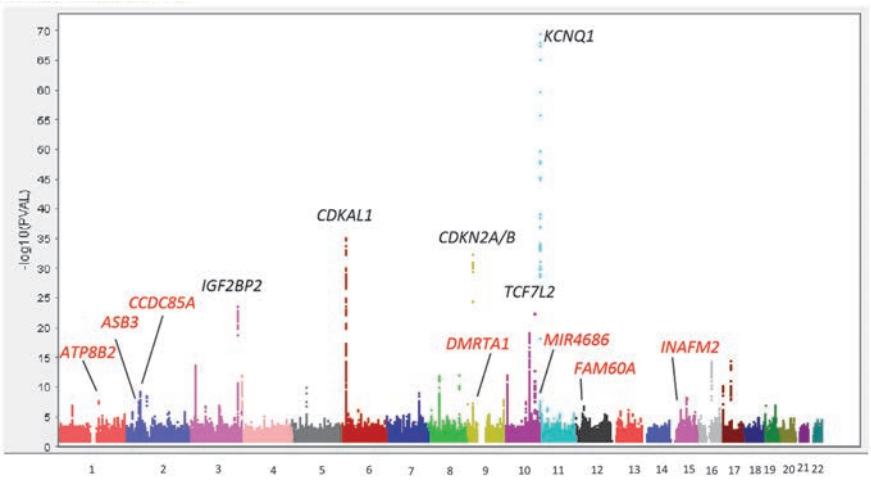


Figure: Results of GWAS meta-analysis for type 2 diabetes in the Japanese population
Names of the previously identified loci are in black and the seven novel loci identified in this study are shown in red.

Our primary focus is on establishing the genetic contribution to type 2 diabetes (T2D) susceptibility in the Japanese population. To that end, we are working directly with the state-of-the-art genetic resources generated by Biobank Japan, which includes GWAS data for more than 35,000 T2D subjects. Our recent analyses of a subset of these GWAS data identified seven novel regions of the genome associated with T2D (Fig). Of the more than 90 T2D loci reported as of 2016, we have increased the number of loci reported from the Japanese population to 14 in total. We are currently expanding this effort to the full set of Biobank Japan. We have also investigated genomic regions associated with microvascular complications of T2D, namely, diabetic retinopathy and nephropathy. GWAS for these complications have been performed by several groups, but worldwide efforts to identify susceptibility to these diabetic complications have not met with clear success. We are strengthening our ties with the neighboring collaborators by contributing our T2D association data to the Asian Genetic Epidemiology Network (AGEN) Consortium as well as to the world-wide DIAMANTE Consortium.

Recent Major Publications

Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN, Fernandez-Tajes J, Feenstra B, van Zuydam NR, Gaulton KJ, Grarup N, Bradfield JP, Strachan DP, Li-Gao R, Ahluwalia TS, Kreiner E, Rueedi R, Lyytikäinen LP, Cousminer DL, Wu Y, Thiering E, Wang CA, Have CT, Hottenga JJ, Vilor-Tejedor N, Joshi PK, Boh ET, Ntalla I, Pitkänen N, Mahajan A, van Leeuwen EM, Joro R, Lagou V, Nodzenski M, Diver LA, Zondervan KT, Bustamante M, Marques-Vidal P, Mercader JM, Bennett AJ, Rahmioglu N, Nyholt DR, Ma RC, Tam CH, Tam WH; CHARGE Consortium Hematology Working Group., Ganesh SK, van Rooij FJ, Jones SE, Loh PR, Ruth KS, Tuke MA, et al. Genome-wide associations for birth weight and correlations with adult disease. ***Nature*** 538, 248–252 (2016)

Fuchsberger C, Flannick J, Teslovich TM, Mahajan A, Agarwala V, Gaulton KJ, Ma C, Fontanillas P, Moutsianas L, McCarthy DJ, Rivas MA, Perry JR, Sim X, Blackwell TW, Robertson NR, Rayner NW, Cingolani P, Locke AE, Fernandez Tajes J, Highland HM, Dupuis J, Chines PS, Lindgren CM, Hartl C, Jackson AU, Chen H, Huyghe JR, van de Bunt M, Pearson RD, Kumar A, Müller-Nurasyid M, Grarup N, Stringham HM, Gamazon ER, Lee J, Chen Y, Scott RA, Below JE, Chen P, Huang J, Go MJ, Stitzel ML, Pasko D, Parker SC, Varga TV, Green T, Beer NL, Day-Williams AG, Ferreira T, Fingerlin T, Horikoshi M, et al. The genetic architecture of type 2 diabetes. ***Nature*** 536, 41–47 (2016)

Imamura M, Takahashi A, Yamauchi T, Hara K, Yasuda K, Grarup N, Zhao W, Wang X, Huerta-Chagoya A, Hu C, Moon S, Long J, Kwak SH, Rasheed A, Saxena R, Ma RC, Okada Y, Iwata M, Hosoe J, Shojima N, Iwasaki M, Fujita H, Suzuki K, Danesh J, Jørgensen T, Jørgensen ME, Witte DR, Brandslund I, Christensen C, Hansen T, Mercader JM, Flannick J, Moreno-Macías H, Burtt NP, Zhang R, Kim YJ, Zheng W, Singh JR, Tam CH, Hirose H, Maegawa H, Ito C, Kaku K, Watada H, Tanaka Y, Tobe K, Kawamori R, Kubo M, Cho YS, Chan JC, et al. Genome-wide association studies in the Japanese population identify seven novel loci for type 2 diabetes. ***Nat Commun*** 7, 10531 (2016)

Laboratory for Respiratory and Allergic Diseases

Team Leader: Mayumi Tamari

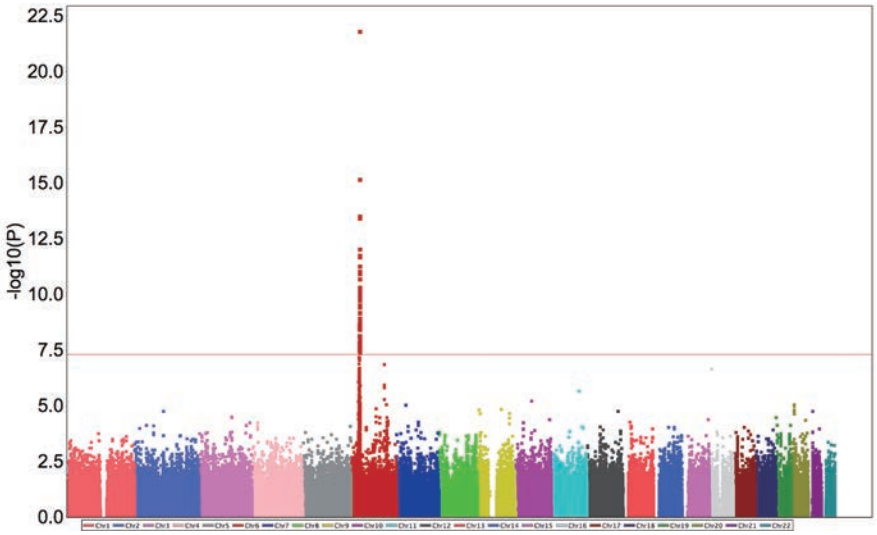


Figure: Manhattan plot of the wheat-dependent exercise-induced anaphylaxis (WDEIA) due to HWP
GWAS of WDEIA induced by HWP-containing facial soap "Cha-no-shizuku". Manhattan plot showing the –log10 P values in the GWAS for cases and controls plotted against their respective positions on autosomes. The red line shows the genome-wide significance threshold for this study ($P = 5 \times 10^{-8}$).

The aim of our project is to explore genetic components of respiratory and allergic diseases. Genome-wide association study (GWAS) is a method to comprehensively assess genes underlying susceptibility to human disorders. Food allergy (FA) is a problem throughout the world, but the genetic factors affecting FA susceptibility in children remain largely unexplored. We recruited patients with FA who were diagnosed by pediatric specialists based on positive oral food challenge or a definitive clinical history after food intake. We performed GWAS for childhood FA in Japanese populations, and identified a total of three loci with genome-wide significance. One of the loci contained a gene that plays an essential role in Th2 cytokine expression. Wheat-dependent exercise-induced anaphylaxis (WDEIA) is severe food allergy that usually develops after ingestion of wheat products followed by physical exercise. Hydrolysed wheat gluten protein (HWP) is used as an additive for facial soap. In Japan, a total of 2026 cases of immediate wheat allergy and WDEIA due to HWP have been reported. Most patients seemed to be sensitized to HWP (Glupearl 19S®) through the use of the facial soap "Cha-no-shizuku". Glupearl 19S® is a degraded gluten made from the direct resolution of wheat by hydrochloric acid. We conducted GWAS of WDEIA induced by HWP-containing facial soap of 464 cases and 3,099 controls. SNPs at a region on chromosome 6 were associated with WDEIA induced by HWP-containing facial soap (Figure). We are conducting a replication study in an independent sample set.

We participate in the Global Alliance between the U.S. NIH Pharmacogenetics Research Network (PGRN) and RIKEN IMS. A pharmacogenetic study of asthma is ongoing now in this international collaboration.

Recent Major Publications

Yatagai Y, Hirota T, Yamada H, Masuko H, Kaneko Y, Iijima H, Naito T, Noguchi E, Tamari M, Kubo M, Takahashi A, Konno S, Makita H, Nishimura M, Hijikata M, Keicho N, Homma S, Taguchi Y, Azuma A, Kudoh S, Hizawa N. Variants near the HLA complex group 22 gene confer increased susceptibility to late-onset asthma in Japanese populations. ***J Allergy Clin Immunol*** 138, 281–283 (2016)

EArly Genetics and Lifecourse Epidemiology (EAGLE) Eczema Consortium. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. ***Nat Genet*** 47, 1449–1456 (2015)

Tokunaga T, Sakashita M, Haruna T, Asaka D, Takeno S, Ikeda H, Nakayama T, Seki N, Ito S, Murata J, Sakuma Y, Yoshida N, Terada T, Morikura I, Sakaida H, Kondo K, Teraguchi K, Okano M, Otori N, Yoshikawa M, Hirakawa K, Haruna S, Himi T, Ikeda K, Ishitoya J, Iino Y, Kawata R, Kawauchi H, Kobayashi M, Yamasoba T, Miwa T, Urashima M, Tamari M, Noguchi E, Ninomiya T, Imoto Y, Morikawa T, Tomita K, Takabayashi T, Fujieda S. Novel scoring system and algorithm for classifying chronic rhinosinusitis: the JESREC Study. ***Allergy*** 70:995–1003 (2015)

Invited Presentations

TTamari M. "Genetic Study of Allergic Diseases" Graduate School Seminar in Fukui University (Fukui, Taiwan) December, 2016

Tamari M. "Genomics in Allergic Disease" Symposium, The 44th meeting of society for inhalation therapy. (Tokyo, Japan) November, 2016

Tamari M. "Gene to Environmental Interactions in Asthma" Joint Congress of APAAACI AND APAPARI 2016. (Kuala Lumpur, Malaysia) October, 2016

Tamari M. "Genomics in Allergic Disease" Genomic Medicine Promotion Forum in Nippon Medical School. (Tokyo, Japan) July, 2016

Tamari M. "Genetic Study of Allergic Diseases" PGRN-RIKEN IMS Strategic Alliance Meeting. (San Francisco, USA) April, 2016

Program for Medical Innovations

Six original projects for clinical applications have been performed in Program for Medical Innovations. 1) A biochemical drug using a PEGylated Cryj-1/2 fusion recombinant protein for Cedar Pollinosis has been developed by the Torii pharmaceutical company and IMS and introduced to the Japanese market. 2) A chemical compound recently developed using the α-galactosylceramide (α-GalCer) analog, RCAI-X, selectively induces apoptosis of IgE but not IgG B cells and preferentially suppresses IgE production. It therefore potentially could be applied to any type of allergic disorder, such as pollinosis, food allergy, as well as allergic asthma. 3) NKT cell-targeted therapy for head and neck tumors has been done in collaboration with Chiba University, and was authorized by the Japanese government as Advanced Medical Treatment B. 4) The artificial adjuvant vector cell as an anti-tumor vaccine project has been accepted by the translational research network program and developed. This vaccine can be dosed with tumor antigen mRNA together with α-GalCer, so that it activates both innate and acquired protective immunity and also induces long-term memory. 5) The human iPS project for clinical use of *in vitro*-generated NKT cells has been accepted as a Center for Clinical Application Research (Type B) in the Research Center Network for Realization of Regenerative Medicine, Japan. 6) A humanized mouse model for MLL gene-rearranged leukemia was established. Also, certain genes were identified that are differentially expressed between normal stem cells and leukemic stem cells.

Laboratory for Immune Regulation

Group Director: Masaru Taniguchi

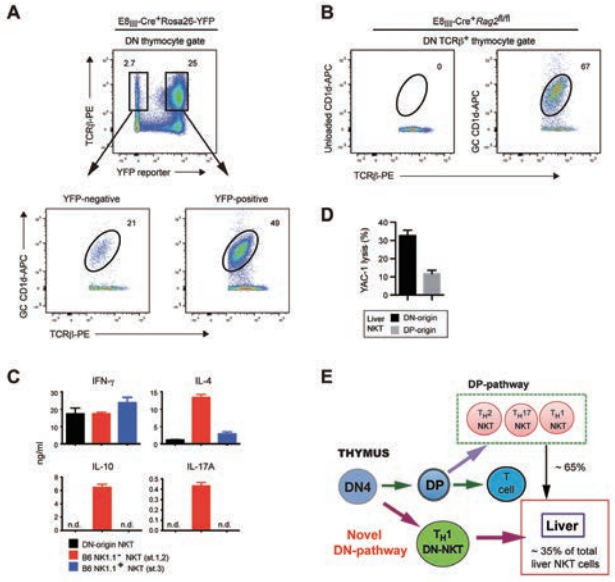


Figure: Identification of an alternative pathway for the development of the Vα14 invariant NKT cell lineage directly from DN thymocytes that bypasses the DP stage
(A) Demonstration of the DN-stage-thymocyte origin of NKT cells by a novel fate-mapping approach. Flow cytometry of cells from $E8_{III}$-Cre^{+}Rosa26-YFP mice. **(B)** Presence of NKT cells developed directly from DN-stage-thymocytes in the $E8_{III}$-Cre^{+}*Rag2*$^{fl/fl}$ mouse that lacks the rearranged *Trav11Traj18* mRNA at the DP stage but not at the DN4 stage thymocytes. **(C)** DN-stage-thymocyte origin NKT cells possess T_H1-type-biased cytokine secretion, and **(D)** show superior cytotoxicity against YAC-1 targets compared to DP-stage-thymocyte origin cells. **(E)** Proposed model of an alternative developmental pathway of NKT cell lineage cells. Our data demonstrate a presence of a previously unknown NKT cell developmental pathway that originates from DN stage thymocytes, i.e., before the DP stage of thymopoiesis. This novel developmental pathway gives rise to a subset of DN-NKT cells with highly potent T_H1-type effector functions that are mainly found in the liver. Our data also suggest that the acquisition of diverse functional characteristics by NKT cells might be dependent on the timing of TCR expression in precursor cells undergoing positive selection.

NKT cells represent a unique subset of innate-like T lymphocytes expressing an invariant T cell receptor (TCR) α chain encoded by rearranged *Trav11-Traj18* gene segments in mice, and *TRAV10-TRAJ18* in humans. This receptor is used preferentially by NKT cells but not by conventional T cells, defining NKT cells as a distinct lineage from conventional T cells. NKT cells recognize glycolipid ligands in conjunction with the monomorphic MHC-like molecule CD1d, and mediate intermediary functions that link the innate and acquired immune systems by their rapid secretion of large amounts of cytokines such as IL-4, IL-17A and IFN-γ after activation. Since the discovery of this unique cell lineage, the developmental aspect of NKT cells has been one of the most intriguing topics. Although it is widely believed that NKT cells are generated from CD4^{+}CD8^{+} double-positive thymocytes, a process termed the DP pathway, it was still not fully understood whether NKT cells develop exclusively by the DP pathway in a manner closely resembling that of conventional T cells, or whether alternatives to this pathway exist. We have recently tested this long-standing question by generating mice with DP stage-specific ablation of *Rag2* gene expression and by a fate-mapping approach with the use of novel $E8_{III}$-Cre^{Tg} mice. Our results demonstrated definitive genetic evidence for the existence of an alternative developmental pathway through which a fraction of DN NKT cells with strong T_H1-biased and cytotoxic characteristics develops directly from late DN4 stage thymocytes that bypasses the DP stage. These finding provide new insights into our understanding of NKT cell development.

Also, our group focuses on translational research, The Japan Agency for Medical Research and Development (AMED), on the establishment of an immunotherapy method using a novel NKT cell glycolipid ligand RK-X that shows superior antitumor and adjuvant effects compared to the widely used glycolipid ligand αGalCer.

Recent Major Publications

Dashtsoodol N, Shigeura T, Aihara M, Ozawa R, Kojo S, Harada M, Endo T, Watanabe T, Ohara O, Taniguchi M. Alternative pathway for the development of Vα14^{+} NKT cells directly from CD4^{-}CD8^{-} thymocytes that bypasses the CD4^{+}CD8^{+} stage. ***Nat Immunol*** 18, 274–282 (2017)

Yamada D, Iyoda T, Vizcardo R, Shimizu K, Sato Y, Endo T, Kitahara G, Okoshi M, Kobayashi M, Sakurai M, Ohara O, Taniguchi M, Koseki H, Fujii S. Efficient regeneration of human V alpha 24^{+} invariant natural killer T cells and their anti-tumor activity in vivo. ***Stem Cells*** 34, 2852–2860 (2016)

Dashtsoodol N, Shigeura T, Ozawa R, Harada M, Kojo S, Watanabe T, Koseki H, Nakayama M, Ohara O, Taniguchi M. Generation of Novel Traj18-Deficient Mice Lacking Vα14 Natural Killer T Cells with an Undisturbed T Cell Receptor α-Chain Repertoire. ***PLOS ONE*** 11, e0153347 (2016)

Invited Presentations

Dashtsoodol N. "Identification of novel alternative pathway of NKT cell development" 5th International Conference "Current Advances in Microbiology and Immunology 2016" (Ulaanbaatar, Mongolia) September, 2016

Taniguchi M. "Overview on development and function of NKT cells" Immunology Summer School 2016 (Onuma, Japan) July, 2016

Laboratory for Immunotherapy

Team Leader: Shin-ichiro Fujii

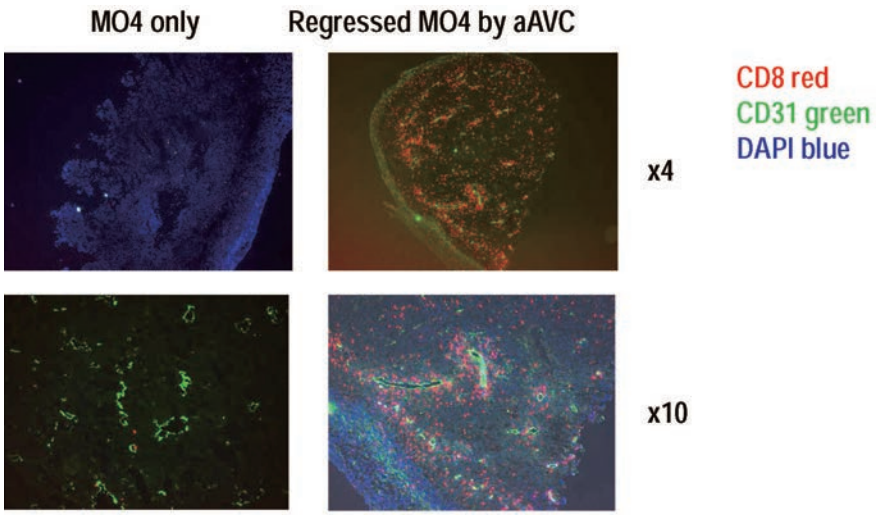


Figure: Trafficking of CTL to tumor site by aAVC therapy
C57BL/6 mice were inoculated with MO4 (OVA-expressing B16 melanoma) subcutaneously. A week later, the mice were treated with aAVC-OVA (α-GalCer loaded, CD1d-expressing NIH3T3 cells transfected with OVA mRNA). Vaccinating the animals with aAVC-OVA caused shrinkage of the otherwise aggressive tumors. $CD8^+$ T cells (red) were accumulated in the tumor. CD31 (green) identifies blood vessel endothelial cells and DAPI (blue) stains DNA. Most of the CD8 T cells were verified as $tetramer^+$ antigen-specific T cells by FACS analysis.

The aims of the laboratory are to extend basic studies for advancing immunotherapy and translational research, from basic studies back and forth to the bedside in the field of cancer and the control of other diseases. For this purpose, we have been focusing on the following 5 projects related to NKT cells. The synthetic glycolipid, α-galactosylceramide (α-GalCer) as an NKT cell ligand is presented by CD1d molecules to invariant NKT lymphocytes. When NKT cells are activated in this way, they show unique immunostimulatory features for innate immunity. First, as a basic study, we have recently identified memory-like $KLRG1^+$NKT cells and have been characterizing them in terms of intrinsic and extrinsic factors (project 1). Additionally, we have been developing translational research projects to establish therapeutic strategies to generate strong antitumor immunity. We previously reported the full maturation of DCs soon after the activation of NKT cells *in vivo*. Based on this observation, we have developed and established artificial adjuvant vector cells (aAVC) as a new type of cell-based drug delivery system composed of NKT cell ligand and tumor-associated antigen. In this year, we found that anti-tumor CTL were recruited to tumor sites (Figure) (project 2). Since this project has also been accepted by the RIKEN translational program and Translational Research center program at Tokyo University, we are making efforts in translational research toward eventual clinical trials (project 3). As project 4, we work toward the establishment of iPS-NKT cells to develop an iPS-NKT cell transfer strategy, in a collaborative study with the RIKEN iPS-group. In this project, our group plays a role in the preparation of primary NKT cells as the starting material for generating NKT cell-derived iPS cells, and in analyzing the function of the iPS-derived NKT cells. As a final project (project 5), we have been working on a joint clinical phase I /IIa study of NKT cell therapy for early stage post-operative lung cancer patients with the National Hospital Organization (NHO). In this study, our role is the analyses of immune cells in the trial and also, in collaboration with RIKEN IMS Genomic Medicine, SNP analysis of responder and poor-responder groups to find suitable biomarkers.

Recent Major Publications

Yamada D, Iyoda T, Vizcardo R, Shimizu K, Sato Y, Endo TA, Kitahara G, Okoshi M, Kobayashi M, Sakurai M, Ohara O, Taniguchi M, Koseki H, Fujii S. Efficient regeneration of Human $Vα24^+$ invariant NKT cells and their anti-tumor activity in vivo. ***Stem Cells*** 34, 2852–2860 (2016)

Yamasaki S, Shimizu K, Kometani K, Sakurai M, Kawamura M, Fujii S. In vivo dendritic cell targeting cellular vaccine induces $CD4^+$ Tfh cell-dependent antibody against influenza virus. ***Sci Rep*** 6, 35173 (2016)

Shimizu K, Yamasaki S, Shinga J, Sato Y, Watanabe T, Ohara O, Kuzushima K, Yagita H, Komuro Y, Asakura M, and Fujii S. Systemic DC activation modulates the tumor microenvironment and shapes the long-lived tumor-specific memory mediated by $CD8^+$ T cells. ***Cancer Res*** 76, 3756–3766 (2016)

Invited Presentations

Fujii S. "Cancer Immunotherapy using tumor antigen-derived mRNA and natural killer T cell ligand" The 31st Transfusion Medical Conference (Hayama, Japan) January, 2017

Fujii S. "Development of multifunctional cancer vaccine, artificial adjuvant vector cells (aAVC)" The 2nd RIKEN symposium "Engineering Research at RIKEN" (Wako, Japan) November, 2016

Fujii S. "Development of cancer vaccine using innate immunity" Seminar in Graduate School of Medicine School of Medicine, Chiba University. (Chiba, Japan) August, 2016

Fujii S. "Development of multifunctional cancer vaccine, artificial adjuvant vector cells (aAVC)" The 20th Annual Meeting of Japanese Association of Cancer Immunology (Osaka, Japan) July, 2016

Fujii S. "Systemic DC activation modulates the tumor microenvironment and shapes the long-lived tumor specific memory mediated by $CD8^+$ T cells" RIKEN IMS-JSI International Symposium on Immunology 2016 (Yokohama, Japan) June, 2016

Drug Discovery Antibody Platform Unit

Unit Leader: Toshitada Takemori

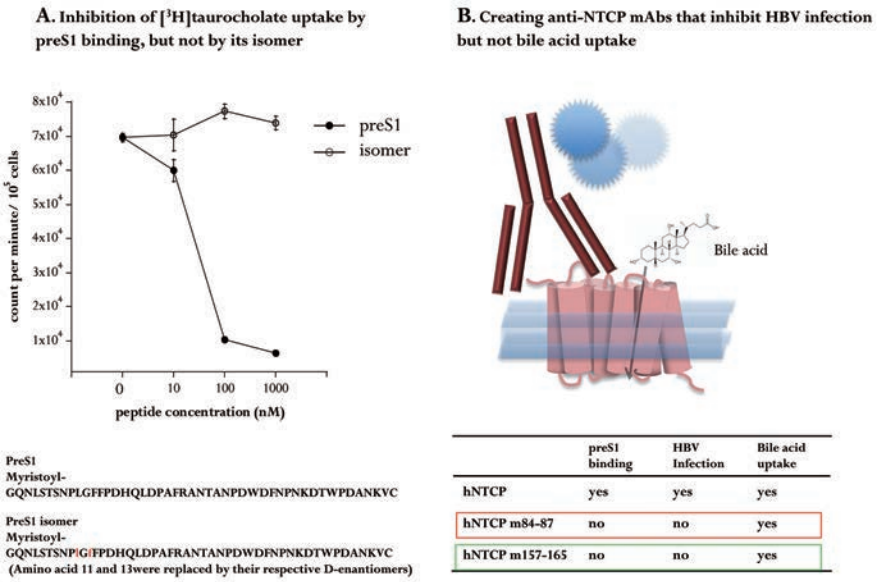


Figure: Effects of liposomal RK-141 (RK-569) and Figure: Toward establishment of HBV entry inhibitors in host cells

A. Binding of the pre-S1 domain to NTCP interferes with bile acid transport by NTCP

Flag-NTCP-expressing HepG2 cells were incubated with preS1 or preS1 isomer peptides (0-1000nM) for 60 min at 37°C, followed by assays for bile acid uptake.

Bile acid uptake was carried out by incubating cells with 1000nM [^{3}H]taurocholate dissolved in sodium ion$^+$ for 15 min at 37°C. After washing, cell lysates were prepared using 1% Triton X-100 in H2O and transferred into liquid scintillation tubes and mixed with liquid scintillation cocktail.

B. NTCP recognition by the pre-S1 is mediated or modulated by two regions of NTCP, aa 84 to 87 and aa 157 to 165. Amino acids 157 to 165 may be critical for maintaining the correct conformation of NTCP. The substitutions corresponding to murine (m) NTCP regions abrogate HBV infection, but not bile acid uptake. NTCP structure or its amino acid residues that are susceptible to viral infection, but not taurocholate uptake activity, (Yan et al. 2013) could be a useful target(s) for mAbs that selectively block viral infection with no or minimal interference in bile salt transport.

Recent Major Publications

Miyauchi K, Sugimoto-Ishige A, Harada Y, Adachi Y, Usami Y, Kaji T, , Hasegawa H, Watanabe T, Hijikata A, Fukuyama S, Maemura T, Ohara O, Kawaoka Y, Takahashi Y, Takemori T, Kubo M. Protective neutralizing influenza antibody response in the absence of T follicular helper cells. ***Nat Immuno***l 17, 1447–1458 (2016)

Kaji T, Hijikata A, Ishige A, Kitami T, Watanabe T, Ohara O, Yanaka N, Okada M, Shimoda M, Taniguchi M, Takemori T. CD4 memory T cells develop and acquire functional competence by sequential cognate interactions and stepwise gene regulation. ***Int Immunol*** 28, 267–282 (2016)

Nance JP, Belanger S, Johnston RJ, Hu JK, Takemori T, Crotty S. Bcl6 middle domain repressor function is required for T follicular helper cell differentiation and utilizes the coreceptor MTA3. ***Proc Natl Acad Sci U S A*** 112, 13324–13329 (2015)

Invited Presentations

Takemori T. "How T and B cells cooperate to establish immunological memory" (Overview talk) The 45th annual meeting of Japanese Society for Immunology. (Okinawa, Japan) December, 2016

We develop antibodies attractive for clinical cancer therapy and prevention of infectious diseases. We are now developing mAbs for AML stem cells and novel entry inhibitors for the treatment of Hepatitis B virus infections. In addition, we are analyzing a mechanism of killing B lymphoma cells upon HLA-DR engagement by mAb.

Chronic Hepatitis B virus (CHBV) chronically infects approximately 250 million people in the world. CHBV infection can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). Several antiviral agents have been recommended as first-line anti-HBV drugs for excellent viral suppression with a low risk of antiviral resistance. However, their cost and the need for essentially life-long treatment are considerable problems. Furthermore, none of these current treatments can eradicate the intracellular virus. Therefore, there is still a medical need for an effective HBV cure.

As HBV binds via the preS1-domain of the viral L protein to the apical sodium-acid dependent bile acid transporter (NTCP) (Yan et al. ***J Virol*** 2013), NTCP is an ideal target for HBV entry inhibition. Entry inhibition both during the acute and persistent phase of the infection could protect naive hepatocytes from uptake, replication, and dissemination of the virus. Long-term treatment during persistent infections may lead to eventual viral clearance due to the natural or immune-mediated turnover of the infected hepatocytes.

As an entry inhibitor, a chemically synthesized lipopeptide derived from the pre-S1domain was developed and is now in a Phase2a clinical trial in Russia. However, binding of the pre-S1 domain to NTCP interferes with bile acid transport by NTCP (Figure A).

It has been recently suggested that human NTCP regions, aa 84 to 87 and aa 157 to 165 may be useful targets for selectively blocking viral infection with no or minimal interference with bile salt transport (Yan et al. ***J Virol*** 2014). According to this possibility, we are currently attempting to establish mAbs against human NTCP that block viral interaction, but not bile acid uptake (Figure B). Successful development of such mAbs may contribute to the development of novel entry inhibitors for the treatment of acute and chronic HBV infections.

YCI Laboratory for Immune Regeneration

Young Chief Investigator: Tomokatsu Ikawa

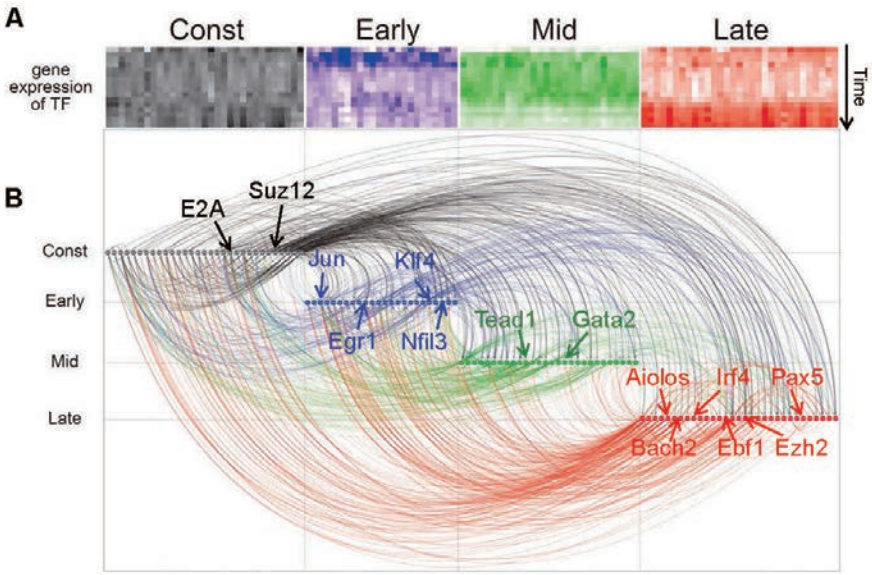


Figure: Transcriptional networks during B cell commitment
(A) Gene expression profiles of 115 Transcription factors (TFs) (33 constant, 27 early, 23 mid and 32 late) which comprise the transcriptional network during B cell commitment. Each column represents the expression pattern of the factor shown in the node of network (B).
(B) Inter-regulation between time phase-specific TFs. Frequently associated interactions (detected in over 70% of ChIP-seq datasets) are represented as an edge. Representative genes are shown by arrows.

B lymphocytes are generated from pluripotent hematopoietic stem cells (HSCs) through a successive series of lineage restriction processes. Although many essential transcription factors (TFs), such as Ikaros, PU.1, E2A, EBF1 and Pax5 have been implicated in regulating B lineage cell fate choice, molecular mechanisms underlying the generation of these patterns during cell fate determination remain unexplored because of the absence of suitable experimental systems.

We have recently established an ideal system that can be used to examine gene regulatory networks during lymphoid lineage specification from HSCs. We overexpressed Id3 protein fused with ERT2 (Estrogen receptor) protein, whose nuclear translocation is induced by 4-hydroxytamoxifen (4-OHT), in hematopoietic progenitors and cultured them in B cell differentiation conditions. B cell differentiation of Id3-transduced cells was blocked at an early developmental stage, but the cells grew enormously and maintained multipotency in the presence of 4-OHT (Ikawa et al. Stem Cell Reports, 2015). We named these multipotent progenitors induced leukocyte stem (iLS) cells.

This novel system enabled the analysis of a large set of regulatory molecules that control the generation of T and B lymphocytes. We have recently discovered the transcriptional network operative during B lineage commitment (Figure). This system can also be applied for *ex vivo* expansion of human hematopoietic stem/progenitors, which will be required for immune cell therapy or transplantation of HSCs. Thus, the aims of our study are 1) from a basic science perspective, to elucidate the mechanisms that orchestrate cell fate specification, commitment and differentiation during lymphocyte development and 2) from a clinical medicine perspective, to establish a novel method to expand human hematopoietic stem/progenitors for the development of HSC transplantation as a clinical strategy.

Recent Major Publications

Ikawa T*, Masuda K, Endo TA, Endo M, Isono K, Koseki Y, Nakagawa R, Kometani K, Takano J, Agata Y, Katsura Y, Kurosaki T, Vidal M, Koseki H and Kawamoto H*. Conversion of T cells to B cells by inactivation of polycomb-mediated epigenetic suppression of the B-lineage program. ***Genes Dev*** 30, 2475–2485 (2016)

Ikawa T*, Masuda K, Huijskens MJAJ, Satoh R, Kakugawa K, Agata Y, Miyai T, Germeraad WTV, Katsura Y, Kawamoto H*. Induced developmental arrest of early hematopoietic progenitors leads to the generation of leukocyte stem cells. ***Stem Cell Reports*** 5, 716–727 (2015)

Inoue T, Morita M, Hijikata A, Fukuda-Yuzawa Y, Adachi S, Isono K, Ikawa T, Kawamoto H, Koseki H, Natsume T, Fukao T, Ohara O, Yamamoto T, Kurosaki T*. CNOT3 contributes to early B cell development by controlling Igh rearrangement and p53 mRNA stability. ***J Exp Med*** 212, 1465–1479 (2015)

Invited Presentations

Ikawa T. "Gene regulatory networks that orchestrate T and B lymphocyte development." Liaison Laboratory Seminar (Kumamoto, Japan) February, 2016

Ikawa T. "Transcriptional networks that control B cell fate determination." BMB2015 (Kobe, Japan) December, 2015

YCI Laboratory for Cellular Bioenergetic Network

Young Chief Investigator: **Toshimori Kitami**

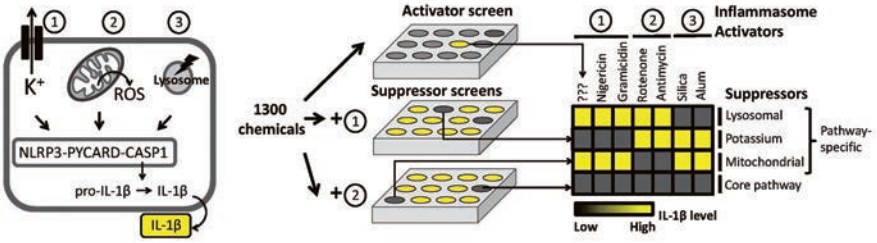


Figure: Searching for chemicals that modulate mitochondrial function using the NLRP3 inflammasome as a disease pathway model
In order to identify chemicals that could activate as well as suppress the NLRP3 inflammasome pathway, we performed multiple layers of screens using IL-1β as a readout of the pathway activity.

Recent Major Publications
Kaji T, Hijikata A, Ishige A, Kitami T, Watanabe T, Ohara O, Yanaka N, Okada M, Shimoda M, Taniguchi M, Takemori T. CD4 memory T cells develop and acquire functional competence by sequential cognate interactions and stepwise gene regulation. ***Int Immunol*** 28, 267–282 (2016)

The overarching goal of our laboratory is to understand the role of cellular metabolism in the pathogenesis of complex diseases. Research over the past decades has shown that monogenic mutations in metabolic pathways cause a wide variety of human diseases. However, more recent studies have highlighted the role of cellular metabolism in the development of a wide variety of complex human diseases. Our laboratory in particular has been studying the function of mitochondrial energy metabolism, which is associated with neurodegeneration, cardiovascular disease, type 2 diabetes, and aging. We hope to identify novel pathways that restore or improve mitochondrial function through genetic and chemical screens and to examine their potential therapeutic value using genetically engineered mouse models and unique chemical probes.

Towards our goal, we have begun to explore the role of mitochondria in an innate immune pathway called the NLRP3 inflammasome, which is involved in variety of age-associated diseases. The NLRP3 inflammasome is activated by cell physiological changes, including mitochondrial damage, although the molecular players involved have not been fully elucidated. To systematically identify key players in this complex pathway, we performed a series of chemical screens and have successfully identified chemicals that can activate as well as suppress the NLRP3 inflammasome via mitochondria. Our analysis of these unique chemicals highlighted an important role of mitochondrial energy metabolism in maintaining intracellular ion homeostasis and preventing NLRP3 activation. Moreover, two outputs of the mitochondria, ATP and reactive oxygen species, both compete for activation and suppression of the NLRP3 inflammasome.

In the coming year, we hope to gain more in-depth quantitative understanding of the interplay between mitochondria and the key interacting molecular players we identified from the screen. In addition, we hope to understand the mechanism by which suppressors of the NLRP3 inflammasome pathway alter or restore the function of mitochondria.

Central Facilities

Central Facilities in IMS provide all researchers in the Center with access to the most advanced equipment and technologies. Central Facilities consist of four sections; the FACS Laboratory managed by Dr. Takashi Saito, the Confocal Laboratory managed by Dr. Takaharu Okada, the Genomics Laboratory managed by Dr. Osamu Ohara, and the Animal Facility managed by Dr. Haruhiko Koseki.

FACS Laboratory

The FACS Laboratory provides a range of support for flow cytometry and cell sorting, techniques that are essential for nearly all immunological experiments. The FACS Lab has upgraded all FACS Arias and added an Aria Fusion. In addition to FACS machines, the lab installed ImageStreamX, a device that combines flow cytometry with the visual detail of microscopy in a single platform, and upgraded CyTOF2, a mass-spectrometry-based cytometer that has the potential for analyzing more than 30 markers simultaneously with metal-labeled antibodies.

In 2016, 1611 analytical and 1963 sorting experiments were performed in the lab. For the users of the FACS machines (cell analyzers and cell sorters), two staff members offer various services: (1) *Technical support and training*: In 2016, the facility offered 11 technical courses (7 for cell sorting and 4 for cell analysis). Courses were held at 3 different levels, Calibur basic, Canto II and Aria basic. A total of 60 researchers took the courses in 2016. (2) *Cell sorting operation service*: The FACS Lab provides a cell sorting operation service, in which researchers can ask an experienced operator to conduct the sorting experiment. In 2016, the lab provided 205 such operation services. Special cell sorting techniques, such as single cell sorting, have also been performed. (3) *Management/maintenance of FACS machines*: FACS machines are available for registered users 24 hours a day and reservations are accepted up to one month in advance through an internal website. In addition to the in-house FACS Lab staff, engineers from Becton Dickinson visit once a week to provide maintenance and technical support.

Table: Instruments in the FACS Lab

Machine types	Machines	# of machines
FACS cell analyzer	Calibur Canto II	4 2
FACS cell sorter	Aria II Aria III Aria Fusion	3 2 1
Mass-cytometer	CyTOF2	1
Imaging flow cytometer	ImageStreamX	1

Confocal Laboratory

The Confocal Laboratory provides equipment for cell and tissue imaging, and coordinates technical support. There are eight fluorescence microscopes available to researchers at IMS.

1. Inverted Leica SP5 system with visible lasers for single-photon excitation including a 405 violet laser.
2. Inverted Leica SP2 system with visible lasers for single-photon excitation including a 405 violet laser. This microscope is equipped with a chamber system that controls CO2 concentration, temperature and humidity for live cell imaging.
3. Inverted Leica SP8 system with two femtosecond Ti:Sa lasers for multiphoton excitation. This system is equipped with two types of scanners (resonant and galvano) and hybrid detectors with high sensitivity and low background noise. One of the two Ti:-Sa lasers is connected to an optical parametric oscillator (OPO) that enables two-photon imaging by long wavelength excitation.
4. Upright Leica SP5 system with two femtosecond Ti:Sa lasers for multiphoton excitation. This system utilizes resonant scanners that enable high-speed acquisition of large z-stacks for live tissue imaging.

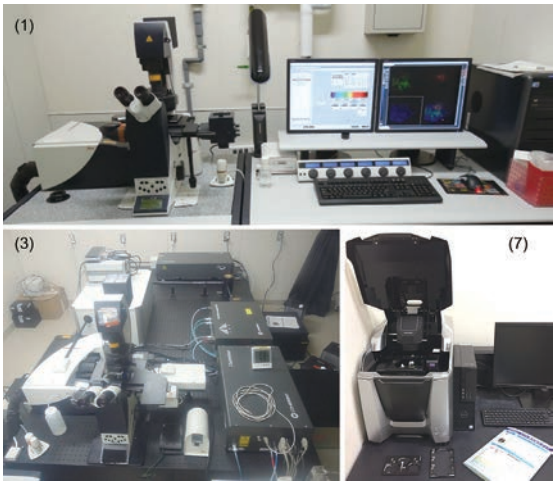


Photo: Inverted Leica SP5 confocal (1), inverted Leica SP8 multiphoton (3), and Keyence BZ-X700 (7) microscopes

5. Inverted Olympus FV1200 system with visible lasers for single-photon excitation.
6. Inverted Nikon N-SIM/N-STORM super-resolution microscope for dual color imaging.
7. Keyence BZ-X700 all-in-one fluorescence microscope.
8. GE Healthcare DeltaVision Elite system.

Genomics Laboratory

We are a technical support service lab that provides genome- and proteome-wide analysis for research groups in the Center for Integrative Medical Sciences (IMS). We offer a variety of services to suit the needs of different labs. These include DNA sequencing, proteomics analysis, multiplex suspension array, cDNA/Genomic clone distribution, and Primer/labeled probe distribution for qRT-PCR analysis of immune cells (TABLE attached). Supplying advanced technologies on demand, we provide comprehensive interrogation of the nucleic-acid based information in a cell at single-base resolution with the Illumina HiSeq1500 and as well as proteomic approaches using the AB SCIEX TripleTOF 5600. Using this unbiased sequencing approach, we have interrogated: transcription units, mapping/genome annotation, alternative splice sites, and transcription factor binding sites. Our mass spectrometry system will make it possible to use quantitative proteomic approaches in various immunological studies. These technologies will help to reveal additional hidden features of the dynamic genomic and proteomic landscape that are regulated by both genetic and epigenetic pathways in all organisms.

Table: Central services provided by the Genomics Lab in 2016

Next-generation DNA sequencing	**# of samples**	**# of teams**
RNA-sequencing	1,525	29
Chip-sequencing	444	9
Others (Exome etc)	148	7
Proteomics	**# of samples**	**# of teams**
Mass Spectrometry Analysis	1	1
Multiplex suspension array	1,451	7
Sanger DNA sequencing	**# of samples**	**# of teams**
36cm capillary	7,728	19
50cm capillary	4,848	15
cDNA clone delivery	**# of samples**	**# of teams**
	14	2
Primer/labeled probe delivery	**# of samples**	**# of teams**
	17	1

Animal Facility

We continue to maintain over 50,000 mice in the SPF area, and 1,500 mice in an isolated area. The SPF area also contains 550 germ-free or gnotobiotic mice in Vinyl Isolator rooms, and in Vinyl Isolator bio-bubble rooms. The former is used by several IMS research groups, in particular the mucosal immunologists, and the latter is for "humanized mice". We introduce mouse lines into the SPF area via a combination of *in vitro* fertilization (IVF) and embryo transfer methods, and have also generated cryostocks of genetic resources for 711 lines. We also maintain relatively large colonies of several commonly used strains such as NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice, *Rag1* KO and Cre deleters, and provide them to users on demand. We have also provided technical assistance to generate knockout and transgenic mice (62 lines). In addition, we made KO and KI mice (83 lines) using the CRISPR/Cas system, and have created 13 lines of germ-free mice.

We generated mice to improve the efficacy of transplantation of human hematopoietic stem cells into NSG mice by better "humanizing" the host strain. For this purpose, we have introduced large genomic fragments containing human genes encoding MHC, cytokines, adhesion molecules, virus receptors and others into the NSG mice. We maintain transgenic mice and knock-in mice with confirmed expression of human genes on a C57BL/6 background and have begun backcrossing these mice onto the NSG background using the speed-congenic method.

Photo: Breeding equipment for germ-free mice and gnotobiotic mice
Lower Left: stainless-isolator
Lower right: vinyl-isolator standard size
Upper right: vinyl-isolator half size

Award winners 2016

Name of the awardee	Name of the award	Date of the announcement
Masaru Taniguchi, Group Leader, Laboratory for Immune Regulation	The Order of the Sacred Treasure	May, 2016
Masayuki Amagai, Team Leader, Laboratory for Skin Homeostasis	International Member of the National Academy of Medicine	Oct, 2016
Masayuki Amagai, Team Leader, Laboratory for Skin Homeostasis	International Honorary Member of American Dermatological Association	Oct, 2016
Kenya Honda, Team Leader, Laboratory for Gut Homeostasis	The 53rd Baelz Prize (1st prize)	Nov, 2016
Kenya Honda, Team Leader, Laboratory for Gut Homeostasis	Academic Award of the Mochida Memorial Foundation (Mochida Memorial Foundation for Medical and Pharmaceutical Research)	Nov, 2016
Hiroshi Ohno, Group Director, Laborator for Intestinal Ecosystem	The 53rd Bälz Award (2nd prize)	Nov, 2016
Hiroshi Ohno, Group Director, Laborator for Intestinal Ecosystem	Hot Topics Award (Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry)	Apr, 2016
Hiroshi Ohno, Group Director, Laborator for Intestinal Ecosystem	Momofuku Ando Award (Grand Prize)	Mar, 2016
Hidewaki Nakagawa, Team Leader, Laboratory for Genome Sequencing Analysis	JCA (Japanese Cancer Association) -Mauvernay Award	Sep, 2016
Makoto Arita, Team Leader, Laboratory for Metabolomics	Lands Award (Japan Society for Lipid Nutrition)	Sep, 2016
Hilde Cheroutre, Team Leader, Laboratory for ImmuneCrosstalk	National Institutes of Health (NIH) Mucosal Immunology Studies Team (MIST) (U01) Award	Jun, 2016
Artem Lysenko, Postdoctoral Reasearcher, **Piotr J. Kamola**, Visiting Researcher, **Keith A. Boroevich**, Technical Staff and **Tatsuhiko Tsunoda**, Group Director, Laboratory for Medical Science Mathematics	Dream Challenges Certificate of Best Performance - Disease Module Identification DREAM Challenge Sub-Challenge 2	Nov, 2016
Tetsuya Kubota, Senior researcher, Laboratory for Metabolic homeostasis	Best Presentation Award (Japan Society for Adaptation Medicine)	Dec, 2016
Yasutaka Motomura, Special Postdoctoral Researcher, Laboratory for Innate immune systems	Best Presentation Award (The 45th Annual Meeting of the Japanese Society for Immunology)	Dec, 2016
Eiji Miyauchi, Visiting Scientist, Laborator for Intestinal Ecosystem	Best Presentation Award (The 44th Annual Meeting of the Japanese Society for Immunology)	Feb, 2016
Shunichi Kosugi, Research Scientist, Laboratory for Statistical Analysis	KAKENHI Reviewers Award	Sep, 2016
Alok Sharma, Research Scientist, Laboratory for Medical Science Mathematics	VC's Research Award (University of the South Pacific)	Oct, 2016
Masato Akiyama, Research Associate, Laboratory for Statistical Analysis	Charles J. Epstein Trainee Awards for Excellence in Human Genetics Research (semifinalist) (2016 American Society of Human Genetics)	July, 2016
Masahiro Nakajimo, Research Associate, Laboratory for Bone and Joint Diseases	Young Investigator Award (Japan Society of Human Genetics)	Jun, 2016
Yuki Aoki, Student Trainee, Laboratory for Human Disease Models	Young Investigator Award (The Japanese Society of Pediatric Hematology/ Oncology)	Dec, 2016
Yuki Aoki, Student Trainee, Laboratory for Human Disease Models	Young Investigator Award (Japan Pediatric Society)	May, 2016
Toshimori Kitami, Senior Scientist, YCI Laboratory for Cellular Bioenergetic Network	Poster Award (The 13th Conference of Asian Society for Mitochondrial Research and Medicine and the 16th Conference of Japanese Society of Mitochondrial Research and Medicine)	Oct, 2016
Shinnosuke Matsueda, Student Trainee, Laboratory for Metabolomics	Best Poster Award (Lipoquality, JSPS Scientific Research on Innovative Area)	Jul, 2016
Ryohei Aoyagi, Junior Research Associate, Laboratory for Metabolomics	Poster Award (Lipoquality, JSPS Scientific Research on Innovative Area)	Jul, 2016
Taiga Kato, Student Trainee, Laboratory for Metabolomics	Poster Award (Lipoquality, JSPS Scientific Research on Innovative Area)	Jul, 2016
Tomomitsu Hirota, Research Scientist, Laboratory for Respiratory and Allergic Diseases	Kenji Mano Travel Grant (Joint Congress of APAAACI and APAPARI 2016)	Oct, 2016
Piotr J. Kamola, Visiting Researcher, Laboratory for Medical Science Mathematics	ISCB Travel Award	Oct, 2016

RIKEN International Program Associate (IPA)

IMS accepted four international students as RIKEN International Program Associates (IPA). Under this IPA program, IMS lab heads host international students from collaborating graduate schools and supervise their Ph.D. program as Joint Supervisors. The students receive a daily living allowance and housing costs for up to a maximum of three years.

The IPA students who studied at IMS in 2016 were
Krutula Nair (Graduate School of Frontier Biosciences, Osaka University) from India studied in the Laboratory for Transcriptional Regulation.
Mei Suen Kong (University of Science, Malaysia) studied in the Laboratory for Cell Signaling.
Chanyoung Shin (Tokyo Institute of Technology) from Korea studied in the Laboratory for Inflammatory Regulation.
Sufeng Chiang (Genome and Systems Biology Degree Program, National Taiwan University) from Taiwan studied in the Laboratory for Integrated Cellular Systems

RIKEN Foreign Postdoctoral Researcher (FPR) Program

The RIKEN Foreign Postdoctoral Researcher (FPR) program offers aspiring young foreign researchers with creative ideas and who show promise of becoming internationally active in the future the opportunity to pursue innovative research at RIKEN under the direction of a RIKEN laboratory head. The FPR Program is one of RIKEN's initiatives to open up its facilities and resources to the forefront of global science and technology.

In 2016, two young researchers studied at IMS as RIKEN FPRs.

Michelle Kendle Maslowski studied in the Laboratory for Intestinal Ecosystem.
Ealey Nequan Kafi studied in the Laboratory for Innate Immune Systems.

RIKEN Junior Research Associate (JRA) Program

The Junior Research Associate Program was launched in 1996 to encourage young scientists with fresh ideas and youthful enthusiasm to collaborate with, and learn from, senior scientists with years of experience. This program provides part-time positions at RIKEN for young researchers enrolled in university Ph.D. programs. The JRA program serves the dual purpose of fostering the development of these young scientists while also energizing RIKEN with their innovative thinking.

This year, 24 JRA students studied in IMS.

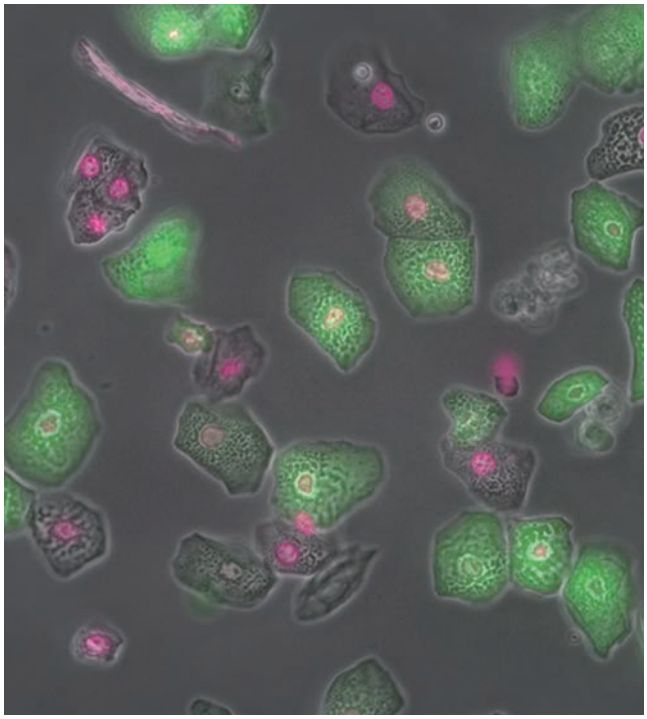
Junichiro Takano (Laboratory for Developmental Genetics)
Yoshihiro Ito (Laboratory for Skin Homeostasis)
Eiichiro Watanabe (Laboratory for Gut Homeostasis)
Satoko Yokoyama (Laboratory for Intestinal Ecosystem)
Ryohei Aoyagi (Laboratory for Metabolomics)
Yuki Furuichi (Laboratory for Skin Homeostasis)
Tadashi Takeuchi (Laboratory for Intestinal Ecosystem)
Rintaro Ono (Laboratory for Human Disease Models)
Yujiro Yamamoto (Laboratory for Genotyping Development)
Yurina Miyajima (Laboratory for Innate Immune Systems)
Takato Kobayashi (Laboratory for Innate Immune Systems)
Takaaki Kawaguchi (Laboratory for Gut Homeostasis)
Kensuke Yamaguchi (Laboratory for Autoimmune Diseases)
Yuma Sakamoto (Laboratory for Bone and Joint Diseases)
Keiko Usui (Laboratory for Skin Homeostasis)
Natsuko Otaki (Laboratory for Innate Immune Systems)
Mamoru Ogawa (Laboratory for Metabolomics)
Hiroki Sugishita (Laboratory for Developmental Genetics)
Hiroe Tetsu (Laboratory for Innate Immune Systems)
Manabu Nagayama (Laboratory for Gut Homeostasis)
Daisuke Hisamatsu (Laboratory for Developmental Genetics)
Yoshiki Momiuchi (Laboratory for Innate Immune Systems)
Ari Morimoto (Laboratory for Skin Homeostasis)
Tomoko Yoshihama (Laboratory for Pharmacogenomics)

RIKEN Special Postdoctoral Researcher (SPDR) Program

RIKEN's Special Postdoctoral Researcher Program was instituted to provide young and creative scientists the opportunity to be involved in autonomous and independent research in line with RIKEN objectives and research fields. The positions are competitive, but if selected, researchers receive salaries and research budgets (1 million yen) from RIKEN and they are able to conduct their research at one of its laboratories.

This year, seven postdocs conducted their research at IMS through the SPDR program.

Juan Guillermo Betancur Medina (Laboratory for Developmental Genetics)
Takeshi Tanoue (Laboratory for Gut Homeostasis)
Yosuke Isobe (Laboratory for Metabolomics)
Yasutaka Motomura (Laboratory for Innate Immune Systems)
Eiji Miyauchi (Laboratory for Intestinal Ecosystem)
Tatsuro Naganuma (Laboratory for Metabolomics)
Alexis Vogelzang (Laboratory for Mucosal Immunity)



Part 3

Research Projects

iPS project

Induced pluripotent stem (iPS) cells possess tremendous therapeutic potential in many areas, including regenerative medicine and immune therapy. We have begun an activity to apply iPS technology to both mouse and human immunology research and to develop therapeutics. On a collaborative basis with individual RCAI-IMS research laboratories, the core facility for iPS research is engaged in developing efficient protocols to reprogram various types of lymphocytes into iPS cells as well as to induce differentiation of iPS cells into a variety of lymphoid lineage cells. This activity is partly supported by the Research Center Network for Realization of Regenerative Medicine from the Japan Agency for Medical Research and Development (AMED) and CREST, Japan Science and Technology Agency.

This year, the facility investigated whether iPS-Vα24$^+$iNKT cells could exert anti-tumor activity *in vivo*. They demonstrated tumor-directed cytotoxicity by iPS-Vα24$^+$iNKT cells in tumor-bearing mice and adjuvant activity to activate autologous NK cells in humanized mice. The anti-tumor activity of iPS-Vα24$^+$iNKT cells *in vivo* validates their clinical potential in cancer immunotherapy. This study was published in Stem Cells (34, 2852–2860 (2016)). They also have operated an IMS Cell Manufacturing Unit (CMU) to prepare iPS-Vα24$^+$iNKT cells under the GMP (Good Manufacturing Practice)/GCTP (Good Gene, Cellular, and Tissue-based Products Manufacturing Practice) guidelines and have started preclinical studies on the safety of these iPS-Vα24$^+$iNKT cells.

Reference

Yamada D, Iyoda T, Vizcardo R, et al. Efficient Regeneration of Human Vα24$^+$ Invariant Natural Killer T Cells and Their Anti-Tumor Activity In Vivo. ***Stem Cells*** 34, 2852–2860 (2016)

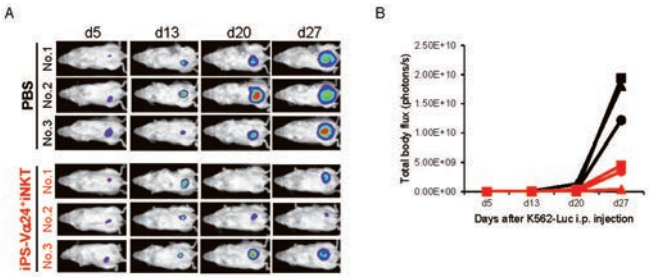


Figure: Antitumor activity of iPS-Vα24$^+$iNKT cells in a NOG mouse model
(A) Imaging of NOG mice that were inoculated with K562-Luc cells. Starting five days later, the mice were treated with 3 x 10^6 human iPS-Vα24$^+$iNKT cells or PBS as control, a procedure repeated 5 times at 2 day intervals. Tumor growth was assessed by IVIS at the indicated time points. (B) Total body flux (photons/s) for each mouse. PBS-treated mice are indicated in black and iPS-Vα24$^+$iNKT cell-treated mice are indicated in red.

Modeling skin diseases

Atopic dermatitis (AD) is regarded as a multifactorial, heterogeneous disease. A number of animal model studies of AD have been developed to improve our understanding of the clinical implications, but as yet there has been limited success in better treatment for AD patients. AD develops and progresses through highly complex processes and these underlying pathological mechanisms are different in each patient. To overcome the pathological complexity, we have constructed center-wide projects and integrated analysis for multimodal data from several dermatitis model mice. Furthermore, we aim to provide "reverse translation" research, applying patient-based findings to animal models.

We take advantage of several AD/chronic dermatitis model mice, including *Spade*, SOCS3 cKO, STAT3 cKO, Tmem79 KO, and FLG KO and analyze RNA-seq-based gene expression profiles to understand pre-symptomatic events occurring in the skin, beginning from birth to the onset of dermatitis. Using such samples, Dr. Ohara's group has conducted transcriptomic analysis, and Dr. Kawakami performed comparative analysis for each animal model by using these transcriptomic data. Dr. T. Okada examines peripheral nerve activation in inflamed skin by confocal and two-photon excitation microscopy.

In addition to animal model data analysis, we are establishing a system for sharing human clinical data and AD animal model data, in collaboration with the medical innovation hub project team (MIH) and the Dermatology Department of Keio University School of Medicine. Dr. Amagai and Dr. Kubo have been working to compare the findings in mouse and human AD in order to understand which processes are common in AD pathogenesis in the two species. We then plan to select a suitable animal model for each variety of human AD.

There is cooperation in these studies between the experimental labs led by Dr. Kubo, T. Okada and Amagai, and the computational analysis/modeling labs led by Dr. Ohara and MIH members.

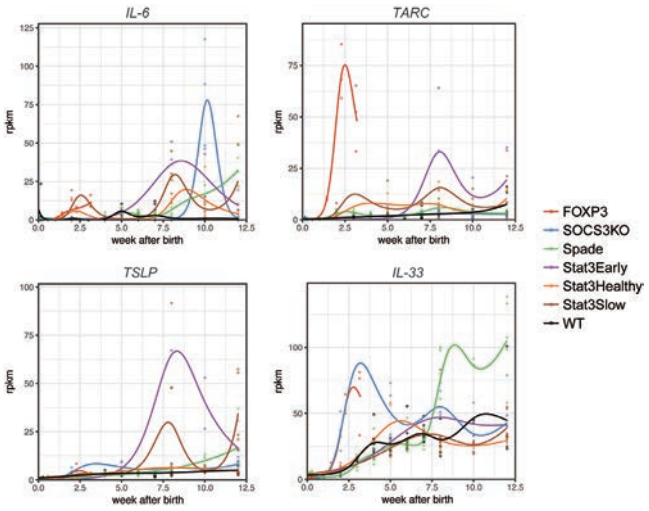


Figure: Each of the dermatitis model mice presented a clearly different immune status.
We performed sequential transcriptomic analysis in the skin of each dermatitis model mouse [*Spade*, SOCS3 cKO, STAT3 cKO (early developed, slowly developed, healthy), FOXP3 mut] and wild-type (WT) mice. Although all AD model mice showed visible inflammation, scratching behavior and epidermal barrier dysfunction, parameters reflecting their immune status were very distinctive.

Integrated omics approach to elucidate the impact of the gut microbiome on the pathogenesis of type 2 diabetes

Recent findings in gut microbiome studies indicate that the loss of microbial diversity, or dysbiosis, is not the consequence but rather the cause of various diseases including type 2 diabetes (T2D). As an IMS Center project, we are applying a comprehensive multiple omics approach to elucidate the impact of the gut microbiome on the pathogenesis of T2D. This is a collaborative effort with Professors Takashi Kadowaki and Tsutomu Yamazaki from the University of Tokyo Hospital. From RIKEN IMS, the Laboratories for Metabolic Homeostasis, Intestinal Ecosystem, Microbiome Sciences, Metabolomics, Integrative Genomics, and Integrated Bioinformatics are mainly involved in this project. Fecal metagenomic, metatranscriptomic and metabolomic data, as well as plasma and urine metabolomic data are obtained from three groups of volunteers undergoing a complete medical checkup at the University of Tokyo Hospital (n=100 each): 1) no abnormal examination outcome, 2) obesity (BMI ≧ 25), and 3) glucose intolerance (fasting blood glucose ≥110 mg/dl and HbA1c ≧6.0%). In addition, exomes and SNPs of T2D susceptibility genes are being analyzed. The goal of the project is to identify T2D risk factors, such as certain bacteria and/or their metabolites, by analyzing the metadata from the comprehensive multiple omics analyses, combined with clinical and genetic datasets. One hundred volunteers have already been recruited for all groups, and data acquisition is underway.

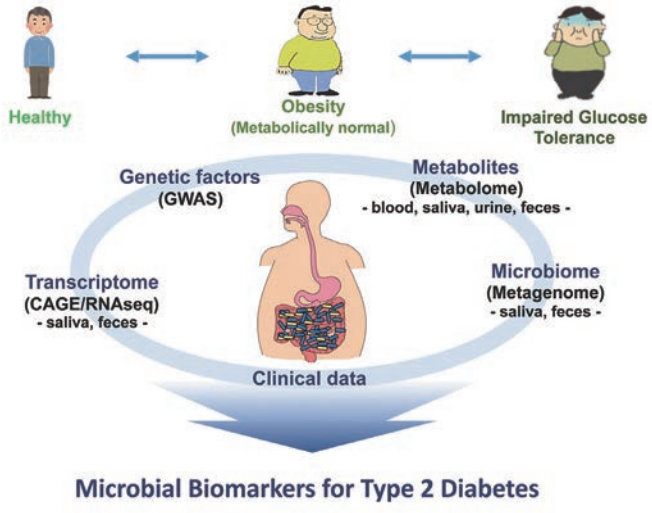


Figure: Analytical scheme to identify microbial biomarker(s) for type 2 diabetes
Metadata sets shown in the figure are collected from the volunteers (three groups: healthy, obese without metabolic abnormality, and those with impaired glucose tolerance; 100 each) and comprehensively analyzed to identify differential microbial and/or microbial metabolic factors among the groups, which should be candidate biomarkers for the pathogenesis of type 2 diabetes.

Humanized mouse

To help overcome the problem of limited availability of human samples and to understand kinetics and behavior of the human immune system *in vivo*, we have developed humanized mice by injecting cord blood-derived hematopoietic stem/progenitor cells into immune-deficient NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) newborn mice. Their immune-compromised status affects the engraftment levels of human hematopoietic and immune cells. Through a collaborative effort between the Jackson Laboratory and RIKEN, we have generated several new strains of NSG mice lacking mouse immunity and expressing human genes encoding, e.g, cytokines, adhesion molecules, and MHC molecules. By using these new humanized mouse models, we can determine the differentiation capacity of human cells of interest. We are trying to correlate gene expression signatures and the *in vivo* fate of human cells.

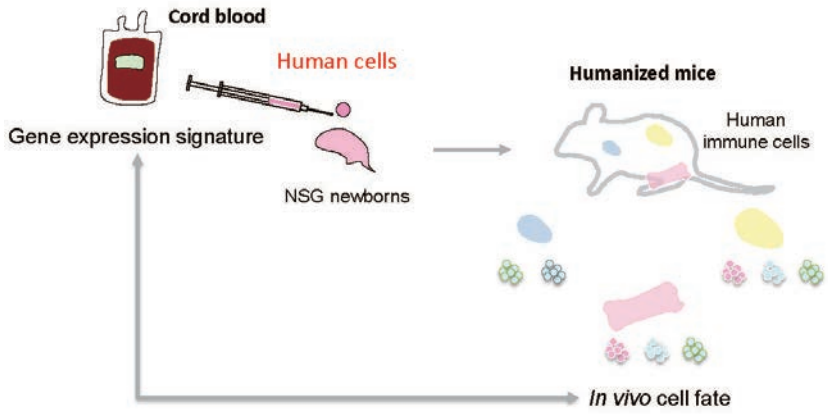


Figure: Using humanized mice to identify the differentiation capacity of human cells
Human CD8$^+$ T cells were purified from a humanized mouse. By injecting purified human cord blood cells into immune-deficient newborn mice, we can analyze to what extent the cells engraft in multiple organs and which immune subsets are generated. By assessing gene expression of the donor cells together with functional characteristics of the cells they generate *in vivo*, we can identify gene expression signatures defining the developmental capacity of the human cells.

NKT Cell Projects

NKT cells have the capacity to enhance subsequent immune responses. When an NKT cell ligand is loaded onto $CD1d^+$ cells, such as dendritic cells (DCs) or CD1d transfectants, NKT cells can respond and produce IFN-γ. The medical innovation groups in IMS have launched projects aimed at application of NKT cell therapy to cancer as translational research. Here we introduce four NKT cell-related projects aimed at cancer treatment.

In the first project, we have been collaborating with 15 National Hospital Organization (NHO) hospitals on clinical therapeutic studies using the NKT glycolipid, α-galactosylceramide (α-GalCer) pulsed autologous DCs (DC/Gal) in a randomized phase IIa trial in early stage lung cancer. At present, 39 patients are entered in this trial. In our part of this study, we perform immunological analyses of the DC/Gal-treated and control cancer patients, including NKT cell functional analyses. As a second project, IMS was selected to become part of the research center network for realization of regenerative medicine. We have successfully established human iPS-NKT cells and published a paper in which iPS-NKT cells were shown to have the potential to produce more IFN-γ and to have strong antitumor effects *in vivo* (Figure 1). In addition, we verified that iPS-NKT cells behave as an adjuvant for NK cells. This iPS-NKT cell project has been planned as a clinical application research project. Third, as new type of cancer vaccine, we established artificial adjuvant vector cells, which contain tumor antigen mRNA and α-GalCer, leading to activation of both innate and adaptive immunity. We have almost completed the preclinical studies through discussions with the Pharmaceuticals and Medical Devices Agency (PMDA). Fourth we developed several new NKT cell ligand candidates and have been investigating the function of one of them. We have been examining its efficacy in preclinical studies for next generation NKT cell ligand cancer immunotherapy. These three projects (iPS-NKT, new NKT ligand and aAVC therapy) have been supported by the Japan Agency for Medical Research and Development (AMED) and are also supported by the RIKEN Drug Discovery and Medical Technology Platforms (DMP).

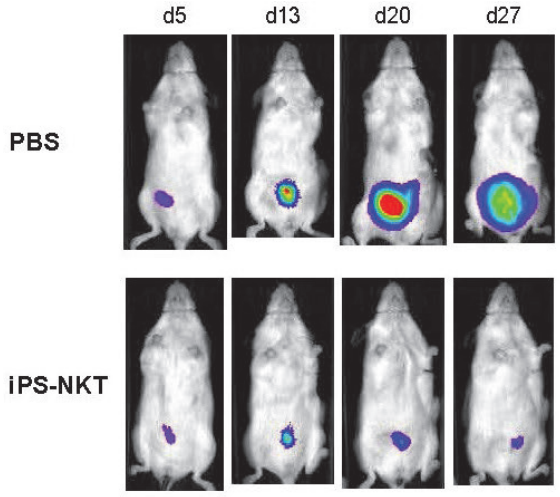


Figure 1: iPS-Vα24⁺iNKT cells elicit antitumor activity in an NOG mouse model
NOD/Shi-scid IL-2rγ^{null} (NOG) immuno-deficient mice were inoculated with K562-Luc cells and five days later, 3x10^6 iPS-Vα24⁺iNKT cells or PBS were given 5 times at 2 day intervals. Tumor growth was assessed by IVIS at the indicated time points.

Linkage to RIKEN Program for Drug Discovery and Medical Technology Platforms (DMP)

IMS collaborates with DMP to develop innovative new pharmaceuticals and medical technologies by facilitating the transfer of basic research within the institute. The DMP was founded in RIKEN in 2010 in order to support all phases of development of new therapeutics, from the discovery of promising targets to the identification of potential lead compounds, such as small molecules and antibodies, and the acquisition of intellectual property rights to drugs and technologies that can then be brought to the development phase.

To achieve effective progress in this area, DMP established 8 Drug Discovery Basic Units, in which the types of studies being performed are organized according to the expertise of each PI. IMS contributes to this effort in several ways, including by setting up a facility for the development of antibody drugs, as the Drug Discovery Antibody Platform Unit. In addition, IMS now has seven collaborative programs with DMP, including Artificial adjuvant vector cells (Shin-ichiro Fujii), Cancer therapy with iPS-derived NKT cells (Haruhiko Koseki), Cancer therapy with NKT cells (Masaru Taniguchi), Drugs for allergic diseases (Yasushi Ishii), Leukemia treatment drugs targeting leukemic stem cells (Fumihiko Ishikawa), mAb therapy for lymphomas (Yasushi Ishii), and a novel inhibitor for HBV infection (Daiki Miki). The preclinical study in the Artificial adjuvant vector cell project for cancer therapy has been completed and, therefore, Fujii et al. are preparing for an investigator initiated-clinical trial.

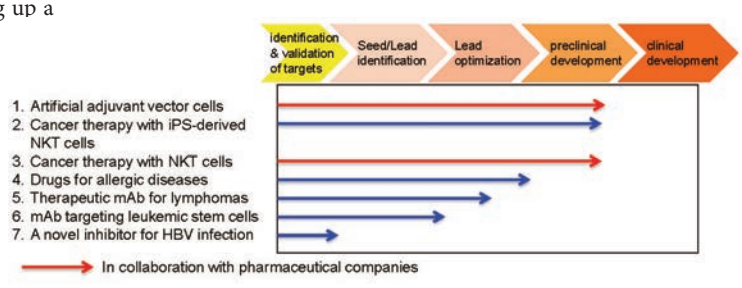


Figure: Collaboration with IMS and DMP for the development of innovative new pharmaceuticals and medical technologies

PGRN-RIKEN Project

The U.S. NIH Pharmacogenomics Research Network (PGRN) is a consortium of research groups funded as individual cooperative agreements with the NIH. PGRN investigators are top researchers from U.S. academic institutions and conduct studies of variation in human genes relevant to drug metabolism, pharmacokinetics and pharmacodynamics, and the relationship of these genetic variation to drug responses. Principal investigators of the PGRN and RIKEN Center for Genomic Medicine (now RIKEN IMS Core for Genomic Medicine: CGM) held a series of discussions on the need to accelerate discoveries in pharmacogenomics (PGx) and launched the Global Alliance of Pharmacogenetics (GAP) in 2008. Currently, the PGRN-RIKEN Project is conducted under a PGRN-Hub, a newly-formed resource established to enhance scientific exchange between the PGRN and the scientific community at large.

In this international collaboration, the PGRN has been successfully assembling a very large collection of DNA samples from well-phenotyped patients receiving specific drugs and drug combinations in clinical trials conducted in the U.S. The CGM focuses on high-throughput genome-wide SNP scans and targeted sequencing of selected genes or regions using next generation sequencing (NGS). We also provide technological and methodological expertise to identify genetic factors associated with drug responses, risk of severe adverse drug reactions and non-response to medications. Together, the PGRN-RIKEN Project capitalizes on these strengths to advance discoveries in PGx research. To date, we have initiated 46 collaborative projects and have 54 publications and over 52,000 DNA samples genotyped through this collaboration, which will lead to development of better and safer medications and realize the dream of global precision medicine.

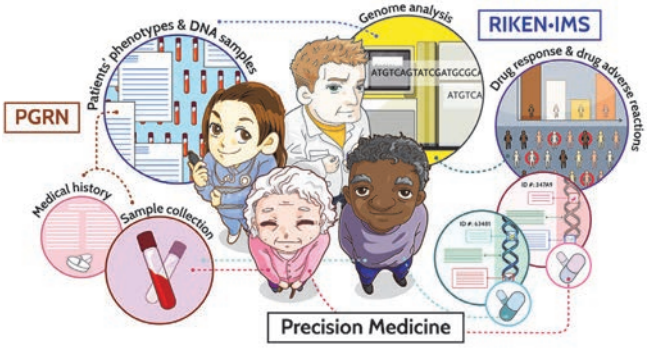


Figure: The Pharmacogenomics Research Network (PGRN)-RIKEN IMS strategic alliance
Please visit http://www.pgrn.org/pgrn-riken.html

Collaboration with Asian institutes and SEAPharm

It has been noticed that severe cutaneous adverse drug reactions (ADRs), including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), occur at a much higher frequency in East Asian and Southeast Asian populations, and several genetic factors have been reported to be associated with the risk of cutaneous ADRs. In the case of the anti-epileptic drug carbamazepine, the US FDA now recommends HLA-B*15:02 screening prior to the administration of this drug for Han Chinese and other Asian populations with a high prevalence of HLA-B*15:02, due to the risk of ADR associated with this allele. To tackle this problem regionally, in 2012 we established the South East Asian Pharmacogenomics Research Network (SEAPharm) together with five other Asian countries (Korea, Indonesia, Malaysia, Taiwan, and Thailand). Membership has been steadily increasing, with Singapore participating in 2014, and Vietnam in 2016. The aim of the collaborative efforts is to identify significant ADRs in the region so that we can identify genomic biomarkers associated with their risk, information that could lead to a reduction in severe ADRs. We are now focusing on the identification of genomic biomarkers associated with cutaneous ADRs induced by the anticonvulsant phenytoin and the antibiotic co-trimoxazole, as well as with hepatic injury induced by anti-tuberculosis agents. In addition, we also aim to understand how the identified genomic biomarkers lead to the ADRs by performing functional studies. It is hoped that the discoveries from our collaborative efforts will identify useful biomarkers that can be used to predict the risk of ADRs, leading to the establishment of "stratified medicine" based on pharmacogenomic-guided drug therapeutics.

Figure: South East Asian Pharmacogenomics Research Network (SEAPharm)

Project

International Cancer Genome Consortium (ICGC)

Laboratory for Genome Sequencing Analysis　Laboratory for Medical Science Mathematics　Laboratory for Digestive Diseases

The ICGC was organized to launch and coordinate a large number of research projects that have the common aim of comprehensively elucidating the genomic changes present in many types of cancers. Its primary goals are to generate comprehensive catalogues of genomic abnormalities in ~25,000 cases of different cancer types and to make the data available to the entire research community with minimal restrictions. At the end of 2016, 78 cancer genome projects across 16 countries and the EU were ongoing, and the ICGC released genomic data from 16,246 donors as Release 23 (December, 2016). The RIKEN group has been involved with virus-related liver cancer, which is one of the most common and deadly cancers worldwide, especially in Japan and Asia. We performed whole genome sequencing (WGS) and RNA-Seq on 300 liver cancer samples and called their somatic mutations by using our in-house pipeline (Nat Genet 2016). ICGC also launched a "pan-cancer" whole genome project (PCAWG) in 2014, in which WGS data + RNA-Seq of 2834 donors were analyzed in uniform pipelines within the same computational environment. Approximately 1000 researchers and technical staff are involved world-wide in 16 theme working groups. We are contributing to this ambitious project as a member of a technical working group arranging ten "cloud" data centers worldwide, PI and researchers for driver gene analysis, mutational signatures, immunogenomics, and mitochondrial genomics, as well as by providing sample data from 270 liver cancers to the PCAWG (10% contribution 270/2834), which is the most productive within the ICGC/TCGA (Figure). In 2016, we completed the alignment and mutation calls by the three pipeline approach and established and validated certain pipelines for immunogenomic profiling from WGS and RNA-Seq, such as HLA genotyping, HLA mutation, and neo-antigen prediction, which were performed in collaboration with the group from The University of Tokyo. More than 95% of donors had their unique HLA genotype determined from whole genome sequencing data (Figure)

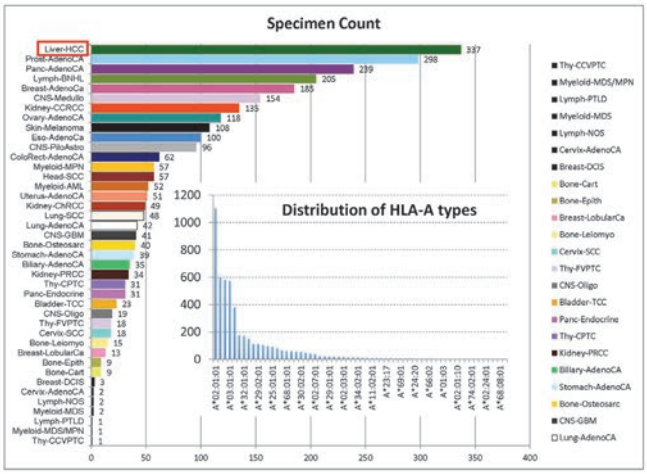


Figure: Specimen count of PCAWG ("Pan-CAncer" Whole Genome project) samples in ICGC/The Cancer Genome Atlas (TCGA) and distribution of their HLA-A genotypes

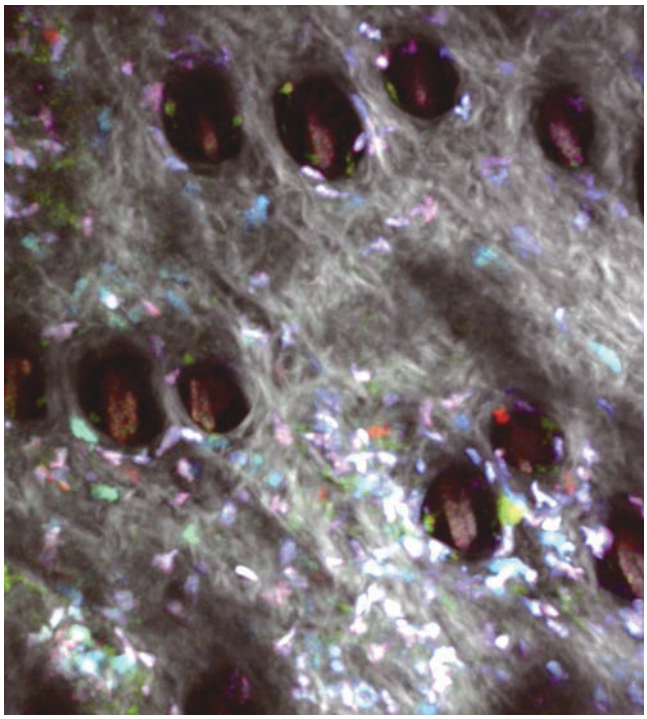
WGS data + RNA-Seq of 2834 donors were analyzed in PCAWG. RIKEN data (270 liver cancer samples) were the most abundant in the PCAWG sample set. The RIKEN HLA/immunogenomics working group determined unique HLA genotypes of more than 95% of donors of 2834 WGS of PCAWG donors.

Commissioned Research

The Biobank Japan Project

In 2013, the Japanese government started a new initiative for life sciences called Healthcare and Medical Strategy. This initiative consists of nine top priority research fields: 1) Drug development, 2) Development of medical devices, 3) Translational research, 4) Regenerative medicine, 5) Genomic medicine, 6) Cancer, 7) Brain science, 8) Infectious diseases, and 9) Rare diseases. For the genomic medicine research field, in 2015 the government decided to promote rapid and publically visible clinical applications of genomic research findings in parallel with strengthening of the basic infrastructure of human genome research. Based on this concept, the government is planning to establish a network of existing biobanks [Biobank Japan (BBJ), Tohoku Medical Megabank (Tohoku MMB), and National Center Biobank Network (NCBN)]. The government also established priority disease areas in 2015. Rare (hereditary) diseases, cancer, dementia, infectious diseases and pharmacogenomics were selected as the first priority disease areas because these diseases are thought to be very close to implementation of genomic information for actual medical practice. Common multifactorial diseases such as diabetes and cardiovascular diseases are positioned as the second priority disease area, which needs further basic genomic research to precisely elucidate the relationships between genetic variations and disease.

The Biobank Japan project was started as a MEXT-commissioned project in 2003, and the final goal of this project is the implementation of personalized medicine. To pursue this aim, the project constructed a large disease biobank, named Biobank Japan, in the Institute of Medical Science, the University of Tokyo. This biobank has already collected DNA and clinical information from 253,000 patients suffering from 51 target diseases. The IMS Core for Genomic Medicine has been working as the main infrastructure of genomic research for this project since the beginning. Since the primary area of this project is common multifactorial disease, we are performing large-scale genomic research including GWAS and whole-genome sequencing-based association studies using Biobank Japan samples. We are also collaborating with various international research groups using this important resource.



Part 4

Events

RIKEN IMS Summer Program (RISP) 2016

IMS was delighted to again successfully organize the 11th RISP (RIKEN IMS Summer Program). RISP began as the RCAI International Summer Program (RISP) in 2006 and has been continued by IMS, beginning 3 years ago. The aim of this activity is to provide networking opportunities on a broad international scale for young scientists, as well as to encourage future collaboration and postdoctoral training experiences in Japan. Due to the broadened research activities of the new IMS, the RISP 2016 program was expanded to include topics in genomic studies and chemical biology to understand human health and diseases, in addition to its original focus on immunology. The internship program, in which the participants perform research in IMS laboratories, has also been maintained. RISP 2016 was co-organized by the Chiba University Leading Graduate School Program.

We were pleased to welcome 31 graduate students and postdoctoral fellows from thirteen countries, together with 9 students from Chiba University and 2 students from Tokyo University, and 1 student from Okinawa Institute of Science and Technology Graduate University (OIST), who gathered at Yokohama from June 10 to 17. The scientific sessions were composed of 16 lectures, including IMS PIs and other invited senior scientists from Japan and abroad. RISP students also presented their research in both oral and poster sessions. The RISP program ended with participation in a two day International Symposium on Immunology, co-organized by IMS and the Japanese Society for Immunology.

RISP2016 was again a success; we received excellent feedback comments in the evaluation survey, and all students indicated that they would recommend this program to colleagues. From the other perspective, many lecturers commented on how impressed they were with the quality of the RISP students. IMS will again organize RISP in 2017, including broader topics as necessary to keep pace with recent developments in this rapidly moving life science field.

The IMS-JSI International Symposium on Immunology 2016

The IMS-JSI International Symposium on Immunology, hosted by the RIKEN Center for Integrative Medical Sciences (IMS) in conjunction with the Japanese Society for Immunology (JSI), was held June 16-17 at the Pacifico Yokohama Conference Center. The symposium, entitled "Immune homeostasis and diseases", included 18 internationally-recognized speakers presenting their cutting-edge research and attracted close to 400 participants. There were four sessions: (1) Microbiota, Fecal bacteriotherapy, (2) Cancer immunology, (3) Genome and epigenome, and (4) Cell death and inflammation. In the "Microbiota, Fecal bacteriotherapy" session, it became clear that microbiota changes, for example, by transfer of fecal bacteria, alter populations of macrophages as well as T cells. These observations may provide clues to understand various human diseases and provide a rational basis for their therapy. The "Cancer immunology" session updated our knowledge of checkpoint inhibitors, newly developed tools such as chimeric antigen receptor (CAR) T cells, and cell-based therapy. The "Genome and epigenome" session broadened our view by revisiting non-coding SNP GWAS and linking it to tissue- or cell type- specificity. Identification of super-enhancers in Treg-specifying genes provided important insight into autoimmune diseases. A combination of single-cell mRNA sequencing and CyTOF defined a unique dendritic cell population, allowing more precise lineage mapping of potential target cells for therapy. In the "Cell death and inflammation" session, the dynamics and regulation of the plasma membrane during cell death turned out to be a key for clearance of apoptotic cells. Dysregulation of DNA sensing impacts the immune system and can lead to autoimmune diseases. Studies of programed necrotic cell death, called pyroptosis, are bringing new insight into inflammatory diseases. A unique role of resident $CD169^{+}$ macrophage as guards during tissue injury in the experimental colitis model was revealed.

13th PGRN-RIKEN Strategic Alliance Meeting

The Global Alliance for Pharmacogenomics (GAP), a collaborative program between the former RIKEN Center for Genomic Medicine (now RIKEN IMS) and the US National Institute of Health (NIH) Pharmacogenomics Research Network (PGRN), was formed in 2008, with the objective of identifying the relationship between genetic variants and individual responses to drugs, including efficacy and side effects. PGRN-RIKEN strategic alliance meetings, held alternately in Japan and the United States, allow for face-to-face discussions about the progress of ongoing projects and future directions for the PGRN-RIKEN collaboration.

On April 20-21, 2016, the 13th PGRN-RIKEN Strategic Alliance Meeting was held at the Hyatt at Fisherman's Wharf, in San Francisco, United States, hosted by PGRN. At the opening of the meeting, Prof. Kathy Giacomini, PGRN Program Leader, introduced the importance of the collaboration, noting that PGRN-RIKEN contributed to as many as 41 out of all 150 Pharmacogenomics GWAS studies conducted by NIH from 2005 to June 2015.

The meeting provided a valuable forum for exchanging information on ongoing collaborative activities: "Carboplatin-paclitaxel with bevacizumab (ICON-7 study) in ovarian cancer", "Response to inhaled corticosteroids in large patients population with asthma", and "Functional genomic studies from two large clinical trials involving aromatase inhibitors for breast cancer". From the RIKEN side, Dr. Mayumi Tamari, an IMS Team Leader, presented the genetics of allergic diseases.

In addition to these presentations, participants also explored four new research proposals from PGRN and RIKEN, ultimately deciding to adopt three of them as additional collaboration projects, and then the meeting concluded successfully.

IMS Advisory Council 2016

The IMS Advisory Council (AC) was held on September 28–30, 2016. The AC began with an Assembly Meeting during which overall Center Activities were described. Dr. Tadashi Yamamoto gave an overview of the Center and this was followed by a description of Core Projects for Genomic Medicine by Dr. Michiaki Kubo and Representative Programs in the Core for Homeostatic Regulation by Dr. Haruhiko Koseki. Finally Dr. Yamamoto gave a description of IMS Future Plans. This session was followed by Block reviews, where individual laboratories were evaluated. Dr. Yamamoto also explained the Terms-of-reference from the new President of RIKEN, Dr. Hiroshi Matsumoto. At the end of the meeting, Dr. Max Cooper as chair of the AC provided oral comments and advice, summarizing the discussions and opinions of the AC panel.

In general, the AC commented that IMS is a thriving, international recognized research institute, particularly in genomic medicine and immunology. IMS PIs continue to publish seminal papers in top quality journals, participate actively in international collaborations and are invited to prestigious international scientific meetings. There are however external and internal forces that may be disruptive of this success. First, the AC was very concerned by the continued decrease in the IMS budget. The second issue was the integration of genomic and biological science at IMS. These are two quite different scientific cultures, the former thriving on team science to answer large-scale questions and the latter on individual/small group science to investigate basic issues that may have clinical applications. The merger of these two groups was expected to be difficult and there have been some small successes. Dr. Yamamoto has inherited these two problems and he is very committed to efforts that will increase the IMS budget and to promoting the successful merger of the genomic and biologic scientific cultures.

Harvard Summer School 2016

IMS offers a summer internship program for undergraduate students from Harvard University. In this program students do a research internship in IMS laboratories, have basic biomedical sciences lectures by IMS PIs and attend a Japanese language course. They also participate in the RIKEN IMS Summer Program (RISP) and the RIKEN IMS-JSI International Symposium on Immunology. The participants receive a letter grade from IMS and course credit from Harvard. In 2016 from June 6 to August 14, there were four students from Harvard University, Jessica Yamada, Andrew Chang, Christopher Tse, and Bovey Rao.

Ms. Yamada conducted her research project in the Laboratory for Disease Systems Modeling (Dr. Kitano), Mr. Chang in the Laboratory for Developmental Genetics (Dr. Koseki), Mr. Tse in the Laboratory for Skin Homeostasis (Dr. Amagai), and Mr. Rao in the YCI Laboratory for Immune Regeneration (Dr. Ikawa). During their internships, the students had numerous discussions with IMS researchers and, at the end of the program, they gave oral presentations describing their research results.

In addition, they visited Yokohama Science Frontier High School and met with students who want to become scientists. On this occasion, they also experienced Japanese culture and learned Sado (tea ceremony) from the local high school students.

RIKEN IMS-IITU Joint Workshop

The first joint workshop between RIKEN IMS and the Institute of Immunology in Tsinghua University (IITU) was held on September 13–14, 2016. Three IMS Group Directors, Drs. Tomohiro Kurosaki, Hiroshi Ohno and Ichiro Taniuchi visited IITU to introduce IMS and discussed possible future collaborations with researchers and students.

Three months later, on December 2–3, 2016, eleven IITU researchers visited RIKEN IMS for the second RIKEN IMS-IITU workshop. The meeting started with the introduction of each institution by IMS Director, Dr. Tadashi Yamamoto, and IITU Director, Dr. Chen Dong. The introductions were followed by presentations from 16 researchers, eight PIs from IITU and eight researchers from IMS. The topics covered wide areas of immunology, including Th17, IL-17, Tfh, DC, SLE, rheumatology, mucosal immunity, skin biology, metabolism, inflammation and neuroinflammatory responses.

On the second day, there were two sessions of one-on-one discussions. Researchers had time to discuss their interests with each other and seek further collaborations. From these discussions, three projects were launched under a collaboration grant from RIKEN IMS. By the end of the workshop, Directors Chen Dong and Tadashi Yamamoto agreed to expand research collaborations in 2017 and meet again at the third joint workshop in Beijing.

The Academia Sinica IBMS - RIKEN IMS Joint Workshop 2016

The Academia Sinica IBMS - RIKEN IMS Joint Workshop, hosted by the RIKEN Center for Integrative Medical Sciences (IMS), was held on March 23 at the RIKEN Yokohama Campus. Six leading scientists from the Institute of BioMedical Sciences (IBMS) of Academia Sinica, the national academic institute of Taiwan, including Dr. Fu-Tong Liu, the current director of IBMS and the vice president of Academia Sinica, visited IMS and gave lectures about their cutting-edge research. Six IMS scientists also gave lectures. The topics broadly covered the fields of immunology, infection and inflammation, ranging from viral host modulation, lipid biology, glycoprotein biology, allergic and autoimmune diseases, microbiome and mucosal immunity, and innate lymphoid cells. During and after the lectures, active discussions took place between both groups of scientists, not only about the data presented in the lectures but also about potential collaborations. This Joint Workshop was a valuable opportunity to begin fostering international cooperation in education and research between RIKEN IMS and the Academia Sinica IBMS.

Adjunct Professorship Programs

IIMS collaborates with and accepts graduate students from 8 domestic university graduate schools. There are now a total of 28 adjunct professors/associate professors in IMS (Table), and 72 students studied at IMS in 2016. On September 10th, IMS held a briefing session on adjunct graduate school programs to provide an opportunity for students to visit and talk directly with lab leaders and to consider their future directions.

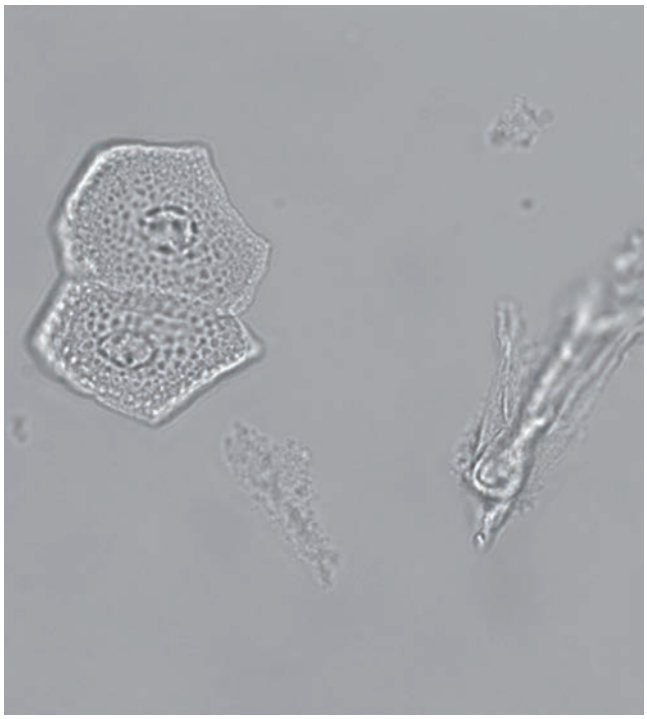
Table: Joint graduate school programs

Graduate Program	Affiliated IMS Investigator
Graduate School of Medicine, Osaka University	Takashi Saito (Visiting Professor), Takashi Tanaka (Visiting Professor)
Department of Immunology, Graduate School of Medicine, Chiba University	Takashi Saito (Visiting Professor), Haruhiko Koseki (Visiting Professor), Hiroshi Ohno (Visiting Professor), Ichiro Taniuchi (Visiting Professor), Shin-ichiro Fujii (Visiting Professor), Fumihiko Ishikawa (Visiting Professor)
School of Biomedical Science, Tokyo Medical and Dental University	Takashi Saito (Visiting Professor)
Graduate School of Medicine, Yokohama City University	Michiaki Kubo (Visiting Professor), Shiro Ikegawa (Visiting Professor), Mayumi Tamari (Visiting Professor), Hidewaki Nakagawa (Visiting Professor), Taisei Mushiroda (Visiting Professor), Yukihide Momozawa (Visiting Associate Professor), Yoichiro Kamatani (Visiting Associate Professor)
Graduate School of Medical Life Science, Yokohama City University	Hiroshi Ohno (Visiting Professor), Makoto Arita (Visiting Professor), Takaharu Okada (Visiting Professor), Kazuyo Moro (Visiting Professor), Hidehiro Fukuyama (Visiting Associate Professor)
Research Institute of Biological Sciences, Tokyo University of Science	Masato Kubo (Professor), Shohei Hori (Visiting Associate Professor), Katsuyuki Shiroguchi (Visiting Associate Professor)
Graduate School of Medicine, Kyoto University	Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Medicine, Keio University	Masayuki Amagai (Professor), Kenya Honda (Professor), Shigeo Koyasu (Visiting Professor), Haruhiko Koseki (Visiting Professor)

Guest lectures 2016

Table: Guest Lectures Jan–Dec, 2016

Date	Speaker	Affiliation	Country	Title
4-Jan-16	Dr. Ruka Setoguchi	Graduate School of Medicine, Kyoto University	Japan	IL-15 reverses functionally compromised state of human memory $CD8^+$ T cells
8-Jan-16	Dr. Yasuo Mori	Graduate School of Engineerin, Kyoto University	Japan	Redox-Sensitive TRP channels: their Roles in Normal Physiological Functions and Diseases
12-Jan-16	Dr. Robert Feil	Medical Institute of Bioregulation, Kyushu University	Japan	Epigenetic mechanisms in mammalian genomic imprinting
14-Jan-16	Dr. Cornelis Murre	University of California San Diego	USA	The contraction of space and time in immunity and gene regulation
19-Jan-16	Dr. Tri Glang Phan	Garvan Institute of Medical Research	Australia	Localisation and reactivation of memory T and B cells in subcapsular proliferative foci in the lymph node
28-Jan-16	Prof. Jean Pieters	Biozentrum, University of Basel	Switzerland	Naïve T cells, autoimmunity and resistance against intracellular pathogens; maintaining the balance
5-Feb-16	Dr. Masashi Maekawa	Ehime University Graduate School of Medicine	Japan	A novel cholesterol biosensor, D4H, reveals mutual requirements of phosphatidylserine (PS) and cholesterol in the plasma membrane
5-Feb-16	Prof. Mamoru Watanabe	Tokyo Medical and Dental University	Japan	Adult tissue stem cell therapy in inflammatory bowel disease
17-Feb-16	Dr. Shinya Ikematsu	Okinawa National College of Technology	Japan	Potential usage of biological resources in Okinawa for increasing average life expectancy
17-Feb-16	Dr. Elena Ezhkova	Icahn School of Medicine at Mount Sinai	USA	Epigenetic regulation of skin development and skin stem cells: Focus on the Polycomb complex
22-Feb-16	Dr. Antoine H.F.M. Peters	Friedrich Miescher Institute for Biomedical Research (FMI)	Switzerland	Histone variants and proteolysis during spermatogenesis
22-Feb-16	Dr. Kiyotsugu Yoshikawa	Graduate School of Medicine, Kyoto University	Japan	Targeting epithelial-mesenchymal transition from breast cancer to glioblastoma
4-Mar-16	Dr. Kazuya Iwamoto	Graduate School of Medical Sciences, Kumamoto University	Japan	Somatic mutations and epigenetic alterations in the postmortem brains of patients with psychiatric disorders
14-Mar-16	Dr. Mikael Sigvardsson	Linkoping University	Sweden	Shaping up a cellular lineage: Lessons from normal and malignant B-lymphocyte development
18-Mar-16	Dr. Tamiki Komatsuzaki	Research Institute for Electronic Science, Hokkaido University	Japan	Molecular data science perspectives for individuality covering from molecules to cells
18-Mar-16	Dr. John R. Stanley	University of Pennsylvania School of Medicine	USA	Proteomic analysis of pemphigus autoantibodies indicates divergent and polyclonal repertoires with changing expression over time
31-Mar-16	Dr. Hideyuki Yoshida	Harvard Medical School	USA	Transcriptional network of interferon and its disease associations -new insights from a global view-
1-Apr-16	Dr. Tomoharu Yasuda	Max Delbrück Center for Molecular Medicine	Germany	Mouse model of a fatal infectious disease and CRISPR/Cas9-mediated gene therapy
11-Apr-16	Dr. Koichi S Kobayashi	Texas A&M Health Science Center College of Medicine	USA	NLRC5/CITA: a critical regulator of MHC class I and cancer
13-Apr-16	Dr. Yumiko Imai	Akita University Graduate School of Medicine	Japan	Novel therapeutic targets in the severe respiratory RNA virus infection from the aspect of the virus-host nuclear interactions
14-Apr-16	Dr. Adrian Bracken	Trinity College Dublin	UK	The PRC2 complex in cellular proliferation and cancer
8-Jun-16	Dr. Jun R. Huh	University of Massachusetts Medical School	USA	Maternal IL17 pathway promotes autism-like phenotypes in offspring
9-Jun-16	Dr. Toru Suzuki	Okinawa Institute of Science and Technology Graduate University	Japan	Cell fate determination by mRNA decay in various biological processes
20-Jun-16	Dr. Takahisa Nakamura	Cincinnati Children's Hospital Medical Center	USA	Role of dsRNA pathways and miRNA-regulatory machinery in obesity
20-Jun-16	Dr. Tomoaki Hishida	Salk Institute for Biological Studies	USA	Cancer initiation and progression from $Sox2^+$ cells
9-Aug-16	Dr. Kazuki Okuyama	Linköping University	Sweden	Proximity interactome analysis of transcription factors involving in hematopoietic cell fate determination
23-Aug-16	Prof. Shin-ichiro IMAI	Washington University School of Medicine	USA	Achieving productive aging in Japan: the systemic regulatory mechanism of mammalian aging and longevity and anti-aging intervention
2-Sep-16	Prof. Kenji Kabashima	Graduate School of Medicine, Kyoto University	Japan	Mechanism of skin immune responses to external stimuli and homeostatic Ig extravasation: Proposal of inducible skin-associated lymphoid tissue (iSALT)
26-Oct-16	Dr. Akiyoshi Uezumi	Institute for Comprehensive Medical Science, Fujita Health University	Japan	Role of mesenchymal progenitor cells in the muscle stroma for the skeletal muscle homeostasis
28-Oct-16	Dr. Nozomu Yachie	Research Center for Advanced Science and Technology (RCAST), The University of Tokyo	Japan	Let's break the walls of measuring cellular and molecular dynamics using DNA barcodes
1-Nov-16	Prof. James Di Santo	Institute Pasteur	France	Transcriptional regulation of innate lymphoid cell development and plasticity
7-Nov-16	Dr. Shinichi Tomizawa	School of Medicine Medical Course Histology and Cell Biology, Yokohama City University	Japan	Epigenome dynamics during mammalian germ cell differentiation
7-Nov-16	Dr. Mayumi Oda	Keio University School of Medicine	Japan	Gene body epigenetic domains and gene-length play a role for cell type-specific gene expression
7-Nov-16	Dr. Hisato Kobayashi	NODAI Genome Research Center, Tokyo University of Agriculture	Japan	DNA methylome analysis in mouse and rat germ cells
7-Nov-16	Dr. Ferdinand von Meyenn	Babraham Institute	UK	Global epigenetic reprogramming: Mechanistic insights from human and mouse ESCs and PGCLCs
11-Nov-16	Dr. Ryo Yamada	Graduate School of Medicine and Faculty of Medicine, Kyoto University	Japan	Genetics for immune system
25-Nov-16	Dr. Katsuhiko Shirahige	Research Center for Epigenetic Disease, Institute of Molecular and Cellular Biosciences, The University of Tokyo	Japan	How Cohesin regulates transcription? –Human genetic diseases link cohesin to transcription machinery–
29-Nov-16	Dr. Emilia Dimitrova	University of Oxford	UK	Role of the CXXC protein Fbxl19 in regulation of gene expression
29-Nov-16	Dr. Julie Brind'Amour	University of British Columbia	Canada	Impact of maternally inherited histone modifications on DNA methylation maintenance and enhancer activation in the early embryo
1-Dec-16	Dr. Chyi-Song Hsieh	Washington University School of Medicine	USA	Interactions of the immune system with commensal bacteria
1-Dec-16	Dr. Markus R Wenk	National University of Singapore	Singapore	Natural variation of blood plasma lipids in healthy Asian individuals
1-Dec-16	Dr. Kenta Yashiro	Graduate School of Medicine, Osaka University	Japan	GFRA2, a GDNF receptor family member, identifies cardiac progenitors and mediates cardiomyocyte differentiation via an alternative signal pathway
15-Dec-16	Dr. Takeshi Egawa	Washington University School of Medicine	USA	Regulation of lymphocyte clonal expansion and tumor-suppression by a c-MYC initiated transcription factor cascade



Part 5

Data and Statistics

Publlications 2016

Table: IMS Publlications Jan-Dec, 2016

Journal	IF (2015)	Number of Papers 2016
Nat Rev Drug Discov	47.1	1
Nat Rev Immunol	39.4	1
Nature	38.1	4
JAMA	37.7	1
Science	34.7	2
Nat Genet	31.6	4
Nat Med	30.4	1
Cell	28.7	1
Lancet Oncol	26.5	1
Cell Stem Cell	22.4	2
Nat Immunol	19.4	6
Nat Cell Biol	18.7	1
Gastroenterology	18.2	1
Eur Heart J	15.1	1
Mol Cell	14.0	1
Mol Psychiatry	13.3	1
Trends Biochem Sci	12.8	1
J Clin Invest	12.6	1
J Allergy Clin Immunol	12.5	3
Ann Rheum Dis	12.4	2
Blood	11.8	2
Nat Commun	11.3	11
Genome Biol	11.3	1
J Exp Med	11.2	2
Biol Psychiatry	11.2	1
Am J Hum Genet	10.8	4
J Hepatol	10.6	1
Genes Dev	10.0	1
Immunol Rev	9.5	2
Proc Natl Acad Sci U S A	9.4	6
Nucleic Acids Res	9.2	2
Diabetes	8.8	1
Clin Cancer Res	8.7	2
Cancer Res	8.6	4
Elife	8.3	1
Cell Rep	7.9	2
Kidney Int	7.7	1
Clin Pharmacol Ther	7.3	4
PLoS Pathog	7.0	1
Haematologica	6.7	1
Cancer Immunol Res	6.7	1
PLoS Genet	6.7	4
Biotechnol Biofuels	6.4	1
J Neurol Neurosurg Psychiatry	6.4	1
J Infect Dis	6.3	1
Allergy	6.3	1
Diabetologia	6.2	1
Mucosal Immunol	6.1	1
Development	6.1	2
Hum Mol Genet	6.0	6
Arterioscler Thromb Vasc Biol	6.0	1
Others		183
TOTAL		**289**

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Budget, personnel and patents

IMS Budget FY2016

IMS Budget FY2016	JPY Million
Government funding for operations	2,656
Commissioned research	896
External competitive funding	805
Total	**4,357**

Patents

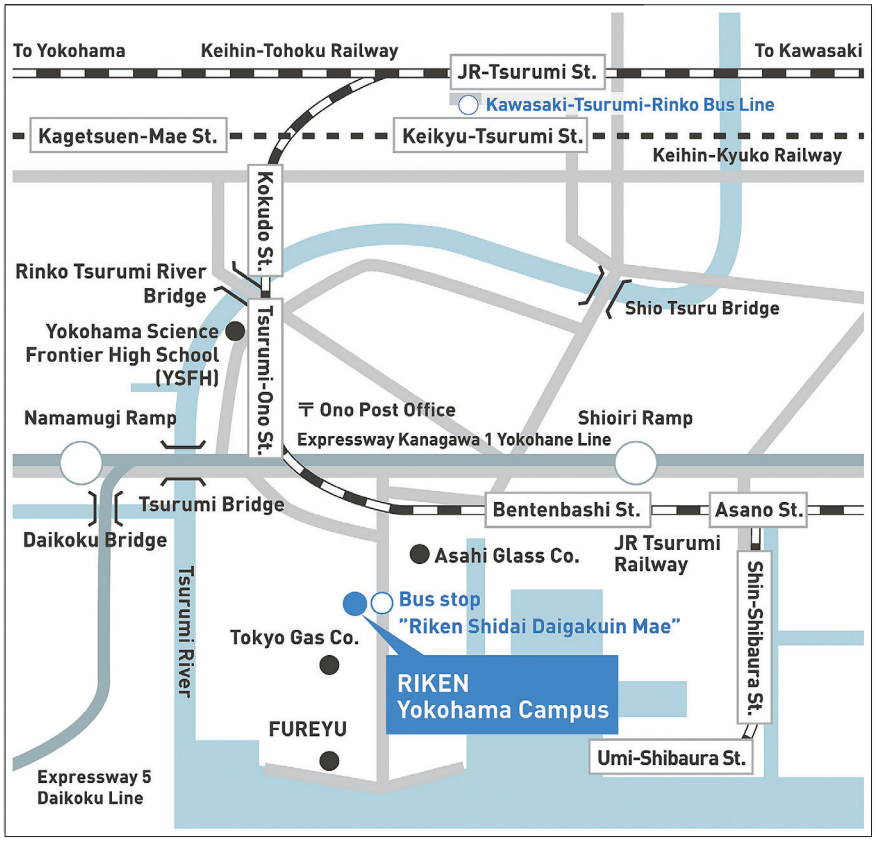
There were 28 patents filed from January to December in 2016.

Patents	Total	International patents (PCT)	Domestic patents (Japan)
2016	28	16	12

Personnel FY2016

Category	Number
Director	1
Senior Advisor	2
Deputy Director	2
Group Director	9
Team Leader	22
Coordinator	2
Partnership-Promotion Coordinator	1
Deputy Team Leader	3
Senior Scientist	22
Research Scientist	39
Postdoctoral Researcher	11
Special Postdoctoral Researcher	6
Foreign Postdoctoral Researcher	2
Research Fellow	6
Senior Technical Scientist	3
Technical Scientist	11
Technical Staff I	56
Technical Staff II	61
International Program Associate	3
Research Associate	9
Junior Research Associate	24
Student Trainee	68
Assistant	28
Part-time Staff	24
Research Consultant	3
Senior Visiting Scientist	9
Visiting Scientist	170
Visiting Technical Scientist	3
Visiting Researcher	2
Senior Research Scientist	1
Temporary Staffing	7
Total	**610**

Access to RIKEN Yokohama Campus



Local Access

By Bus
Take the #08 bus from Platform 8 at the East Exit of Tsurumi Station (also accessible from the West Exit of Keikyu Tsurumi Station) and get off at the RIKEN Shidai Daigakuin Mae bus stop. The institute is across the street. All buses from this platform are bound for Fureyu.

Buses depart Tsurumi every 5-15 minutes. It takes about 15 minutes to arrive at RIKEN Yokohama. The fare is 220 yen.

By Train
A 15-minute walk from JR Tsurumi-Ono Station (JR Tsurumi Line), which is directly accessible by transfer from JR Tsurumi Station.

Trains run about every 10 minutes during morning and evening rush hour, but less frequently at other times.

Searchable train timetables in English are available at http://www.hyperdia.com/en/

By Taxi
Use the taxi stand at the East Exit of JR Tsurumi Station or the West Exit of Keikyu Tsurumi Station. The trip takes about 10 minutes and costs around 1,200 yen.

From the Airport

From Haneda Airport

Route 1
Take the Keikyu Railways Airport Express* (blue kanji sign) for Yokohama and get off at Keikyu Tsurumi Station (27-29 minutes). Airport Express trains run every 10-15 minutes between 9:30 a.m. and 9:30 p.m. Next, follow the Local Access directions above to get to RIKEN Yokohama.

Route 2
Take any train marked with a green (express), red or dark grey kanji sign to Keikyu Kamata Station. Transfer to the Keikyu Main Line and take a local train* toward Yokohama until Keikyu Tsurumi Station* (12 minutes).

*Only Airport Express (blue kanji sign) and local trains (dark grey kanji sign) stop at Keikyu Tsurumi Station. Note that Keikyu Tsurumi Station and JR Tsurumi Station are two different railway stations and are separated by a bus rotary (the stations are about 150 meters apart).

From Narita Airport

From Narita Airport Station take the JR Sobu Line (Rapid Express), Airport Limousine Bus or JR Narita Express to JR Shinagawa Station. (JR Sobu Line is the most inexpensive option and takes about 1 hour and 15 minutes). From JR Shinagawa Station take the JR Keihin Tohoku Line (Yokohama direction) to JR Tsurumi Station (18 minutes). Next, follow the Local Access directions above to get to RIKEN Yokohama.

* A reserved seat express that requires payment of a surcharge in addition to train fare.

Searchable train timetables in English are available at http://www.hyperdia.com/en/

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RIKEN 2017-030