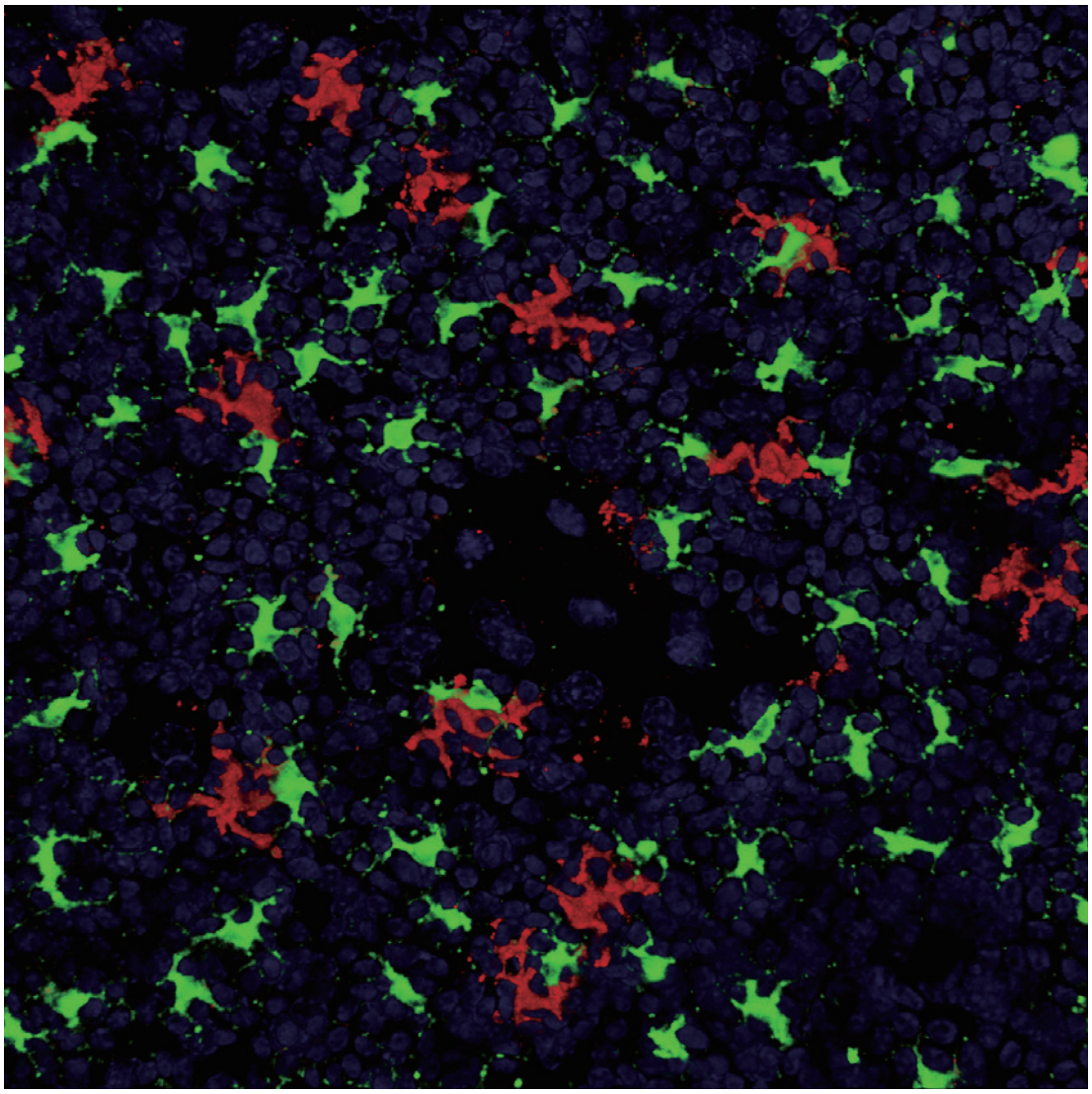
RIKEN IMS Annual Report 2021

RIKEN Center for Integrative Medical Sciences



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Director's Report

As we emerge from a second year of the COVID-19 pandemic, I am truly inspired by the resilient professionals at RIKEN IMS. In spite of the challenges imposed by SARS-CoV-2 and its life-threatening variants, our researchers continued to make significant scientific advances that will benefit not only Japan but many regions around the world.

With travel restrictions still preventing in-person international collaborations in 2021, IMS expanded its efforts to take crucial scientific exchanges online. The 5th IMS-Stanford ISCBRM Joint Symposium and the RIKEN-Luxembourg Scientific Symposium invited lively discussions from both our local and international colleagues. Continuing these interactions during this unpredictable time is paramount for both fostering bonds between IMS and our collaborators and inspiring scientific discovery.

Locally, IMS hosted the inaugural RIKEN IMS Joint Mouse and Human Cell Atlas meeting. During the single-day event, close to 100 scientists congregated online to share valuable information on innovations and ideas to advance single-cell research in mice and, ultimately, humans. RIKEN is proud to spearhead Asia's efforts toward the Human Cell Atlas project, which aims to develop a reference map of the diverse cell types in the human body. The RIKEN IMS Joint Mouse and Human Cell Atlas meeting will bolster the institute's leading role in this initiative.

IMS scientists continued to publish world-class research in 2021, including several in high-impact journals. Sidonia Fagarasan and colleagues' discovery of a novel role for the well-known brain chemical GABA in the immune system, and Hiroshi Ohno and colleagues' report on the influence of the major metabolite acetate on specific intestinal bacteria, both of which were published in Nature, are just some examples. These studies are excellent reminders of the complexity of our body systems and the need to think outside the box to uncover the intricate underpinnings of our physiology.

To further the institute's capabilities, IMS received several substantial grants in 2021. These include funding from the Japan Agency for Medical Research and Development to conduct genome-wide sequencing of Japanese patients with dementia and to develop equipment for immunophenotype monitoring. IMS also received external funds to enhance the institute's capacity to perform proteomic studies, which will be an important complement to our already established strength in genomics and transcriptomics. This, together with novel lipidomic technologies being developed by Makoto Arita's team, will expand IMS's breadth in multi-omics research.

The emergence of COVID-19 in 2020 prompted several teams at IMS to contribute to the urgent need to understand and tackle this new threat. The institute's focus on immunology, genomics, and human genetics places many of its laboratories in prime position to do so. In 2021, some of these studies began to bear fruit, as exemplified by Shinichiro Fujii and colleagues' publication on memory immune killer T cells and their recognition of HLA-A24—the human leukocyte antigen commonly found in Japanese. A wide range of other projects are still underway, including work from Hidehiro Fukuyama's team on vitamin D3 adjuvants with potential applications in vaccines and several collaborative projects with the RIKEN Program for Drug Discovery and Medical Technology Platforms.

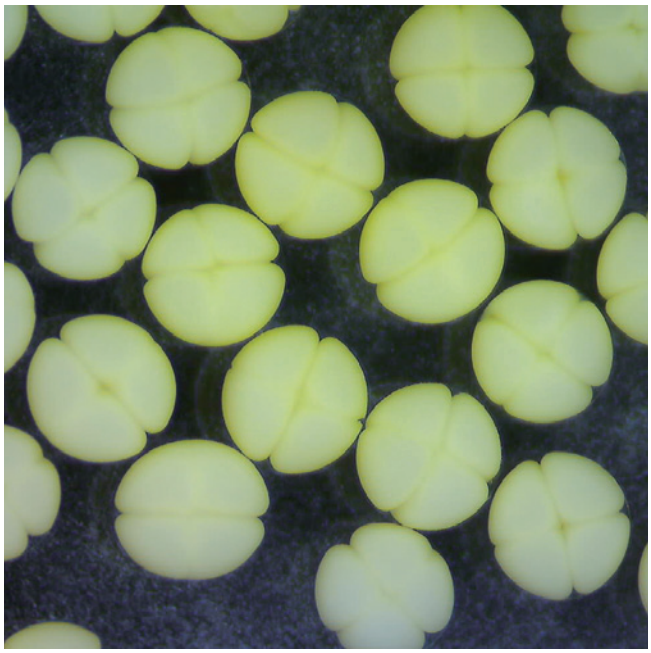
The pandemic has also shone a light on numerous under-the-radar issues. Among these is the importance of human-derived samples in immunological research. My hope is that the innovative technologies and ideas emerging from IMS laboratories will promote the integration of data- and hypothesis-driven science to propel a movement towards human-centred life science initiatives. Of course, human-based projects come with great responsibility to protect personal information. RIKEN will face the challenge of building a secure cloud-computing system to store the monumental amounts of data to come; I hope IMS will play a major role in this endeavour.

Although it is impossible to predict what 2022 will hold, my hope is that we will soon be able to safely recommence scientific meetings both locally and internationally. Such mixing of scientific minds is vital for promoting mutual understanding and collaboration to foster scientific discovery and fortify the excellent research at IMS.

Kazuhiko Yamamoto
Director
RIKEN Center for Integrative Medical Sciences

Part 1

Research Highlights



A protein to help T cells remember

Shin-ichiro Fujii

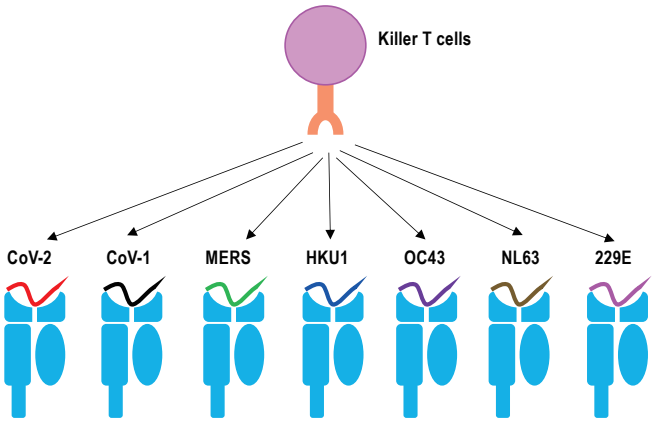


Figure: Killer T cells that specifically recognize a peptide on SARS-CoV-2 can cross-react with other coronaviruses
A peptide on SARS-CoV-2 called QYI activates killer T cells in blood samples taken from HLA-A24-positive unexposed healthy donors. As QYI shares sequence similarities with several other coronaviruses, QYI-specific T cells can cross-react with these family members (SARS-CoV-1, MERS, HKU1, OC43, NL63, and 229E). This suggests that T cells may "remember" sequences that are common between SARS-CoV-2 and other human coronaviruses, even in people who have not been exposed to the new virus.

T cells exposed to seasonal coronaviruses can recognise SARS-CoV-2 in uninfected individuals harbouring the immune factor HLA-A24—including a large proportion of Japanese.

Two years into the COVID-19 pandemic, the disease has now spread to almost all corners of the globe. Its impact, however, has been disproportionate. Some parts of Asia, including Japan, for example, have experienced lower rates of death and serious illness compared to several European nations and minority racial groups in the US. Understanding the cause of this disparity could provide ways to boost the immunity of vulnerable populations.

One biological factor linked to disease susceptibility is the human leukocyte antigen (HLA) subtype HLA-A24, a cell-surface protein that activates T cells with "killer" activity in the event of infection. Intriguingly, HLA-A24 is found in about 60% of Japanese compared to just 10% to 20% of Europeans and Americans.

Shin-ichiro Fujii, Team Leader of the Laboratory of Immunotherapy at IMS, surmised that studying the immune responses of HLA-A24-positive individuals may provide clues about how Asian populations like Japanese have largely avoided severe COVID-19. "Specifically, we wanted to know if pre-existing T cells in these people can cross-react with SARS-CoV-2 because this could lead to a more rapid immune response," Fujii said.

In their study published in Communications Biology, Fujii and his colleagues answered this question by first performing an *in silico* analysis to predict sequences located on the SARS-CoV-2 spike protein that can bind strongly to HLA-A24. The team identified six epitopes to put to the test.

When exposed to immune cells from healthy HLA-A24-positive individuals who had never been infected with SARS-CoV-2, only one of the six epitopes, called QYI, activated killer T cells. A more fine-grained comparison of the epitopes showed that QYI was also the only one to share sequence similarities with several seasonal coronaviruses.

The researchers' findings suggest that T cells may "remember" sequences that are common between SARS-CoV-2 and its family members, even in people who have not been exposed to the new virus. In fact, the team went on to find a whole library of epitopes in the region surrounding QYI that both share sequences with seasonal coronaviruses and stimulate strong T cell activity.

When the group performed the same tests on immune cells from HLA-A24-positive patients with haematological malignancies, who have a higher risk of COVID-19 infection and death, they found much lower levels of cross-reactivity. All hope is not lost, however, because while only 14.8% of patients showed T cell activity against QYI, more than 65% mounted a response to the library of epitopes the team identified on the spike protein.

"A mixture of the immunodominant epitopes we identified could be used to immunise immunocompromised patients," Fujii said, offering hope of better protecting susceptible populations against COVID-19. Currently, however, these findings are only applicable to individuals with HLA-A24. The next step, Fujii says, will be to look at other HLA subtypes.

Original paper:
Shimizu K, Iyoda T, Sanpei A, Nakazato H, Okada M, Ueda S, Kato-Murayama M, Murayama K, Shirouzu M, Harada N, Hidaka M and Fujii S. Identification of TCR repertoires in functionally competent cytotoxic T cells cross-reactive to SARS-CoV-2. ***Commune Biol*** 4, 1365 (2021)

Brain chemical plays immune modulator

Sidonia Fagarasan

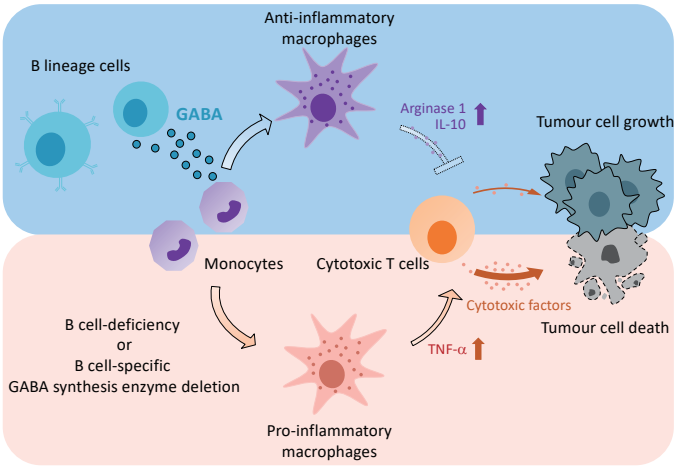


Figure: B cells produce GABA, whose "calming" effect accelerates tumour growth
(Top) GABA generated by B cells promotes the development of macrophages that secrete the anti-inflammatory cytokine IL-10. IL-10 in turn inhibits cytotoxic "killer" T cells, whose role is normally to destroy tumour cells, leading to rapid tumour growth.
(Bottom) In the absence of GABA, macrophages that produce pro-inflammatory molecules such as TNF-alpha dominate. TNF-alpha in turn activates the destruction of tumour cells via T cell activity, leading to tumour death.

New evidence that B cells produce the well-known neurotransmitter GABA suggests there may be another mode of communication in the immune system.

Ever since life began, singled-celled organisms like bacteria have used cues in their environment to grow, communicate and survive. These signals often take the form of metabolites, small water-soluble molecules produced as cells break down larger compounds. While we know that some multicellular processes also sense and exchange information through metabolites, the details and extent of this mechanism in mammals are just beginning to be unearthed.

Recent work from Sidonia Fagarasan, Team Leader of the Laboratory for Mucosal Immunity at IMS, and her group suggests that metabolites may play a role in the mammalian immune system. "We discovered that activation of the immune system changes the metabolome profoundly," she said, implying that immune cells are consuming and utilising metabolites.

Fagarasan hypothesized that, like the classic system of cytokines and receptors, metabolites may act as a mode of communication. In a new study published in Nature, her team took a multidisciplinary approach, combining immunology with biochemistry and metabolomics, to identify these molecules and their roles.

The researchers first inoculated mice to activate the immune system and compared the profiles of over 200 metabolites in activated and non-activated lymph nodes from the same animals. In complementary studies, they also compared compounds produced in mice lacking T cells, B cells, or both. To their surprise, they discovered that B cells produced GABA—a well-known inhibitory brain chemical.

To probe the function of this metabolite in the immune system, the team took the first ever look at its effect on tumours. When they implanted a GABA-releasing pellet into a mouse cancer model lacking B cells, the group found that tumour growth accelerated, suggesting that, like in the brain, GABA may play a suppressive role in the immune system.

Indeed, the team went on to show that GABA promoted the development of macrophages that produce the typical anti-inflammatory cytokine IL-10, but inhibited expression of the pro-inflammatory molecule TNF-alpha. GABA also inhibited "killer" T cells, which normally destroy tumours.

"I think the GABA produced by B cells is educating macrophages to become anti-inflammatory," Faragasan said. She added that while this is beneficial for the normal repair of tissues, it could be harmful when a more active immune response is required, such as in the presence of a tumour.

According to Fagarasan, we may have inherited this metabolite communication method from our simpler ancestors, noting that bacteria and plants produce GABA as a signalling molecule. However, to truly appreciate how and why GABA is being used in the mammalian immune system, the next step is to uncover the *modus operandi*.

"If we understand how the cells are working and communicating, then we can turn on and off that dialogue," Fagarasan said, a feat that could open the door to novel treatments.

Original paper:
Zhang B, Vogelzang A, Miyajima M, Sugiura Y, Wu Y, Chamoto K, Nakano R, Hatae R, Menzies RJ, Sonomura K, Hojo N, Ogawa T, Kobayashi W, Tsutsui Y, Yamamoto S, Maruya M, Narushima S, Suzuki K, Sugiya H, Murakami K, Hashimoto M, Ueno H, Kobayashi T, Ito K, Hirano T, Shiroguchi K, Matsuda F, Suematsu M, Honjo T, Fagarasan S. B cell-derived GABA elicits IL-10^{+} macrophages to limit anti-tumour immunity. ***Nature*** 599, 471-476 (2021) doi: 10.1038/s41586-021-04082-1

A conductor of gut bacteria revealed

Hiroshi Ohno

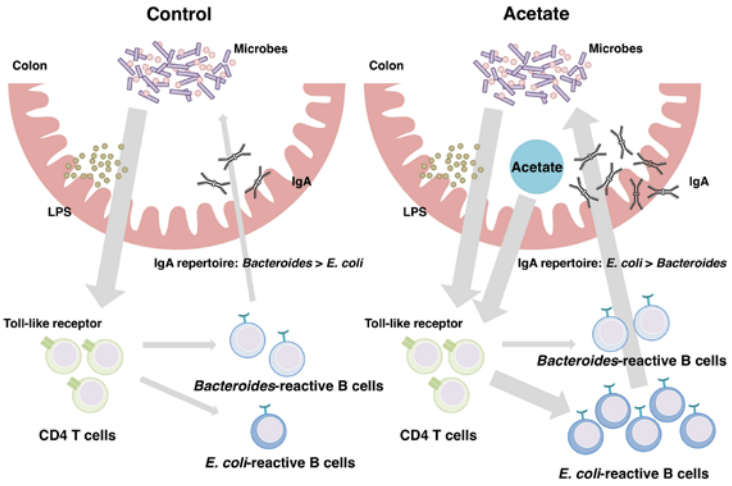


Figure: Acetate orchestrates interactions between IgA and bacteria in the gut
(Left) In steady state, IgA nonpreferentially interacts with a beneficial strain such as *Bacteroides thetaiotaomicron* and a potentially harmful one such as *Escherichia coli*. (Right) The presence of high acetate both increases IgA levels and redirects the immunoglobulin's preference to *E. coli*.

Research at IMS finds that the metabolite acetate orchestrates interactions between IgA and selective intestinal micro-organisms—a finding that has important implications for health.

Anyone who has had a health check knows that their blood can say a lot about their well-being. Perhaps surprisingly, so too can the unique community of microbes inhabiting each individual. An imbalance in gut bacteria, for example, has been linked to conditions as wide ranging as obesity and Alzheimer's disease. Understanding how microbial populations are regulated could provide crucial insight into ways to both maintain health and treat disease.

Bacteria in the gut play a plethora of roles, from breaking down complex molecules derived from the food we eat to programming the immune system. Researchers like Hiroshi Ohno, Team Leader of the Laboratory of Intestinal Ecosystem at IMS, have theorised that these seemingly disparate actions may actually converge to maintain bacterial balance.

One way that this could transpire is through metabolites called short-chain fatty acids (SCFAs), which regulate various actions of the immune system, including the production of IgA. As well as being the most abundant immunoglobulin in the body, IgA is known to regulate the composition and function of gut bacteria.

"We hypothesized that SCFAs affect IgA in the intestines, which could have important downstream implications on resident bacteria," Ohno said.

In their study published in Nature, Ohno and colleagues tested this theory by exploring the effects of three major SCFAs in the intestine: propionate, butyrate and acetate. After feeding the rodents special diets that selectively increased each SCFA in the large intestine, they found that only one—acetate—had an effect on IgA.

In addition to increasing IgA levels, acetate also altered the binding capacity of the immunoglobulin to bacterial cells. The latter action proved surprisingly specific. Experiments in mice devoid of all micro-organisms except for either a beneficial strain called *Bacteroides thetaiotaomicron* or a potentially harmful one called *Escherichia coli* revealed that acetate directs IgA binding only to the latter species.

The purpose of acetate's influence on interactions between IgA and selective microbes could be to control the latter's entry into the body. By imaging fluorescently-labelled *E. coli*, the researchers indeed found that binding of IgA prevents the harmful bacteria from colonising and infiltrating the cells lining the intestinal tract.

Given that metabolites like acetate can be derived directly from the food we eat, especially indigestible dietary fibres, these findings suggest that our diet is a simple yet effective portal through which to rebalance our intestinal microbiota. This could be especially important for the elderly, patients with irritable bowel syndrome and undernourished children, who have altered populations of IgA-bound bacteria.

However, given the sparse research on metabolites to date, Ohno plans to focus on further understanding the interactions between these small molecules and the body's micro-organisms and their consequences on health.

Original paper:
Takeuchi T, Miyauchi E, Kanaya T, Kato T, Nakanishi Y, Watanabe T, Kitami T, Taida T, Sasaki T, Negishi H, Shimamoto S, Matsuyama A, Kimura I, Williams IR, Ohara O, Ohno H. Acetate differentially regulates IgA reactivity to commensal bacteria. ***Nature*** 595, 560-564 (2021)

Expanding the genetic atlas of disease

Yukinori Okada

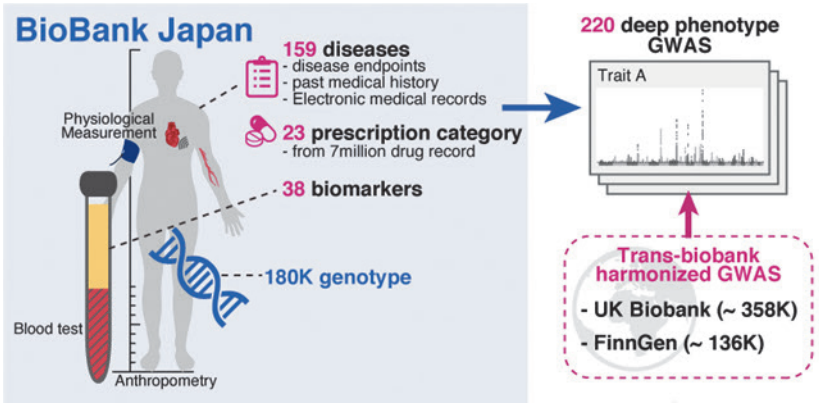


Figure: Trans-biobank genome-wide association study (GWAS) meta-analysis of over 200 human phenotypes

More than 200 phenotypes were extracted for analysis based on patient medical history, electronic medical records and data on biomarkers and drug use available through BioBank Japan. Subsequent cross-population meta-analysis of Japanese data with those from Europeans in UK Biobank and FinnGen repositories revealed many shared genetic variants.

The GWAS summary statistics are publicly available at https://pheweb.jp/

Large-scale genome-wide association studies that incorporate deep phenotype and cross-population analyses could enhance disease classification, IMS researchers show.

Completion of the Human Genome Project in 2003 paved the way for numerous genome-wide association studies (GWAS), which aim to find links between genetic variants and visible traits. Many are probing the genetic basis of disease, which could improve a myriad of clinical practices, including how medical conditions are characterised.

While historically based on symptoms and affected organs, disease categories are regularly being updated with insights derived from emerging diagnostic technologies such as imaging and biomarker assays. GWAS could help further refine these classifications and offer novel criteria by which to group new diseases or difficult-to-categorise syndromes.

However, despite their wide adoption, GWAS lack comprehensiveness. Most studies have been conducted in Europeans, for example, leaving large gaps in our knowledge of Asian genomes. To expand the atlas of genetic associations, a study highlighted on the cover of the October 2021 issue of Nature Genetics and led by Yukinori Okada, Team Leader of the Laboratory for Systems Genetics at IMS, introduced two key features to the analysis.

"The first is deep phenotyping, which means exploring many phenotypes. The second is conducting trans-biobank GWAS," Okada explained.

First, the researchers trawled through data from BioBank Japan, a repository of biological and clinical data on patients with 47 target diseases, to extract over 200 features, including information on biomarkers and drug use, for analysis. These included 108 phenotypes on which GWAS has never before been conducted in East Asian populations.

Next, the team performed cross-population meta-analyses to compare corresponding traits between Japanese and Europeans based on data from UK Biobank and FinnGen repositories. They showed that the two populations share many common genetic variants, which, while expected, was only able to be confirmed thanks to the large-scale nature of their cross-biobank study, Okada noted.

In addition to extracting disease-related variants, the analysis can also be useful for redefining or reclassifying disease. To show this, the researchers used a mathematical model to extract latent components characteristic of a two-dimensional matrix of phenotypes and their associated variants.

"We interpreted the biological meaning of these components by projecting many bioresources," Okada explained, such as biomarker and metabolome GWAS data.

When a set of allergic diseases were categorised based on these components, the groupings reflected existing classifications, suggesting that genetics-driven hypotheses can complement current disease categorisation strategies.

This ambitious study, according to Okada, was made possible by the efforts of co-first authors Saori Sakaue, whose ideas drove the project, and Masahiro Kanai, whose computing prowess has enable the team to share their findings in an open source web resource called BioBank Japan PheWeb (https://pheweb.jp/).

In the future, the team hope to integrate GWAS with data from other omics and single-cell analyses to continue to expand the landscape of associations between genetics and disease.

Original paper:

Sakaue S, Kanai M, Tanigawa Y, Karjalainen J, Kurki M, Koshiba S, Narita A, Konuma T, Yamamoto K, Akiyama M, Ishigaki K, Suzuki A, Suzuki K, Obara W, Yamaji K, Takahashi K, Asai S, Takahashi Y, Suzuki T, Shinozaki N, Yamaguchi H, Minami S, Murayama S, Yoshimori K, Nagayama S, Obata D, Higashiyama M, Masumoto A, Koretsune Y; FinnGen, Ito K, Terao C, Yamauchi T, Komuro I, Kadowaki T, Tamiya G, Yamamoto M, Nakamura Y, Kubo M, Murakami Y, Yamamoto K, Kamatani Y, Palotie A, Rivas MA, Daly MJ, Matsuda K, Okada Y. A cross-population atlas of genetic associations for 220 human phenotypes. ***Nat Genet*** 53, 1415-1424 (2021)

How acquired DNA marks are inherited

Azusa Inoue

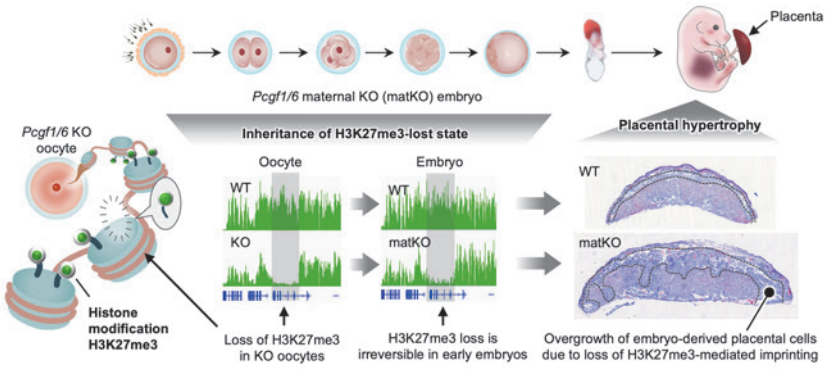


Figure: Inheritance of H3K27me3 marks in egg cells has important implications for embryo and placenta development
A region-specific loss of H3K27me3 in egg cells, induced by knocking out (KO) two proteins (Polycomb group ring finger 1 and 6 (Pcgf1/6)), is irreversibly inherited by embryos and causes loss of H3K27me3-mediated imprinting, partial prenatal lethality, and placental enlargement.

Understanding the mechanisms that drive the inheritance of acquired DNA-modifying marks from female mouse eggs is critical for ensuring healthy embryo and placenta development.

When French zoologist Jean-Baptiste Lamarck first proposed that acquired traits could be inherited in 1809, his idea was met with scepticism. This doubt endured and the theory was largely brushed aside for nearly two centuries. Today, insights from epigenetics are reviving the concept of acquired inheritance, showing that our behaviours and environment can prompt physical modifications to our DNA—some of which can in fact be passed on.

Changes to the genetic code primarily involve the addition of chemical groups either directly to the DNA or to proteins called histones around which the sequence wraps. These "marks" affect how nearby genes are expressed without altering the underlying code.

For example, a histone modification called H3K27me3 plays a key role in imprinting, a phenomenon in which expression of a gene differs depending on its parent of origin. By acting to suppress copies of genes obtained from the mother, H3K27me3-mediated imprinting leads to expression of only the paternally-derived allele.

"We previously showed that acquired H3K27me3 marks can be passed on in mammals," said Azusa Inoue, Young Chief Investigator of the Laboratory for Metabolic Epigenetics at IMS. In their new study published in Nature Genetics, Inoue and colleagues investigated how this happens and what role it plays in embryo development.

Based on previous evidence that H3K27me3 interacts with a modification called H2AK119ub1, the researchers hypothesized that the latter may be involved in the formation of the former. To test this theory, they used a technique called low-input CUT&RUN to find the locations at which the marks bind.

Looking first in egg cells, the team noted that the two modifications overlapped with one another. They saw similar patterns in fertilised eggs and at several stages across embryo development, with any addition or loss of H2AK119ub1 preceding that of H3K27me3.

Suspecting that H2AK119ub1 may act as an instruction telling H3K27me3 where and when to form, the researchers next deleted two proteins needed to make H2AK119ub1. Indeed, egg cells missing H2AK119ub1 also lacked H3K27me3 at select genes. These genes were consequently more highly expressed than in control situations, an indication that normal H3K27me3-mediated imprinting of maternally-derived genes had been impacted.

Surprisingly, the team found that the loss of H3K27me3 was irreversibly inherited by the next generation, an effect that had several adverse repercussions. In addition to loss of H3K27me3-related imprinting, the missing mark increased the risk of prenatal death and placenta enlargement.

"These findings have important clinical implications because they suggest that changes to histone modifications in egg cells that arise before pregnancy cannot be undone once the eggs are fertilized," Inoue explained.

In follow-up studies, Inoue wants to find out how defects in histone-based inheritance compromise late development and what impact environmental changes and disease have on inheritable histone modifications.

Original paper:
Mei H, Kozuka C, Hayashi R, Kumon M, Koseki H, Inoue A. H2AK119ub1 guides maternal inheritance and zygotic deposition of H3K27me3 in mouse embryos. ***Nat Genet*** 53, 539-550 (2021)

Two steps to broader immunity

Masato Kubo

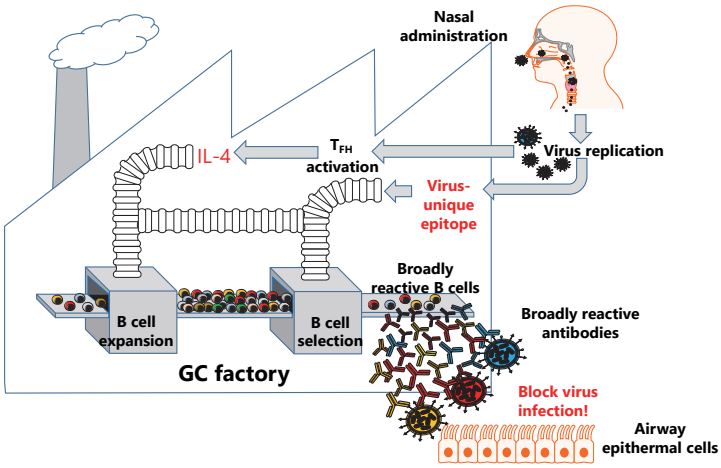


Figure: IL-4 acts as an essential fuel to expand minor B cell populations in the germinal center (GC) factory
Virus infection induces the production of broadly reactive antibodies through two steps: (1) The virus enters the respiratory system and promotes viral replication deep in the lungs. This process induces structural changes in the virus hemagglutinin (HA) protein, causing it to expose rare antigenic epitopes; (2) In the germinal center (GC), follicular helper T cell (T_{FH})-derived IL-4 plays a crucial role in the expansion of minor B cell populations that recognize common HA epitopes.

IMS researchers have discovered a two-step process that is essential for generating a broad antibody response against influenza viruses.

The rapid rollout of COVID-19 vaccines has been a saving grace in the ongoing pandemic. However, frequent coverage of the efficacy of the wide variety of available shots has offered a reminder that not all vaccines are created equal.

This reality also holds true for influenza. Although influenza shots come in two main types: live attenuated and inactivated, the latter is preferred in many countries including Japan because it causes fewer side effects. However, inactivated vaccines only stimulate the immune system to produce protective antibodies against the strains used for inoculation. Live attenuated vaccines, on the other hand, like natural infection, lead to a broader immune response against multiple related strains.

Exactly how this broad immunity arises, however, is unclear. "Infection gives rise to a totally different type of antibody response compared to inactivated vaccines," said Masato Kubo, Team Leader of the Laboratory of Cytokine Regulation at IMS. "We wanted to know why."

To unravel this mystery, Kubo and his colleagues studied the phenomenon in mice. In their study published in Nature Communications, the team first checked that the animals in fact exhibit the narrow versus broad antibody response by either inoculating them with an inactivated vaccine or infecting them through the nose with one strain of the influenza virus, before exposing them to the same or a different virus two weeks later. As expected, vaccination only protected against exposure to the same virus, whereas natural infection led to the additional production of antibodies against another strain of the same subtype.

Through a series of assays and cell and tissue analyses, the team discovered that two processes were essential for generating this broad antibody response. The first starts with viral replication in the lungs. This causes structural changes in a mushroom-shaped protein called hemagglutinin (HA) located on the surface of influenza, causing it to expose normally-hidden antigenic epitopes that are shared across different virus strains.

Intriguingly, the team found that the mice already harboured existing B cells that recognise these newly exposed epitopes. However, these cells are normally rare and need to be amplified to have an effect.

In the second step, a subset of immune cells known as follicular helper T cells located in specialised lymph node structures called germinal centers multiply and secrete the cytokine IL-4. "IL-4 is key to expanding these rare B cell populations," Kubo said.

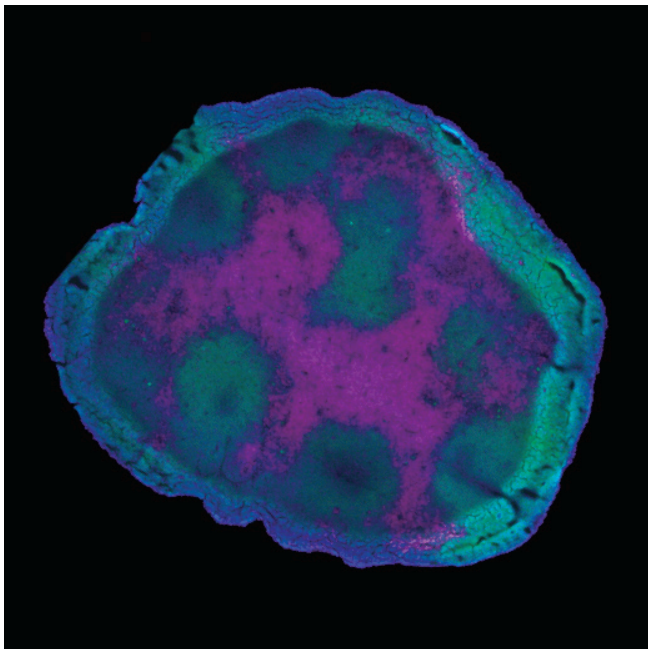
These findings could lead to new strategies for inoculation that not only offer broader protection but also cause fewer side effects. However, Kubo emphasizes that more details of the mechanism need to be resolved first.

In addition to ironing out these specifics, the team is now applying a similar strategy to study coronaviruses, with the hope of finding an effective way to protect against upcoming variants.

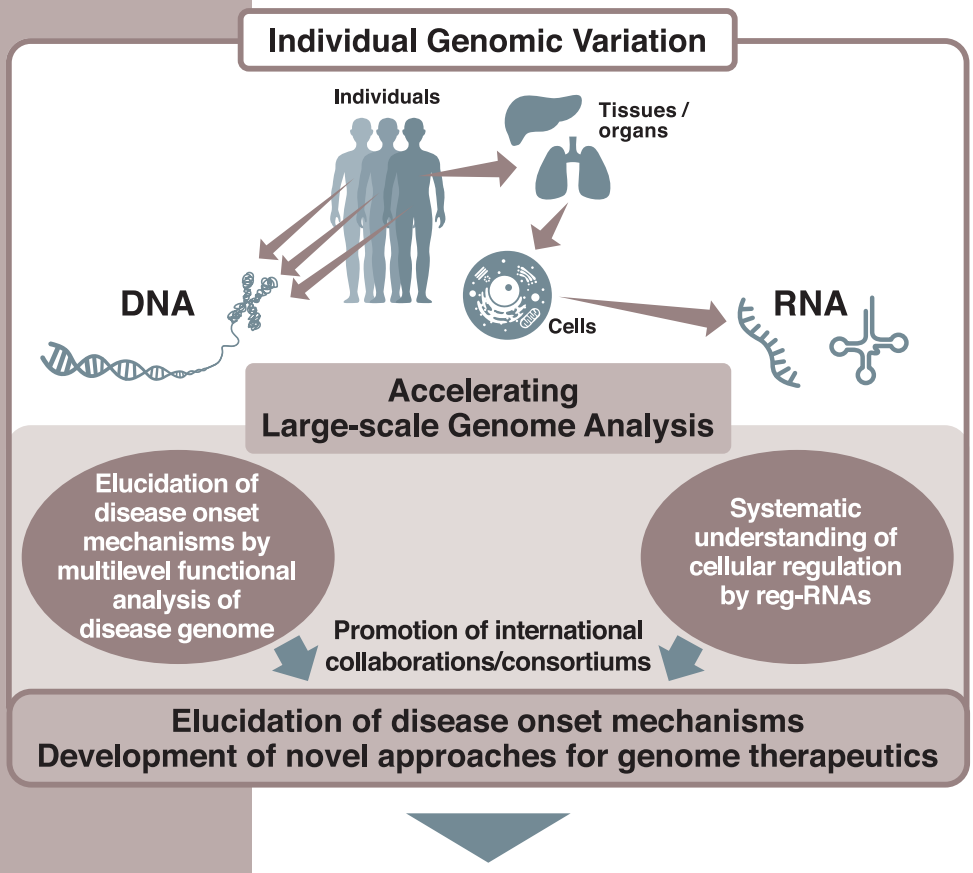
Original paper:
Miyauchi K, Adachi Y, Tonouchi K, Yajima T, Harada Y, Fukuyama H, Deno S, Iwakura Y, Yoshimura A, Hasegawa H, Yugi K, Fujii S, Ohara O, Takahashi Y, and Kubo M. Influenza virus infection expands the breadth of antibody responses through IL-4 signal in B cells. ***Nat Commun*** 12, 3789 (2021)

Part 2

Lab Activities



Division of Genomic Medicine



Division of Genomic Medicine will develop new methods for genome-based drug discovery and produce supporting evidence for the realization of genomic medicine.

Laboratory for Transcriptome Technology

Team Leader: Piero Carninci

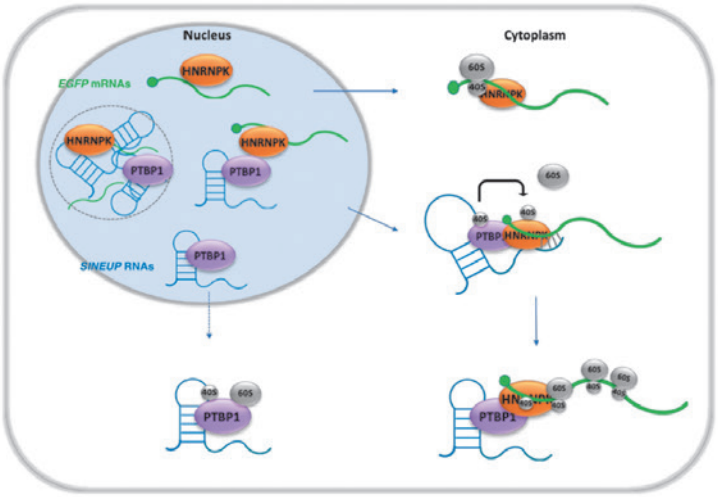


Figure: Model of SINEUP RNA and SINEUP RNA binding protein (RBP) interactions
SINEUP RBPs (PTBP1 and HNRNPK) participate in SINEUP RNA localization both in the nucleus and the cytoplasm. In the nucleus, some RNAs form RNA–protein granules (dotted circle) where immature transcripts with non-proper conformations likely accumulate to cluster, and mature transcripts including EGFP mRNA and SINEUP RNA form complexes with SINEUP RPBs. These complexes may then be shuttled into the cytoplasm. In the cytoplasm, SINEUP RNAs co-operate with the SINEUP RBPs, likely remodeling SINEUP RNA structure, and recruit ribosomal subunits to efficiently supply them to EGFP mRNA, resulting in the enhancement of EGFP mRNA translation. EGFP mRNA can be exported into the cytoplasm by itself, but its translation is initiated more efficiently when SINEUP-GFP mRNA is present.

Recent Major Publications

Toki N, Takahashi H, Sharma H, Valentine MNZ, Rahman FM, Zucchelli S, Gustincich S, Carninci P. SINEUP long non-coding RNA acts via PTBP1 and HNRNPK to promote translational initiation assemblies. ***Nucleic Acids Res*** 48, 11626-11644 (2020)

Toki N, Takahashi H, Zucchelli S, Gustincich S, Carninci P. Synthetic *in vitro* transcribed lncRNAs (SINEUPs) with chemical modifications enhance target mRNA translation. ***FEBS Lett.*** 594, 4357-4369 (2020)

Ohyama T, Takahashi H, Sharma H, Yamazaki T, Gustincich S, Ishii Y, Carninci P. An NMR-based approach reveals the core structure of the functional domain of SINEUP lncRNAs. ***Nucleic Acids Res.*** 48, 9346-9360 (2020)

Invited presentations

Carninci P. "Identification of non-coding genome elements at single-cell resolution for fine modulation of gene activity" Computational biology and artificial intelligence for personalized medicine (Russia/Online) September 2021

Carninci P. "Why map every cell in the human body?" PNEUMOTRIESTE 2021 (Trieste, Italy/Online) September 2021

Carninci P. "Growing role of regulatory RNAs in genomic medicine" SCIENCE & LAW International Law Answers to Scientists' Current Challenges (Trieste, Italy/Online) September 2021

Carninci P. "Long non-coding RNAs: from interactome to function" Noncoding RNA World: From Mechanism to Therapy (Switzerland/Online) July 2021

Carninci P. "Cellular networks and their regulation using antisense lncRNAs" School of Biotechnology & Biomolecular Sciences at the University of New South Wales Online Seminar (Sydney, Australia/Online) April 2021

Our laboratory focuses on elucidating the functions of long non-coding RNAs (lncRNAs). We previously discovered a type of antisense lncRNA called SINEUPs that work specifically to enhance the production of proteins by binding to their target mRNAs. SINEUPs can be designed to amplify a specific protein so that they are possible tools for developing therapies for diseases where a particular protein is insufficiently synthesized.

In order to understand the molecular mechanisms of SINEUP activity, we investigated the interactions of SINEUPs with other molecules. Using RNA FISH, we found that SINEUPs localize to both the nucleus and cytoplasm, while mRNAs mainly localize to the cytoplasm, and that SINEUPs and mRNAs co-localize in the cytoplasm. Then we performed ChIRP-MS analysis of proteins that bind to SINEUPs and mRNAs and the result showed that RNA binding proteins (RBPs), HNRNPK and PTBP1, form a complex at the co-localized sites. Overexpression of these RBPs enhanced the target protein production only when SINEUPs were present, suggesting that HNRNPK and PTBP1 form a complex with SINEUPs and functionally enhance the target protein production. From these results and loss- and gain-of-function studies of the RBPs, we found that the complex formation of SINEUPs and the RBPs enables the translocation of the SINEUPs from the cell nucleus to the cytoplasm, facilitates the initiation of the mRNA translation, and promotes the synthesis of the target proteins.

The molecular structure of RNA is also important for elucidating its mechanism of function. Using NMR in collaboration with Dr. Yoshitaka Ishii's group at RIKEN SPring-8 Center, we furthermore identified the secondary structure and the active site of a 167-nt inverted SINE B2, which is present in the effector domain of SINEUPs.

These findings will promote a better understanding of SINEUPs and contribute to therapeutic advances.

Laboratory for

Cellular Function Conversion Technology

Team Leader: **Harukazu Suzuki**

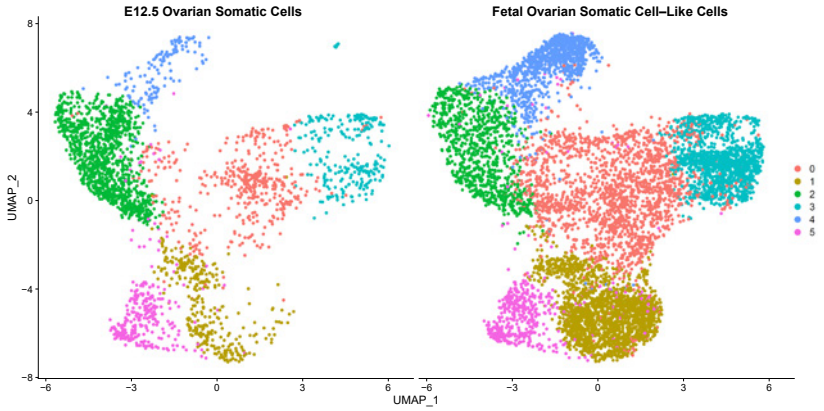


Figure:
UMAP projection showed similar pattern between ovarian somatic-like cells generated from pluripotent stem cells (right) and E12.5 ovarian somatic cells (left). Each color represents cell clusters.

Recent Major Publications

Yoshino T, Suzuki T, Nagamatsu G, Yabukami H, Ikegaya M, Kishima M, Kita H, Imamura T, Nakashima K, Nishinakamura R, Tachibana M, Inoue M, Shima Y, Morohashi K, Hayashi K. Generation of ovarian follicles from mouse pluripotent stem cells. ***Science*** 373, eabe0237 (2021)

Suzuki T, Abe T, Ikegaya M, Suzuki K, Yabukami H, Sato T, Komeya M, Ogawa T. Global inflammatory response in *in vitro* organ cultured testes using single-cell RNA-sequencing. ***bioRxiv*** https://doi.org/10.1101/2021.12.01.470873 (2021)

Ozturk M, Chia JE, Hazra R, Saqib M, Maine RA, Guler R, Suzuki H, Mishra BB, Brombacher F, Parihar SP. Evaluation of Berberine as an Adjunct to TB Treatment. ***Front Immunol*** 12, 656419 (2021)

DNA methylation is an important epigenetic modification to regulate mammalian gene expression. In order to explore transcription factors (TFs) associated with DNA methylation changes, we validated a subset of the bioinformatically selected TFs using an *in vitro* assay and found that 28 of 49 TFs from various TF families had DNA-demethylation-promoting activity. In sex determination of gonads at E10.5-12.5, Nr5a1 and Gata4 were suggested to regulate DNA demethylation. Furthermore, we confirmed that the RUNX1 TF is essential for differentiation from iPS cells to pre-HSPC II cells ($CD44^{High}$ $cKit^{Neg}$). Moreover, this differentiation was significantly reduced by a RUNX1 mutant lacking DNA-demethylation-promoting activity, suggesting the importance of RUNX1-mediated DNA demethylation in hematopoietic differentiation.

We also analyzed disorders associated with aberrant DNA methylation. Ten-Eleven Translocation-2 (Tet2) is one of the most commonly mutated genes in myeloproliferative neoplasms (MPN). Using single-cell RNA sequencing analysis of progenitors isolated from hematopoietic cell-specific Tet2-deficient mice, we identified a novel group of progenitors that may contribute to MPN pathogenesis. In familial platelet disorder (FPD), we found abnormalities of DNA methylation related to platelet functions in hematopoietic progenitor cells with FPD mutations, suggesting that DNA methylation abnormalities are involved in FPD pathology. Furthermore, we have begun the analysis of the "second-hit" (second mutation) for tumor genesis in FPD and an epigenetic biomarker search for endometrial cancer.

We analyzed the generation of ovarian follicles from mouse pluripotent stem cells using single-cell RNA sequencing (Figure). This technology was also applied to *in vitro* organ cultured testes to analyze the global inflammatory response.

Using large-scale CRISPR-Cas9 screening, we identified several potential epigenetic regulators including MEN1 during epithelial-to-mesenchymal transition (EMT). Furthermore, we found that EMT preferentially occurred at the peripheral edge of solid tumors, where proteasomal regulation of the ZEB1 TF was suggested to play an important role in this process.

Laboratory for

Genome Information Analysis

Team Leader: Chung-Chau Hon

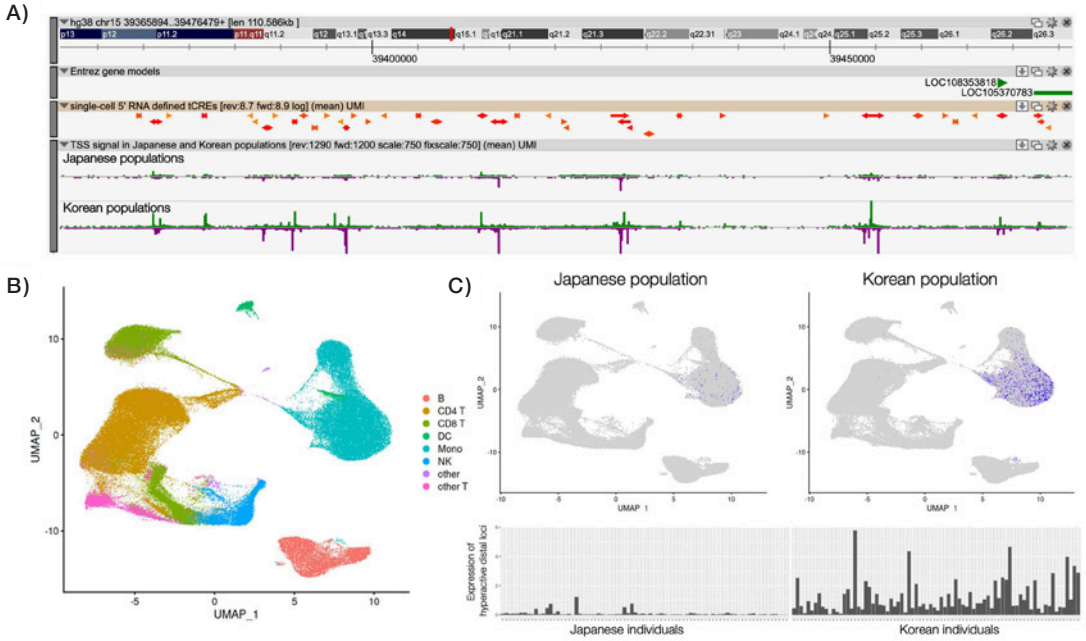


Figure: Ethnic-specific and cell-type specific tCRE activities at an intergenic hyperactive locus

A) An example of an intergenic hyperactive distal locus with clusters of highly active distal tCREs, defined by the transcription start site (TSS) signal, in peripheral blood mononuclear cells of Japanese and Korean populations. B) A UMAP showing the peripheral blood mononuclear cells collected from Japanese and Korean populations. C) The expression levels of the hyperactive distal locus among Japanese and Korean single-cells (top) and individuals (bottom).

Recent Major Publications

Woogeng IN, Kaczkowski B, Abugessaisa I, Hu H, Tachibana A, Sahara Y, Hon CC, Hasegawa A, Sakai N, Nishida M, Sanyal H, Sho J, Kajita K, Kasukawa T, Takasato M, Carninci P, Maeda A, Mandai M, Arner E, Takahashi M, Kime C. Inducing human retinal pigment epithelium-like cells from somatic tissue. ***Stem Cell Reports*** 17, 289-306 (2022)

Syed KM, Hon CC. Heterogeneity among enhancer RNAs: origins, consequences and perspectives. ***Essays Biochem*** 65, 709-721 (2021)

Sun M, Wang Y, Zheng C, Wei Y, Hou J, Zhang P, He W, Lv X, Ding Y, Liang H, Hon CC, Chen X, Xu H, Chen Y. Systematic functional interrogation of human pseudogenes using CRISPRi. ***Genome Biol*** 22, 240 (2021)

Our mission is to understand the roles of the non-coding genome in human diseases in terms of dysregulation of gene expression through integration of transcriptomic, epigenomic and genetic data. Expression of genes is primarily controlled by the activities of their cognate *cis*-regulatory elements (CREs, mostly promoters and enhancers), and our team focuses on analyzing their activities in various cell-type-specific and disease-specific contexts on a population level scale using single cell technologies. These contexts include variations of CRE activities in immune cells across Asian populations, dysregulation of gene regulatory networks in hematopoietic stem cells in Acute Myeloid Leukemia and disease-specific responses in iPSC-derived motor neurons in Amyotrophic Lateral Sclerosis. We use single-cell RNA-5'end-sequencing to profile the activities of transcribed CREs (tCREs) in these systems, enabling us to interrogate the context-specific activities of distal tCREs, which are crucial in gene regulation (Figure). We also develop tools for identification of authentic tCREs from these data (https://github.com/chung-lab/SCAFE) and adopt deep learning methods to predict the effects of genetic variants on gene expression using these data. Our ultimate goal is to integrate these context specific CRE activities with the genetic data to pinpoint the crucial genetic variants contributing to predisposition to disease.

Laboratory for

Applied Computational Genomics

Team Leader: **Michiel de Hoon**

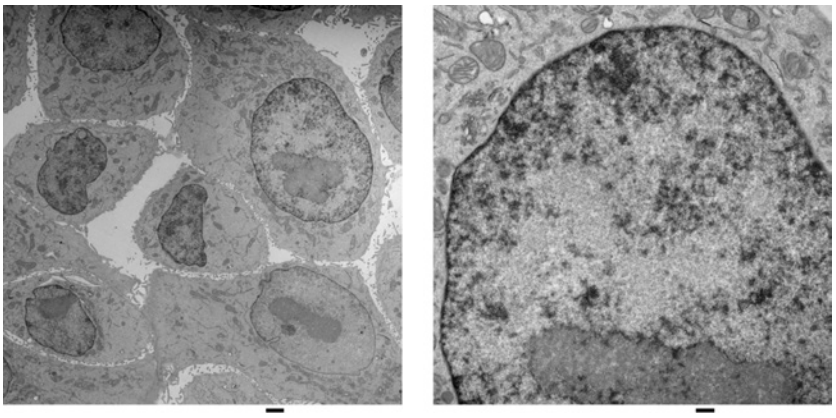


Figure:
Electron microscope image of MCF-10A human mammary epithelial cells (in collaboration with the IMS Laboratory for Cellular Epigenomics, the IMS Laboratory for Skin Homeostasis and Tokyo University of Technology). Chromatin was stained with osmium and can be observed in the electron micrographs as dark areas. The scale bars correspond to 2 µm (left) and 500 nm (right).

More than 98% of the human genome is non-coding, generating a wide variety of long non-coding RNAs (lncRNAs) depending on cell type. The biological function of most lncRNAs is currently not well defined and their systematic annotation is challenging due to their low expression, rapid degradation, high cell type-specificity, poor conservation across organisms, and lack of families of functionally orthologous lncRNAs. As most genetic variants associated with disease are located in non-coding regions, characterizing the function of lncRNAs is of fundamental importance for understanding human disease.

As lncRNAs are enriched in the cell nucleus, they may have regulatory functions by acting directly on chromatin. We analyzed the 3D structure of chromatin in the nucleus using Hi-C, which captures pairs of DNA segments close to each other in 3D space and characterized each lncRNA by the functional categories of protein-coding genes in its vicinity. Functional annotations of 13,534 lncRNAs, generated by our analysis in 18 different cell types and tissues, were disseminated publicly using ZENBU, an integrated visualization and data analysis system.

Complementary to these sequencing-based strategies, we are developing imaging methods for electron microscopy to visualize chromosome conformation at nanometer-scale resolution and to landmark specific genomic regions such as promoters and enhancers, as well as other biomolecules, in these images. Our long-term aim is to understand the structure of chromatin as the biophysical basis of gene regulation in the nucleus.

In response to the ongoing SARS-CoV-2 pandemic, we collaborate with multiple laboratories in our Center to better understand the response of human cells to infection by different strains of the virus by analyzing and comparing the transcriptome following infection.

Recent Major Publications

Hashimoto M, Saito Y, Nakagawa R, Ogahara I, Takagi S, Takata S, Amitani H, Endo M, Yuki H, Ramilowski JA, Severin J, Manabe R, Watanabe T, Ozaki K, Kaneko A, Kajita H, Fujiki S, Sato K, Honma T, Uchida N, Fukami T, Okazaki Y, Ohara O, Shultz LD, Yamada M, Taniguchi S, Vyas P, De Hoon M, Momozawa Y, Ishikawa F. Combined inhibition of XIAP and BCL2 drives maximal therapeutic efficacy in genetically diverse aggressive acute myeloid leukemia. ***Nat Cancer*** 2, 340-356 (2021)

Ramilowski JA, Yip CW, Agrawal S, Chang JC, Ciani Y, Kulakovskiy IV, Mendez M, Ooi JLC, Ouyang JF, Parkinson N, Petri A, Roos L, Severin J, Yasuzawa K, Abugessaisa I, Akalin A, Antonov IV, Arner E, Bonetti A, Bono H, Borsari B, Brombacher F, Cameron CJ, Cannistraci CV, Cardenas R, Cardon M, Chang H, Dostie J, Ducoli L, Favorov A, Fort A, Garrido D, Gil N, Gimenez J, Guler R, Handoko L, Harshbarger J, Hasegawa A, Hasegawa Y, Hashimoto K, Hayatsu N, Heutink P, Hirose T, Imada EL, Itoh M, Kaczkowski B, Kanhere A, ..., De Hoon M, Shin JW, Carninci P. Functional annotation of human long noncoding RNAs via molecular phenotyping. ***Genome Res*** 30, 1060-1072 (2020)

Alam T, Agrawal S, Severin J, Young RS, Andersson R, Arner E, Hasegawa A, Lizio M, Ramilowski JA, Abugessaisa I, Ishizu Y, Noma S, Tarui H, Taylor MS, Lassmann T, Itoh M, Kasukawa T, Kawaji H, Marchionni L, Sheng G, Forrest ARR, Khachigian LM, Hayashizaki Y, Carninci P, De Hoon MJL. Comparative transcriptomics of primary cells in vertebrates. ***Genome Res*** 30, 951-961 (2020)

Laboratory for

Large-Scale Biomedical Data Technology

Team Leader: Takeya Kasukawa

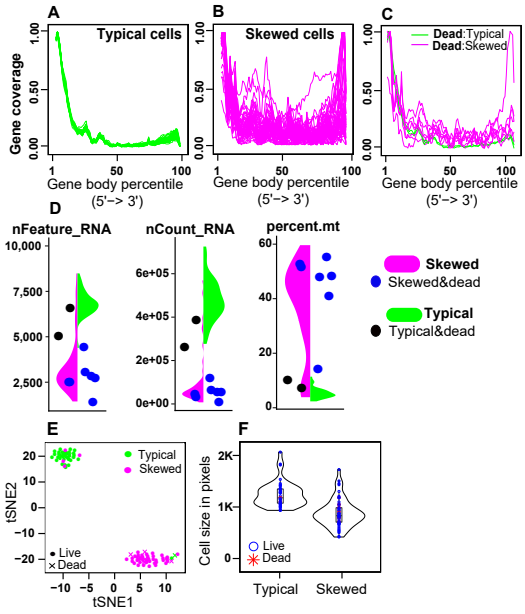


Figure: An example of a SkewC single-cell RNA-seq quality assessment

Typical/skewed cells: QC annotation with SkewC; Dead/live cells: QC annotation by the original data producers. (A-B) Gene body coverage plots for typical and skewed cells. (C) The gene body coverage plot for dead cells (colored by typical and skewed cells with SkewC). (D) Violin plots showing Seurat QC metrics. The violin plot split by the SkewC annotations and dead cells, shown as black and blue circles, respectively. (E) The t-SNE clustering plots with typical and skewed cell annotations. Dead and live cells are highlighted by symbols. (F) The distribution of cell sizes between typical and skewed cells, dead and live are highlighted by symbols.

Recent Major Publications

Abugessaisa I, Ramilowski JA, Lizio M, Severin J, Hasegawa A, Harshbarger J, Kondo A, Noguchi S, Yip CW, Ooi JLC, Tagami M, Hori F, Agrawal S, Hon CC, Cardon M, Ikeda S, Ono H, Bono H, Kato M, Hashimoto K, Bonetti A, Kato M, Kobayashi N, Shin J, de Hoon M, Hayashizaki Y, Carninci P, Kawaji H, Kasukawa T. FANTOM enters 20th year: expansion of transcriptomic atlases and functional annotation of non-coding RNAs. ***Nucleic Acids Res*** 49, D892-D898 (2021)

Ramilowski JA, Yip CW, Agrawal S, Chang JC, Ciani Y, Kulakovskiy IV, Mendez M, Ooi JLC, Ouyang JF, Parkinson N, Petri A, Roos L, Severin J, Yasuzawa K, Abugessaisa I, Akalin A, Antonov IV, Arner E, Bonetti A, Bono H, Borsari B, Brombacher F, Cameron CJ, Cannistraci CV, Cardenas R, Cardon M, Chang H, Dostie J, Ducoli L, Favorov A, Fort A, Garrido D, Gil N, Gimenez J, Guler R, Handoko L, Harshbarger J, Hasegawa A, Hasegawa Y, Hashimoto K,…, Forrest ARR, Guigó R, Hoffman MM, Hon CC, Kasukawa T, Kauppinen S, Kere J, Lenhard B, Schneider C, Suzuki H, Yagi K, de Hoon MJL, Shin JW, Carninci P. Functional annotation of human long noncoding RNAs via molecular phenotyping. ***Genome Res*** 30,1060-1072 (2020)

Bonetti A, Agostini F, Suzuki AM, Hashimoto K, Pascarella G, Gimenez J, Roos L, Nash AJ, Ghilotti M, Cameron CJF, Valentine M, Medvedeva YA, Noguchi S, Agirre E, Kashi K, Samudyata, Luginbühl J, Cazzoli R, Agrawal S, Luscombe NM, Blanchette M, Kasukawa T, de Hoon M, Arner E, Lenhard B, Plessy C, Castelo-Branco G, Orlando V, Carninci P. RADICL-seq Identifies General and Cell Type-Specific Principles of Genome-Wide RNA-chromatin Interactions. ***Nat Commun*** 11, 1018 (2020)

Invited presentations

Kasukawa T. "FANTOM project and data resource for understanding the transcription in the mammalian genomes" BioC Asia (Online) November 2021

Because of rapid improvements in sequencing technologies, many types of transcriptomic, genomic and epigenomic data have been generated and made publicly available. Such data resources are potentially useful for the elucidation of biological systems and the development of medical tools by performing large-scale integrative analyses. Our mission is promoting such data-driven studies in the biomedical field and developing component technologies to efficiently reuse large-scale biomedical data by employing data engineering technologies.

For this purpose, we have several ongoing research projects. One of them is the development of a QC pipeline and methods for reusing and evaluating public single-cell RNA-seq data and the construction of a public database named "SCPortalen" (https://single-cell.riken.jp/). Our new QC method (SkewC) can identify "skewed cells," which can negatively affect further downstream analyses with single-cell RNA-seq data (See figure). With this method, we found that such cells are present in most publicly available single-cell RNA-seq data. Another project is the development of a reference set of transcription start sites (refTSS: https://reftss.riken.jp/), which can be used for the quantification of 5'-end RNA-seq data, and also can be used as a platform for integrating many types of transcriptome and epigenome data to promote the study of transcriptional regulation. We are also working on studies targeting human pathology/disease: the transcriptome analysis of human blood samples from aged patients with frailty phenotype and the transcriptome analysis to develop a diagnostic tool for mycetoma, an infectious disease on the WHO listing of neglected tropical diseases.

Along with these research projects, we provide and support the information infrastructure for several IMS laboratories.

Laboratory for

Advanced Genomic Circuit

Team Leader: **Jay W. Shin**

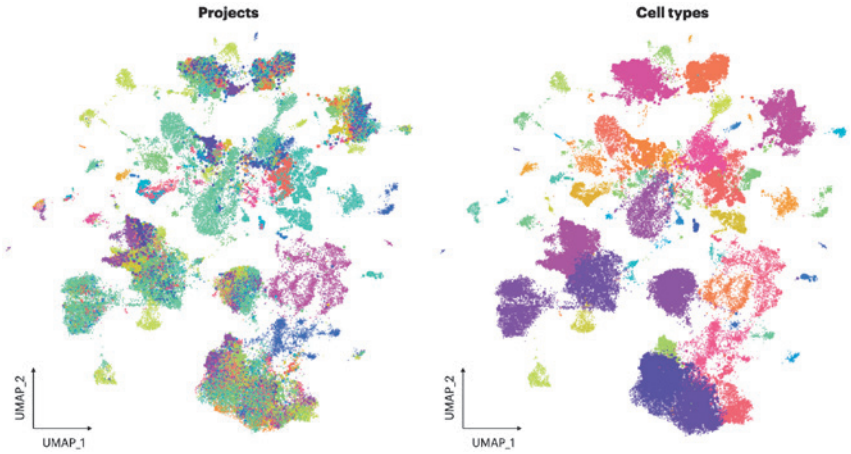


Figure: Single Cell Clustering Across 15 Human Tissues Based on 5' RNA-seq
Single cells are clustered based on similarities in cis-regulatory elements (UMAP projection). For each tissue, distinct cell types are highlighted, revealing a vast diversity of human cells.

The human genome is a complex entanglement of DNA strands with exquisite coordination amongst gene regulatory elements to switch a gene on and off. Understanding how our genome can sense the correct regulatory partners to trigger gene activation or repression – especially in a highly compact environment – requires a comprehensive profiling of DNA, RNA and protein features and their interactions with one another. Unraveling this mystery will shed light on novel ways to decipher the regulatory elements in the human genome, enabling us to correctly identify the causality of genetic disorders, to rectify cellular malfunctions, to reengineer cells, and possibly to extend the lifespan of vital organs.

One of our strategies involves the implementation of single-cell 5' RNA-seq to profile the coding and non-coding regulatory activities in human cells. As part of the Single Cell Medical Network Program, we profiled thousands of single cells from various human tissues, including the colon, kidney, muscles, lung, and blood and we systematically annotated *cis*-regulatory elements across hundreds of cell types and states. The lab further explored these gene regulatory elements by genetic associations, including single-cell eQTL, as part of the Human Cell Atlas Asia project, and chromatin-chromatin interactions using the third-generation DNA sequencer to elucidate long-range chromatin interactions at single-cell resolution. We specialize in gene-targeting tools, such as CRISPR-interference and antisense oligonucleotides, to elucidate the functional involvement of *cis*-regulatory RNAs and elements in human stem cell differentiation, brain development and reprogramming.

Recent Major Publications

Luginbühl J, Kouno T, Nakano R, Chater TE, Sivaraman DM, Kishima M, Roudnicky F, Carninci P, Plessy C, Shin JW. Decoding neuronal diversification by multiplexed single-cell RNA-seq. ***Stem Cell Rep*** 16, 810-824 (2021)

Ducoli L, Agrawal S, Sibler E, Kouno T, Tacconi C, Hon CC, Berger SD, Müllhaupt D, He Y, Kim J, D'Addio M, Dieterich LC, Carninci P, de Hoon MJL, Shin JW*, Detmar M*. LETR1 is a lymphatic endothelial-specific lncRNA that governs cell proliferation and migration through KLF4 and SEMA3C. ***Nat Commun*** 12, 925 (2021)

Rozenblatt-Rosen O*, Shin JW*, Rood JE, Hupalowska A; Human Cell Atlas Standards and Technology Working Group, Regev A, Heyn H. Building a high-quality Human Cell Atlas. ***Nat Biotechnol*** 39, 149-153 (2021)

Invited presentations

Shin JW. "Decoding Neuronal Diversification by Multiplexed Single Cell RNA-seq" Keio University - Stanford University Joint Seminar (Tokyo, Japan/Online) October 2021

Shin JW. "Human Cell Atlasing the Transcribed Cis-Regulatory Elements" CZI Single-Cell Biology Annual Meeting (Paolo Alto, USA/Online) October 2021

Shin JW. "Functional Elucidation of Gene Regulatory Elements in Brain Development" The 64th Japanese Society of Neurochemistry Annual Meeting (Nara, Japan/Online) September 2021

Shin JW. "Building the Human Cell Regulatory Atlas" The 19th Protein Island Matsuyama International Symposium (Ehime, Japan/Online) September 2021

Shin JW. "Decoding Neuronal Diversification by Multiplexed Single Cell RNA-seq" International Society for Stem Cell Research - Computational Workshop (Online) March 2021

Laboratory for
Cellular Epigenomics

Team Leader: **Aki Minoda**

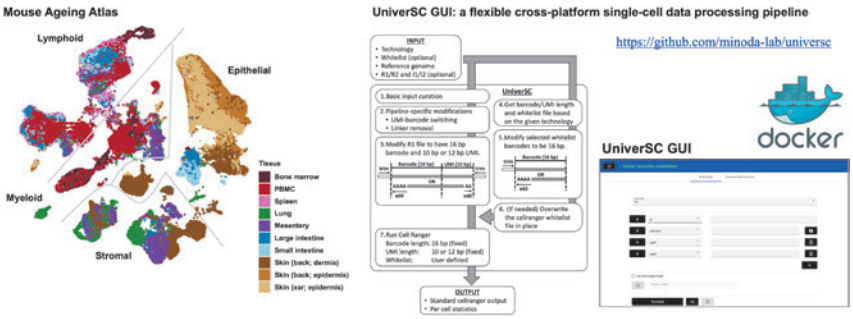


Figure: Construction of a Mouse Ageing Atlas with single-cell genomics and UniverSC: a flexible cross-platform single-cell data processing pipeline
Left: 5' scRNA-seq data from 8 mouse tissues obtained from young (2 months) and old (19 months) SPF (specific pathogen-free) or GF (germ-free) mice are combined and displayed.
Right: The pipeline and the GUI for UniverSC are shown.

Recent Major Publications

Kelly ST, Battenberg K, Hetherington NA, Hayashi M, Minoda A. UniverSC: a flexible cross-platform single-cell data processing pipeline. ***BioRxiv*** doi: https://doi.org/10.1101/2021.01.19.427209 (2021)

Augusto RC, Rey O, Cosseau C, Chaparro C, Vidal-Dupiol J, Allienne JF, Duval D, Pinaud S, Tönges S, Andriantsoa R, Luquet E, Aubret F, Dia Sow M, David P, Thomson V, Joly D, Gomes Lima M, Federico D, Danchin E, Minoda A, Grunau C. A simple ATAC-seq protocol for population epigenetics. ***Wellcome Open Res*** 5, 121 (2021)

Ramilowski JA, Yip CW, Agrawal S, Chang JC, Ciani Y, Kulakovskiy IV, Mendez M, Ooi JLC, Ouyang JF, Parkinson N, Petri A, Roos L, Severin J, Yasuzawa K, Abugessaisa I, Akalin A, Antonov IV, Arner E, Bonetti A, Bono H, Borsari B, Brombacher F, Cameron CJ, Cannistraci CV, Cardenas R, Cardon M, Chang H, Dostie J, Ducoli L, Favorov A, Fort A, Garrido D, Gil N, Gimenez J, Guler R, Handoko L, Harshbarger J, Hasegawa A, Hasegawa Y, Hashimoto K, Hayatsu N, Heutink P, Hirose T, Imada EL, Itoh M, Kaczkowski B, Kanhere A, ..., De Hoon M, Shin JW, Carninci P. Functional annotation of human long noncoding RNAs via molecular phenotyping. ***Genome Res*** 30, 1060-1072 (2020)

Invited presentations

Minoda A. "Determining the state of type 2 innate immune response in the lung in old age" Japanese Society for Immunology (Online) December 2021

Minoda A. "Mouse Ageing Promoter Atlas: effect of the microbiota on ageing" Human Cell Atlas Asia (Online) November 2021

Minoda A. "Single cell RNA-seq analysis towards understanding ageing" The 57th Annual Meeting of Liver Cancer Study Group of Japan (Online) July 2021

Minoda A. "Tissue ageing dissected by single cell RNA-seq" Okinawa Institute of Science and Technology Graduate University (Okinawa, Japan) March 2021

Minoda A. "Tissue ageing dissected by single cell RNA-seq" Biology Departmental Seminar, University of Alabama at Birmingham (Online) February 2021

Our lab aims to determine epigenomic and transcriptomic changes in a comprehensive manner in various models by applying the most advanced available technologies, such as single-cell genomics. Such information will be utilized to gain insights into various biological questions at the molecular level.

Construction of a Mouse Ageing Atlas with single-cell genomics

Inflammation is a major hallmark of ageing. To determine whether the presence of the microbiota is contributing to the increase in inflammation that is observed with age (termed 'inflammaging'), we are generating single-cell genomic (5' scRNA-seq and scATAC-seq) datasets of various tissues from both SPF and germ-free mice at various ages, as well as lipidomics (in collaboration with the Arita lab, IMS), metabolomics and microbiome (in collaboration with the Ohno lab, IMS). Such a rich collection of multi-omics datasets will likely provide us with an unbiased insight at many different levels, including the effect of the microbiome, into the complex biological phenomena of ageing. Our preliminary analysis of the completed datasets from 8 different tissues captures increased inflammation signatures with old age at the transcriptomic level, which are in fact reduced in the germ-free mice that completely lack microbes. This indeed suggests that the microbiota contributes to inflammaging.

Development of UniverSC: a flexible cross-platform single-cell data processing pipeline

Single-cell RNA-sequencing analysis to quantify RNA molecules in individual cells has become popular as it can obtain a large amount of information from each experiment. We have developed UniverSC (https://github.com/minoda-lab/universc), a universal single-cell RNA-seq data processing tool that supports any UMI-based platform. Our command-line tool enables consistent and comprehensive integration, comparison and evaluation across data generated from a wide range of platforms. In an effort to democratize single-cell analysis, UniverSC is also available through Docker as well as GUI (graphical user interface).

Laboratory for

Comprehensive Genomic Analysis

Team Leader: Yasushi Okazaki

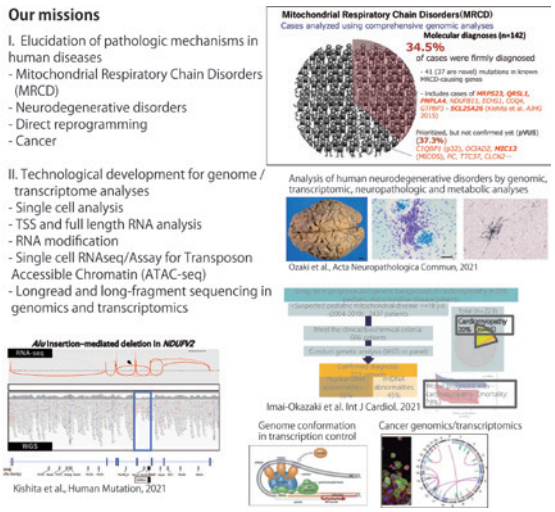


Figure: Future plans
We will continue to apply omics and functional analyses to mitochondrial and neurological diseases, taste receptors, direct reprogramming, and other themes in molecular medicine and biology. In addition, we will continue technological development for genomic and transcriptomic analyses. We continue to utilize state-of-the-art technologies such as Nanopore and PacBio long-read sequencers, as well as high-quality short-read sequencers from Illumina and MGI, through the IMS genome platform, to solve biological/medical problems. Especially, because we recently are focusing on the development of single-cell technologies, we will apply these technologies to various unsolved important questions in molecular medicine.

Recent Major Publications

Ozaki K, Irioka T, Uchihara T, Yamada A, Nakamura A, Majima T, Igarashi S, Shintaku H, Yakeishi M, Tsuura Y, Okazaki Y, Ishikawa K, Yokota T. Neuropathology of SCA34 showing widespread oligodendroglial pathology with vacuolar white matter degeneration: a case study. ***Acta Neuropathol Commun*** 9, 172 (2021)

Kishita Y, Shimura M, Kohda M, Fushimi T, Nitta KR, Yatsuka Y, Hirose S, Ideguchi H, Ohtake A, Murayama K, Okazaki Y. Genome sequencing and RNA-seq analyses of mitochondrial complex I deficiency revealed Alu insertion-mediated deletion in NDUFV2. ***Hum Mutat*** 42, 1422-1428 (2021)

Imai-Okazaki A, Matsunaga A, Yatsuka Y, Nitta KR, Kishita Y, Sugiura A, Sugiyama Y, Fushimi T, Shimura M, Ichimoto K, Tajika M, Tominaga M, Ebihara T, Matsuhashi T, Tsuruoka T, Kohda M, Hirata T, Harashima H, Nojiri S, Takeda A, Nakaya A, Kogaki S, Sakata Y, Ohtake A, Murayama K, Okazaki Y. Long-term prognosis and genetic background of cardiomyopathy in 223 pediatric mitochondrial disease patients. ***Int J Cardiol*** 341, 48-55 (2021)

Invited presentations

Okazaki Y. "Genetic analyses of mitochondrial diseases and development to therapeutics" The 20th annual meeting of the Japanese Society of Mitochondrial Research and Medicine (Tokyo, Japan) December 2021

Okazaki Y. "Research and Diagnosis of mitochondrial cardiomyopathy in Japan" Mitochondrial Medicine Workshop Beijing 2021 (Beijing, China/Online) October 2021

Okazaki Y. "Genetic diagnosis of mitochondrial diseases" 37th Japanese Pediatric Liver Disease Research Meeting (Chiba, Japan) June 2021

From FY2016 to FY2017, we provided research support for RIKEN and outside researchers at a facility named Genome Network Analysis Support (GeNAS). Beginning in FY 2018, the Laboratory for Comprehensive Genomic Analysis (CGA) was formed. CGA not only took over some of the research and development missions from GeNAS, but also began to develop its own separate research activities.

The CGA laboratory conducts omics analyses to elucidate the pathophysiology of human diseases. We do so in order to understand the mechanisms of diseases that disrupt the homeostatic function of various cells and tissues and to discover new drug targets. Specifically, we focus on the identification and characterization of novel causative genes for human hereditary disorders such as mitochondrial diseases, neurological diseases and other rare intractable diseases. As a part of a collaborative project aiming to identify causative variants in "rare diseases undiagnosed by exome sequencing", we analyze such challenging clinical cases with our genome/transcriptome technologies using long- and short-read sequencing. We also are trying to discover new potential drug targets for the realization of personalized medicine in a high-throughput screening system using zebrafish. In addition, we study the above described medical themes from the viewpoint of evolutionary biology. Finally, we also study the taste receptor system using single-cell analyses.

Furthermore, we continue to contribute to collaborative research projects through the "IMS genome platform". We provide our special genome and transcriptome analysis technologies, such as Cap Analysis of Gene Expression (CAGE) as well as single-cell RNAseq and single-cell ATAC-seq. Some of these essential technologies have been developed by us and we continuously strive to improve them and create new ones. We are working with several dozen collaborators utilizing these technologies. We also are clarifying molecular mechanisms underlying direct reprogramming of fibroblasts into other distinctively differentiated cell types.

Preventive Medicine and Applied Genomics Unit

Unit Leader: **Hideya Kawaji**

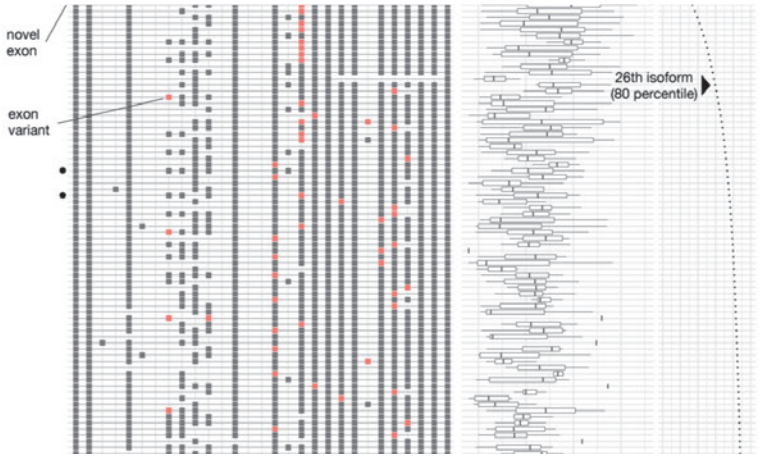


Figure: Integrated view of transcriptome and epigenetic marks provided by the FANTOM5 web resource
A partial listing of the *TACC2* mRNA isoforms discovered by using a long-read sequencer. Gray squares and lines listing indicate exons and introns, respectively, and the adjacent boxplots indicate frequencies of individual exons and isoforms. Only the top 50 isoforms are shown. (The figure is from Sci. Rep. 11:16835, 2021).

Remarkable progress has recently been made in molecular profiling technologies, including genome-wide technologies developed at RIKEN, and their effective use is one of the major interests in life science research, in particular to solve medical problems. The RIKEN Preventive Medicine and Diagnosis Innovation Program (RIKEN PMI) coordinates translational studies that utilize RIKEN technologies to solve clinical problems. Our unit was established to conduct or support such studies with PMI funding, in particular from the perspective of information sciences or computational genomics. Our projects are roughly classified into three categories: identification of cell markers required for regenerative medicine, exploration of diagnostic markers useful in patient treatment and our own advances to assist in such translational as well as basic science research.

Our collaborative research with RIKEN PMI led to several biomedical research publications: Molecular characterization of cancer-associated fibroblasts in non-small cell lung cancers (Iwai M., *et al.* Mol Oncol. 2021), Gene expression signatures of patient-derived mononuclear cells cultured for vasculogenic wound healing (Tanaka R., Stem Cells Transl Med. 2021), Single-cell profiling of patient-derived multiple myeloma cells (Hirabayashi S. *et al.* Biochem Biophys Res Commun. 2021), and Potential diagnostic biomarkers of myeloproliferative neoplasms (Morishita S., *et al.* Cancer Sci. 2021). Furthermore, we uncovered more than 200 mRNA isoforms derived from a single gene locus, *TACC2*, by using a long-read sequencer (Ito Y., *et al.* Sci Rep. 2021). This level of isoform variety has not been seen before, which motivates further exploration of transcriptome complexity. We also provided an important foundation for safety assessment of oligonucleotide therapeutics in a pre-clinical study, i.e., a high-quality genome assembly of the crab-eating macaque (Jayakumar V., *et al.* Sci Data. 2021). This has been employed as the reference genome assembly of the species and is also a major contribution to basic science as well as biomedical studies.

Recent Major Publications

Jayakumar V, Nishimura O, Kadota M, Hirose N, Sano H, Murakawa Y, Yamamoto Y, Nakaya M, Tsukiyama T, Seita Y, Nakamura S, Kawai J, Sasaki E, Ema M, Kuraku S, Kawaji H, Sakakibara Y. Chromosomal-scale de novo genome assemblies of Cynomolgus Macaque and Common Marmoset. ***Sci Data*** 8, 159 (2021)

Ito Y, Terao Y, Noma S, Tagami M, Yoshida E, Hayashizaki Y, Itoh M, Kawaji H. Nanopore sequencing reveals TACC2 locus complexity and diversity of isoforms transcribed from an intronic promoter. ***Sci Rep*** 11, 9355 (2021)

Abugessaisa I, Ramilowski JA, Lizio M, Severin J, Hasegawa A, Harshbarger J, Kondo A, Noguchi S, Yip CW, Ooi JLC, Tagami M, Hori F, Agrawal S, Hon CC, Cardon M, Ikeda S, Ono H, Bono H, Kato M, Hashimoto K, Bonetti A, Kato M, Kobayashi N, Shin J, de Hoon M, Hayashizaki Y, Carninci P, Kawaji H, Kasukawa T. FANTOM enters 20th year: expansion of transcriptomic atlases and functional annotation of non-coding RNAs. ***Nucleic Acids Res*** 49, D892-D898 (2021)

RIKEN-IFOM Joint Laboratory for Cancer Genomics

Team Leader: Yasuhiro Murakawa

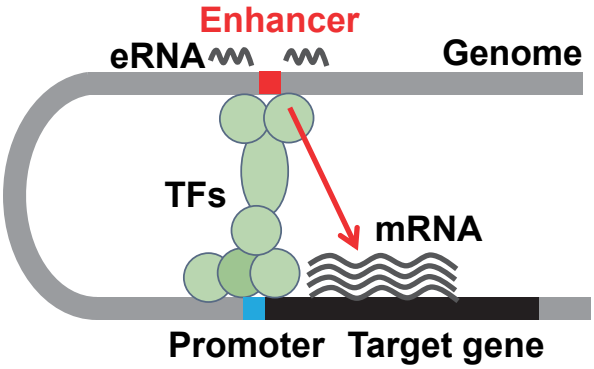


Figure: Enhancer-mediated gene regulation
Enhancers are small segments of distal *cis*-regulatory DNA elements that significantly enhance the expression of target genes and play key roles in the establishment of cell-type-specific function and identity.

Recent Major Publications

Sato Y, Oguchi A, Fukushima Y, Masuda K, Toriu N, Taniguchi K, Yoshikawa T, Cui X, Kondo M, Hosoi T, Komidori S, Shimizu Y, Fujita H, Jiang L, Kong Y, Yamanashi T, Seita J, Yamamoto T, Toyokuni S, Hamazaki Y, Hattori M, Yoshikai Y, Boor P, Floege J, Kawamoto H, Murakawa Y, Minato N, Yanagita M. CD153-CD30 signaling promotes age-dependent tertiary lymphoid tissue expansion and kidney injury. ***J Clin Invest*** 132, e146071 (2021)

Jayakumar V, Nishimura O, Kadota M, Hirose N, Sano H, Murakawa Y, Yamamoto Y, Nakaya M, Tsukiyama T, Seita Y, Nakamura S, Kawai J, Sasaki E, Ema M, Kuraku S, Kawaji H, Sakakibara Y. Chromosomal-scale de novo genome assemblies of Cynomolgus Macaque and Common Marmoset. ***Sci Data*** 8, 159 (2021)

Sasaki K, Oguchi A, Cheng K, Murakawa Y, Okamoto I, Ohta H, Yabuta Y, Iwatani C, Tsuchiya H, Yamamoto T, Seita Y, Saitou M. The embryonic ontogeny of the gonadal somatic cells in mice and monkeys. ***Cell Rep*** 35, 109075 (2021)

Invited presentations

Murakawa Y. "Functional characterization of human disease pathways using high-resolution chromatin contact maps" Immuno UK: In-Person (London, UK) October 2021

The body-wide transcriptome is generated by the spatiotemporal orchestration of *cis*-regulatory elements such as promoters and enhancers. In particular, enhancers are distal *cis*-regulatory DNA elements that are crucial for the establishment of cell-type-specific function and identity (Figure). We aim to decipher the *cis*-regulatory code that governs the transcriptional landscapes of malignancies, thereby gaining fundamental insight into cancer development and maintenance.

To investigate the *cis*-regulatory code, we have developed a simple and robust technology, NET-CAGE, to determine globally the 5'-ends of nascent RNAs, thereby sensitively detecting even unstable transcripts, including enhancer-derived RNAs. NET-CAGE enabled ultra-sensitive detection of a number of enhancers at single-nucleotide resolution (Hirabayashi *et al.* Nature Genetics, 2019).

We are applying our original NET-CAGE technology to describe the active *cis*-regulatory landscape across hundreds of diverse tumors, discovering differentially regulated enhancers, genes and long non-coding RNAs. Furthermore, using our unique atlas of active enhancer regions at single-nucleotide resolution, we further aim to develop a series of original technologies to investigate connectivity and functionality of *cis*-regulatory elements at both population and single-cell levels. We believe in the importance of developing novel technologies that can solve biomedical mysteries that cannot be otherwise solved.

Lastly, through integrated analysis of (epi) genomic data with clinical information, we explore molecular therapeutic targets and biomarkers.

Laboratory for Genotyping Development

Team Leader: Yukihide Momozawa

Figure: Clinical characteristics of patients with renal cell carcinoma who carry pathogenic variants in statistically associated genes

We performed a case-control study using 1,532 patients with renal cell carcinoma and 5,996 controls. We separately analyzed two major histological subtypes, clear cell renal cell carcinoma (ccRCC) and non-clear cell renal cell carcinoma (nccRCC). Each vertical box in this figure indicates each patient. The purple and yellow boxes in the upper part represent loss of function and missense variants, respectively. The lower part indicates the clinical characteristics of each patient.

Recent Major Publications

Sekine Y, Iwasaki Y, Aoi T, Endo M, Hirata M, Kamatani Y, Matsuda K, Sugano K, Yoshida T, Murakami Y, Fukui T, Akamatsu S, Ogawa O, Nakagawa H, Numakura K, Narita S, Habuchi T, Momozawa Y. Different risk genes contribute to clear cell and non-clear cell renal cell carcinoma in 1,532 Japanese patients and 5,996 controls. ***Hum Mol Genet***, (2021) In press

Hashimoto M, Saito Y, Nakagawa R, Ogahara I, Takagi S, Takata S, Amitani H, Endo M, Yuki H, Ramilowski JA, Severin J, Manabe R, Watanabe T, Ozaki K, Kaneko A, Kajita H, Fujiki S, Sato K, Honma T, Uchida N, Fukami T, Okazaki Y, Ohara O, Shultz LD, Yamada M, Taniguchi S, Vyas P, Hoon MJ, Momozawa Y, Ishikawa F. Combined inhibition of XIAP and BCL2 drives maximal therapeutic efficacy in genetically diverse aggressive acute myeloid leukemia. ***Nat Cancer*** 2, 340-356 (2021)

Saiki R, Momozawa Y, Nannya Y, Nakagawa MM, Ochi Y, Yoshizato T, Terao C, Kuroda Y, Shiraishi Y, Chiba K, Tanaka H, Niida A, Imoto S, Matsuda K, Morisaki T, Murakami Y, Kamatani Y, Matsuda S, Kubo M, Miyano S, Makishima H, Ogawa S. Combined landscape of single-nucleotide variants and copy number alterations in clonal hematopoiesis. ***Nat Med*** 27, 1239-1249 (2021)

Invited presentations

Momozawa Y. "Large-scale genome sequencing of hereditary cancer genes across 14 cancer types in Japan" X Jubilee International Science-Practical Conference Molecular Diagnostics (Online) December 2021

Momozawa Y. "Pan-cancer analysis of hereditary cancer genes" The 80th Annual Meeting of the Japan Neurosurgical Society (Yokohama, Japan) October 2021

Momozawa Y. "Large-scale germline sequencing of hereditary cancer genes in >60,000 cancer patients and controls" 2021 JCA-AACR Precision Cancer Medicine International Conference (Online) September 2021

Momozawa Y. "Current situation about hereditary cancer genes revealed by Japanese large-scale genome data" The 80th Annual Meeting of the Japanese Cancer Association (Yokohama, Japan) September 2021

Momozawa Y. "Characterics of Japanes hereditary prostate cancer" The 30th Annual Meeting of the Preventive Nephrology and Urology (Fukuoka, Japan) July 2021

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 various laboratories in IMS, and 2) to support each laboratory in library preparation, sequencing and data analysis related to sequencing as the Genome Platform.

Our laboratory published 22 papers in 2021. The main achievements this year are:

- A case-control study of 14 renal cell carcinoma (RCC)-related genes and 26 cancer-predisposing genes was performed using 1,563 Japanese patients with RCC and 6,016 controls. For clear cell RCC, 52 of 1,283 (4.05%) patients carried pathogenic variants mainly in the cancer-predisposing genes such as *TP53* ($P = 1.73 \times 10^{-4}$; OR, 5.8; 95% CI, 2.2–15.7). Approximately 80% of patients with pathogenic variants in *TP53* had p.Ala189Val, which was specific in the East Asian population. For non-clear cell RCC, 14 of 249 (5.62%) patients carried pathogenic variants mainly in the RCC-related genes such as *BAP1* and *FH* ($P = 6.27 \times 10^{-5}$; OR, Inf; 95% CI, 10.0–Inf). Patients with pathogenic variants in associated genes were diagnosed 15.8 years earlier and had a stronger family history of RCC (OR, 20.0; 95% CI 1.3-237.4) than non-carriers. Our study showed different and population-specific contributions of risk genes between ccRCC and nccRCC in Japanese for better-personalized medicine [Hum Mol Genet (in press)].
- We also contributed to other key findings about acute myeloid leukemia (Nat Cancer 2:340–356) and clonal hematopoiesis (Nat Med 27:1239-1249) within/outside of RIKEN by using our high-throughput targeted sequencing.

We will continue to work as a research hub for large-scale genomic analyses to contribute to the implementation of personalized medicine.

Laboratory for

Statistical and Translational Genetics

Team Leader: **Chikashi Terao**

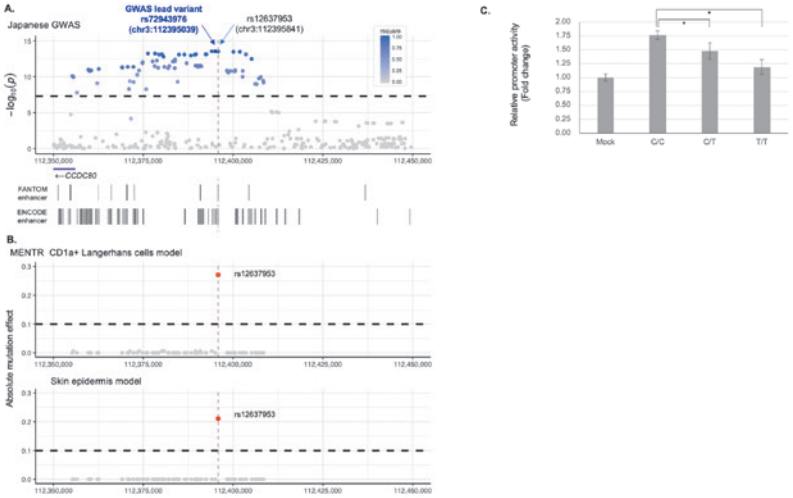


Figure: Successful fine-mapping of the *CCDC80* region in atopic dermatitis
MENTR, our own prediction method of transcripts including non-coding RNA based on machine-learning, could pinpoint the causal variant altering enhancer RNA levels in Langerhans cells and skin epidermis.

Recent Major Publications

Tanaka N, Koido M, Suzuki A, Otomo N, Suetsugu H, Kochi Y, Tomizuka K, Momozawa Y, Kamatani Y; Biobank Japan Project, Ikegawa S, Yamamoto K, Terao C*. Eight novel susceptibility loci and putative causal variants in atopic dermatitis. ***J Allergy Clin Immunol*** 148, 1293-1306 (2021)

Boer CG, Hatzikotoulas K, Southam L, Stefánsdóttir L, Zhang Y, Coutinho de Almeida R, Wu TT, Zheng J, Hartley A, Teder-Laving M, Skogholt AH, Terao C, Zengini E, Alexiadis G, Barysenka A, Bjornsdottir G, Gabrielsen ME, Gilly A, Ingvarsson T, Johnsen MB, Jonsson H, Kloppenburg M, Luetge A, Lund SH, Mägi R, Mangino M, Nelissen RRGHH, Shivakumar M, Steinberg J, Takuwa H, Thomas LF, Tuerlings M; arcOGEN Consortium; HUNT All-In Pain; ARGO Consortium; Regeneron Genetics Center, Babis GC, Yin Cheung JP, Kang JH, Kraft P,... Ikegawa S, Hveem K, Esko T, Wilkinson JM, Meulenbelt I, Michael Lee MT, van Meurs JBJ, Styrkársdóttir U, Zeggini E. Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. ***Cell*** 184, 6003-6005 (2021)

Yin X, Kim K, Suetsugu H, Bang SY, Wen L, Koido M, Ha E, Liu L, Sakamoto Y, Jo S, Leng RX, Otomo N, ...Suzuki A, Sumida T, Okada Y, Matsuda K, Matsuo K, Kochi Y; Japanese Research Committee on Idiopathic Osteonecrosis of the Femoral Head, Kottyan LC, Weirauch MT, Parameswaran S, Eswar S, Salim H, Chen X, Yamamoto K, Harley JB, Ohmura K, Kim TH, Yang S, Yamamoto T, Kim BJ, Shen N, Ikegawa S, Lee HS, Zhang X, Terao C*, Cui Y, Bae SC. Meta-analysis of 208370 East Asians identifies 113 susceptibility loci for systemic lupus erythematosus. ***Ann Rheum Dis*** 80, 632-640 (2021)

Invited presentations

Terao C. "Clarification of the mechanism underlying leukemia using SNP array data" TCCSG webinar (Tokyo, Japan/Online) July 2021

Terao C. "Research on rheumatic diseases with a view to clinical application" The 9th Yokohama-Kawasaki Rheumatism Wakakusa/Cosmos Meeting (Tokyo, Japan) June 2021

Terao C. "Construction of database and analyses of data for rheumatic diseases under the viewpoint of physician scientist" Meet the Expert, Japanese College of Rheumatology (JCR) symposium (Japan/Online) April 2021

Terao C. "Genetic basis on clonal hematopoiesis" The 3rd Fukushima Onco-Cardiology Research Meeting (Fukushima Japan) March 2021

Terao C. "Genetics disentangling the basics of rheumatoid arthritis and autoimmune diseases" The 11th Rheumatology Education Inter League REIL (Osaka, Japan) January 2021

We focus on the identification of genetic susceptibility variants associated with complex traits and on understanding their biological roles by using integrative analyses of epigenome, transcriptome and chromatin accessibility data. We are also interested in acquired mutations as strong driving forces of phenotypes. We will then deliver these genetic findings to patients as part of our ongoing translational research efforts.

In 2021, we expanded the biological associations of somatic chromosomal alterations in autosomes (mosaic chromosomal alterations, mCAs) from DNA microarray data. We reported that mCAs are a risk for infection or severity of infection including with SARS-CoV-2 and that mCAs and clonal hematopoiesis with point mutations synergistically increase the risk of blood cancers. These findings illustrate how critical somatic mutations are for diseases and phenotypes and how important it is to take acquired (non-innate) and dynamic components into consideration to evaluate human health and predict future outcomes. Our lab played central roles in genetic analyses of atopic dermatitis, systemic lupus erythematosus, epilepsia, and adolescent idiopathic scoliosis. Especially for atopic dermatitis, we successfully fine-mapped the CCDC80 locus using our own machine-learning-based transcription prediction method, MENTR, to reveal a causal variant that would alter an enhancer level in skin-relevant tissues/cells. This prediction was experimentally validated. We also contributed to four big papers in Nature and Cell – genetic studies of ovarian aging, osteoarthritis and lipids, and an expression quantitative trait loci study of comprehensive white blood cell subpopulations.

We are moving into two new major research fields. One is to focus on somatic events and to use whole-genome sequencing analysis to detect rare variants, which represent strong candidates to explain population differences in the genetics of the traits, especially somatic events. The second is to employ deep learning techniques to predict the biological consequences of trait-relevant variants.

Laboratory for Pharmacogenomics

Team Leader: Taisei Mushiroda

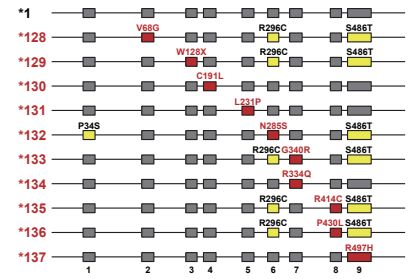
Figure: Determination of novel CYP2D6 haplotypes using the targeted next-generation sequencing panel, PKseq

Pharmacokinetic (PK) variabilities in intestinal absorption, hepatic drug metabolism, and biliary and renal excretion are often responsible for inter-individual differences in drug efficacy and risk of adverse drug reactions. PKseq is a highly efficient and accurate next-generation sequencing (NGS) platform for the resequencing of PK-related genes. PKseq identified 14 novel CYP2D6 variants, and ten novel haplotypes were registered as CYP2D6*128 to *137 alleles in the Pharmacogene Variation Consortium (PharmVar) database. Based on the *in vitro* Vmax/Km value of each allele, *128, *129, *130, *131, *132, and *133 were predicted to be non-functional alleles.

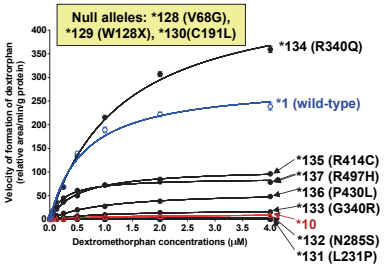
100 important pharmacokinetics-related genes, including 62 drug-metabolizing enzymes and 37 drug transporters

ABCB1	CYP1A1	CYP2D6	CYP4B1	DPYD	NAT1	SLC19A1	SLC22A12	SLC47A2	UGT1A3
ABCB4	CYP1A2	CYP2E1	CYP4F2	FMO1	NAT2	SLC22A1	SLC28A1	SLCO1B1	UGT1A4
ABCB11	CYP1B1	CYP2J2	CYP4F3	FMO2	NUDT1	SLC22A2	SLC28A2	SLCO1B3	UGT1A5
ABCC1	CYP2A6	CYP2S1	CYP4F8	FMO3	NUDT15	SLC22A3	SLC28A3	SLCO2B1	UGT1A6
ABCC2	CYP2A13	CYP2W1	CYP4F12	FMO4	POR	SLC22A4	SLC29A1	SLUT1A1	UGT1A7
ABCC3	CYP2B6	CYP3A4	CYP4Z1	FMO5	SLC10A1	SLC22A5	SLC29A2	SLUT1A2	UGT1A8
ABCC4	CYP2C8	CYP3A5	CYP11A1	GSTA1	SLC10A2	SLC22A6	SLC29A3	SLUT1E1	UGT1A9
ABCG2	CYP2C9	CYP3A7	CYP17A1	GSTM1	SLC15A1	SLC22A8	SLC31A1	SLUT2B1	UGT1A10
CES1	CYP2C18	CYP3A43	CYP19A1	GSTP1	SLC15A2	SLC22A9	SLC46A1	TPMT	UGT2B7
CES2	CYP2C19	CYP4A11	CYP26A1	GSTT1	SLC16A7	SLC22A11	SLC47A1	UGT1A1	VKORC1

Novel CYP2D6 haplotypes identified in 990 Japanese subjects



Michaelis-Menten plots of novel CYP2D6 variant proteins involved in the metabolism of dextromethorphan



Individual responses to drugs vary widely. Lack of drug efficacy can lead to inadequate disease control and is furthermore a waste of resources; conversely, adverse drug reactions (ADRs) are frequent and often unpredictable. Many germline polymorphisms, which are called pharmacogenomics (PGx) biomarkers, have been identified in genes that affect efficacy or ADR risk for various drugs. In Japan, the National Health Insurance System currently covers only three germline genetic tests, for *UGT1A1*, *NUDT15* and *CYP2C9*, to predict drug responses prior to drug administration. We conduct genomic analyses for the identification of PGx biomarkers useful for predicting drug responses.

A next-generation sequencing (NGS) panel, PKseq, can comprehensively and accurately analyze common and rare variants of 100 pharmacokinetics (PK)-related genes with higher sensitivity and specificity compared to whole-genome and whole-exome sequencing. Indeed, when we applied the PKseq technology to the determination of haplotypes of CYP2D6, very important drug-metabolizing enzymes for clinical therapeutics, in 990 Japanese subjects, 14 novel variants and 10 novel haplotypes were identified that affected the *in vitro* metabolic activities of CYP2D6. Using our experience in developing PKseq, we recently developed a novel targeted NGS panel, "corePGseq" consisting of 14 clinically important PK-related genes and 4 HLA genes associated with the risk of developing serious ADRs. In fact, the corePGseq panel allowed us to identify the HLA-DPB1*02:01:02 allele as associated with the risk of wheat-dependent exercise-induced anaphylaxis (WDEIA). Compared to PKseq, corePGseq also reduces the analysis burden and cost of reagents and is expected to be applied to pharmacogenetic testing in clinical settings.

Recent Major Publications

Fukunaga K, Chinuki Y, Hamada Y, Fukutomi Y, Sugiyama A, Kishikawa R, Fukunaga A, Oda Y, Ugajin T, Yokozeki H, Harada N, Suehiro M, Hide M, Nakagawa Y, Noguchi E, Nakamura M, Matsunaga K, Yagami A, Morita E, Mushiroda T. Genome-wide association study reveals an association between the HLA-DPB1*02:01:02 allele and wheat-dependent exercise-induced anaphylaxis. ***Am J Hum Genet*** 105, 1540-1548 (2021)

Fukunaga K, Kato K, Okusaka T, Saito T, Ikeda M, Yoshida T, Zembutsu H, Iwata N, Mushiroda T. Functional Characterization of the Effects of *N*-acetyltransferase 2 Alleles on *N*-acetylation of Eight Drugs and Worldwide Distribution of Substrate-Specific Diversity. ***Front Genet*** 12, 652704 (2021)

Fukunaga K, Hishinuma E, Hiratsuka M, Kato K, Okusaka T, Saito T, Ikeda M, Yoshida T, Zembutsu H, Iwata N, Mushiroda T. Determination of novel CYP2D6 haplotype using the targeted sequencing followed by the long-read sequencing and the functional characterization in the Japanese population. ***J Hum Genet*** 66, 139-149 (2021)

Invited presentations

Mushiroda T. "Current status and challenges of clinical implementation of pharmacogenomics" The 42nd Annual Scientific Meeting of the Japanese Society of Clinical Pharmacology and Therapeutics (Sendai, Japan) December 2021

Mushiroda T. "Pharmacogenomics in non-psychiatric disorders" The 117th Annual Meeting of the Japanese Society of Psychiatry and Neurology (Kyoto, Japan) September 2021

Mushiroda T. "Prediction of risk of severe adverse drug reactions based on pharmacogenomics" The 28th Annual Meeting of Non-Profit Organization Human and Animal Bridging Research Organization (Online) June 2021

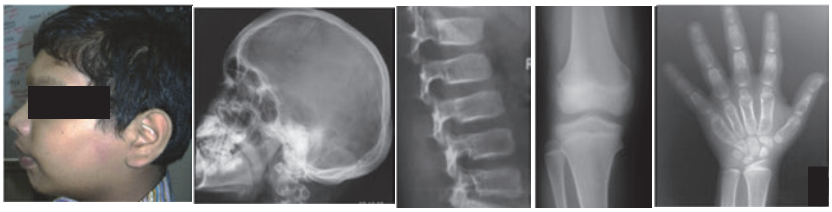
Mushiroda T. "A recent update of pharmacogenetic tests" The 4th Pharmacogenomics Seminar (Online) June 2021

Hikino K. "Individualized drug therapy and pharmacogenomics in pediatrics" The 47th Annual Meeting of the Japan Society of Developmental Pharmacology and Therapeutics (Online) Match 2021

Laboratory for
Bone and Joint Diseases

Team Leader: **Shiro Ikegawa**

Figure:
Clinical features of craniotubular dysplasia, Ikegawa type, caused by bi-allelic loss of function mutations in *TMEM53*. The patients have dysplasias of the spine and the long and short tubular bones.



1) Genomic Study of Common Diseases

Common bone and joint diseases are serious worldwide problems for health and the economy, as exemplified by the WHO initiative "Bone and Joint Decade" (2000-2010) and the "Locomotive syndrome 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 clarify their disease-causing mechanisms at the molecular level. Using the genome information obtained from these studies, we will realize our final goal of "personalized medicine". GWASs for OA, LDD/LDH, adolescent idiopathic scoliosis, OPLL, and ANF are in progress and we have succeeded in the identification of many susceptibility genes. Functional studies of the genes *in vitro* and using animal models are underway.

2) Genomic Study of Skeletal Dysplasia

Skeletal dysplasia is a group of heritable (monogenic) disorders affecting the skeleton, and more than 450 diseases belong to this category. Skeletal dysplasia is an intractable disease, so many patients are waiting for an effective treatment. We are engaged in clinical and basic studies of these difficult diseases. By large-scale mutation screening, including exome sequencing, we are identifying the disease-causative genes. By now, we have identified 30 novel genes including *TMEM53* for craniotubular dysplasia, Ikegawa type, and *SLC4A2* for osteopetrosis, Ikegawa type.

Through the analyses of phenotypes and diseases genes, we seek to understand the molecular mechanisms of bone and joint formation and the pathogenesis of common bone and joint diseases, as well as to contribute to the diagnosis and treatment of rare intractable diseases. Using the disease genes for skeletal dysplasia as candidate genes, we perform association studies for common bone and joint diseases corresponding to skeletal dysplasia, the so-called "rare to common" approach.

Recent Major Publications

Guo L, Iida A, Bhavani GS, Gowrishankar K, Wang Z, Xue JY, Wang J, Miyake N, Matsumoto N, Hasegawa T, Iizuka Y, Matsuda M, Nakashima T, Takechi M, Iseki S, Yambe S, Nishimura G, Koseki H, Shukunami C, Girisha KM, Ikegawa S. Deficiency of TMEM53 causes a previously unknown sclerosing bone disorder by dysregulation of BMP-SMAD signaling. ***Nat Commun*** 6, 2046 (2021)

Boer CG, Hatzikotoulas K, Southam L, Stefánsdóttir L, Zhang Y, Coutinho de Almeida R, Wu TT, Zheng J, .., Cheah KSE, Ikegawa S, Hveem K, Esko T, Wilkinson JM, Meulenbelt I, Lee MTM, van Meurs JBJ, Styrkársdóttir U, Zeggini E. Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. ***Cell*** 184, 4784-4818 (2021)

Xue JY, Grigelioniene G, Wang Z, Nishimura G, Iida A, Matsumoto N, Tham E, Miyake N, Ikegawa S, Guo L. SLC4A2 deficiency causes a new type of osteopetrosis. ***J Bone Miner Res*** 37, 226-235 (2021)

Invited presentations

Ikegawa S. "Skeletal Dysplasia from the Basics" The 32nd Annual Meeting of the Japan Pediatric Orthopedic Society (Okayama, Japan/Online) December 2021

Ikegawa S. "Genomic Study of Bone and Joint Diseases–present and future" The 36th Annual Meeting of the Japan Orthopedic Society Basic Research Meeting (Tokyo, Japan/Online) October 2021

Ikegawa S. "Progress in skeletal dysplasia diagnosis and experience in Japan" The Asia-Pacific Skeletal Dysplasia Meeting (Hong Kong/Online) May 2021

Ikegawa S. "Genomic Study of Rare Diseases of Skeleton" APAC MPS Summit 2021 (Taipei/Online) May 2021

Laboratory for

Genomics of Diabetes and Metabolism

Team Leader: Momoko Horikoshi

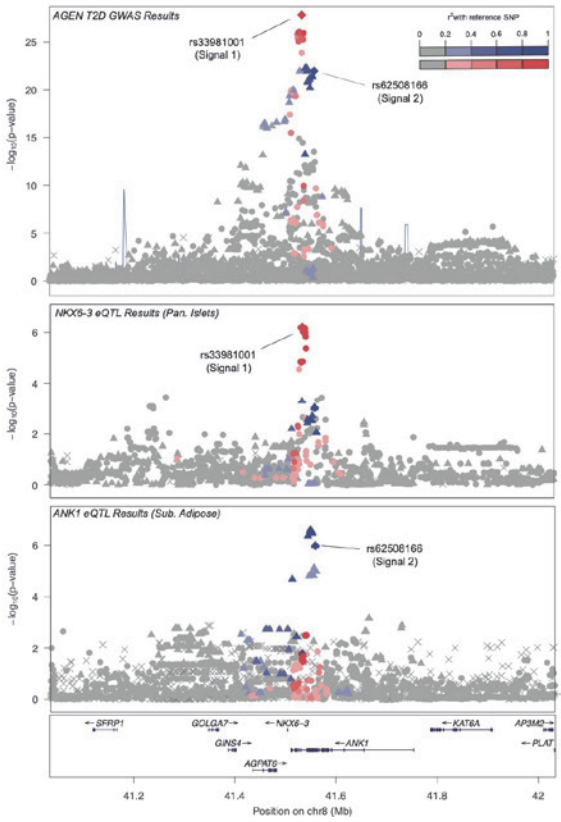


Figure: Two distinct T2D-association signals at the *ANK1-NKX6-3* locus are associated with expression levels of two transcripts in two tissues

Top, Regional association plot for East Asian sex-combined BMI-unadjusted meta-analysis at the *ANK1-NKX6-3* locus highlighting three distinct T2D-association signals ($P<1x10^{-5}$): signal 1, rs33981001; signal 2, rs62508166; signal 3, rs144239281. Variants are colored red and blue according to the strength of East Asian linkage disequilibrium (LD) with the lead variants of first and second signals, respectively. Middle, Variant rs12549902, which is in high LD (EAS LD r^2=0.80) with signal 1 rs33981001, shows the strongest association with the expression level of *NKX6-3* in pancreatic islets (n=118). Bottom, Variant rs516946, which is in high LD (EAS LD r^2=0.96) with signal 2 rs62508166, shows the strongest association with the expression level of *ANK1* in subcutaneous adipose tissue (n=770).

Our lab is interested in investigating the genetic background of diabetes and related metabolic traits that may help us better understand the underlying disease mechanisms. We have been focusing on the genetic contribution to type 2 diabetes (T2D) susceptibility in the Japanese population by using the rich genetic resources generated by Biobank Japan (BBJ). By using the full BBJ collection, we conducted a single population genome-wide association study (GWAS) of T2D in 191,764 Japanese. In addition to the then established >150 T2D loci, we identified 28 novel loci. We joined this effort with our international collaborators in the Asian Genetic Epidemiology Network for T2D (AGEN-T2D) to include 433,540 East Asian individuals in the GWAS meta-analysis. We identified 301 distinct association signals at 183 loci. Previously undescribed associations included signals in or near genes and a microRNA cluster that affects the differentiation of muscle and adipose tissues, which are essential in the development of T2D. Interestingly, expression quantitative trait loci at two overlapping T2D signals affect two genes in different tissues (Figure). Association studies in diverse populations demonstrated the benefit of identifying additional loci and elucidating disease-associated genes, biology and pathways. We are currently combining these results with those investigated in other ethnicities.

Recent Major Publications

Chen J*, Spracklen CN*, Marenne G*, Varshney A*, Corbin LJ*, Luan J, Willems SM, Wu Y, Zhang X, Horikoshi M, Boutin TS, Mägi R, Waage J, Li-Gao R, Chan KHK, Yao J, Anasanti MD, Chu AY, Claringbould A, Heikkinen J, Hong J, Hottenga JJ, Huo S, Kaakinen MA, Louie T, März W, Moreno-Macias H, Ndungu A, Nelson SC, Nolte IM, North KE, Raulerson CK, Ray D, Rohde R, Rybin D, Schurmann C, Sim X, Southam L, Stewart ID, Wang CA, Wang Y, Wu P, Zhang W, AhIuwalia TS, Appel EVR, Bielak LF, Brody JA, Burtt NP, Cabrera CP, Cade BE, Chai JF *et al.* The trans-ancestral genomic architecture of glycemic traits. ***Nat Genet*** 53, 840-860 (2021)

Imamura M, Takahashi A, Matsunami M, Horikoshi M, Iwata M, Araki SI, Toyoda M, Susarla G, Ahn J, Park KH, Kong J, Moon S, Sobrin L; International Diabetic Retinopathy and Genetics CONsortium (iDRAGON), Yamauchi T, Tobe K, Maegawa H, Kadowaki T, Maeda S. Genome-wide association studies identify two novel loci conferring susceptibility to diabetic retinopathy in Japanese patients with type 2 diabetes. ***Hum Mol Genet*** 30, 716-726 (2021)

Spracklen CN*, Horikoshi M*, Kim YJ*, Lin K*, Bragg F, Moon S, Suzuki K, Tam CHT, Tabara Y, Kwak SH, Takeuchi F, Long J, Lim VJY, Chai JF, Chen CH, Nakatochi M, Yao J, Choi HS, Iyengar AK, Perrin HJ, Brotman SM, van de Bunt M, Gloyn AL, Below JE, Boehnke M, Bowden DW, Chambers JC, Mahajan A, McCarthy MI, Ng MCY, Petty LE, Zhang W, Morris AP, Adair LS, Akiyama M, Bian Z, Chan JCN, Chang LC, Chee ML, Chen YI, Chen YT, Chen Z, Chuang LM, Du S, Gordon-Larsen P, Gross M, Guo X, Guo Y, Han S, Howard AG *et al.* Identification of type 2 diabetes loci in 433,540 East Asian individuals. ***Nature*** 582, 240-245 (2020)

*These authors jointly contributed to the work

Invited presentations

Horikoshi M. "Large-scale genome-wide association study of type 2 diabetes" The 64th Annual Meeting of the Japan Diabetes Society (Toyama, Japan/Online) May 2021

Horikoshi M. "Large-scale genome-wide association study of type 2 diabetes" The 11th Annual Meeting of Obesity and Digestive disease (Tokyo, Japan/Online) April 2021

Horikoshi M. "Large-scale genome-wide association study of type 2 diabetes" The 94th Annual Meeting of the Japan Endocrinology Society (Online) April 2021

Laboratory for

Cardiovascular Genomics and Informatics

Team Leader: **Kaoru Ito**

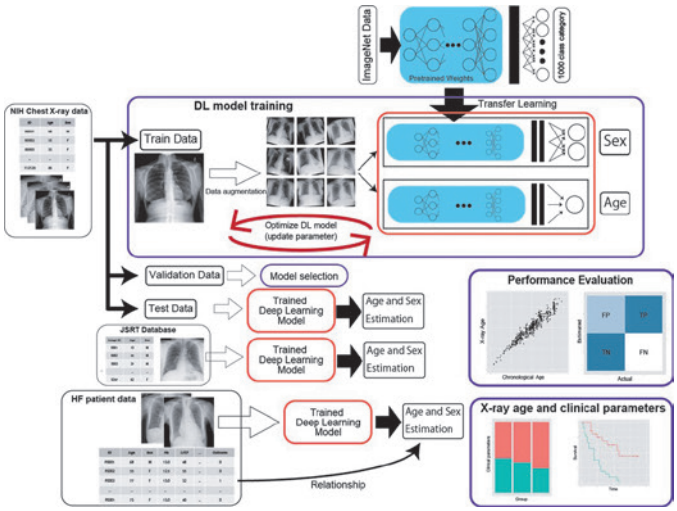


Figure: Deep learning-based cardiovascular age for clinical applications

The chest X-ray (CXR) dataset was randomly divided into training, validation and test datasets. Our deep neural network (DNN) models were trained to estimate age and sex using the training dataset. The weights of the models were initialized with pre-trained weights on ImageNet data and trained using transfer learning and fine-tuning techniques. Various models with different architectures were separately trained. Validation data were only used to tune the hyperparameters and to select the final model. The accuracy of the deep learning model was estimated using a hold-out test dataset. The independent dataset was also used to estimate the performance to verify the generalizability of the trained DNN in an independent population. The trained DNN was applied to CXRs of heart failure patients to evaluate the association between the estimated age (X-ray age) and various clinical parameters and the clinical outcomes of heart failure.

Recent Major Publications

Patel PN, Ito K, Willcox JAL, Haghighi A, Jang MY, Gorham JM, DePalma SR, Lam L, McDonough B, Johnson R, Lakdawala NK, Roberts A, Barton PJR, Cook SA, Fatkin D, Seidman CE, Seidman JG. Contribution of Noncanonical Splice Variants to TTN Truncating Variant Cardiomyopathy. ***Circ Genom Precis Med*** 14, e003389 (2021)

Hartiala JA, Han Y, Jia Q, Hilser JR, Huang P, Gukasyan J, Schwartzman WS, Cai Z, Biswas S, Trégouët DA, Smith NL; INVENT Consortium; CHARGE Consortium Hemostasis Working Group; GENIUS-CHD Consortium, Seldin M, Pan C, Mehrabian M, Lusis AJ, Bazeley P, Sun YV, Liu C, Quyyumi AA, Scholz M, Thiery J, Delgado GE, Kleber ME, März W, Howe LJ, Asselbergs FW, van Vugt M, Vlachojannis GJ, ... Ito K, Koyama S, Kamatani Y, Komuro I; Biobank Japan, Stolze LK, Romanoski CE, Khan MD, Turner AW, Miller CL, Aherrahrou R, Civelek M, Ma L, Björkegren JLM, Kumar SR, Tang WHW, Hazen SL, Allayee H. Genome-wide analysis identifies novel susceptibility loci for myocardial infarction. ***Eur Heart J*** 42, 919 (2021)

Miyazawa K, Ito K. The Evolving Story in the Genetic Analysis for Heart Failure. ***Front Cardiovasc Med*** 8, 646816 (2021)

Invited presentations

Ito K. Frontiers of Genomic Research on Lifestyle-related Diseases "Frontiers of Genomic Analysis of Cardiovascular Diseases" The 66th Annual Meeting of the Japan Society of Human Genetics and the 28th Annual Meeting of the Japanese Society for Gene Diagnosis and Therapy - Joint Conference 2021 (Yokohama/Online) October 2021

Ito K. Frontiers of Heart Failure Genomic Medicine "Heart Failure Research as a 'Common' Disease from a Genomic Perspective" The 25th Annual Scientific Meeting of the Japanese Heart Failure Society (Online) October 2021

Ito K. "How Should We Apply Omics on Cardiovascular Diseases to Clinical Medicine?" CVCT (Cardiovascular Clinical Trialist) - Asia-Pacific Forum (Online) July 2021

Ito K. The Promise and Peril of Next-Generation sequencing "Interpreting the Mendelian Genetic Disease Variant of Unknown Significance Using AI" The 67th Annual Meeting of the Japanese Heart Rhythm Society (Online) July 2021

Ito K. Genetic Cardiovascular Disease - From Mechanism to Phenotype and GWAS "Complex Genetic Architecture of Coronary Artery Disease Revealed by GWAS and Machine Learning" The 85th Annual Scientific Meeting of the Japanese Circulation Society (Yokohama/Online) March 2021

Cardiovascular diseases continue to be the leading cause of death worldwide. Therefore, understanding the pathogenesis of these diseases, applying it to clinical practice and identifying new therapeutic targets are important for world health. To this end, we are conducting research to elucidate the precise genetic mechanisms underlying these diseases and to promote the clinical applications of genomic information in clinical practice, using cutting-edge technologies such as whole-genome sequencing and machine learning in addition to statistical genetics.

Among these cardiovascular diseases, our team mainly targets not only common diseases such as atherosclerotic diseases, arrhythmias and heart failure, but also rare diseases such as Kawasaki disease, chronic thromboembolic pulmonary hypertension and cancer treatment-related cardiac dysfunction. At present, we are: 1) Conducting large-scale studies to understand the genetic underpinnings of ischemic heart disease, the most common atherosclerotic disease, and atrial fibrillation, the most common arrhythmia, as well as identifying the genetic differences between Japanese and Europeans, in collaboration with international consortia. 2) Developing and validating a new genetic analysis method based on a machine learning algorithm that solves the "P greater than N" scenario, where the sample size is small but the number of variants to be analyzed is large. 3) Elucidating of the mechanism of rare cardiovascular diseases using human omics data from multi-center patients in Japan. 4) Performing prospective cohort studies to examine the possibility of clinical application of genomic information. 5) Developing a functional analysis system using massively parallel *in vitro* assays with artificial intelligence. 6) Developing and validating deep learning-based cardiovascular age for clinical applications. We also play an important role in genomic analyses of AMED GRIFIN and AMED intractable disease projects for cardiovascular diseases.

Our ultimate goal is to provide better genome-informed diagnostic/management/treatment approaches to patients suffering from cardiovascular diseases and to medical professionals fighting on the front lines of clinical practice.

Laboratory for Systems Genetics

Team Leader: Yukinori Okada

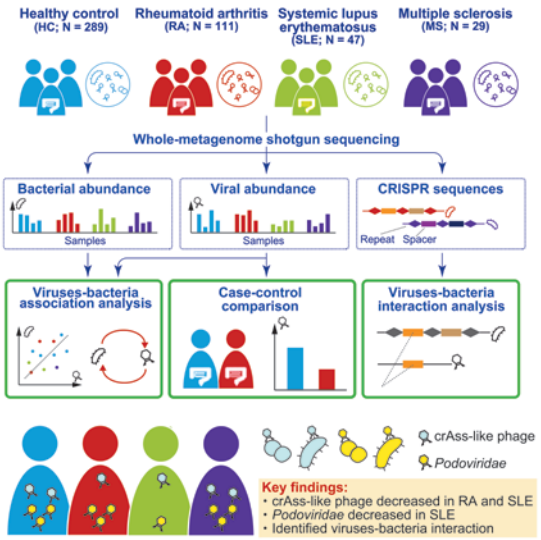


Figure: Whole gut virome analysis of autoimmune diseases
We constructed *in silico* pipelines to quantify the human gut virome from metagenome shotgun sequencing data. Application of the pipeline to the shotgun sequencing of 476 Japanese identified novel features of the gut virome in autoimmune diseases, including decreased crAss-like phages in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Recent Major Publications

Tomofuji Y, Kishikawa T, Maeda Y, Ogawa K, Nii T, Okuno T, Oguro-Igashira E, Kinoshita M, Yamamoto K, Sonehara K, Yagita M, Hosokawa A, Motooka D, Matsumoto Y, Matsuoka H, Yoshimura M, Ohshima S, Nakamura S, Inohara H, Mochizuki H, Takeda K, Kumanogoh A, Okada Y. Whole gut virome analysis of 476 Japanese revealed a link between phage and autoimmune disease. ***Ann Rheum Dis*** 81, 278-288 (2022)

Okada Y, Wang QS. A massive effort links protein-coding gene variants to health. ***Nature*** 599, 561-563 (2021)

Sakaue S, Kanai M, Tanigawa Y, Karjalainen J, Kurki M, Koshiba S, Narita A, Konuma T, Yamamoto K, Akiyama M, Ishigaki K, Suzuki A, Suzuki K, Obara W, Yamaji K, Takahashi K, Asai S, Takahashi Y, Suzuki T, Shinozaki N, Yamaguchi H, Minami S, Murayama S, Yoshimori K, Nagayama S, Obata D, Higashiyama M, Masumoto A, Koretsune Y; FinnGen, Ito K, Terao C, Yamauchi T, Komuro I, Kadowaki T, Tamiya G, Yamamoto M, Nakamura Y, Kubo M, Murakami Y, Yamamoto K, Kamatani Y, Palotie A, Rivas MA, Daly MJ, Matsuda K, Okada Y. A cross-population atlas of genetic associations for 220 human phenotypes. ***Nat Genet*** 53, 1415-1424 (2021)

Invited presentations

Okada Y. "Statistical genetics, disease biology, drug discovery, and personalized medicine" The 50th Annual Meeting of The Japanese Society for Immunology (Nara, Japan) November 2021

Okada Y. "Big data analysis of autoimmune diseases" 41st Annual Scientific Meeting of Korean College of Rheumatology 2021 (Seoul, Korea/Online) October 2021

Okada Y. "Cross-population Mendelian randomization analysis and application for novel drug discovery" Mendelian Randomization Conference 2021 (Bristol, England/Online) July 2021

Okada Y. "Drug discovery through genomic analysis" The 65th Annual General Assembly and Scientific Meeting of the Japan College of Rheumatology (Kobe, Japan) April 2021

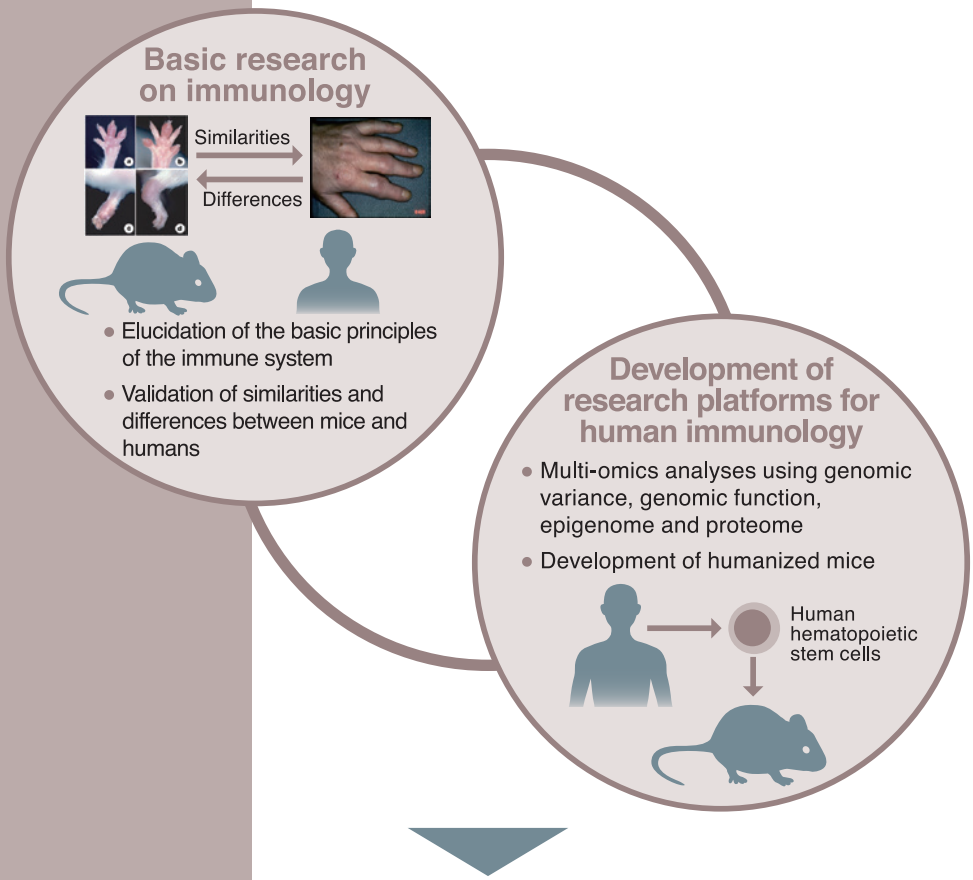
Okada Y. "Statistical genetics, disease biology, and drug discovery" Complex Disease Genetics Day 2021 (Helsinki, Finland/Online) January 2021

The genome is a blueprint of humans. The goal of our team is to decipher the blueprint and elucidate hidden biology. We focus on large-scale human genome, omics, and phenome data and the underlying high-dimensional network. By developing novel methodologies in statistical genetics and machine learning, we redefine human biology as systems of genetics. Our team aims to elucidate disease biology, leading to novel drug discovery and implementation of genomic personalized medicine.

In 2021, our team conducted a series of large-scale human genomics studies by utilizing newly developed computational methodologies. Cross-population genome-wide association study (GWAS) meta-analysis integrating global biobanks (Biobank Japan, UK Biobank and FinnGen) identified thousands of genetic variants associated with a wide range of human phenotypes. Deconvolution of the phenome-wide GWAS summary statistics using Truncated Singular Value Decomposition (TSVD) revealed the hidden biology of human complex diseases (Sakaue S and Kanai M *et al. Nat Genet* 2021).

The microbiome is the genetic material of all the microbes that live on and inside the human body and plays essential roles in a variety of human diseases. As exploitation of new human omics layers, we developed *in silico* pipelines to quantify the entire human gut virome. Based on the shotgun sequencing of 476 Japanese, we conducted a case-control comparison of viral abundance in multiple autoimmune diseases. We found that crAss-like phages, one of the main components of a healthy gut virome, were significantly decreased in the gut of patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). A quantitative virus-bacterium association analysis identified symbiosis between *Podoviridae* and *Faecalibacterium*. Our study suggested that the gut virome can affect our body either directly or via bacteria and presented new candidates that contribute to the development of autoimmune diseases (Tomofuji Y *et al. Ann Rheum Dis* 2022).

Division of Human Immunology



Division of Human Immunology will elucidate the principles of the immune system and develop a research platform for human immunology.

Laboratory for
Autoimmune Diseases

Team Leader: Kazuhiko Yamamoto

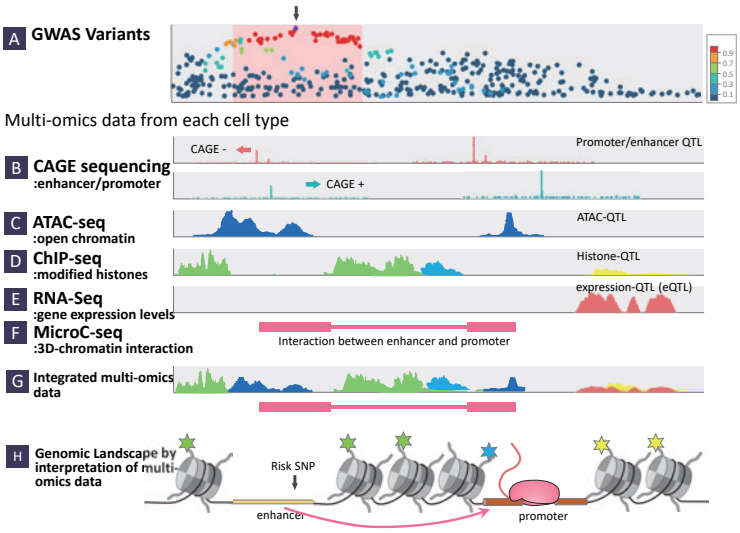


Figure: Functional genetics of autoimmune diseases

Recent Major Publications

Sakaue S, Kanai M, Tanigawa Y, Karjalainen J, Kurki M, Koshiba S, Narita A, Konuma T, Yamamoto K, Akiyama M, Ishigaki K, Suzuki A, Suzuki K, Obara W, Yamaji K, Takahashi K, Asai S, Takahashi Y, Suzuki T, Shinozaki N, Yamaguchi H, Minami S, Murayama S, Yoshimori K, Nagayama S, Obata D, Higashiyama M, Masumoto A, Koretsune Y, FinnGen; Ito K, Terao C, Yamauchi T, Komuro I, Kadowaki T, Tamiya G, Yamamoto M, Nakamura Y, Kubo M, Murakami Y, Yamamoto K, Kamatani Y, Palotie A, Rivas MA, Daly MJ, Matsuda K, Okada Y. A cross-population atlas of genetic associations for 220 human phenotypes. ***Nat Genet*** 53, 1415-1424 (2021)

Ota M, Nagafuchi Y, Hatano H, Ishigaki K, Terao C, Takeshima Y, Yanaoka H, Kobayashi S, Okubo M, Shirai H, Sugimori Y, Maeda J, Nakano M, Yamada S, Yoshida R, Tsuchiya H, Tsuchida Y, Akizuki S, Yoshifuji H, Ohmura K, Mimori T, Yoshida K, Kurosaka D, Okada M, Setoguchi K, Kaneko H, Ban N, Yabuki N, Matsuki K, Mutoh H, Oyama S, Okazaki M, Tsunoda H, Iwasaki Y, Sumitomo S, Shoda H, Kochi Y, Okada Y, Yamamoto K, Okamura T, Fujio K. Dynamic landscape of immune cell-specific gene regulation in immune-mediated diseases. ***Cell*** 184, 3006-3021 (2021)

Suzuki A, Guerrini MM, Yamamoto K. Functional genomics of autoimmune diseases. ***Ann Rheum Dis*** 80, 689-697 (2021)

Invited presentations

Yamamoto K. "Dynamic Landscape of immune cell-specific gene regulation" The 13th International Forum on Rheumatoid Arthritis (IFRA2021) (Beijing, China/Online) September 2021

The majority of the disease-susceptibility genetic variants revealed by genome-wide association study (GWAS) function as expression quantitative trait loci (eQTLs) that are involved in gene expression. The eQTL effect is frequently found in a cell type-specific manner. Such cell specificity is largely due to epigenomic alterations, such as cell type-specific histone modifications and DNA methylation in specific regions of chromosomes. It has been further reported that disease susceptibility variants significantly overlap with the location of histone modifications in promoters and active enhancers of specific immune cell subsets. Therefore, it is possible to provide causal information to the intermediate phenotypes between genetic information and disease by qualitatively and quantitatively measuring the functional molecules of immunocompetent cells in various immunological diseases, focusing on disease-susceptible genetic variants, gene expression (mRNA), epigenome, and proteins.

We are performing functional genomic strategies for integrating GWAS results with the current understanding of specific diseases, mainly focusing on promoters, enhancers, and long non-coding RNAs. We are isolating nearly 30 different lymphocyte subsets from human peripheral blood mononuclear cells of healthy individuals and analyzing genotypes, gene expression, and open chromatin regions. Cells from healthy individuals are expected to exhibit the least biased gene expression and thus can provide a baseline for comparison with cells from patients with immunological diseases. Since chromosomes are transmitted to offspring in certain blocks and many neighboring genetic variants behave similarly, it is not easy to determine which among them is the true causal variant. Using several methods including CAGE analysis, we are tracking down the targets.

We are also performing several analyses using recently developed single-cell technologies. By integrating these fragments of information, we can understand the physiological and pathological changes of the whole immune system of an individual and the ways for intervening when there are unfavorable conditions.

Laboratory for
Cell Signaling

Team Leader: **Takashi Saito**

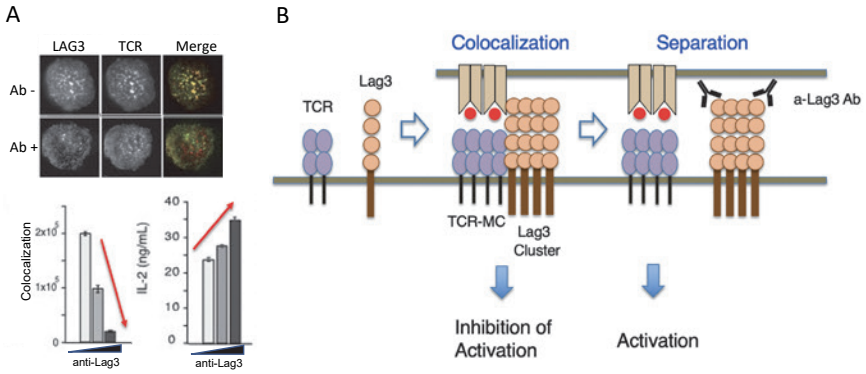


Figure: Negative regulation of T cell activation through LAG3 colocalized with TCR microclusters
A. LAG3 generates clusters colocalized with TCR-microcluster (MC) upon T cell activation. The LAG3 mAb inhibited the colocalization between TCR-MC and LAG3 clusters while enhancing IL-2 production. B. Schematic illustration showing that colocalization of LAG3 clusters with a TCR-MC is critical for LAG3 to suppress T cell activation, which is inhibited by the LAG3 mAb.

Recent Major Publications

Kumagai A, Nara T, Uematsu M, Kakinuma Y, Saito T, Masuda K. Development and characterization of a unique anti-IgE monoclonal antibody cross-reactive between human and canine IgE. ***Immun Inflamm Dis*** 9, 1740-1748 (2021)

Imanishi T, Unno M, Kobayashi W, Yoneda N, Akira S, Saito T. mTORC1 signaling controls TLR2-mediated T cell activation by inducing TIRAP expression. ***Cell Reports*** 32, 107911 (2020)

Imanishi T, Saito T. T cell co-stimulation and functional modulation by innate signals. ***Trends Immunol*** 41, 200-212 (2020)

Invited presentations

Saito T. "Regulation of adhesion and activation of T cells at Immune synapse" The 44th Annual Meeting of Molecular Biology Society of Japan 2021 (Yokohama, Japan/Online) December 2021

Saito T. "Inhibition of T cell activation through PD-1/LAG-3 clusters and their modulation by checkpoint inhibitors" OIST Conference (Okinawa, Japan/Online) February 2021

The objective of our team is to determine the molecular mechanisms of T cell activation, differentiation and function. Ultimately, we wish to elucidate the onset of immune diseases and to modulate T cell function/activation to prevent immune diseases such as autoimmunity and allergic inflammation. For this purpose, we have analyzed the regulation of T cell activation/function from a signaling perspective.

Our finding that TCR-microclusters (MC) initiate T cell activation led us to analyze the dynamics of signaling molecules at the immune synapse. Similar to our previous studies of CTLA4 and PD-1, we have analyzed the dynamic regulation of another inhibitory co-stimulation receptor, LAG3. This inhibitory receptor was also colocalized with the TCR-MC upon TCR stimulation to mediate inhibition of T cell activation. Since this association is critical for the inhibitory function, a LAG3 mAb induced separation of the LAG3 cluster from the TCR-MC and, consequently, enhancement of T cell activation. Our analyses provide a dynamic view of signal regulation to define inhibitory mechanisms (Figure).

We have analyzed negative regulation of T cell activation, particularly by the autoimmune-related protein tyrosine phosphatase PTPN22. Its deficiency resulted in enhanced activation and an increase in effector/memory T cells. Analysis of the associated proteins revealed that PTPN22 was recruited to the TCR-MC to comprise an "inhibitory complex" with other inhibitory molecules to inhibit activation. A PTPN22 mutant causing susceptibility to autoimmune diseases was defective in recruitment to the TCR-MC. These studies help define the autoimmune susceptibility caused by the mutation.

We have also analyzed the modulation of T cell function by innate-like signals. During this analyses, we found that Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) plays a critical role in T cell function and differentiation. We are analyzing T cell-specific RIPK1-deficient mice to determine its role in T cells on metabolism and aging.

Laboratory for

Lymphocyte Differentiation

Team Leader: **Tomohiro Kurosaki**

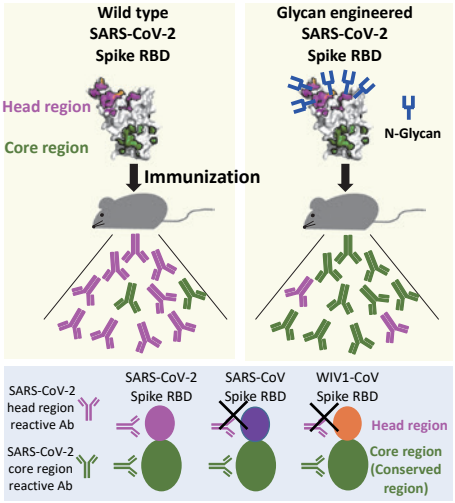


Figure: How to construct protective vaccines against not only SARS-CoV-2 but also SARS-CoV and WIV1-CoV viruses

In order to induce antibodies predominantly against regions that are structurally conserved in the RBD among SARS-related viruses, glycan engineering was performed to mask the dominant epitope on the RBD head region, which is a structurally non-conserved region. When mice are immunized with this modified RBD vaccine, as expected, antibodies are predominantly induced that recognize the structurally conserved core-RBD region of not only SARS-CoV-2 but also other related SARS-related viruses such as SARS-CoV and WIV1-CoV and were also highly protective against these SARS-related viruses.

Recent Major Publications

Shinnakasu R, Sakakibara S, Yamamoto H, Wang PH, Moriyama S, Sax N, Ono C, Yamanaka A, Adachi Y, Onodera T, Sato T, Shinkai M, Suzuki R, Matsuura Y, Hashii N, Takahashi Y, Inoue T, Yamashita K, Kurosaki T. Glycan engineering of the SARS-CoV-2 receptor-binding domain elicits cross-neutralizing antibodies for SARS-related viruses. ***J Exp Med*** 218, e20211003 (2021)

Inoue T, Shinnakasu R, Kawai C, Ise W, Kawakami E, Sax N, Oki T, Kitamura T, Yamashita K, Fukuyama H, Kurosaki T. Exit from germinal center to become quiescent memory B cells depends on metabolic reprograming and provision of a survival signal. ***J Exp Med*** 218, e20200866. (2021)

Fukuyama H, Shinnakasu R, Kurosaki T. Influenza vaccination strategies targeting the hemagglutinin stem region. ***Immunol Rev*** 296, 132-141 (2020)

Invited presentations

Kurosaki T. "Strategy of Vaccine Development for Variant Viruses" Biopharma EXPO 2021 (Chiba, Japan) December 2021

Kurosaki T. "Function of memory B cells and their generation mechanism" FIMSA 2021, 8th FIMSA Congress (Busan, Korea/Online) October 2021

Kurosaki T. "When designing dream vaccines, consider the memory B cell behavior" Seminar at Okinawa Institute of Science and Technology Graduate University (Okinawa, Japan) April 2021

Kurosaki T. "Immune regulation through B lymphocytes" Seminar at Osaka University (Suita, Japan) March 2021

The coronavirus disease 2019 (COVID-19) pandemic, caused by the β-coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global health crisis. In this year, we carried out three major studies: 1) In order to obtain future effective therapeutic antibodies, we identified the antibody repertoire for SARS-CoV-2 viruses that was generated in infected individuals; 2) In order to make broadly protective vaccines against SARS-related coronaviruses, we designed new types of vaccines and tested whether they work in a mouse model system; 3) In addition, we applied our new type of adjuvant for use in COVID-19 vaccines by expanding our work on influenza vaccine development. This new adjuvant is designed for use in a patch vaccine in the future.

SARS-related coronaviruses may cause future outbreaks, therefore broadly protective vaccines are urgently needed. When looking carefully at the structure of the receptor-binding region (RBD) of the spike protein of the SARS-CoV-2 virus, we see that it is composed of the head and the core sub-region, which is directly involved in the entry of SARS-CoV-2 and contributes to the overall structural stability of the RBD region, respectively. Given that the core sub-domain is structurally well conserved among SARS-related viruses, we intended to make new types of vaccines to elicit neutralizing antibodies against the core sub-domain. For this purpose, we introduced N-linked glycans onto the SARS-CoV-2 RBD surfaces and used them as immunogens in a mouse model. These types of vaccines indeed elicited significant neutralizing activity for not only SARS-CoV-2 but also SARS-related viruses such as bat WIV1-CoV. Apart from antigens, adjuvants are also very important for efficient humoral responses. As mentioned above, we had already developed a new type of adjuvant that facilitates the Tfh-B cell axis, thereby inducing high-quality and quantity antibodies. Combining this novel type of adjuvant with glycan-engineered antigens for SARS-related viruses, we are attempting to add this new adjuvant for maximizing the production of broadly neutralizing antibodies.

Laboratory for

Transcriptional Regulation

Team Leader: **Ichiro Taniuchi**

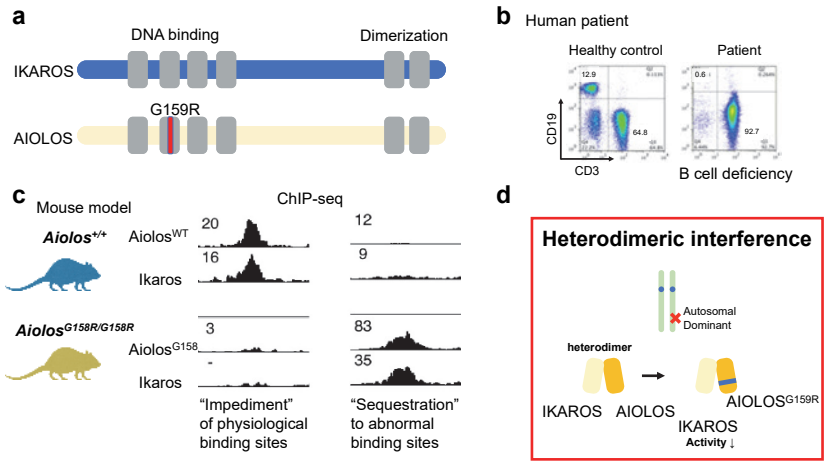


Figure: A heterodimeric interference is a novel pathogenic mechanism for inborn errors of immunity (IEI)

(a) Structure of IKAROS and AIOLOS proteins. IKZF family proteins possess four Zinc -finger and two Zinc-finger domains at the middle and C-terminus, respectively. We isolated an *AIOLOS*^{G159R} missense variant from a human IEI family case. (b) Flow cytometry analyses of peripheral blood showing a lack of CD19^{+} B lymphocytes in the patient. (c) ChIP-seq analyses using thymocytes from a mouse harboring the corresponding mutation *Aiolos*^{G158R} revealed impaired DNA binding of wild type Ikaros when Ikaros formed a heterodimer with mutant Aiolos^{G158R} protein. (d) Graphic summary of the novel pathogenic mechanism, heterodimeric interference, for autosomal dominant genetic disorders. IKAROS function is hijacked by the AIOLOS^{G159R} variant.

Recent Major Publications

Yamashita M, Kuehn HS, Okuyama K, Okada S, Inoue Y, Mitsuiki N, Imai K, Takagi M, Kanegane H, Takeuchi M, Shimojo N, Tsumura M, Padhi AK, Zhang KYJ, Boisson B, Casanova JL, Ohara O, Rosenzweig SD, Taniuchi I, Morio T. A variant in human AIOLOS impairs adaptive immunity by interfering with IKAROS. ***Nat Immunol*** 22, 893-903 (2021)

Kuehn HS, Chang J, Yamashita M, Niemela JE, Zou C, Okuyama K, Harada J, Stoddard JL, Nunes-Santos CJ, Boast B, Baxter RM, Hsieh EWY, Garofalo M, Fleisher TA, Morio T, Taniuchi I, Dutmer CM, Rosenzweig SD. T and B cell abnormalities, pneumocystis pneumonia, and chronic lymphocytic leukemia associated with an AIOLOS defect in patients. ***J Exp Med*** 218, e20211118 (2021)

Andrews LP, Vignali KM, Szymczak-Workman AL, Burton AR, Brunazzi EA, Ngiow SF, Harusato A, Sharpe AH, Wherry EJ, Taniuchi I, Workman CJ, Vignali DAA. A Cre-driven allele-conditioning line to interrogate CD4^{+} conventional T cells. ***Immunity*** 54, 2209-2217 (2021)

Invited presentations

Taniuchi I. "Novel pathogenesis of human primary immunodeficiency by missense variants in transcription factors" 14th International Symposium on Nanomedicine (Matsue, Japan/Online) November 2021

Okuyama K. "Transcriptional regulation and chromatin remodeling by a zinc finger protein, Bcl11b" 17th Primate Research Forum (Tsukuba, Japan) November 2021

Taniuchi I. "Aging-related changes in thymic microenvironment in mice" RIKEN IMS Joint Human and Mouse Cell Atlas Meeting (Yokohama, Japan/Online) October 2021

The immune system consists of many types of hematopoietic cells. The development of those immune cells from hematopoietic stem cells is regulated by the precise decoding of genomic information by transcription factors. My laboratory has been addressing how transcription factors control immune cell development. Lymphocytes with a variety of antigen-specificities are the main players in the adaptive immune system. The primary developmental program of T lymphocytes that occurs in the thymus has been evolved to select useful and non-self-reactive immune soldiers using a sophisticated nuclear program that integrates environmental cues sensed by T cell antigen receptors (TCR). Another research aim in my laboratory is to understand how TCR signals are sensed and are coupled with cell fate determination programs in the nucleus by using the helper-versus cytotoxic-lineage choice as a model.

Inborn errors in immunity (IEI), also known as primary immunodeficiencies (PID), are genetic disorders that result in increased susceptibility to infectious disease, autoinflammatory disease and autoimmunity. IEIs are often caused by genetic variants in a single gene. Animal models serve as a powerful resource to unravel the pathogenesis of IEIs. By characterizing two familial IEI cases, we identified two *AIOLOS* variants, *AIOLOS*^{G159R} and *AIOLOS*^{N160S}, as causal for the IEIs. IKAROS family proteins, which consist of six members, function as homo- or heterodimers. Using mouse models harboring the corresponding human variant *AIOLOS*^{G159R}, we revealed that *AIOLOS*^{G159R} protein hijacks IKAROS function at least by sequestration of IKAROS protein from its normal binding genomic regions. We designate this novel pathogenic mechanism caused by autosomal dominance as "heterodimeric interference", a concept that could be applied to a wide range of diseases caused by a disorder of multimer protein complexes.

Laboratory for

Immune Cell Systems

Team Leader: Shigeo Koyasu

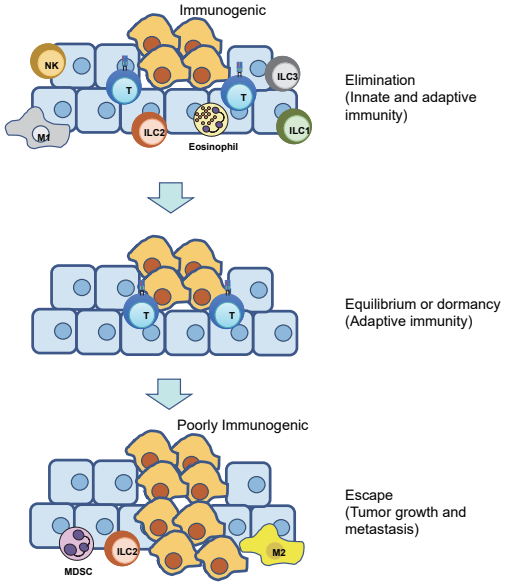


Figure: Concept of cancer immunoediting by innate lymphoid cells (ILCs)
Cancer immunoediting is composed of three phases: elimination, equilibrium, and escape. In the elimination phase, ILCs alone or in combination with several different components of the innate and adaptive immune system may protect the host from tumor formation. However, if this process is not successful, tumor cells may enter the equilibrium phase, in which they may be either maintained chronically or immunologically sculpted by the components of the adaptive immunity to produce new populations of tumor variants. These variants may eventually acquire the ability to circumvent recognition and destruction by ILCs through a variety of mechanisms and become clinically detectable during the escape phase. Abbreviations: M1, pro-inflammatory M1 macrophages; M2 anti-inflammatory M2 macrophages; MDSC, myeloid-derived suppressor cells.

Recent Major Publications

Wagner M, Koyasu S. Innate lymphoid cells in skin homeostasis and malignancy. ***Front Immunol*** 12, 758522 (2021)

Ishihama H, Ishii K, Nagai S, Kakinuma H, Sasaki A, Yoshioka K, Kuramoto T, Shiono Y, Funao H, Isogai N, Tsuji T, Okada Y, Koyasu S, Toyama Y, Nakamura M, Aizawa M, Matsumoto M. An antibacterial coated polymer prevents biofilm formation and implant-associated infection. ***Sci Rep*** 11, 3602 (2021)

Sumiyoshi M, Kotani Y, Ikuta Y, Suzue K, Ozawa M, Katakai T, Yamada T, Abe T, Bando K, Koyasu S, Kanaho Y, Watanabe T, Matsuda S. Arf1 and Arf6 synergistically maintain survival of T cells during activation. ***J Immunol*** 206, 366-375 (2021)

We have been studying the function of group 2 innate lymphoid cells (ILC2) capable of producing large amounts of type 2 cytokines, such as IL-4, IL-5 and IL-13. Although ILC2s are rare in secondary lymphoid organs relative to other immune cells, they harbor a unique location within non-lymphoid tissues, especially skin, mucosal barriers and adipose tissues. Despite intensive studies on the role of ILC2 in allergic disorders, their role in anti-tumor immunity has been obscure and controversial. Cancer immunoediting comprises three phases: elimination (immunosurveillance by immune cells), equilibrium (preventing tumor outgrowth and sculpting tumor immunogenicity by adaptive immune cells) and escape (expression of suppressing ligand such as PD-L1 by tumor cells and recruitment of immunosuppressive cells). The study of ILCs in cancer is still in its infancy. Indeed, ILCs have been separately associated with tumor-promoting as well as tumor-suppressing activities and involvement of ILCs in cancer immunoediting has been obscure. We have shown that ILC2s play a tumor-suppressing role in a mouse melanoma system. In humans, expression of *IL33* (activation of ILC2) and *SIGLEC8* (recruitment of eosinophils) are associated with better overall survival in melanoma patients, whereas no correlation was observed between the expression levels of *IL33* and *SIGLEC8* and overall survival for some other tumors, such as lung squamous cell carcinoma and pancreatic adenocarcinoma. Among ILCs, NK cells are well known for their antiviral and antitumor activities. ILC1s produce IFN-γ, which induces proinflammatory M1 macrophages. ILC2s recruit and activate eosinophils through the release of IL-5, which displays suppressing activity for tumors such as melanoma. On the other hand, ILC2s induce anti-inflammatory M2 macrophages. The presence of ILC3s correlates with the density of tertiary lymphoid structures that are associated with favorable prognosis in some tumors such as non-small cell lung carcinoma. A more comprehensive understanding of how these mechanisms develop will be crucial in harnessing the power of immunotherapy to treat a variety of tumors.

Laboratory for

Innate Immune Systems

Team Leader: Kazuyo Moro

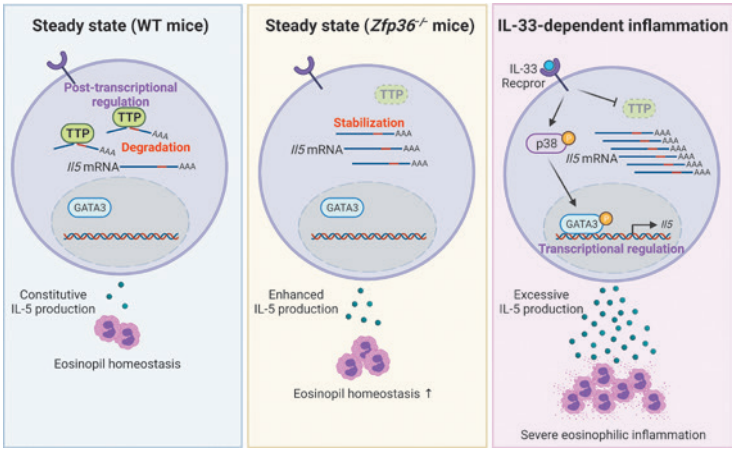


Figure: Posttranscriptional regulation of ILC2 homeostatic function via TTP
IL-33 induces a large amount of IL-5 from activated-ILC2s by transcriptional regulation. On the other hand, in steady-state ILC2s, excessive cytokine production is suppressed by TTP-mediated posttranscriptional regulation.

Recent Major Publications

Hikichi Y, Motomura Y, Takeuchi O, Moro K. Posttranscriptional regulation of ILC2 homeostatic function via tristetraprolin. ***J Exp Med*** 218, e20210181 (2021)

Momiuchi Y, Motomura Y, Suga E, Mizuno H, Kikuta J, Morimoto A, Mochizuki M, Otaki N, Ishii M, Moro K. Group 2 innate lymphoid cells in bone marrow regulate osteoclastogenesis in a reciprocal manner via RANKL, GM-CSF and IL-13. ***Int Immunol*** 33, 573-585 (2021)

Sudo T, Motomura Y, Okuzaki D, Hasegawa T, Yokota T, Kikuta J, Ao T, Mizuno H, Matsui T, Motooka D, Yoshizawa R, Nagasawa T, Kanakura Y, Moro K, Ishii M. Group 2 innate lymphoid cells support hematopoietic recovery under stress conditions. ***J Exp Med*** 218, e20200817 (2021)

Invited presentations

Moro K. "Fibroblast-derived IL-33 causes pulmonary fibrosis via activation of ILC2" The 50th Annual Meeting of the Japanese Society for Immunology (Kyoto, Japan) December 2021

Moro K. "Fibroblast-derived IL-33 causes pulmonary fibrosis via activation of ILC2s" British Society for Immunology Congress 2021 (Edinburgh, UK/Online) November 2021

Moro K. "Single-cell analysis and the innate lymphoid cells" The 25th Congress of the Asian Pacific Society of Respirology (Kyoto, Japan/Online) November 2021

Moro K. "Single cell RNA sequence analysis in ILC2s-related diseases" The Human Cell Atlas-Asia 2021 meeting (Nedlands, Australia/Online) November 2021

Moro K. "Group 2 and 3 innate lymphoid cells drive spontaneous pulmonary fibrosis" The 27th International Symposium on Molecular Cell Biology of Macrophages (Osaka, Japan) June 2021

We have investigated the remarkable properties of innate immunity through studying group 2 innate lymphoid cells (ILC2), an innate lymphocyte lineage that we identified in 2010. ILC2 contribute to immune responses by secreting effector cytokines such as IL-5 and IL-13 and regulate the functions of both immune and non-immune cells. ILC2 play a pathogenic role in allergic diseases in barrier tissues including lungs, intestines, and skin. Aiming at advancing therapeutic strategies, we dissect how ILC2 form communication networks with other cells and how these networks malfunction in disease.

ILC2s are unique in their ability to produce low levels of type 2 cytokines at steady-state. However, it was unknown how this constitutive cytokine production is regulated. We reported that tristetraprolin (TTP/*Zfp36*), an RNA-binding protein that induces mRNA degradation, is highly expressed in naive ILC2s and downregulated following IL-33 stimulation. In ILC2s from *Zfp36*$^{-/-}$ mice, constitutive IL-5 production is elevated owing to the stabilization of its mRNA, which results in an increased number of eosinophils in the intestine. A luciferase assay demonstrated that TTP directly regulates *Il5* mRNA stability and that overexpression of TTP markedly suppresses IL-5 production by ILC2s, even under IL-33 stimulation. Collectively, TTP-mediated posttranscriptional regulation acts as a deterrent of excessive cytokine production in steady-state ILC2s to maintain body homeostasis.

ILC2s are tissue-resident cells that play different roles in different organs by sensing environmental factors in their surroundings. Initially, it was thought that ILC2s in bone marrow (BM) are progenitors for systemic ILC2s, which migrate to other organs and acquire effector functions. However, accumulating evidence that ILC2s differentiate in peripheral tissues suggests that BM ILC2s may play a specific role in the BM as a unique effector per se. We demonstrated that BM ILC2s highly express RANKL, a robust cytokine for osteoclast differentiation and activation, and that RANKL expression on ILC2s is up-regulated by IL-2 and IL-7. BM ILC2s co-cultured with BMMs in the presence of IL-7 induce the differentiation of TRAP-positive osteoclasts in a RANKL-dependent manner. In contrast, BM ILC2s stimulated with IL-33 down-regulate RANKL expression and convert BMM differentiation into M2 macrophage-like cells rather than osteoclasts by GM-CSF and IL-13 production. These results suggest that ILC2s regulate osteoclast activation and contribute to bone homeostasis in both steady-state and IL-33-induced inflammation.

Laboratory for

Immune Homeostasis

Team Leader: Taishin Akiyama

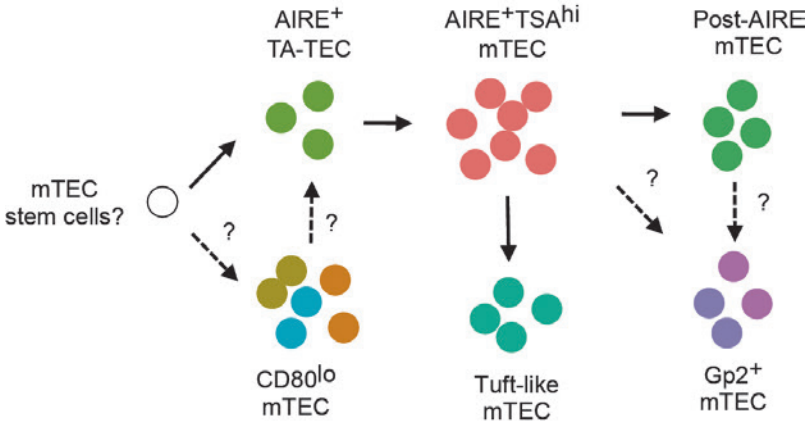


Figure: A proposed mechanism for the differentiation of mTECs in adult thymus
Transit amplifying AIRE$^+$ mTECs (TA-TEC) differentiate into AIRE$^+$ mTEC and subsequently other mature types of mTEC (Post-AIRE mTEC, Tuft-like mTEC and Gp2$^+$ mTEC). The stem/progenitor cells of adult mTECs remain elusive.

Recent Major Publications

Akiyama T, Yamamoto T. Regulation of Early Lymphocyte Development via mRNA Decay Catalyzed by the CCR4-NOT Complex. ***Front Immunol*** 12, 715675 (2021)

Akiyama T, Suzuki T, Yamamoto T. RNA decay machinery safeguards immune cell development and immunological responses. ***Trends Immunol*** 42, 447 (2021)

Ishikawa T, Akiyama N, Akiyama T. In Pursuit of Adult Progenitors of Thymic Epithelial Cells. ***Front Immunol*** 12, 621824 (2021)

Invited presentations

Akiyama T. "Single Cell RNA-seq Analysis of Human Thymic Epithelial Neoplasms" RIKEN IMS Joint Human and Mouse Cell Atlas Meeting (Yokohama, Japan/Online) October 2021

Akiyama T. "Mechanisms of thymic T cell selection and onset of autoimmune diseases" Rheumatology Conference 2021 (Tokyo, Japan/Online) June 2021

Induction of T cell self-tolerance in the thymus is controlled by medullary thymic epithelial cells (mTECs), which express thousands of Tissue-specific Antigens (TSAs), regulated by the AIRE transcription factor. TSAs are directly or indirectly presented to T cells in the thymus. When recognizing TSAs with high affinity, developing T cells undergo apoptosis or are converted into regulatory T cells in the thymus.

mTEC turnover is homeostatic in the adult thymus with a duration of approximately 2 weeks. However, cellular and molecular mechanisms underlying the maintenance of adult mTECs remain elusive. We performed an integrative analysis of single-cell assays for transposase accessible chromatin (scATAC-seq) and single-cell RNA sequencing (scRNA-seq) of adult murine TECs. Data analysis suggested that AIRE-expressing (AIRE$^+$) mTECs are separated into two subsets with distinct gene expression profiles and chromatin accessibility. One subset expresses a high level of proliferation markers and may be equivalent to transit-amplifying cells (TACs). In general, TACs are defined as a proliferative cell population linking stem cells and differentiated cells. In addition, TACs are short-lived and undergo differentiation after a few cell divisions. In order to verify the presence of TACs of mTECs (TA-TECs) in adult thymus, we isolated the proliferating mTEC subset expressing AIRE as a TA-TEC candidate by utilizing the Fucci technology, which allows visualization of cell cycle progression in living cells. This proliferating Aire$^+$ mTEC subpopulation showed very low expression of TSAs regulated by AIRE, in contrast to quiescent AIRE$^+$ mTECs. Moreover, studies using *in vivo* BrdU pulse-labeling and *in vitro* reaggregated thymic organ culture suggested that the proliferating AIRE$^+$ mTECs are short-lived and that they differentiate into quiescent AIRE$^+$ mTECs and later differentiation stages of mTECs. Accordingly, we conclude that these proliferating AIRE$^+$ mTECs are TACs for mTECs expressing TSAs. Consequently, we propose a cellular mechanism for mTEC differentiation in the adult thymus.

As disturbance of TECs provokes immunodeficiency and autoimmunity, this study will aid the development of novel therapeutic strategies against such TEC-associated diseases.

Laboratory for
Immune Crosstalk

Team Leader: Hilde Cheroutre

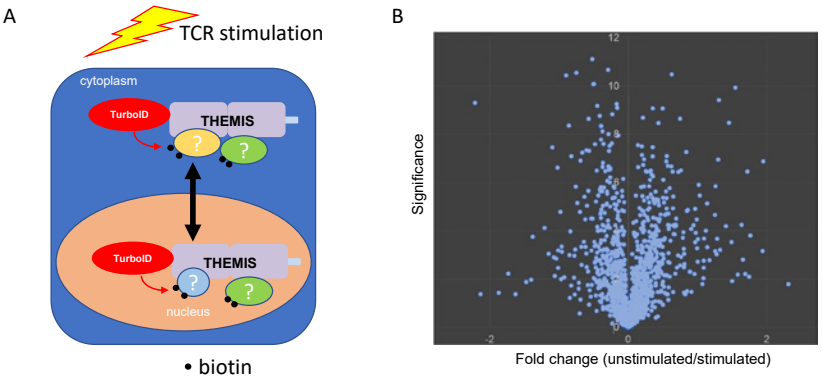


Figure: Identification of proteins proximal to THEMIS by proteomics

A. We introduced cDNA encoding a TurboID-Themis fusion protein. TurboID is a biotinylation enzyme that adds biotin to proximal proteins.

B. Volcano plot showing fold change and significance of biotinylated proteins. We compared biotinylated proteins in the lysates from an unstimulated and TCR-stimulated T cell line.

Recent Major Publications

Liu W, Chou TF, Garrett-Thomson SC, Seo GY, Fedorov E, Ramagopal UA, Bonanno JB, Wang Q, Kim K, Garforth SJ, Kakugawa K, Cheroutre H, Kronenberg M, Almo SC. HVEM structures and mutants reveal distinct functions of binding to LIGHT and BTLA/CD160. ***J Exp Med*** 218, e20211112 (2021)

Invited presentations

Cheroutre H. "New Members in an Old Club: TCR signalosome Revised" Distinguished Lecture at the Research Forum of the Oklahoma Medical Research Foundation (OMRF), University of Oklahoma Medical Center (Oklahoma City, USA) November 2021

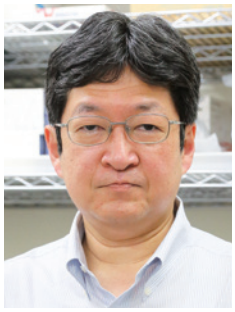
Cheroutre H. "How and Why: do CD4 Th Cells Convert to CTL in the Intestine?" Kenneth Rainin Foundation 2021 Innovation Symposium (Oakland, USA/Online) July 2021

Cheroutre H. "Oral Non-Pathogen Antigens Induce Protective Tolerance in the Intestine" MIST NIH/NIAID (Rockville, USA) February 2021

We discovered *THEMIS* as an indispensable gene for T cell development. Many GWAS studies revealed that the *THEMIS* locus associates with T cell-driven autoimmune diseases such as Celiac Disease, Multiple Sclerosis, Rheumatoid Arthritis, and Atopic Dermatitis. THEMIS is involved in T cell receptor (TCR) signaling as an adapter molecule able to modulate signal strength. THEMIS-deficiency results in a reduction of conventional mature T cells in mice. However, the mechanisms used by THEMIS to control TCR signaling remain unclear and controversial. Although functions of cytoplasmic THEMIS have been studied intensively, THEMIS is also present in the nucleus but its nuclear function is poorly understood.

To investigate the importance of nuclear THEMIS function, we established ERT2-*Themis* knock-in mice. In these mice, without tamoxifen, THEMIS remains exclusively in the cytoplasm, which results in a severe deficiency in conventional T cell development similar to germline *Themis* knock-out mice. Tamoxifen administration *in vivo*, induced import of THEMIS protein into the nucleus and resulted in total recovery of normal T cell development and maturation. These results clearly indicate that THEMIS in T cells plays essential and non-redundant roles in the cytoplasm and nucleus and that dynamic subcellular localization is important for its function.

We next aimed to identify THEMIS interacting proteins in the cytoplasm and nucleus with/without TCR stimulation by biotinylating enzyme and mass spectrometry in collaboration with Dr. Yibo (IMS, YCI Laboratory for Next-Generation Proteomics). We introduced a TurboID-*Themis* fusion construct into the mouse T cell hybridoma, 2B4, and compared the array of biotinylated proteins after TCR stimulation with those of unstimulated 2B4 and found that, besides cytoplasmic components, several important nuclear proteins were biotinylated. These include histone modification enzymes, components of the nuclear pore complex and chromatin-remodeling proteins. These findings suggest roles of nuclear THEMIS in epigenetic modulation and gene transcription in response to TCR stimulation.



Laboratory for
Inflammatory Regulation

Team Leader: Takashi Tanaka

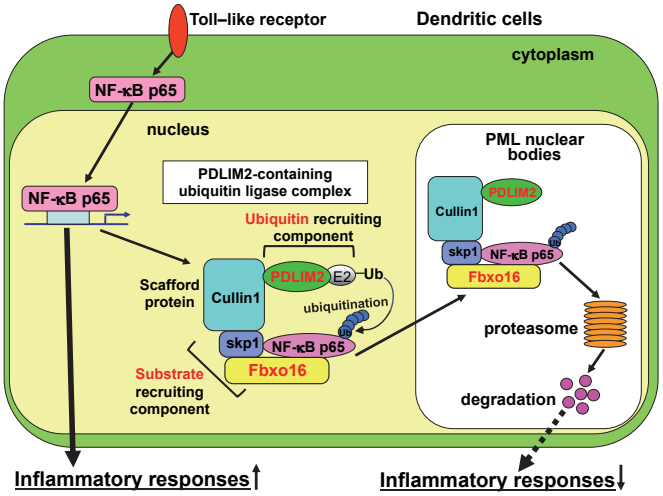


Figure: PDLIM2 and Fbxo16 form a functional ubiquitin E3 ligase complex targeting NF-κB p65 for degradation

PDLIM2 binds to E2, a ubiquitin conjugating enzyme, and forms a functional ubiquitin E3 ligase complex with Cullin1, a scaffold protein providing a platform consisting of the complex, Fbxo16, a component to bind to substrates, and skp1, an adaptor protein, and cooperatively promotes polyubiquitination and proteasomal degradation of the p65 subunit of the NF-κB transcription factor, thereby suppressing NF-κB-mediated inflammatory responses.

Recent Major Publications

Takemori T, Sugimoto-Ishige A, Nishitsuji H, Futamura Y, Harada M, Kimura-Someya T, Matsumoto T, Honma T, Takana M, Yaguchi M, Isono K, Koseki H, Osada H, Miki D, Saito T, Tanaka T, Fukami T, Goto T, Shirouzu M, Shimotohno K, Chayama K. Establishment of a monoclonal antibody against human NTCP that blocks HBV infection. ***J Virol*** JV10168621 (Epub ahead of print) (2022)

Sugimoto-Ishige A, Harada M, Tanaka M, Terooatea T, Adachi Y, Takahashi Y, Tanaka T, Burrows PD, Hikidda M, Takemori T. Bim establishes the B cell repertories from early to late in the immune response. ***Int Immunol*** 33, 79-90 (2020)

Jodo A, Shibazaki A, Onuma A, Kaishi T, Tanaka T. PDLIM7 synergizes with PDLIM2 and p62/Sqstm1 to inhibit inflammatory signaling by promoting degradation of the p65 subunit of NF-κB. ***Front Immunol*** 11, 1559 (2020)

The inflammatory response is an important host defense mechanism, initiated by dendritic cells, to eliminate invading microbial pathogens. However, these responses must be terminated at the appropriate time, otherwise excessive responses may cause massive damage to the host and lead to autoimmune diseases, indicating that negative regulatory systems for inflammation are critical to prevent immunopathology. Our research goal is to identify a series of key negative regulators of inflammation and clarify the complete picture of the molecular mechanisms for regulating inflammatory responses.

We previously identified PDLIM2 (PDZ and LIM domain-containing protein-2), a nuclear protein that belongs to a large family of LIM proteins, as a key factor negatively regulating inflammatory responses. PDLIM2 is a ubiquitin E3 ligase for the p65 subunit of NF-κB in dendritic cells that negatively regulates NF-κB-mediated inflammation. In general, ubiquitin E3 ligase binds to E2, a ubiquitin-conjugating enzyme, and forms a functional ubiquitin ligase complex with Cullin, a scaffold protein. This complex provides a platform onto which an F-box-containing protein can be added to bind to substrates. By screening using siRNA for F-box-containing proteins, we have identified Fbxo16 as a substrate-binding component in the PDLIM2-containing ubiquitin E3 ligase complex. Fbxo16 binds to PDLIM2 and synergistically promotes polyubiquitination and degradation of p65 together with PDLIM2. Notably, knockdown of Fbxo16 by siRNA in dendritic cells impaired PDLIM2-mediated degradation of p65 and thus enhanced LPS-induced production of proinflammatory cytokines such as IL-6 and IL-12. Moreover, Fbxo16 also binds to IRF3/7 transcription factors and promotes their proteasome-dependent degradation, thereby suppressing IRF3/7-mediated signaling. Consistently, knockdown of Fbxo16 in dendritic cells augmented Toll-like receptor-mediated expression of Type I interferon, IFNβ/α. These results delineate a novel role of Fbxo16, as a component of a ubiquitin E3 ligase complex, in negatively regulating NF-κB- and IRF3/7-mediated innate immune responses in dendritic cells, cooperatively with PDLIM2.

Laboratory for
Cytokine Regulation

Team Leader: **Masato Kubo**

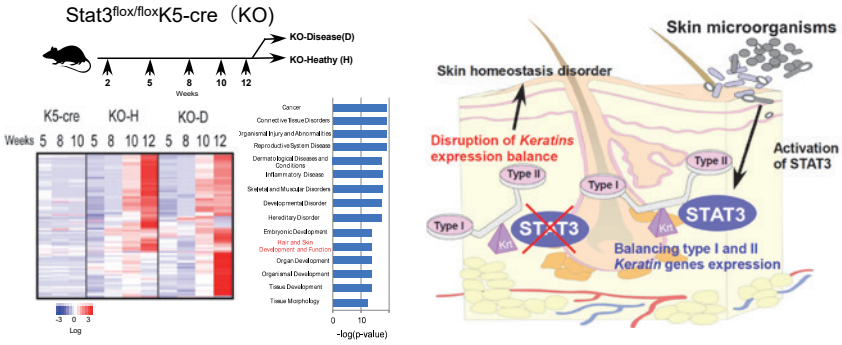


Figure: Role of STAT3 signaling in maintaining homeostasis of skin keratinocytes
Left: skin transcriptome was analyzed in K5-cre (control) and Stat3 KO mice, and the KO group were further divided into two groups based on disease onset as follows: healthy with no dermatitis (KO-H) and diseased mice (KO-D). Heat mapping of enhanced gene in KO mice and Gene enrichment analysis. The results indicated strong association with hair and skin development and function. Right: A schematic diagram shows that STAT3 signaling is required for balancing type I and II *Keratin* genes expression, which is disturbed by microorganisms, to maintain skin homeostasis.

Recent Major Publications

Miyauchi K, Ki S, Ukai M, Suzuki Y, Inoue K, Suda W, Matsui T, Ito Y, Honda K, Koseki H, Ohara O, Tanaka JR. Okada-Hatakeyama M, Kubo M. Essential role of STAT3 signaling in hair follicle homeostasis. ***Front Immunol*** 12, 663177 (2021)

Miyauchi K, Adachi Y, Tonouchi K, Yajima T, Harada Y, Fukuyama H, Deno S, Iwakura Y, Yoshimura A, Hasegawa H, Yugi K, Fujii S, Ohara O, Takahashi Y, and Kubo M. Influenza virus infection expands the breadth of antibody responses through IL-4 signal in B cells. ***Nat Commun*** 12, 3789 (2021)

Wang F, Trier AM, Li F, Kim S, Chen Z, Chai JN, Mack MR, Morrison SA, Hamilton JD, Baek J, Yang TB, Ver Heul AM, Xie AZ, Dong X, Kubo M, Hu H, Hsieh CS, Dong X, Liu Q, Margolis DJ, Ardeleanu M, Miller MJ, Kim BS. A basophil-neuronal axis promotes itch. ***Cell*** 184, 422-440.e17 (2021)

Invited presentations

Sasaki T, Kubo M. "A pathogenic role of IL-13 in anaphylaxis" The 50th Annual Meeting of the Japanese Society for Immunology (Nara, Japan) December 2021

Kubo M. "Different immunodominance of influenza virus between infection and vaccination" The 5th International Immunological Memory and Vaccine Forum (Berlin, Germany/Online) April 2021

Our laboratory tries to understand how cytokines contribute to T and B cell immunity, mainly focusing on infectious virus immunity and innate and acquired immunity in allergic responses.

Signal transducer and activator of transcription 3 (STAT) is a critical cytokine signaling molecule that is activated in response to several gp130-related cytokines. STAT3 provides a crucial signal in controlling epithelial proliferation in various tissues, including the skin. A dominant-negative mutation of the *STAT3* gene causes atopic dermatitis (AD)-like dermatitis in Hyper IgE syndrome patients. However, how STAT3 defects in the skin lead to AD remains poorly understood. We established Stat3$^{flox/flox}$ K5-cre mice and found that STAT3 signaling is required to maintain skin homeostasis by negatively controlling the expression of hair follicle-specific keratin genes. These STAT3-dependent keratin genes play a critical role in maintaining the homeostasis of skin, which is constantly exposed to microorganisms. Indeed, the AD onset is observed in specific pathogen-free but not in germ-free conditions. Therefore, the STAT3-dependent process influenced the skin barrier penetration and skin microbes. Our results indicated that STAT3 activation by skin microbes plays a vital role in maintaining skin homeostasis and barrier function throughout life (Figure).

The adaptive immune response to virus infection is multifaceted and complex. Vaccines are the best available countermeasure against virus infection. However, viruses escape from vaccine-induced immune responses owing to their high mutation rate and antigenic flexibility. We have reported that inactivated vaccines against the influenza A virus (IAV) induce IgG2-dominant reactions, but their effectiveness is narrow. In contrast, the IgG responses caused by natural infection with IAV were relatively broader compared to the inactivated vaccine. We demonstrated that the broadly neutralizing antibodies (bnAbs) targeted the conserved conformational epitopes of hemagglutinin and that these bnAbs pre-existed in the germline compartment. We also revealed that the efficient generation of the bnAbs largely depends on IL-4 from T_{FH} cells in the germinal center. Therefore, we conclude that attenuated live-virus vaccination is a valuable strategy for producing effective bnAbs.

Laboratory for

Human Immunogenetics

Team Leader: **Kazuyoshi Ishigaki**

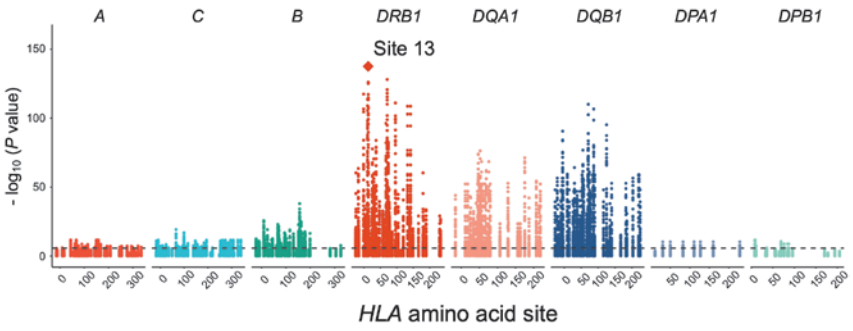


Figure: *P* values of the HLA-CDR3 association analysis
P values for all CDR3 amino acid compositions are plotted at each *HLA* site. The *HLA* site with the lowest *P* value (*HLA-DRB1* site 13) is highlighted by a diamond. The dashed line indicates the significance threshold with the Bonferroni multiple testing correction ($P < 0.05/24{,}360$ total tests).

Our laboratory aims to elucidate the genetic control of immune functions and autoimmunity risk. Our recent research focuses on *human leukocyte antigen* (*HLA*) genetic risks for autoimmunity. Polymorphisms in the *HLA* genes strongly influence autoimmune disease risk. *HLA* risk alleles may influence thymic selection to increase the frequency of T cell receptors (TCRs) reactive with autoantigens (central hypothesis). However, research in human autoimmunity has provided little evidence supporting the central hypothesis. We recently investigated the influence of *HLA* alleles on TCR composition at the highly diverse complementarity determining region 3 (CDR3), which confers antigen recognition. We demonstrated unexpectedly powerful *HLA*-CDR3 associations (see figure below). We showed that the CDR3 features promoted by *HLA* risk alleles were more enriched in candidate pathogenic TCRs than in control TCRs (e.g., citrullinated-epitope-specific TCRs in rheumatoid arthritis patients). We thus provided novel genetic evidence supporting the central hypothesis (Ishigaki K *et al.*, *Nat Genet* 2022).

In another project, we recently developed a novel analytic strategy for TCRs that assesses TCR features of regulatory T cells (T-reg). We showed that the hydrophobicity of CDR3 and the usage of some TCR V genes increase the likelihood that thymic T cells will acquire a T-reg phenotype (Kaitlyn L *et al.*, *Nat Immunol* 2022).

Our laboratory is also committed to multiple new research areas: i) functional genetics studies using CRISPR-based genome editing, ii) development of a novel analytic pipeline to integrate ATAC-seq and RNA-seq data, iii) development of a novel polygenic risk score (PRS) calculation strategy. All of these research directions have the promise of improving our understanding of the genetic basis of autoimmunity risk.

Recent Major Publications

Ishigaki K. Beyond GWAS: from simple associations to functional insights. ***Semin Immunopathol*** 44, 3-14 (2022)

Ishigaki K, Akiyama M, Kanai M, Takahashi A, Kawakami E, Sugishita H, Sakaue S, Matoba N, Low SK, Okada Y, Terao C, Amariuta T, Gazal S, Kochi Y, Horikoshi M, Suzuki K, Ito K, Koyama S, Ozaki K, Niida S, Sakata Y, Sakata Y, Kohno T, Shiraishi K, Momozawa Y, Hirata M, Matsuda K, Ikeda M, Iwata N, Ikegawa S, Kou I, Tanaka T, Nakagawa H, Suzuki A, Hirota T, Tamari M, Chayama K, Miki D, Mori M, Nagayama S, Daigo Y, Miki Y, Katagiri T, Ogawa O, Obara W, Ito H, Yoshida T, Imoto I, Takahashi T, Tanikawa C, *et al.* Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases. ***Nat Genet*** 52, 669–679 (2020)

Amariuta T, Ishigaki K (co-first author), Sugishita H, Ohta T, Koido M, Dey KK, Matsuda K, Murakami Y, Price AL, Kawakami E, Terao C, Raychaudhuri S. Improving the trans-ancestry portability of polygenic risk scores by prioritizing variants in predicted cell-type-specific regulatory elements. ***Nat Genet*** 52,1346-1354 (2020)

Invited presentations

Ishigaki K. "Immune system variation induced by genetic risk of autoimmunity" The 50th Annual Meeting of the Japanese Society for Immunology (Nara, Japan) December 2021

Ishigaki K. "Investigation of HLA genetic risk of autoimmunity" The 8th Japan College of Rheumatology, Basic Research Conference (Tokyo, Japan) November 2021

Infectious Diseases Research Unit

Unit Leader: **Haruhiko Koseki**

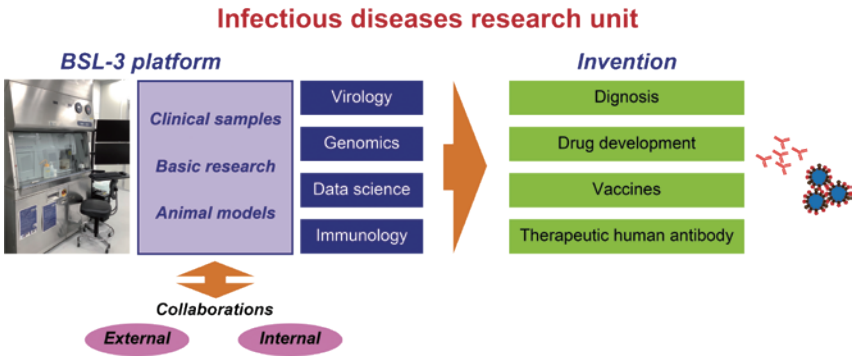


Figure: A schematic view of our research strength and direction
The newly established BSL-3 provided a unique opportunity to perform infectious disease research, leading to inventions.

Recent Major Publications

Miyauchi E, Taida T, Kawasumi M, Ohkusa T, Sato N, Ohno H. Analysis of colonic mucosa-associated microbiota using endoscopically collected lavage. ***Sci Rep*** 12, 1758 (2022)

Takeuchi T, Miyauchi E, Kanaya T, Kato T, Nakanishi Y, Watanabe T, Kitami T, Taida T, Sasaki T, Negishi H, Shimamoto S, Matsuyama A, Kimura I, Williams IR, Ohara O, Ohno H. Acetate differentially regulates IgA reactivity to commensal bacteria. ***Nature*** 595, 560-564 (2021)

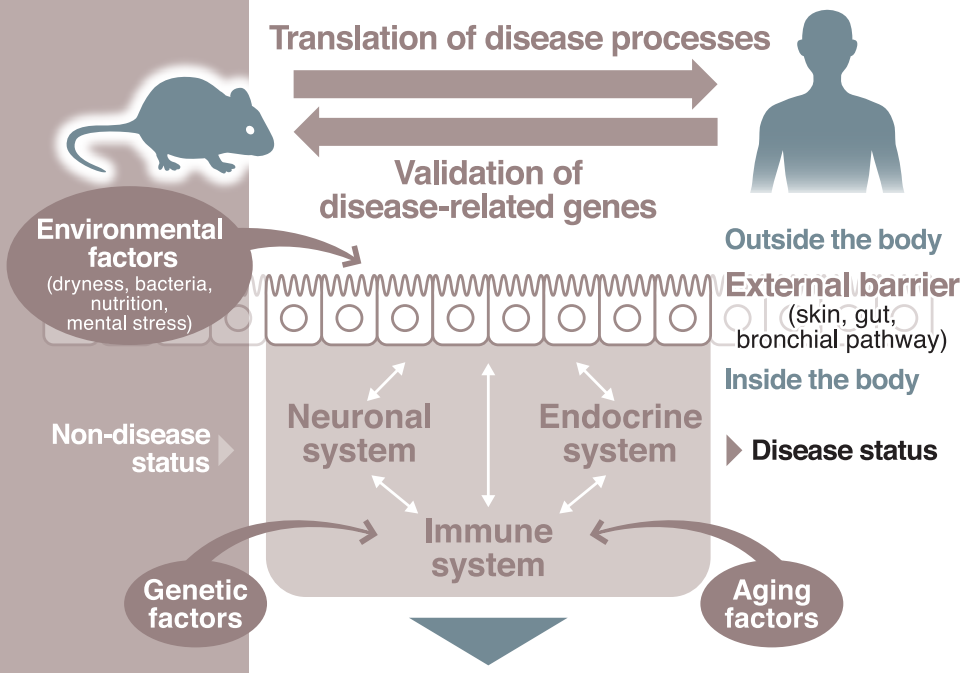
Miyauchi K, Adachi Y, Tonouchi K, Yajima T, Harada Y, Fukuyama H, Deno S, Iwakura Y, Yoshimura A, Hasegawa H, Yugi K, Fujii SI, Ohara O, Takahashi Y, Kubo M. Influenza virus infection expands the breadth of antibody responses through IL-4 signalling in B cells. ***Nat Commun*** 12, 3789 (2021)

Our research unit was established this year in response to the high demand for investigating the extremely contagious disease, COVID-19 (Coronavirus disease 2019) in both public and research communities during the pandemic. We believe that IMS strengths in immunology, genomics and data science, and their established research field network will help to understand what factors exacerbate the disease and to develop next-generation vaccines. Our newly established BSL-3 facilities are capable of handling animal infection experiments, virus characterization and manipulating patient materials.

We have three major achievements: First, in a collaboration with KEIO hospital, we isolated and patent-filed human broadly neutralizing antibodies against several SARS-CoV-2 variants of concern (VOC) from COVID-19 convalescent patients. These antibodies are expected to be used for human immunotherapy in the near future. Second, our recent discovery of a new vaccine adjuvant, vitamin D3, is being applied to the COVID-19 vaccine. The vaccine is designed as a "patch" vaccine to facilitate herd immunity ideally without using conventional needles. Third, we generated several animal models for COVID-19, including one in which the human *ACE2* (Angiotensin-converting enzyme 2) gene is introduced into mice. These model animals enable us to evaluate the efficiency and safety of newly developed vaccines or anti-virus drugs *in vivo*. The rapid establishment of biosafety laboratory infrastructure gave us a unique research opportunity to investigate the mechanism of immune escape during SARS-CoV-2 breakthrough infections by participating in a consortium of COVID-19 research in the Kanto area of Japan, supported by AMED (the Agency for Medical Research and Development).

Our goal is to provide the basis of therapeutic intervention for infectious diseases. Currently ongoing fruitful internal and external collaborations will lead to discoveries that will help achieve this goal.

Division of Disease Systems Biology



Division of Disease Systems Biology will elucidate the regulation of homeostasis and disease onset as a dynamic living system.

Laboratory for
Developmental Genetics

Team Leader: **Haruhiko Koseki**

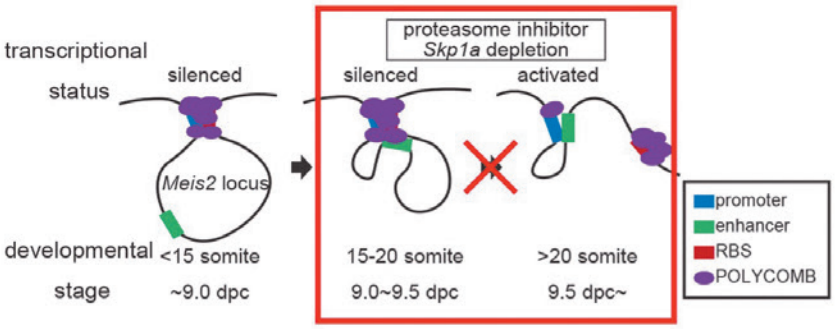


Figure: The role of SKP1A to activate Polycomb-repressed genes
A schematic representation of dynamic changes in PcG association with the *Meis2* gene during midbrain development. In 9.0 dpc midbrain, in which Meis2 is silenced, PcG factors bind to promoter and RBS (RING1B-binding site) and facilitate their association. During 9.0 to 9.5 dpc, the midbrain enhancer region is recruited to promoter/RBS regions in a PcG-dependent manner. In 9.5 dpc midbrain, SKP1A facilitates dissociation of PcG factors and RBS from the *Meis2* promoter in proteasome-dependent manner. This does not occur in the absence of SKP1A (red X).

Recent Major Publications

Ohinata Y, Endo TA, Sugishita H, Watanabe T, Iizuka Y, Kawamoto Y, Saraya A, Kumon M, Koseki Y, Kondo T, Ohara O, Koseki H. Establishment of mouse stem cells that can recapitulate the developmental potential of primitive endoderm. ***Science*** 4;375(6580):574-578 (2022)

Takada Y, Yaman-Deveci R, Shirakawa T, Sharif J, Tomizawa SI, Miura F, Ito T, Ono M, Nakajima K, Koseki Y, Shiotani F, Ishiguro KI, Ohbo K, Koseki H. Maintenance DNA methylation in pre-meiotic germ cells regulates meiotic prophase by facilitating homologous chromosome pairing. ***Development*** 148, dev194605 (2021)

Sugishita H, Kondo T, Ito S, Nakayama M, Yakushiji-Kaminatsui N, Kawakami E, Koseki Y, Ohinata Y, Sharif J, Harachi M, Blackledge NP, Klose RJ, Koseki H. Variant PCGF1-PRC1 links PRC2 recruitment with differentiation-associated transcriptional inactivation at target genes. ***Nat Commun*** 12, 5341 (2021)

Invited presentations

Koseki H. "Adoptive iPS-NKT immunotherapy for cancer" The 18th Annual Meeting of the Society for Reconstruction and Regeneration in Urologic Surgery (Online) June 2021

Proper transcriptional regulation is critical for the life cycle of multicellular organisms. We study a group of repressive chromatin regulatory factors, namely Polycomb group (PcG), that predominantly contributes to transcriptional control of development/differentiation-related genes. Although it is widely accepted that PcG factors primarily contribute to maintain the repressive state of developmental gene expression, our previous studies revealed its impacts on the transition of transcriptional status of target genes that associates with developmental processes. To explore how PcG factors contribute to this transcriptional phase shift, we focused on variant Polycomb repressive complex-1 incorporating PCGF1 (PCGF1-PRC1), which is known to mediate mono-ubiquitination of Lysine 119 of Histone H2A (H2AK119ub1) by the RING1B component. Importantly, PCGF1-PRC1 includes another E3 ubiquitin ligase, SKP1A, via its interaction with F-box of KDM2B. Although SKP1A is known to regulate stability of target proteins, its role in PCGF1-PRC1 has not been elucidated. SKP1A was found to associate with CpG islands (CGIs) in a KDM2B-dependent manner in embryonic stem cells (ESCs) but not to contribute to stable down-regulation of PcG-target genes in ESCs. Instead, PcG-target genes, which are upregulated during ESC-to-embryoid body (EB) differentiation, were found to require SKP1A for their upregulation. We indeed observed that PcG factors failed to be evicted from these target genes during ESC-to-EB differentiation, suggesting a role of SKP1A to strip PcG factors off the target genes, likely by linking PcG-bound CGIs with the proteasome. This issue was further tested during midbrain development, which accompanies PcG-dependent activation of several PcG-target genes including Meis2. We observed that Meis2 activation required SKP1A-dependent eviction of PcG factors from Meis2-associated CGI. In this work, we propose the role of SKP1A for transcriptional activation of PcG-repressed genes.

Laboratory for

Intestinal Ecosystem

Team Leader: Hiroshi Ohno

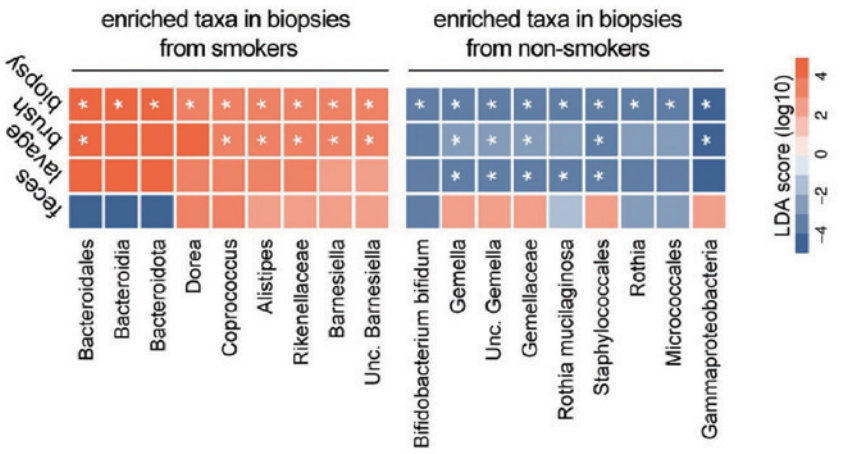


Figure: Heatmap of log 10 linear discriminant analysis (LDA) scores of taxa detected in biopsy samples as discriminants between non-smokers and smokers

The lavage samples showed an identical pattern of LDA scores to biopsy samples, although they were only partially significant. *p < 0.05; Kruskal-Wallis rank sum test. [Taken from Figure 3b of Miyauchi E *et al.* ***Sci Rep*** 12: 1758 (2022)].

Recent Major Publications

Miyauchi E, Taida T, Kawasumi M, Ohkusa T, Sato N, Ohno H. Analysis of colonic mucosa- mucosa-associated microbiota using endoscopically collected lavage. ***Sci Rep*** 12, 1758 (2022)

Takeuchi T, Miyauchi E, Kanaya T, Kato T, Nakanishi Y, Watanabe T, Kitami T, Taida T, Sasaki T, Negishi H, Shimamoto S, Matsuyama A, Kimura I, Williams IR, Ohara O, Ohno H. Acetate differentially regulates IgA reactivity to commensal bacteria. ***Nature*** 595, 560-564 (2021)

Takeuchi T, Ohno H. Reciprocal regulation of IgA and the gut microbiota: a key mutualism in the intestine. ***Int Immunol*** 33, 781-786 (2021)

Invited presentations

Ohno H. "Mucosal immunity and microbiota" The 49th Annual Meeting of the Japanese Society of Clinical Immunology (Tokyo, Japan) October 2021

Ohno H. "Gut microbiota, immunity, and autoimmune diseases" Educational Lecture, The 83rd Annual Meeting of the Japanese Society of Hematology (Tokyo, Japan/Online) September 2021

Ohno H. "Gut Microbiota and Autoimmune Diseases – Type 1 Diabetes and Multiple Sclerosis -" Educational Lecture, The Asian Pacific Association for the Study of the Liver Single Topic Conference, Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application (Osaka, Japan/Online) September 2021

Ohno H. "Gut Microbiota and Autoimmune Diseases" Symposium 4: Mucosal immunology, Dysbiosis, The 27th International Symposium on Molecular Cell Biology of Macrophages (Osaka, Japan/Online) June 2021

Ohno H. "The role of small intestinal microbes for central nervous system inflammation" International Conference on Beneficial Microbes 2021 (Rostov-on-Don, Russia/Online) June 2021

Gut microbiota is the consortium of numerous commensal bacteria residing in our intestines. Our lab has been studying the molecular mechanisms of host-gut microbiota interactions.

The host does not freely allow the residence of those microorganisms. The intestinal immune system can somehow sense the bacteria in the gut lumen and try to contain them. To this end, M cells, a specialized intestinal epithelial cell subset for recognition and uptake of luminal bacteria to initiate intestinal immune responses, have evolved. Our lab has elucidated the molecular mechanisms of M-cell differentiation and function.

IgA is a major class of immunoglobulins produced and secreted in mucosal tissues including the intestine. Last year, we have published a paper showing that the amount and specificity of IgA toward commensal bacteria is regulated by acetate and the type of bacteria present in the intestine.

Host-gut microbiota interactions deeply impact the physiology and pathology of the host. We have been studying this process by applying an integrated omics approach, where exhaustive analyses at different layers of organismal activities are combined, including (meta) genomics, epigenomics, (meta) transcriptomics and metabolomics.

The bacterial composition of the gut lumen and mucosa are distinct and the mucosa-associated bacteria are thought to more deeply impact the host pathophysiology. In the past year, we have evaluated the potential use of colonic lavage samples for mucosal microbiota analysis in humans. Among the different types of colonic mucosal samples collected from healthy volunteers, the lavage samples contained a higher amount of bacterial DNA and were less contaminated with host DNA compared to mucosal brushing and biopsy. Furthermore, differences in mucosal microbes were detectable in lavage samples from non-smokers and smokers. Therefore, the colonic lavage method could promote research on the human mucosal microbiota, especially in gastrointestinal disorders.

Laboratory for Integrative Genomics

Team Leader: Jun Seita

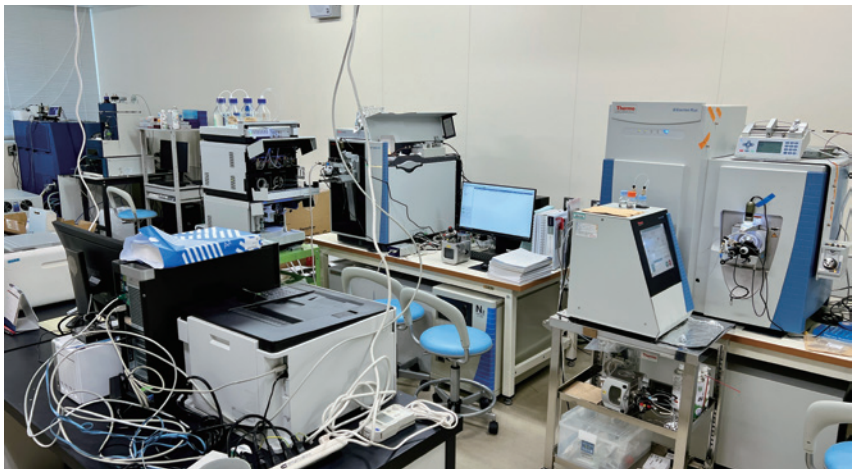


Figure: New environment for proteomics and research automation
Lab space on the 2nd floor of the North building has been transformed to house cutting-edge mass spectrometers and liquid handling robots. The laboratory for Integrative Genomics will provide the latest proteomics technologies and lab automation through this infrastructure.

Following successful launch of the Genome Platform, the laboratory for Integrative Genomics has been evolving toward multi-omics science. In 2021, we built a new lab space for proteomics and lab automation (Figure). This space will house four currently functioning and newly introduced mass spectrometers and several liquid handling robots and will be a foundation to provide another layer of omics technologies to IMS. Indeed, the era of multi-omics is coming and our technologies have provided a comprehensive and detailed viewpoint to many biological projects. For instance, our algorithm for quantitative transcriptome has helped to reveal aging of skeletal stem cells and generation of an inflammatory niche (publication 1). Repertoire analysis using next generation sequencing has unveiled the dynamics of differential regulation on IgA reactivity to commensal bacteria (publication 2). RNA-seq technology has unraveled the molecular mechanisms in aggressive acute myeloid leukemia (publication 3). We believe that multi-layer omics approaches at DNA, RNA, protein, and so on levels, become more and more important for comprehensive understanding of biological systems, thus we will continue developing multi-omics technologies.

Recent Major Publications

Ambrosi TH, Marecic O, McArdle A, Sinha R, Gulati GS, Tong X, Wang Y, Steininger HM, Hoover MY, Koepke LS, Murphy MP, Sokol J, Seo EY, Tevlin R, Lopez M, Brewer RE, Mascharak S, Lu L, Ajanaku O, Conley SD, Seita J, Morri M, Neff NF, Sahoo D, Yang F, Weissman IL, Longaker MT, Chan CKF. Aged skeletal stem cells generate an inflammatory degenerative niche. ***Nature*** 597, 256-262 (2021).

Takeuchi T, Miyauchi E, Kanaya T, Kato T, Nakanishi Y, Watanabe T, Kitami T, Taida T, Sasaki T, Negishi H, Shimamoto S, Matsuyama A, Kimura I, Williams IR, Ohara O, Ohno H. Acetate differentially regulates IgA reactivity to commensal bacteria. ***Nature*** 595, 560-564 (2021)

Hashimoto M, Saito Y, Nakagawa R, Ogahara I, Takagi S, Takata S, Amitani H, Endo M, Yuki H, Ramilowski JA, Severin J, Manabe R, Watanabe T, Ozaki K, Kaneko A, Kajita H, Fujiki S, Sato K, Honma T, Uchida N, Fukami T, Okazaki Y, Ohara O, Shultz LD, Yamada M, Taniguchi S, Vyas P, de Hoon M, Momozawa Y, Ishikawa F. Combined inhibition of XIAP and BCL2 drives maximal therapeutic efficacy in genetically diverse aggressive acute myeloid leukemia. ***Nat Cancer*** 2, 340-356 (2021)

Invited presentations

Seita J. "Basic and application of data-driven medical sciences in RIKEN" Keynote at RIKEN-Kumamoto University Joint Symposium on DX in Biomedical Science (Kumamoto, Japan/Online) December 2021

Seita J. "Potential of Deep Learning in Acute Medicine" Symposium at the 49th Annual Meeting of the Japanese Association for Acute Medicine (Tokyo, Japan) November 2021

Seita J. "Role of hybrid scientist in cancer research" The 80th Annual Meeting of the Japanese Cancer Association (Yokohama/Kanagawa, Japan) October 2021

Seita J. "Can AI make revolution in human genetics?" Symposium at the 66th Annual Meeting of the Japan Society of Human Genetics and the 28th Annual Meeting of the Japanese Society for Gene Diagnosis and Therapy – Joint Conference (Yokohama/Kanagawa, Japan) October 2021

Seita J. "Human lung molecular atlas by single cell RNA-seq" The 61st Annual Meeting of the Japanese Respiratory Society (Tokyo, Japan) April 2021

Laboratory for
Mucosal Immunity

Team Leader: Sidonia Fagarasan

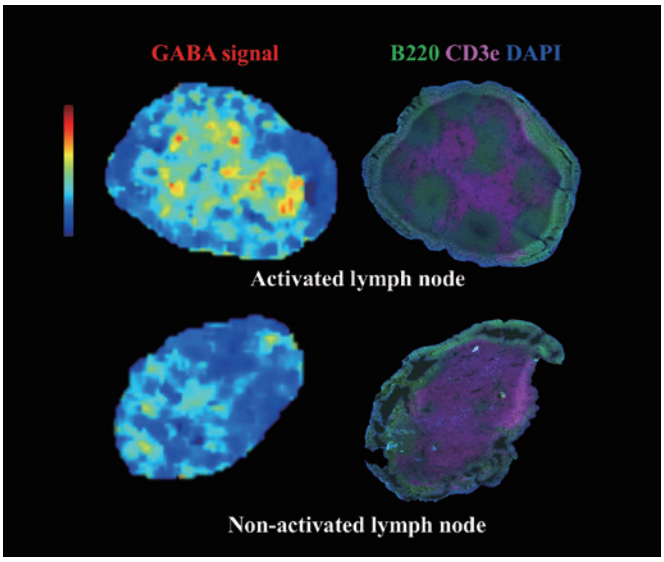


Figure: Visualization of GABA in lymph nodes after foot pad immunization
Imagining mass spectrometry and immunohistochemistry of ipsilateral or draining inguinal lymph node (upper panels) and contralateral, non-draining inguinal lymph (lower panels) showing GABA overlapping with B cell follicles.

Recent Major Publications

Zhang B, Vogelzang A, Miyajima M, Sugiura Y, Wu Y, Chamoto K, Nakano R, Hatae R, Menzies RJ, Sonomura K, Hojo N, Ogawa T, Kobayashi W, Tsutsui Y, Yamamoto S, Maruya M, Narushima S, Suzuki K, Sugiya H, Murakami K, Hashimoto M, Ueno H, Kobayashi T, Ito K, Hirano T, Shiroguchi K, Matsuda F, Suematsu M, Honjo T, Fagarasan S. B cell-derived GABA elicits IL-10^{+} macrophages to limit anti-tumour immunity. ***Nature*** 599, 471-476 (2021) doi: 10.1038/s41586-021-04082-1

Shirakashi M, Maruya M, Hirota K, Tsuruyama T, Matsuo T, Watanabe R, Murata K, Tanaka M, Ito H, Yoshifuji H, Ohmura K, Elewaut D, Sakaguchi S, Fagarasan S, Mimori T, Hashimoto M. Effect of Impaired T Cell Receptor Signaling on the Gut Microbiota in a Mouse Model of Systemic Autoimmunity. ***Arthritis Rheumatol*** 74, 641-653 (2021) doi: 10.1002/art.42016

Akrami M, Menzies R, Chamoto K, Miyajima M, Suzuki R, Sato H, Nishii A, Tomura M, Fagarasan S, Honjo T. Circulation of gut-preactivated naïve CD8^{+} T cells enhances antitumor immunity in B cell-defective mice. ***Proc Natl Acad Sci U S A*** 117, 23674-23683 (2020) doi: 10.1073/pnas.2010981117

Invited presentations

Fagarasan S. Zhang B (talk) "B cell derived GABA elicits anti-inflammatory macrophages limiting anti-tumor responses" Japanese Society for Immunology Annual Meeting (Nara, Japan) December 2021

Fagarasan S. Zhang B (talk) "B cell derived GABA elicits anti-inflammatory macrophages limiting anti-tumor responses" The 19th Awaji International Forum on Infection and Immunity (Online) September 2021

Fagarasan S. "The biochemical dialog between major physiological systems mediated by the immune cells" The 25th Annual Meeting of Japanese Association for Cancer Immunology (Online) July 2021

Fagarasan S. "The biochemical dialog between major physiological systems mediated by the immune cells" The Uehara International Symposium 2021, Brain-Periphery Interactions in Health and Diseases (Online) June 2021

Fagarasan S. "The biochemical dialog between major physiological systems mediated by the immune cells" Kyoto University-University of California San Diego seminars The 9th NIF Winter School on Advanced Immunology (Online) April 2021

Immunometabolism: mapping pathways and identifying metabolites with immune regulatory function

Antigenic stimulation leads to activation, proliferation and differentiation of immune cells into immediate effector or long-term memory cells able to reestablish and maintain homeostasis. The demands for biomass building and synthesis of effector molecules require readjustment of cellular metabolism, during which small molecules are likely produced and secreted. We hypothesized that many such molecules derived from metabolic reprogramming induced by immune cell activation have functions other than simply serving as intermediates in metabolic pathways. For example, they may function as signaling molecules influencing the immune cells function. We found that antigenic stimulation induced upon immunization significantly elevated the levels of gamma-aminobutyric (GABA) in the activated lymph nodes. This was surprising, as GABA is primarily known as a major neurotransmitter produced by neurons in the central nervous system. Moreover, the source of GABA in the lymphoid tissues is essentially unknown. Our experiments revealed that B cells are the main source of GABA in lymphoid tissues, raising the possibility that B cell-derived GABA might participate in regulation of immune responses (Figure).

Mechanistically we demonstrated that B cell-derived GABA promotes monocyte differentiation into anti-inflammatory macrophages secreting IL-10 and inhibits CD8^{+} T cell killer function.

Understanding the divergent metabolic pathways utilized by different immune cell subsets and how the metabolites turn into immunoregulators may, in the future, allow targeted therapeutic approaches that both inhibit tumor cell growth and enhance immunity to cancer, or allow the design of drugs that delicately undermine overactive B cell responses in autoimmunity.

Laboratory for
Gut Homeostasis

Team Leader: **Kenya Honda**

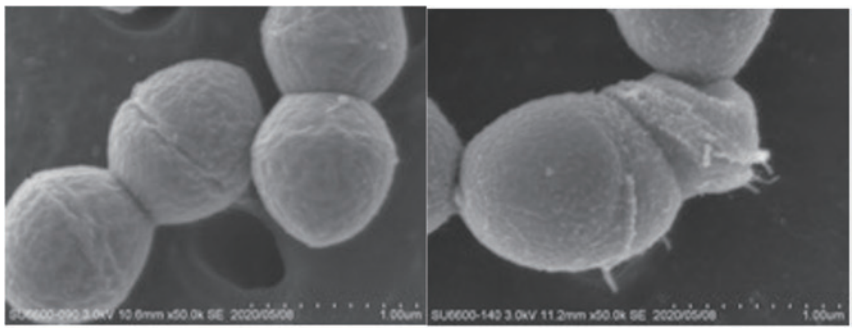


Figure: Bactericidal effect of isoalloLCA on *Enterococcus faecium*

Scanning electron micrographs of drug-resistant bacteria grown in medium without (left) or with (right) the secondary bile acid isoalloLCA. The bacteria grown with isoalloLCA exhibit changes to their shapes.

Recent Major Publications

Sato Y, Atarashi K, Plichta DR, Arai Y, Sasajima S, Kearney SM, Suda W, Takeshita K, Sasaki T, Okamoto S, Skelly AN, Okamura Y, Vlamakis H, Li Y, Tanoue T, Takei H, Nittono H, Narushima S, Irie J, Itoh H, Moriya K, Sugiura Y, Suematsu M, Moritoki N, Shibata S, Littman DR, Fischbach MA, Uwamino Y, Inoue T, Honda A, Hattori M, Murai T, Xavier RJ, Hirose N, Honda K. Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians. ***Nature*** 599, 458-464 (2021)

Li Y, Honda K. Toward the development of defined microbial therapeutics. ***Int Immunol*** 33, 761-766 (2021)

Ito Y, Sasaki T, Li Y, Tanoue T, Sugiura Y, Skelly AN, Suda W, Kawashima Y, Okahashi N, Watanabe E, Horikawa H, Shiohama A, Kurokawa R, Kawakami E, Iseki H, Kawasaki H, Iwakura Y, Shiota A, Yu L, Hisatsune J, Koseki H, Sugai M, Arita M, Ohara O, Matsui T, Suematsu M, Hattori M, Atarashi K, Amagai M, Honda K. Staphylococcus cohnii is a potentially biotherapeutic skin commensal alleviating skin inflammation. ***Cell Rep*** 35, 109052 (2021)

Invited presentations

Honda K. "Mining the gut microbiota and identifying effector microbes to combat pathogen infection" Seminar series at Genentech (USA/Online) September 2021

Honda K. "Microbiota and regulation of intestinal immunity" Principles of Mucosal Immunology Course (USA/Online) July 2021

Honda K. "Identification of unique bile acid-metabolizing bacteria from the microbiome of centenarians" The international centenarian consortium (ICC) conference (Japan/Online) June 2021

Honda K. "Mining the human gut microbiota to identify its beneficial components" Seminar series at Center for Immunity and Infection Lausanne, University of Lausanne (Switzerland/Online) June 2021

Honda K. "Identification of Gut Bacterial Strains Involved in the Regulation of Host Physiological Functions" Keystone symposia on Harnessing the Microbiome for Disease Prevention and Therapy (USA/Online) January 2021

Trillions of microorganisms reside in the intestine and maintain gut homeostasis. Our laboratory has been focusing on identifying commensal bacteria that induce specific branches of immune cells in the intestine. We have succeeded in isolating bacterial consortia that stimulate targeted immune responses, including induction of $CD4^{+}Foxp3^{+}$ regulatory T (Treg) cells, T_H17 cells, T_H1 cells, and CD8 T cells. The 17 Treg cell-inducing consortium is now under evaluation in a Phase 2 study for therapy in patients with ulcerative colitis. The 11 CD8 cell-inducing consortium is now under evaluation in Phase 1/2 studies in the US in combination with anti-PD1 mAb for therapy in patients with checkpoint inhibitor refractory melanoma, gastric cancer and colorectal cancer.

In addition to immune-modulatory bacteria, we identified and isolated bile acid-metabolizing bacteria from centenarians' feces. Centenarians display decreased susceptibility to ageing-associated illness, chronic inflammation and infectious disease. We found that centenarians have a distinct gut microbiome enriched in microbes capable of generating unique secondary bile acids (BAs), including iso-, 3-oxo-, allo-, 3-oxoallo-, and isoallo-lithocholic acid (LCA). Among these BAs, the biosynthetic pathway for isoalloLCA had not been described previously. By screening 68 bacterial isolates from a centenarians' fecal microbiota, we identified Odoribacteraceae strains as effective producers of isoalloLCA, both *in vitro* and *in vivo*. Furthermore, we found that the enzymes 5α-reductase (5AR) and 3β-hydroxysteroid dehydrogenase (3βHSDH) were responsible for isoalloLCA production. IsoalloLCA exerted potent antimicrobial effects against gram-positive, but not gram-negative, multidrug-resistant pathogens, including *Clostridioides difficile* and *Enterococcus faecium*. These findings suggest that specific bile acid metabolism may be involved in reducing the risk of pathobiont infection, thereby potentially contributing to the maintenance of intestinal homeostasis.

Laboratory for

Skin Homeostasis

Team Leader: Masayuki Amagai

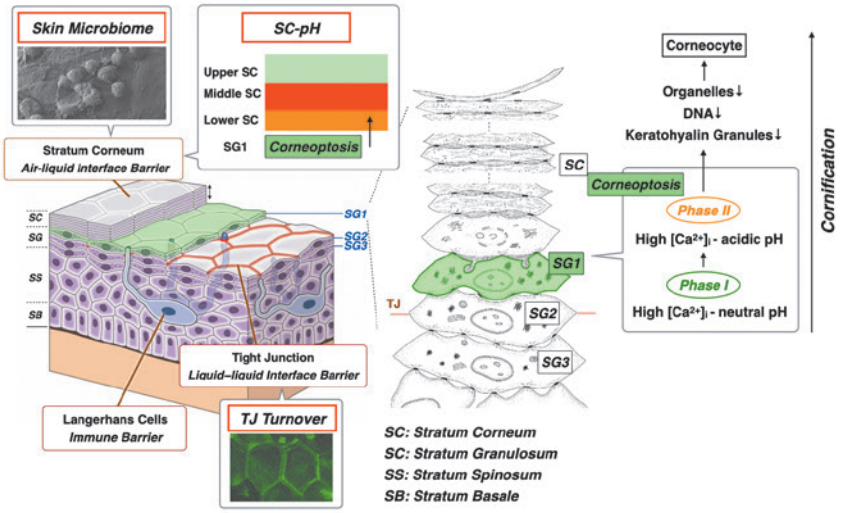


Figure: Comprehensive analysis of skin barrier homeostasis
Our team is working to clarify the mechanisms of skin barrier homeostasis by focusing on the stratum corneum (SC), tight junction (TJ) and SG1 cells. We established a live imaging system, especially focusing on a unique type of SG1 cell death termed 'corneoptosis' and pH changes in the SC. We found that corneoptosis is composed of two phases (phase I and II) by using an optimized plasmid injection method to study the cornification process in mice. We also found that SC has three stepwise pH zones and we are currently studying the host-microbe interactions on skin and intracellular pH in inflammatory skin conditions.

Recent Major Publications

Takahashi H, Nomura H, Iriki H, Kubo A, Isami K, Mikami Y, Mukai M, Sasaki T, Yamagami J, Kudo J, Ito H, Kamata A, Kurebayashi Y, Yoshida H, Yoshimura A, Sun H, Suematsu M, O'Shea JJ, Kanno Y, and Amagai M. Cholesterol 25-hydroxylase is a metabolic switch to constrain T cell-mediated inflammation in the skin. ***Sci Immunol*** 6, eabb6444 (2021)

Matsui T, Kadono-Maekubo N, Suzuki Y, Furuichi Y, Shiraga K, Sasaki H, Ishida A, Takahashi S, Okada T, Toyooka K, Sharif J, Abe T, Kiyonari H, Tominaga M, Miyawaki A, Amagai M. A unique mode of keratinocyte death requires intracellular acidification. ***Proc Natl Acad Sci U S A*** 118, e2020722118 (2021)

Ito Y, Sasaki T, Li Y, Tanoue T, Sugiura Y, Skelly AN, Suda W, Kawashima Y, Okahashi N, Watanabe E, Horikawa H, Shiohama A, Kurokawa R, Kawakami E, Iseki H, Kawasaki H, Iwakura Y, Shiota A, Yu L, Hisatsune J, Koseki H, Sugai M, Arita M, Ohara O, Matsui T, Suematsu M, Hattori M, Atarashi K, Amagai M, Honda K. Staphylococcus cohnii is a potentially biotherapeutic skin commensal alleviating skin inflammation. ***Cell Rep*** 35, 109052 (2021)

Invited presentations

Amagai M. "Corneoptosis, functional cell death of keratinocytes" SLDDDRS Webinar Series 2021-2023, Stanford University (Online) November 2021

Amagai M. "Corneoptosis, unique cell death process of keratinocytes" The 18th International Symposium of the Cutaneous Biology Research Institute, Yonsei University College of Medicine (Online) October 2021

Amagai M. "Peripheral tolerance to desmoglein3, pemphigus vulgaris antigen" 2021 Annual Scientific Meeting of Australasian Society for Dermatological Research (Online) July 2021

Amagai M. "Corneoptosis, unique cell death process of keratinocyte" ISBS 2021 World Congress on Biophysics and Imaging of the Skin, hybrid (Berlin, Germany/Online) June 2021

Amagai M. "Chasing after simple logic behind a complex disease, pemphigus" Aaron Lerner Discovery Lecture 2021, Yale University (Online) April 2021

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.

Epidermis, the outermost component of the skin, is composed of keratinocytes and consists of the stratum basale, stratum spinosum, stratum granulosum (SG), and stratum corneum (SC), from bottom to top. Our group has been focusing on the SC as an air-liquid barrier and the tight junction 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, how it can maintain the barrier, yet still constantly replace and shed cells.

Our group is trying to clarify how epidermal barrier homeostasis is maintained under normal conditions and how impaired barrier function occurs and affects microenvironments of the skin in various disease conditions. Our experimental approaches are comprehensive, combining molecular biology, biochemistry, ultrastructural anatomy, live imaging, microbiology, and systems biology. We show via intravital imaging of mouse skin that SG1 cell death is preceded by prolonged (~60 min) intracellular Ca^{2+} elevation (phase I) and rapid intracellular acidification (phase II). These findings provide an important framework to understand the unique cell death pathway in keratinocytes, which we termed 'corneoptosis'.

Another of our strengths is to be able to go back and forth between our basic science findings in mice and those in clinical science in humans with various skin diseases. Based on the discovery of corneoptosis, we are now examining how corneoptosis and pH regulation in SC are impaired in the presence of inflammation, such as in atopic dermatitis. Our goal is to understand skin barrier homeostasis in health and disease and to provide more targeted therapeutic approaches with fewer side effects to patients suffering from inflammatory skin diseases.

Laboratory for
Tissue Dynamics

Team Leader: **Takaharu Okada**

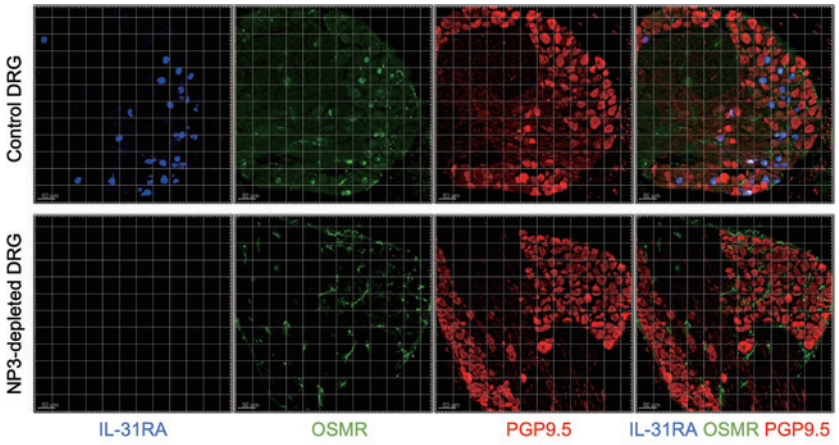


Figure: Immunofluorescence staining of dorsal root ganglia (DRG) sections from control and NP3-depleted mice
IL-31RA and OSMR are the subunits of IL-31 receptor. PGP9.5 is a pan-neuronal marker.

The goal of the laboratory is to understand the molecular and cellular mechanisms that underlie tissue homeostasis and its breakdown during disease development. As a most recent focus, we have been studying the function of primary sensory nerves in the skin, with a particular interest in the itch-transmitting nerves. Previous single-cell RNA-sequencing analyses have suggested that C-fiber neurons that transmit itch can be broadly divided into three subsets called NP1, NP2 and NP3, which express distinct sets of itch-associated receptor genes. However, the roles for each subset in the pruritus and inflammation of atopic dermatitis and other skin diseases are incompletely understood. To tackle this problem, we are generating mouse models in which each of the itch nerve subsets can be specifically manipulated at various stages of disease development. This year we have established and analyzed a mouse strain lacking NP3 sensory neurons, which highly express the receptor for the itch-inducing cytokine IL-31 (Figure). We found that NP3 neurons are important not only for IL-31-induced itch but also for itch induced by other pruritogens, such as the mast cell stimulator compound 48/80. By using these mice, we also determined the contribution of NP3 neurons to chronic inflammatory itch caused by repetitive treatment with the active vitamin D3 analogue MC903. In addition, our data suggest that NP3 neurons may have a regulatory role in inflammation that is exacerbated by the itch-scratch cycle.

Recent Major Publications

Matsui T, Kadono-Maekubo N, Suzuki Y, Furuichi Y, Shiraga K, Sasaki H, Ishida A, Takahashi S, Okada T, Toyooka K, Sharif J, Abe T, Kiyonari H, Tominaga M, Miyawaki A, Amagai M. A unique mode of keratinocyte death requires intracellular acidification. ***Proc Natl Acad Sci USA*** 118, e2020722118 (2021)

Teratani T, Mikami Y, Nakamoto N, Suzuki T, Harada Y, Okabayashi K, Hagihara Y, Taniki N, Kohno K, Shibata S, Miyamoto K, Ishigame H, Chu PS, Sujino T, Suda W, Hattori M, Matsui M, Okada T, Okano H, Inoue M, Yada T, Kitagawa Y, Yoshimura A, Tanida M, Tsuda M, Iwasaki Y, Kanai T. The liver-brain-gut neural arc maintains the T_{reg} cell niche in the gut. ***Nature*** 585, 591-596 (2020)

Invited presentations

Okada T. "Itch induction and regulation of inflammation by IL-31-stimulated sensory neurons" The 51st Annual Meeting of the Japanese Society of Cutaneous Immunology and Allergy (Tokyo, Japan/Online) November 2021

Okada T. "Activation mechanisms of primary sensory neurons transmitting itch" The 30th International Symposium of Itch (Online) November 2021

Laboratory for Metabolomics

Team Leader: Makoto Arita

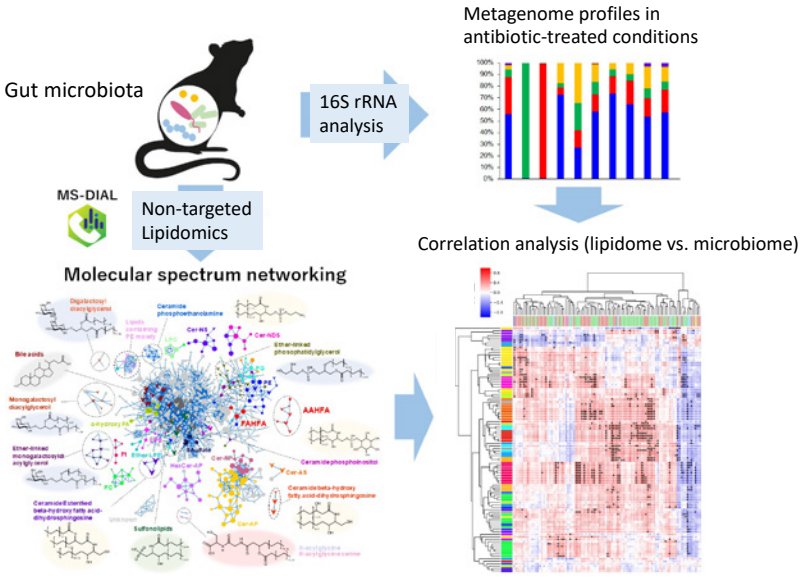


Figure: Global profiling of gut microbiota-associated lipid metabolites in antibiotic-treated mice

We conducted non-targeted lipidomics followed by feature-based molecular MS/MS spectral networking to characterize gut bacteria-dependent lipid subclasses in antibiotic-treated mice. Based on the integrated analysis of 985 lipid profiles and 16S rRNA sequence data providing 2,494 operational taxonomic units, we could successfully predict the bacterial species responsible for the production of a series of microbiota-associated lipids.

Recent Major Publications

Okahashi N, Ueda M, Yasuda S, Tsugawa H, Arita M. Global profiling of gut microbiota-associated lipid metabolites in antibiotic-treated mice by LC-MS/MS-based analyses. ***STAR Protoc*** 2, 100492 (2021)

Hirata T, Yamamoto K, Ikeda K, Arita M. Functional lipidomics of vascular endothelial cells in response to laminar shear stress. ***FASEB J*** 35, e21301 (2021)

Tsugawa H, Ikeda K, Takahashi M, Satoh A, Mori Y, Uchino H, Okahashi N, Yamada Y, Tada I, Bonini P, Higashi Y, Okazaki Y, Zhou Z, Zhu Z, Koelmel J, Cajka T, Fiehn O, Saito K, Arita M, Arita M. A lipidome atlas in MS-DIAL 4. ***Nat Biotechnol*** 38, 1159-1163 (2020)

Invited presentations

Arita M. "Advanced lipidomics technology and its application in biology" 5th RIKEN IMS-Stanford ISCBRM Joint Symposium (Online) November 2021

Arita M. "Omega-3 fatty acid metabolism that controls inflammation and tissue homeostasis" World Congress of OMEGA3 Science and Technology (WCOST) (Online) November 2021

Arita M. "Advanced non-targeted lipidomics and its application to vascular biology" 19th International Symposium on Atherosclerosis (ISA2021) (Kyoto, Japan) October 2021

Arita M. "Advanced non-targeted lipidomics and its application in biology" The 5th JCS Council Forum on Basic Cardiovascular Research (Online) September 2021

Arita M. "Biology of LipoQuality: Advanced lipidomics technology and its application in biology" 9th International Singapore Lipid Symposium (iSLS9) (Online) March 2021

Lipids constitute biological membranes and play a variety of roles as energy sources, signaling molecules and their precursors. It is important to understand at the molecular level the structural diversity of these lipid molecules and how they are recognized and utilized *in vivo*. Since abnormal lipid metabolism is an underlying factor in many diseases and lipids contain many bioactive molecules, this may lead to the discovery of new seeds for drug discovery and medical applications, such as early diagnosis and treatment.

We established a non-targeted lipidomics platform packaged in MS-DIAL4 that derived a catalog of over 8,000 unique structures of 117 lipid class categories. We created a comprehensive database of lipid structures with their mass spectrum properties, such as retention time, collision cross-section, and mass fragmentation pattern to correctly characterize lipids. This database has been published as a "lipidome atlas".

Host-microbiota interactions create a unique metabolic milieu that modulates intestinal environments. By combining non-targeted mass spectrometry with feature-based molecular spectrum networking technology, we revealed that lipids with complex structures are produced by the gut microbiota. Correctly capturing such molecular groups, which have been overlooked by conventional targeted analysis, will lead to an understanding of the significance of bacterial species that correlate with various diseases such as cancer, obesity, and allergy. The method developed in this study is a technology that enables us to observe such changes in the bacterial flora as changes in the lipidome environment from a bird's eye view and is expected to contribute to the identification of functional metabolites that mediate host-bacteria interactions.

Laboratory for
Microbiome Sciences

Team Leader: Hiroshi Ohno

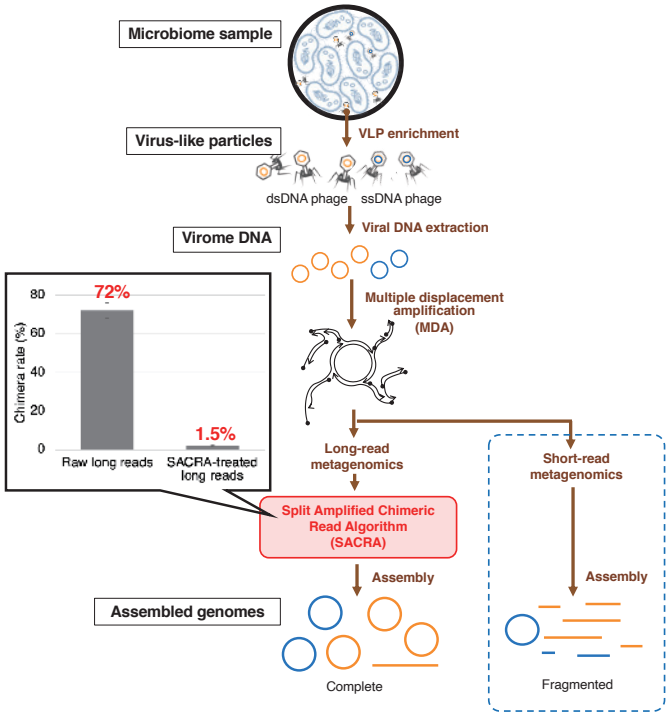


Figure: SACRA-coupled long-read metagenomics for virome analysis
Our method extracts virus-like particles (mainly double and single-strand DNA phages) from human fecal samples and obtains the virome DNA. Then the low-biomass virome DNA is amplified by multiple displacement amplification (MDA) to obtain sufficient DNA for sequencing. Conventionally, the MDA-treated virome DNA is sequenced by a short-read sequencer, but subsequent assembly generates highly fragmented genomes. Our method can reconstruct complete phage genomes using long reads preprocessed by the novel bioinformatics tool SACRA for reducing the chimeric reads generated by MDA.

Recent Major Publications

Furuhashi H, Takayasu L, Isshi K, Hara Y, Ono S, Kato M, Sumiyama K, Suda W. Effect of storage temperature and flash-freezing on salivary microbiota profiles based on 16S rRNA-targeted sequencing. ***Eur J Oral Sci*** 20, e12852 (2022)

Kiguchi Y, Nishijima S, Kumar N, Hattori M, Suda W. Long-read metagenomics of multiple displacement amplified DNA of low-biomass human gut phageomes by SACRA pre-processing chimeric reads. ***DNA Res*** 28, dsab019 (2021)

Cariño R 3rd, Takayasu L, Suda W, Masuoka H, Hirayama K, Konishi S, Umezaki M. The search for aliens within us: a review of evidence and theory regarding the foetal microbiome. ***Crit Rev Microbiol*** 17, 1-13 (2021)

Invited presentations

Suda W. "Research of the Human Gut and Oral Microbiome", OMC Gastroenterology Conference (Online) November 2021

Suda W. "Trends and Examples of Human Microbiome Research", Diabetes Web Lecture Organized by Nippon Boehringer Ingelheim Co., Ltd (Online) October 2021

Suda W. "Trends and Applications of Microbiome Analysis" Educational Lecture, The 120th Annual Meeting of the Japanese Dermatological Association (Yokohama, Japan/Online) June 2021

The Laboratory for Microbiome Sciences has been engaged in the study of the complex interactions between symbiotic microbial ecosystems (composed of bacteria, viruses and fungi) and their hosts. Through the development of new experimental and informatics-based technologies using state-of-the-art sequencers, we aim to comprehensively understand and eventually control these symbiotic microbial ecosystems by clarifying not only the microbiome structure variation among individuals but also the time-series dynamics.

Currently, our team has published 15 papers in FY2021. In one representative paper, we have developed a novel long-read metagenomic method to analyze the viral community (virome) in the human gut (*DNA Res*. 2021 Oct 11;28(6):dsab019.). Our method amplifies the very low amount of double and single-strand viral genomic DNA in a human fecal sample by multiple displacement amplification (MDA) to obtain sufficient DNA for long-read sequencing. A novel bioinformatic method, SACRA, developed in our lab can efficiently correct the artificial chimeric reads generated by MDA to non-chimeric reads. *De novo* assembly using SACRA-treated long reads reconstructs high-quality phage genomes, which are often fragmented in conventional short-read data because of the community's genomic complexity. This technology reveals the previously undetectable characteristics of the human gut virome and contributes to clarifying the role of viruses in the human gut microbiome.

We are also constructing a high-quality genomic database by independently compiling the microbial chromosomes, plasmids, and phages from human gut long-read metagenomics of the Japanese cohort. This database is composed of various novel genetic elements with high completeness and is expected to provide a foundation for diversified human gut microbiome.

Laboratory for

Integrated Cellular Systems

Team Leader: **Katsuyuki Yugi**

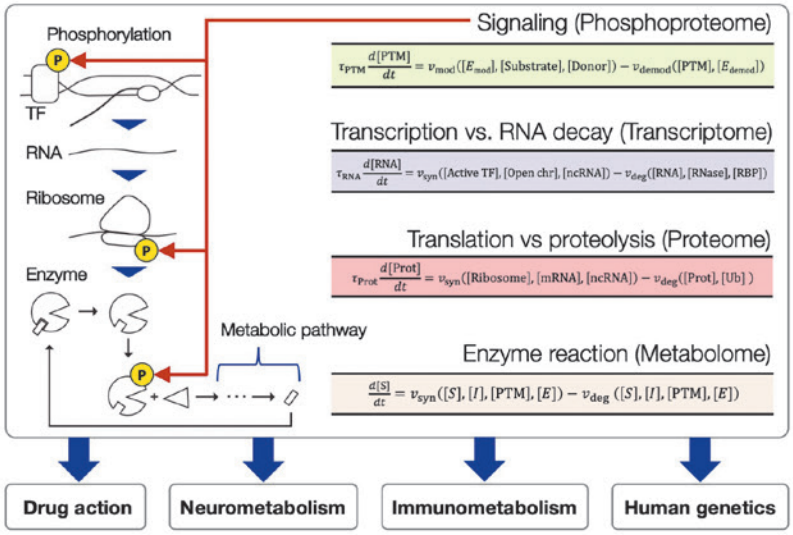


Figure: Differential equation representation of a trans-omic network and its application to related fields

We integrate multiple omic data with postulating a dynamic picture of cellular processes driven by reaction kinetics. Each reaction rate (terms represented by '*v*') is a function of the number of molecules that belong to the same or other omic layers. Characteristic time 'τ' emphasizes time scales for each omic layer (PTM: post-translational modification such as phosphorylation of an enzyme; E_{mod}: modification enzyme; Substrate: substrate for the modification reaction; Donor: chemical group donor such as acetyl-CoA for histone acetylation; E_{demod}: demodification enzyme; Active TF: active transcription factor; Open chr: open chromatin; ncRNA: noncoding RNA; RBP: RNA binding proteins; Prot: protein abundance; Ub: ubiquitin; *S*: reactant metabolites; *I*: activators or inhibitors; *E*: enzyme abundance). We apply this methodology to characterizing complex metabolic regulatory systems related to drug action, the nervous system, the immune system, human genetics, etc.

Recent Major Publications

Miyauchi K, Adachi Y, Tonouchi K, Yajima T, Harada Y, Fukuyama H, Deno S, Iwakura Y, Yoshimura A, Hasegawa H, Yugi K, Fujii S.I, Ohara O, Takahashi Y, Kubo M. Influenza virus infection expands the breadth of antibody responses through IL-4 signalling in B cells. ***Nat Commun*** 12, 3789 (2021)

Egami R, Kokaji T, Hatano A, Yugi K, Eto M, Morita K, Ohno S, Fujii M, Hironaka K, Uematsu S, Terakawa A, Bai U, Pan Y, Tsuchiya T, Ozaki H, Inoue H, Uda S, Kubota H, Suzuki Y, Matsumoto M, Nakayama K.I, Hirayama A, Soga T, Kuroda S. Trans-omic analysis reveals obesity-associated dysregulation of inter-organ metabolic cycles between the liver and skeletal muscle. ***iScience*** 24, 102217 (2021)

Kokaji T, Hatano A, Ito Y, Yugi K, Eto M, Morita K, Ohno S, Fujii M, Hironaka KI, Egami R, Terakawa A, Tsuchiya T, Ozaki H, Inoue H, Uda S, Kubota H, Suzuki Y, Ikeda K, Arita M, Matsumoto M, Nakayama KI, Hirayama A, Soga T, Kuroda S. Transomics analysis reveals allosteric and gene regulation axes for altered hepatic glucose-responsive metabolism in obesity. ***Sci Signal*** 13, eaaz1236 (2020)

Invited presentations

Yugi K. "A data-driven and a hypothesis-driven omics integration for systems biology of metabolism" NARA Institute of Science and Technology (Online) November 2021

Yugi K. "Trans-omic data integration for reconstructing metabolic regulatory networks" Current Trends in Bioinformatics, Yokohama City University (Online) November 2021

Yugi K. "Trans-omics: systems biology based on integration of multiple omic data" Lectures on Molecular Biology, Niigata University, (Online) October 2021

Metabolism is a biological process involved in various diseases, not only metabolic diseases such as obesity and diabetes, but also autoimmune diseases, psychiatric disorders and cancer. Biochemical pathways for metabolism consist of myriad feedback loops, thereby defying simple causation analyses frequently performed in other linear networks and cascades. Furthermore, metabolism undergoes multiplexed regulation from other omic layers, e.g., phosphorylation of enzymes by signal transduction (phosphoproteome), transcriptional regulation (transcriptome), translational regulation (expression proteome), etc. Our research interest is to understand intracellular metabolism and its regulatory mechanisms as a system of biochemical reactions in dynamic, macroscopic and quantitative contexts. We employ the methodology of 'trans-omics' that aims to reconstruct global metabolic regulatory networks spanning multiple omic layers, not as a group of indirect statistical correlations but as chains of direct mechanistic interactions on the basis of reaction kinetics (Yugi *et al.*, *Trends Biotechnol.*, 2016; Yugi and Kuroda, *Cell Syst.*, 2017; Yugi and Kuroda, *Curr. Opin. Syst. Biol.*, 2018; Yugi *et al.*, *Curr. Opin. Syst. Biol.*, 2019; Okatomo *et al.*, *Neurosci Res.*, 2022). Interdisciplinary approaches, such as 'wet' biology experiments, and 'dry' analyses, such as databases and mathematical models, are utilized to characterize the global metabolic regulatory networks. The network reconstruction is performed based on comprehensive measurement data, public databases and a kinetic picture of the cellular processes (Figure). The comprehensive data of multiple omic layers should be measured under identical conditions in a time-series manner so that one can construct mathematical models of the multi-layered network for subsequent systems biological analyses. We eventually aim to reveal the chain of logic from individual biochemical reactions to omics-scale metabolic regulatory systems.

Laboratory for
Metabolic Networks

Team Leader: **Toshimori Kitami**

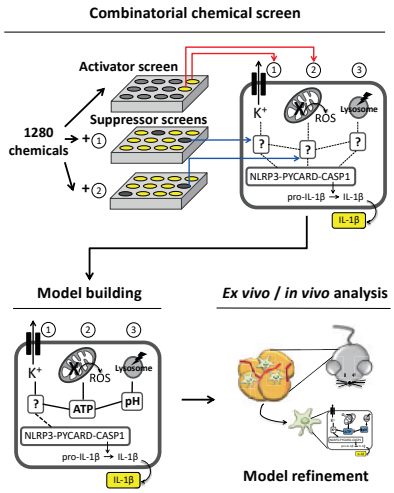


Figure: Schematic of our chemical screening strategy to understand the role of mitochondria in NLRP3 inflammasome activation

Recent Major Publications

Takeuchi T, Miyauchi E, Kanaya T, Kato T, Nakanishi Y, Watanabe T, Kitami T, Taida T, Sasaki T, Negishi H, Shimamoto S, Matsuyama A, Kimura I, Williams IR, Ohara O, Ohno H. Acetate differentially regulates IgA reactivity to commensal bacteria. ***Nature*** 595, 560-564 (2021)

Kitami T, Fukuda S, Kato T, Yamaguti K, Nakatomi Y, Yamano E, Kataoka Y, Mizuno K, Tsuboi Y, Kogo Y, Suzuki H, Itoh M, Morioka MS, Kawaji H, Koseki H, Kikuchi J, Hayashizaki Y, Ohno H, Kuratsune H, Watanabe Y. Deep phenotyping of myalgic encephalomyelitis/chronic fatigue syndrome in Japanese population. ***Sci Rep*** 10, 19933 (2020)

Tran UT, Kitami T. Niclosamide activates the NLRP3 inflammasome by intracellular acidification and mitochondrial inhibition. ***Commun Biol*** 2, 2 (2019)

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 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.

Mitochondria in inflammation

We recently used our screening toolbox to identify chemicals that can activate and suppress an inflammatory pathway called the NLRP3 inflammasome. We discovered niclosamide (mitochondrial uncoupler) as a new activator of this pathway. By pursuing the mechanism of action of niclosamide, we learned how mitochondrial energy metabolism, in partnership with glycolysis, is linked to inflammasome activation.

We are also extending our screening approach to understand how other environmental toxins and particles activate the inflammasome. We are continuing to explore how different key cellular processes, especially in regards to cellular metabolism, work together during inflammasome activation.

Mitochondria in neuronal death

We have begun to study how mitochondrial stress leads to neuronal cell death in the context of Parkinson's disease. In collaboration with our colleagues from the Luxembourg Centre for Systems Biomedicine (LCSB), we are using focused chemical screens and metabolomics to understand how metabolic pathways respond to mitochondrial stress. We hope to understand how mitochondrial perturbation triggers various stress response pathways and to search for ways to delay or avert neuronal cell death.

Drug Discovery Antibody Platform Unit

Unit Leader: Takashi Saito

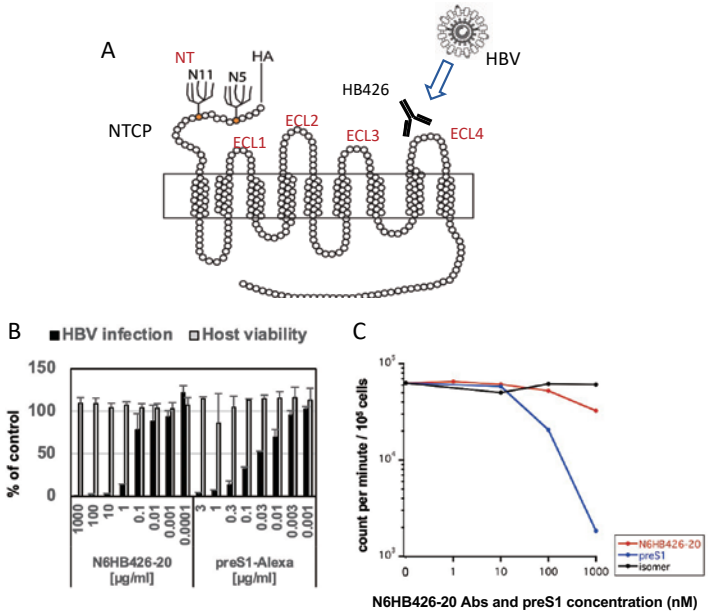


Figure: Established anti-NTCP mAb prevents HBV infection

A) Schematic structure of NTCP with the susceptible region (ECL4) for HBV binding and blocking of HBV entry by the anti-NTCP mAb. B) Inhibition of HBV infection of a liver cell line by the anti-NTCP mAb, N6HB426. C) Whereas the anti-NTCP mAb blocks HBV entry, it did not block bile-acid import.

Recent Major Publications

Takemori T, Sugimoto-Ishige A, Nishitsuji H, Futamura Y, Harada M, Kimura-Someya T, Matsumoto T, Honma T, Tanaka M, Yaguchi M, Isono K, Koseki H, Osada H, Miki D, Saito T, Tanaka T, Fukami T, Goto T, Shirouzu M, Shimotohno K, Chayama K. Establishment of a monoclonal antibody against human NTCP that blocks HBV infection. ***J Virol*** 96, e0168621 (2022) doi: 10.1128/JVI.01686-21

Sugimoto-Ishige A, Harada M, Tanaka M, Terooatea T, Adachi Y, Takahashi Y, Tanaka T, Burrows PD, Hikida M, Takemori T. Bim establishes the B cell repertoire from early to late in the immune response. ***Int Immunol*** 33, 79-90 (2020)

Tanaka M, Ishige A, Yaguchi M, Matsumoto T, Shirouzu M, Yokoyama S, Ishikawa F, Kitabayashi I, Takemori T, Harada M. Development of a simple new flow cytometric antibody-dependent cellular cytotoxicity (ADCC) assay with excellent sensitivity. ***J Immunol Methods*** 464, 74-86 (2019)

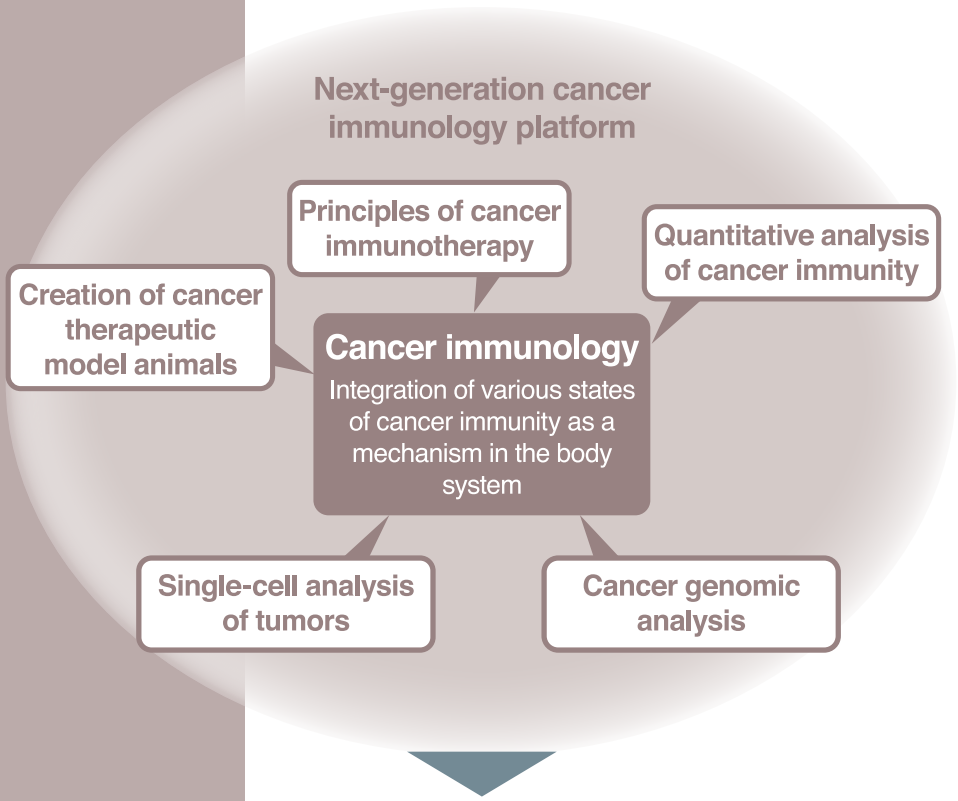
The Drug Discovery Antibody Platform Unit (Ab Platform) is one of nine Drug Discovery Basic Units in the Drug Discovery and Medical Technology Platform (DMP). DMP develops innovative new pharmaceuticals to transfer the basic research performed at the institute to the clinics. Particularly, the Ab Platform creates new monoclonal Abs (mAb) for therapeutic purposes of preventing/modulating various diseases.

During the last few years, we have developed and analyzed a mAb against a human hepatitis B virus (HBV) receptor, NTCP (sodium-taurocholate co-transporting polypeptide). Because the preS1-domain of HBV binds to NTCP, it could be a key target for the development of HBV blocking agents. Indeed, the established mAb inhibits the entry of HBV into human liver cells *in vitro* and inhibits *in vivo* infection in a system using human-liver chimeric mice. Currently available inhibitors of NTCP binding of HBV have the unwanted side effect of also blocking bile acid import, however, this mAb does not. Therefore, the mAb becomes an ideal specific inhibitor of HBV infection without side effects. Now humanized NTCP mAbs are being developed for clinical applications (Figure and publication).

We also established mAbs against CRTAM (Cytotoxic and Regulatory T Cell Molecule). CRTAM is critical for the development/function of $CD4^+$ cytotoxic T cells (CTL), which are thought to be critical for late-phase induction of EAE, experimental autoimmune encephalomyelitis. Indeed, preliminary results showed a significant inhibition of late phase of EAE induction by administration of a CRTAM mAb, suggesting its potential therapeutic use.

More recently, we have established mAbs against human TMPRSS2 for the purpose of inhibiting infection with SARS-CoV2. TMPRSS2 is critical for viral entry and small molecule inhibitors of TMPRSS2 have been shown to inhibit SARS-CoV2 infection, although these compounds also have side effects and instability. Therefore, mAbs against TMPRSS2 may have a more specific inhibitory function and modulate COVID-19. Indeed, some mAbs showed strong inhibition of any SARS-CoV2 variants *in vitro*.

Division of Cancer Immunology



Division of Cancer Immunology will explore novel principles of the immune system, focusing on tumor cells, and promote research for the establishment of novel therapeutics.

Laboratory for Medical Science Mathematics

Team Leader: Tatsuhiko Tsunoda

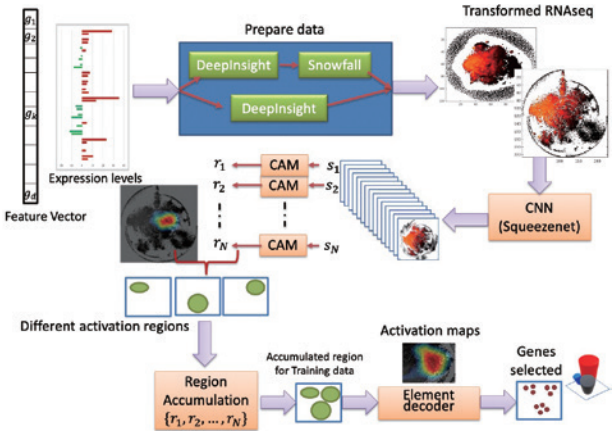


Figure: DeepFeature – deep learning for 'omic data analysis and interpretation
The overall DeepFeature procedure for feature selection using a convolutional neural network.

Recent Major Publications

Sharma A, Lysenko A, Boroevich KA, Vans E, Tsunoda T. DeepFeature: feature selection in nonimage data using convolutional neural network. ***Brief Bioinform*** 22, bbab297 (2021)

Rheinbay E, Nielsen MM, Abascal F, Wala JA, Shapira O, Tiao G, Hornshøj H, Hess JM, Juul RI, Lin Z, Feuerbach L, Sabarinathan R, Madsen T, Kim J, Mularoni L, Shuai S, Lanzós A, Herrmann C, Maruvka YE, Shen C, Amin SB, Bandopadhayay P, Bertl J, Boroevich KA, Busanovich J, Carlevaro-Fita J, Chakravarty D, Chan CWY, Craft D, Dhingra P, Diamanti K, Fonseca NA, Gonzalez-Perez A, Guo Q, Hamilton MP, Haradhvala NJ, Hong C, Isaev K, Johnson TA, ... , Tsunoda T, *et al.* Analyses of non-coding somatic drivers in 2,658 cancer whole genomes. ***Nature*** 578, 102-111 (2020)

Nishino J, Watanabe S, Miya F, Kamatani T, Sugawara T, Boroevich KA, Tsunoda T. Quantification of multicellular colonization in tumor metastasis using exome sequencing data. ***Int J Cancer*** 146, 2488-2497 (2020)

Invited presentations

Tsunoda T. "Deep Learning and Mathematical Sciences for Advancing Genomic Medicine" The 25th Annual Meeting of the Japanese Association for Molecular Target Therapy of Cancer, Symposium 2 "AI" (Japan/Online) May 2021

Tsunoda T. "Genomic Medicine with New Intelligence" The University of Tokyo Institute for Genomic Medicine 2020 Symposium (Japan/Online) March 2021

Tsunoda T. "Exploring etiologies, sub-classification, and risk prediction of diseases based on big-data analysis of clinical and whole omics data in medicine" ERCIM-JST Joint Symposium on Big Data and Artificial Intelligence (Japan/Online) February 2021

Tsunoda T. "Exploring etiologies, sub-classification, and risk prediction of diseases based on big-data analysis of clinical and whole omics data in medicine" CREST International Symposium on Big Data Application (Japan/Online) January 2021

Tsunoda T. "Deep learning opens up new frontiers in genomic medicine" The 16th The Japanese Association for Molecular Target Therapy of Cancer TR Workshop (Japan/Online) January 2021

Effective utilization of rapidly developing 'omic profiling technologies and the introduction of personalized/precision/preventive medicine have recently become major goals of biomedical research. This paradigm shift requires moving away from traditional approaches that do not adequately consider the individual characteristics of each patient. Our laboratory develops new strategies to address these challenges by bringing ideas and methods from mathematics and computational sciences to the medical domain. The first part of our approach is driven by integrative analysis of clinical and omic data and aims to explore the etiologies of intractable diseases. Next, we classify each disease into finer categories, such as based on the types of anti-cancer immune responses, using molecular profiles, and then clarify the underlying causal mechanisms with systems-based approaches. Lastly, we apply mathematical and machine learning techniques to infer optimal therapy for each patient to guide treatment decisions made by their hospital or clinic. Similar approaches can be used for disease prevention based on an individual's medical history. Our research projects include: (1) Investigating the relationship between tumor microenvironment, subclonal diversity, drug response, and patient prognosis in lung, colorectal and liver cancer, (2) Development of novel machine learning methods for cancer immunology multi-omics, (3) Integrative trans-omics modelling of disease-associated genomic variations, (4) Accurate insertion/deletion calling from next-generation sequencing (NGS) data, (5) Whole exome sequencing (WES) analysis to identify intractable disease-causing genes, (6) Cancer whole genome sequencing (WGS) analysis, (7) Development of new clustering methods, (8) Development of cancer classification and prognosis prediction methods based on gene expression data, (9) Prediction of optimal drug combinations for cancer chemotherapy, (10) Drug toxicity prediction with machine learning, (11) Prediction of post-translational amino-acid modifications, protein structure, protein-peptide interactions, molecular recognition features (MoRFs), and protein functions, (12) Discovery of clinically-relevant subtypes for cancer immunotherapy, and (13) Development of explainable AI and deep learning technologies for image and 'omic data analyses.

Laboratory for
Cancer Genomics

Team Leader: **Hidewaki Nakagawa**

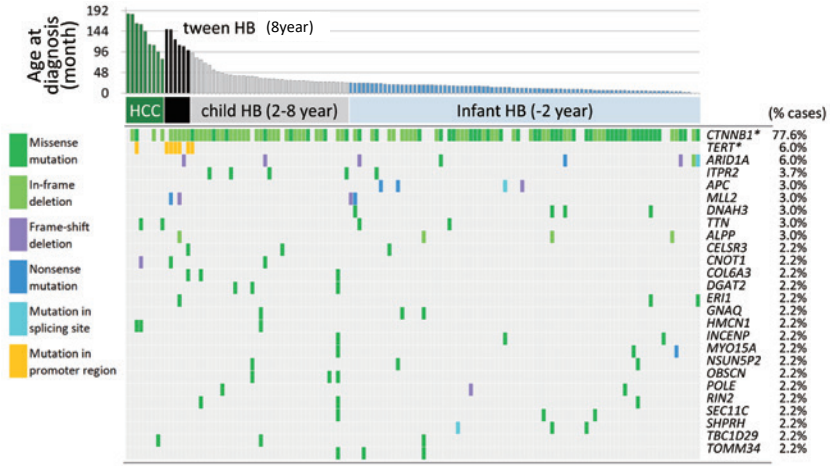


Figure:
Landscape of driver mutations in childhood liver neoplasms.134 cases of childhood hepatoblastoma (HB) and hepatocellular carcinoma (HCC) are sorted by the age at diagnosis. The bar graph in the top panel shows individual age at diagnosis (childhood HCC, tween HB, child HB, infant HB). The middle panel details driver mutations in this cohort. (*) Mutations and in-frame deletions of *CTNNB1* are frequently observed in HBs and *TERT* promoter mutations are prevalent in the tween HBs and HCCs.

Cancer is essentially a "disease of the genome" that develops and evolves with the accumulation of a variety of mutations in its genetically unstable background. Some somatic mutations of driver genes have been successfully targeted for cancer treatment, and germline variants are related to cancer predisposition and risk assessment. Now, genotype-based personalized cancer therapy is in the clinical stage. Understanding of, and attention to, the underlying genetic diversity in cancer is, therefore, likely to increase the success of new cancer treatment modalities. Recent explosive advances in next-generation sequencing (NGS) and bioinformatics enable us to perform systematic, genome-wide identification of all somatic abnormalities by whole genome sequencing (WGS) and RNA sequencing. Furthermore, cancer also has been proven to have features of an immune reaction and, thus, immune therapies targeting immune checkpoints and neo-antigens derived from somatically mutated proteins are also treatment realities. To explore whole genomic and immuno-genomic alterations and their diversity in cancer, we have been applying NGS and new single-cell technologies and analyzing these data through international collaborations such as the International Cancer Genome Consortium (ICGC). These approaches, combined with mathematical analysis and other -omics analyses, can clarify the underlying cancer genesis and cancer immunity and achieve a molecular sub-classification of cancer, which will facilitate the discovery of genomic biomarkers and personalized cancer medicine.

Recent Major Publications

Nagae G, Yamamoto Y, Fujita M, Fujita T, Umeda T, Fukuda S, Tatsuno K, Maejima K, Kurihara S, Kojima M, Hishiki T, Watanabe K, Ida K, Yano M, Hiyama Y, Tanaka Y, Inoue T, Ueda H, Nakagawa H*, Aburatani H*, and Hiyama E*. Genetic and epigenetic basis of hepatoblastoma diversity. ***Nat Commun*** 12, 5423 (2021)

Mizuno S, Yamaguchi R, Hasegawa T, Hayashi S, Fujita M, Zhang F, Koh Y, Lee SY, Yoon SS, Shimizu E, Komura M, Fujimoto A, Nagai M, Kato M, Liang H, Miyano S, Zhang Z, Nakagawa H*, and Imoto S*. Immuno-genomic pan-cancer landscape reveals immune escape mechanisms and immuno-editing histories. ***Sci Rep*** 11, 15713 (2021)

Ebata N, Fujita M, Sasagawa S, Maejima K, Okawa Y, Hatanaka Y, Mitsuhashi T, Oosawa A, Tanaka H, Miyano S, Nakamura T, Hirano S*, and Nakagawa H*. Genomic and transcriptomic landscape of gallbladder cancer for molecular classification and tumor microenvironment characterization. ***Cancers (Basel)*** 13, 733 (2021)

Invited presentations

Nakagawa H. "Genomic Structures and Carcinogenesis of HBV Integrations in Liver Cancer" The Asian Pacific Association for the Study of the Liver (Tokyo, Japan) December 2021

Nakagawa H. "Data Sharing in the International Genome Consortium" Illumina Genomics Summit 2021 (Tokyo, Japan/Online) November 2021

Nakagawa H. "Whole genomics and Precision Oncology for Hepato-biliary Cancer" The Asian Pacific Association for the Study of the Liver (Osaka, Japan) September 2021

Nakagawa H. "Immuno-genomic Analysis of Cancer Tissues to Understand Tumor Immunology" The 25th Annual Meeting of the Japanese Association of Cancer Immunology (Wakayama, Japan) July 2021

Nakagawa H. "Whole Genome Sequencing analysis and Clinical Implication for Liver Cancer" The 57th Annual Meeting of Liver Cancer Study Group of Japan (Kagoshima, Japan) July 2021

Laboratory for Immunotherapy

Team Leader: Shin-ichiro Fujii

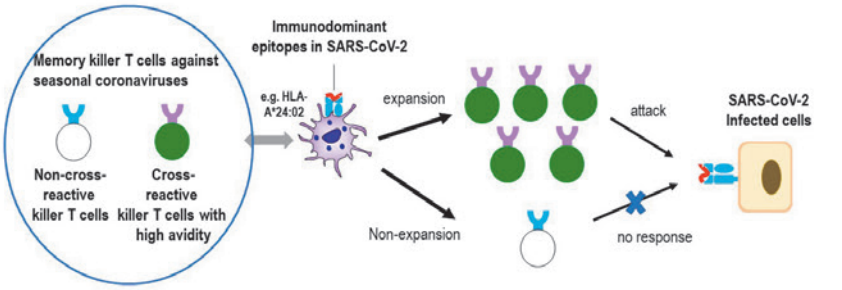


Figure: The role of pre-existing, cross-reactive killer T cells for SARS-CoV-2 in COVID-19
When SARS-CoV-2-cross-reactive, memory killer T cells with high avidity are maintained, they can expand quickly in response to immunodominant epitopes in SARS-CoV-2 and also exert anti-viral effects.

The role of pre-existing, cross-reactive killer T cells for SARS-CoV-2 in COVID-19

We have worked to harness the innate and adaptive immune systems, resulting in the induction of immunological memory against cancer. We have been promoting our artificial adjuvant vector cell (aAVC) project against cancer. NY-ESO-1 is an esophageal squamous cell carcinoma antigen and WT1 is a Wilms' tumor antigen. In the current year, we established NY-ESO-1 expressing aAVC (aAVC-NY-ESO-1) and showed the immunological and anti-tumor effect in a preclinical study in collaboration with Astellas Pharma Inc. For aAVC-WT1, we started a Phase II clinical study in collaboration with the Institute of Medical Science, the University of Tokyo, Chiba University and Kagawa University. The COVID-19 pandemic has been ongoing since 2019. Recent data have reported the insufficient efficacy of the current COVID-19 vaccines in cancer patients. COVID-19 is a thus a serious issue in cancer patients and, since 2020, we therefore started a COVID-19 project to overcome the COVID-19 pandemic. To evaluate the CTL response against SARS-CoV-2, we first focused on identification of immunodominant epitopes in the viral spike protein for HLA-A24, which is major HLA in Japan. We identified the "QYI peptide", which can induce specific CTLs in 80% of HLA-A24$^+$ unexposed healthy donors but in only 14% of HLA-A24$^+$ patients with hematological malignancies. These QYI-specific CTLs are pre-existing memory CTLs against seasonal coronaviruses and cross-reactive against SARS-CoV-2 (Figure). We demonstrated TCR cross-reactivity of these CTLs at the single cell level and obtained crystal structures of the HLA-A24-QYI peptide or the relevant peptides of seasonal coronaviruses. For high-risk groups such as cancer patients, a second line vaccine is needed. To this end, we have developed SARS-CoV-2 spike protein-expressing aAVC-CoV-2 as a COVID-19 vaccine. We examined whether aAVC-CoV-2 can act as a promising second line vaccine for these high-risk groups. In a preclinical study, we obtained solid immunological evidence - aAVC-CoV-2 induced robust multifunctional SARS-CoV-2-specific T cells as well as anti-SARS-CoV-2 antibodies. Especially, we found long-term memory CTLs in aAVC-CoV-2-vaccinated mice.

Recent Major Publications

Fujii S, Yamasaki S, Hanada K, Ueda S, Kawamura M, and Shimizu K. Cancer immunotherapy using artificial adjuvant vector cells to deliver NY-ESO-1 antigen to dendritic cells *in situ*. ***Cancer Sci*** 113, 864-874 (2022)

Shimizu K, Iyoda T, Sanpei A, Nakazato H, Okada M, Ueda S, Kato-Murayama M, Murayama K, Shirouzu M, Harada N, Hidaka M, and Fujii S. Identification of TCR repertoires in functionally competent cytotoxic T cells cross-reactive to SARS-CoV-2. ***Commune Biol*** 4, 1365 (2021)

Miyauchi K, Adachi Y, Tonouchi K, Yajima T, Harada Y, Fukuyama H, Deno S, Iwakura Y, Yoshimura A, Hasegawa H, Yugi K, Fujii S, Ohara O, Takahashi Y, and Kubo M. Influenza virus infection expands the breadth of antibody responses through IL-4 signalling in B cells. ***Nat Commun*** 12, 3789 (2021)

Invited presentations

Fujii S. "A new therapeutic cancer vaccine inducing both innate and adaptive immunity, artificial adjuvant vector cells" The 29th Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics (Online) September 2021

Fujii S. "Dendritic cell- based new immunotherapy" The 28th Annual Meeting of the Japanese Society of Immunotoxicology (Online) September 2021

Fujii S. "A new therapeutic cancer vaccine inducing multifunctional immunity, artificial adjuvant vector cells" 2021 The 1st JCA-AACR Precision Cancer Medicine International Conference (Online) September 2021

Fujii S. "Immune response of first-in-human phase I clinical trial by WT1-expressing artificial adjuvant vector cells (aAVC-WT1)" The 25th Annual Meeting of Japanese Association of Cancer Immunology (Wakayama, Japan) July 2021

Fujii S. "Immune responses of cancer immunotherapy with artificial adjuvant vector cells in Phase I trial" The 13th Annual Meeting of the Japanese Society of Immunotherapy for Hematological disorders (Tokyo, Japan) June 2021

Laboratory for

Human Disease Models

Team Leader: Fumihiko Ishikawa

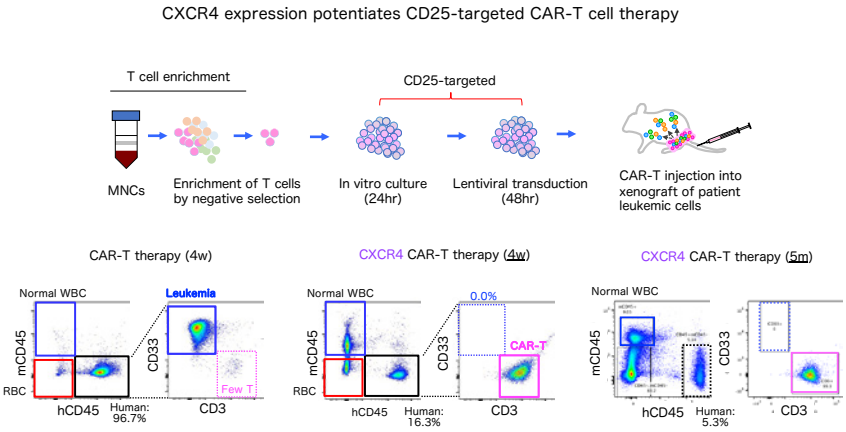


Figure: Engineering of chemokine receptor-expressing CAR-T cells for AML treatment
(Upper) A schematic showing how to prepare CD25-targeted CAR-T cells for injecting into mice bearing an AML patient-derived xenograft. (Lower left) In an AML xenograft mouse injected with CD25-targeted CAR-T cells, we did not observe good therapeutic efficacy with the remaining CD33$^+$ AML cells. (Lower middle) However, additional engineering to cause CXCR4 expression resulted in the complete elimination of AML cells and good recovery of normal hematopoiesis. (Lower right) The potent therapeutic effect against AML was maintained up until 5 months post-injection.

We aim to understand genetic and biological heterogeneity and complexity of poor prognosis leukemia. Through a collaboration with Dr. Shuichi Taniguchi's group at Toranomon Hospital, we have collected hundreds of leukemia patient samples. By extracting DNA and RNA from those samples as well as setting up xenogeneic transplantation using NOD/SCID/Il2rgKO newborns, we identified mutations and chromosomal aberrations critical in leukemia initiation. Furthermore, we discovered non-mutated key survival molecules in mitochondria and in the cytoplasm in a patient-specific manner.

To strengthen the therapeutic effect of small molecules targeting the intracellular vulnerability proteins, we set out to engineer T cells binding particular cell surface antigens and exhibiting potent cytotoxic activity against patient leukemic cells. In AML, considering the lack of or only low-level expression of putative targets in normal hematopoietic stem/progenitor cells and non-hematopoietic tissues, as well as the prognostic impact of the target's expression on patient survival, we chose CD25/IL2RA as one of the therapeutic cell surface molecules. Though CD25-targeted CAR-T cell therapy resulted in variable efficacy in the AML xenograft model, additional engineering resulting in expression of CXCR4 in the CAR-T cells led to complete elimination of human leukemic cells in bone marrow, spleen, liver, and peripheral blood. *In vivo* treatment of normal, non-leukemic humanized mice with the CXCR4-expressing CD25-targeted CAR-T cells did not result in a severe reduction of normal human immune subsets including regulatory T cells. We are currently performing and analyzing single-cell TCR and gene expression profiling as well as mass cytometry, which thus far is indicating that maintenance of central memory or stem cell memory CD4$^+$ T cells and over-represented expression of TCF7, IL-7R and CD226/DNAM1 are crucial cellular and molecular signatures of prevention of cellular exhaustion and supporting successful treatment efficacy of the CAR-T cells.

Recent Major Publications

Hashimoto M, Saito Y, Nakagawa R, Ogahara I, Takagi S, Takata S, Amitani H, Endo M, Yuki H, Manabe R, Watanabe T, Ozaki K, Kaneko A, Kajita H, Fujiki S, Sato K, Honma T, Uchida N, Okazaki Y, Ohara O, Shultz LD, Yamada M, Taniguchi S, Vyas P, de Hoon M, Momozawa Y, Ishikawa F. Maximal vulnerability converges to XIAP and BCL2 in leukemia with diverse genetic aberrations. ***Nat Cancer*** 2: 340-356 (2021)

De Groot A, Saito Y, Kawakami E, Hashimoto M, Aoki Y, Ono R, Ogahara I, Fujiki S, Kaneko A, Watanabe T, Takagi M, Tomizawa D, Koh K, Eguchi M, Ishii E, Ohara O, Shultz LD, Mizutani S, Ishikawa F. Targeting critical kinases and anti-apoptotic molecules overcomes steroid resistance in MLL-rearranged leukemia. ***EBioMedicine*** 64, 103235 (2021)

Saito Y, Shultz L, Ishikawa F. Understanding normal and malignant human hematopoiesis using next-generation humanized mice. ***Trends Immunol*** 41, 706-720 (2020)

Invited presentations

Ari Itoh-Nakadai. "Targeting poor prognosis leukemia with CD25-targeted chemokine receptor expressing CAR T cell therapy" Annual Meeting of Japan Biochemistry Society (online) November 2021

Ishikawa F. "Finding vulnerabilities in genetically-complex hematologic tumors" The 80th Annual Meeting for Japan Cancer Association (Yokohama, Japan) October 2021

Ishikawa F. "Humanized Mice for studying normal and diseased blood and immune systems" Lecture for Graduate School at Yokohama City University (Yokohama, Japan) October 2021

Ishikawa F. "Humanized Mice for Normal and Malignant Human Hematopoiesis" China-Japan High-level Expert Symposium on Animal Models (Beijing, China/Online) August 2021

Ishikawa F. "Identification of vulnerabilities in genetically-complex AML" Annual Meeting of European Hematology Association 2021(Online) June 2021

Special Program for Young Leaders

RIKEN Hakubi Fellows Program

RIKEN offers junior PI (Principal Investigator) positions, the RIKEN Hakubi Fellows, to exceptionally talented researchers for a maximum of 7 years. The RIKEN Hakubi Fellows are expected to engage independently in creative and ambitious research in natural and mathematical sciences, including research areas bordering the humanities and social sciences. An important goal of the RIKEN Hakubi Program is to foster stimulating interactions among Fellows with diverse backgrounds and to create an intellectual hub of scientists with different disciplines within and beyond RIKEN.

"Hakubi" is a phrase derived from classical Chinese story about five siblings in ancient China, all gifted, but the most brilliant one had white (haku) eyebrows (bi).

Young Chief Investigator Program

The Young Chief Investigator Program (YCI) aims to provide a career path for young investigators who conduct multidisciplinary research that will bridge immunology with other research fields. In this program, the selected Young Chief Investigator (age below 40) will head an independent research laboratory, but will have an access to mentoring by multiple senior specialists in related research fields. Mentors provide guidance for experimental design, preparation of papers and presentations, promotion of international visibility, and obtaining research funding. The YCI laboratory will also share space, equipment and facilities with a host laboratory in IMS.

Genome Immunobiology RIKEN Hakubi Research Team

Hakubi Team Leader: **Nicholas Parrish**

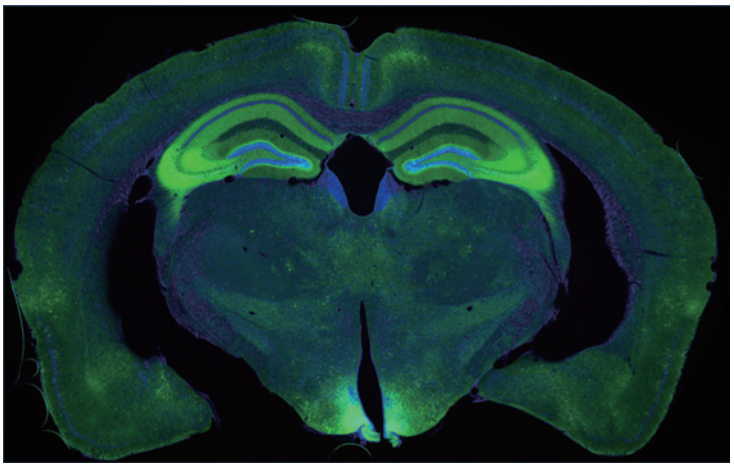


Figure: Coronal section of mouse brain infected with Borna disease virus
Borna disease virus (BoDV) is a re-emerging zoonotic pathogen that causes encephalitis and has a high case fatality rate in humans. We have developed a murine model of Borna disease virus infection. As in humans, the virus replicates abundantly in the hippocampus, which appears bright green in the adjacent photomicrograph due to GFP expressed from a BoDV reporter. The hippocampus contains abundant neural stem cells in adult animals and has often been reported to harbor somatic piRNAs.

We study the anamnestic responses of genomes upon exposure to mobile genetic elements. We focus on endogenous viral elements (EVEs), which are virus-derived sequences integrated into the genomes of their hosts. EVEs are often transcribed and processed into small RNAs called PIWI-interacting RNAs (piRNAs), which can guide RNA interference (RNAi) against complementary sequences. We are testing if piRNA-guided RNAi functions as antiviral immunity in eukaryotes, similar to the CRISPR/Cas adaptive immune system in prokaryotes. We previously showed that human and mouse EVEs derived from relatives of Borna disease virus (BoDV) are transcribed and processed into piRNAs (Parrish NF *et al.*, *RNA*. 2015). While piRNAs are known to guide RNAi against transposons, they have not been shown to function in antiviral immunity against exogenous viruses in mammals. We are using genome engineering to test this hypothesis in a murine model of BoDV infection. In addition, we have characterized EVEs derived from HHV-6 that have been stably co-evolving with human chromosomes since prehistory (Liu X *et al.*, *PLoS Genet*. 2020; Aswad A *et al.*, *Mol Biol Evol*. 2020). We are currently testing whether these EVEs make piRNAs and how they influence human phenotypes. We have also developed new computational tools to determine the genotype of polymorphic human mobile genetic elements, including EVEs, derived from human endogenous retroviruses (HERVs) (Kojima S *et al.*, *PLoS Genet*. 2020). In collaboration with several laboratories in IMS, we are using these tools to probe the influence of variation in EVEs and other mobile genetic elements on human phenotypes, including responses to coronavirus infection.

Recent Major Publications

Takahashi T, Heaton SM, Parrish NF. Mammalian antiviral systems directed by small RNA. ***PLoS Pathog*** 17,e1010091 (2021)

Kojima S, Kamada AJ, Parrish NF. Virus-derived variation in diverse human genomes. ***PLoS Genet*** 17, e1009324 (2021)

Kojima S, Yoshikawa K, Ito J, Nakagawa S, Parrish NF, Horie M, Kawano S, Tomonaga K. Virus-like insertions with sequence signatures similar to those of endogenous nonretroviral RNA viruses in the human genome. ***Proc Natl Acad Sci U S A*** 118, e2010758118 (2021)

Invited presentations

Kojima S. "Variation in human genomes and phenotypes driven by mobile genetic elements" Transposable Elements at the Crossroads of Evolution, Health, and Disease (Whistler, Canada/Online) March 2022

Parrish N.F. "Viruses make us who we are" Vanderbilt University Medical Center Grand Rounds (Nashville, USA) January 2022

Koide R. "A mouse model to test for EVE-derived antiviral activity against Borna disease virus" 68th Japan Society for Virology annual meeting (Kobe, Japan/Online) November 2021

Kamada A.J. "A model system to define the immune genes controlling reactivation of an endogenous virus" 68th Japan Society for Virology annual meeting (Kobe, Japan/Online) November 2021

Kojima S. "Screening for human herpesvirus 6 integrated in human genomes using biobank- scale sequencing data" 68th Japan Society for Virology annual meeting (Kobe, Japan/Online) November 2021

YCI Laboratory for Immunological Transcriptomics

Young Chief Investigator: Hideyuki Yoshida

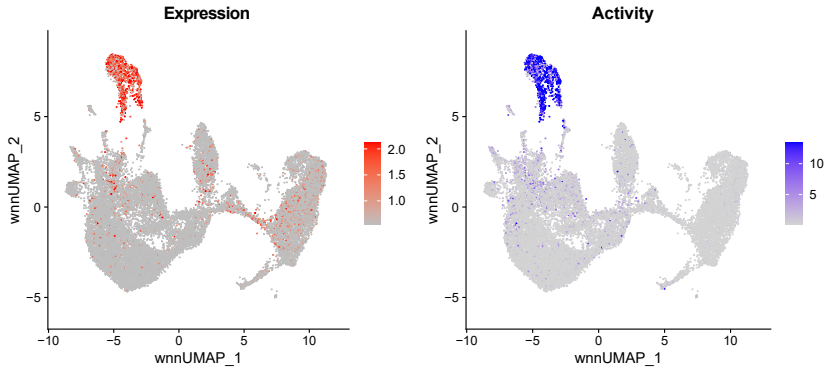


Figure: Activity of a transcription factor (TF) associated with a subpopulation of mTECs
TF expression and activity were examined by single-cell profiling and are shown for a representative TF by a color gradient (expression, left panel; activity, right panel). While TF activity does not always correlate with its expression, this TF is active in the expressed population. As a TF specific for a population can relate to its identity and function, this information is helpful to understand the population.

Recent Major Publications

Morimoto J, Matsumoto M, Miyazawa R, Yoshida H, Tsuneyama K, Matsumoto M. Aire suppresses CTLA-4 expression from the thymic stroma to control autoimmunity. ***Cell Rep*** 38, 110384 (2022)

Nishijima H, Matsumoto M, Morimoto J, Hosomichi K, Akiyama N, Akiyama T, Oya T, Tsuneyama K, Yoshida H, Matsumoto M. Aire Controls Heterogeneity of Medullary Thymic Epithelial Cells for the Expression of Self-Antigens. ***J Immunol*** 208, 303-320 (2022)

Rose SA, Wroblewska A, Dhainaut M, Yoshida H, Shaffer JM, Bektesevic A, Ben-Zvi B, Rhoads A, Kim EY, Yu B, Lavin Y, Merad M, Buenrostro JD, Brown BD, Immunological Genome C. A microRNA expression and regulatory element activity atlas of the mouse immune system. ***Nat Immunol*** 22, 914-927 (2021)

Yoshida H, Lareau CA, Ramirez RN, Rose SA, Maier B, Wroblewska A, Desland F, Chudnovskiy A, Mortha A, Dominguez C, Tellier J, Kim E, Dwyer D, Shinton S, Nabekura T, Qi Y, Yu B, Robinette M, Kim KW, Wagers A, Rhoads A, Nutt SL, Brown BD, Mostafavi S, Buenrostro JD, Benoist C, Immunological Genome P. The cis-Regulatory Atlas of the Mouse Immune System. ***Cell*** 176, 897-912. e20 (2019)

Invited presentations

Yoshida H. "Transcriptional and post-transcriptional regulatory networks in immunity." The 94th Annual Meeting of the Japanese Biochemical Society (Japan/Online) November 2021

Yoshida H. "Data-driven immunology: analysis of regulatory mechanisms in immunocytes by comprehensive profiling." The 29th Molecular Immunology Forum Tokyo (Online) March 2021

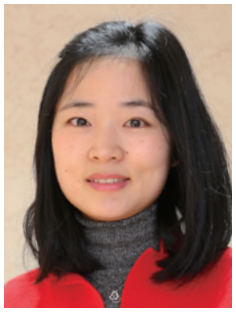
Gene regulation is one of the most fundamental mechanisms governing cell functions and biological processes, including immune cells and the immune system, and has been studied in many contexts. Recent advances in epigenome and transcriptome profiling technologies enable us to investigate gene regulation more comprehensively and precisely than ever to elucidate uncharted mechanisms in biology. Accordingly, transcriptomics has developed as a field of research to challenge biological questions utilizing these profiling approaches in a data-driven manner. In contrast, immunological studies usually start from phenotypes and investigate underlying mechanisms employing a hypothesis-driven approach. As both approaches have their own advantages, integration of immunology and transcriptomics can effectively dissect the immune system in an unprecedented manner. We are utilizing the techniques of cutting-edge transcriptomics to dissect gene regulation in immune cells, which will provide a deeper understanding of immune cell functions and ultimately lead to advances in the treatment of immune disorders. While transcriptomic approaches can be applied for various studies, we are currently engaging two major subjects.

1) Gene regulation underpinning immune tolerance.

Negative selection of self-reactive T cells occurs in the thymus and is an essential mechanism for achieving immune tolerance. Thymic medullary epithelial cells (mTECs) express various self-antigens, including peripheral tissue antigens (PTAs), whose expression is otherwise restricted to specific peripheral tissues. Developing T cells that strongly react with self-antigens are eliminated by apoptosis. Since the disrupted expression of PTAs can result in disturbed negative selection and autoimmune disorders, understanding the mechanisms controlling their expression is important to understand the pathogenesis of autoimmune diseases and to develop new treatments. To thoroughly understand mTECs and PTA expression, we have analyzed mTECs by single-cell RNA-seq and revealed gene regulation and populational heterogeneity (Figure). We are now investigating the immunological significance of our findings by transcriptomic approaches employing mouse models.

2) Data-driven project: systematic analysis of various immunocytes.

Bioinformatics has greatly impacted research on gene regulation and is becoming more powerful with the advent of big data analysis. To promote these data-driven studies, we are collaborating with the worldwide ImmGen group (http://www.immgen.org/).



YCI Laboratory for

Next-Generation Proteomics

Young Chief Investigator: **Yibo Wu**

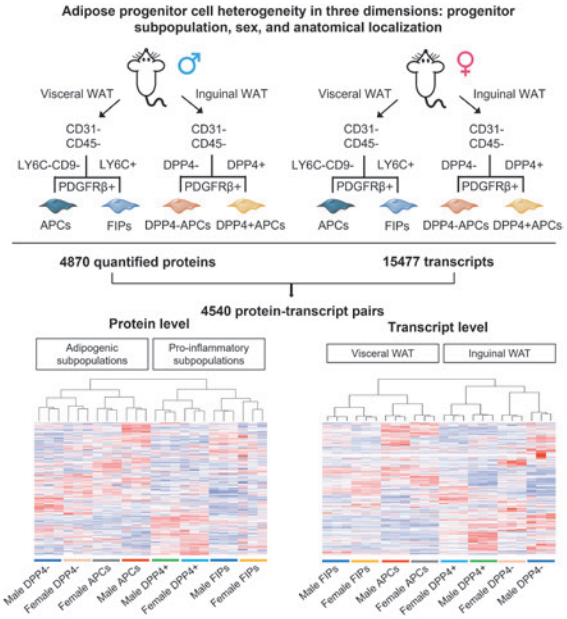


Figure: A multilayered omics analysis reveals sex- and depot-dependent adipose stromal cell heterogeneity

We describe a comprehensive and integrated dataset that details the transcriptomic and proteomic heterogeneity of adipose tissue progenitors in three dimensions: cell type (WAT PDGFR+ stromal cell subpopulations), anatomical localization (visceral and subcutaneous adipose depots), and sex (male and female mice). We have quantified 4870 proteins and 15477 transcripts across 24 samples, including 4540 protein-transcript pairs. Our data unveil molecular signatures defining sex differences in preadipocyte differentiation and identify regulatory pathways that functionally distinguish adipose progenitor subpopulations.

Our laboratory applies state-of-the-art mass spectrometry and computational methods for proteome analysis in complex biological systems, such as white adipose tissue (WAT). We have developed research themes on the molecular mechanism of WAT expansion. Specifically, we are interested in the molecular and functional heterogeneity of adipocyte progenitor populations, as well as how adipocytes, precursors and immune cells coordinately regulate the function of WAT.

To investigate sex- and depot (i.e. visceral or subcutaneous WAT)-dependent adipocyte progenitor cell heterogeneity, we performed a multilayered omics analysis to dissect adipose progenitor cell heterogeneity in three dimensions: progenitor subpopulation, sex, and anatomical localization. We have quantified 4870 proteins and 15477 transcripts. The data are freely accessible as a resource at "Pread Profiler" (http://preadprofiler.net/). Both proteomic and transcriptomic data clearly separated the eight cell populations (Figure). However, it is remarkable that the manner in which the populations can be grouped depends on the layer of analysis. Clustering based on proteomic data highlights the functional similarities and differences between the subgroups of cells, whereas clustering of cell populations based on transcript levels was driven by their inherent depot-dependent differences in gene expression.

In addition, we found that transcript levels are not indicative of cognate protein levels in many scenarios. Thus, changes in a certain transcript may not necessarily be reflected at the protein level. Surprisingly, both sex and depot had an impact on protein-transcript correlation.

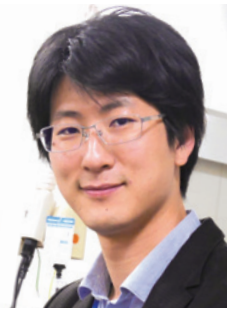
Our data revealed functional pathways that could discriminate cell populations. We found that PPARg phosphorylation underlies sex differences in iWAT APC differentiation. Furthermore, we discovered the importance of glutathione metabolism and AhR signaling in regulating progenitor cell fate and function. This multilayered omics analysis provides unprecedented insights into adipose stromal cell heterogeneity and highlights the benefit of complementary proteomics to support findings from scRNA-seq studies.

Recent Major Publications

Shan B, Barker S. C, Shao M, Zhang Q, Gupta K. R, Wu Y. Multilayered omics reveal Sex- and depot-dependent adipose progenitor cell heterogeneity. ***Cell Metab*** 34, 783-799.e7 (2022)

Zhang B, Vogelzang A, Miyajima M, Sugiura Y, Wu Y, ... Honjo T, Fagarasan S. B cell-derived GABA elicits IL-10^{+} macrophages to limit anti-tumour immunity. ***Nature*** 599, 471-476 (2021)

Shao M, Hepler C, Zhang Q, Shan B, Vishvanath L, Gervaise H. H, Zhao S, Yu A A, Wu Y, Strand W. D, and Rana K. Gupta. Pathologic HIF1α signaling drives adipose progenitor dysfunction in obesity. ***Cell Stem Cell*** 28, 1-17 (2021)



YCI Laboratory for

Metabolic Epigenetics

Young Chief Investigator: **Azusa Inoue**

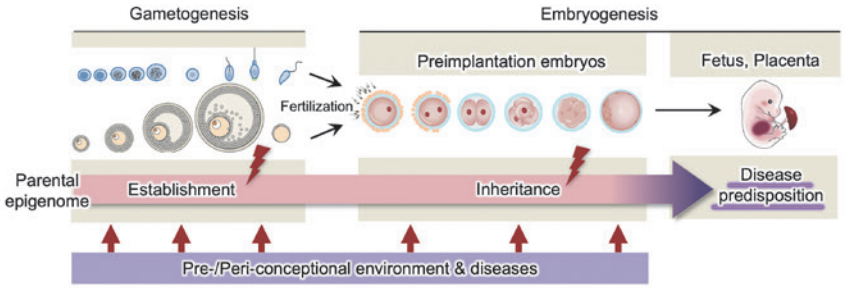


Figure:
Parental programming hypothesis. Parental epigenomes are established during gametogenesis, and some of them are inherited by embryos to regulate gene expression in the fetus and placenta. Our lab investigates the effect of the pre-/peri-conceptional parental environment on epigenetic inheritance and disease predisposition in the offspring.

Recent Major Publications

Mei H, Kozuka C, Hayashi R, Kumon M, Koseki H, Inoue A. H2AK119ub1 guides maternal inheritance and zygotic deposition of H3K27me3 in mouse embryos. ***Nat Genet*** 53, 539-550 (2021)

Invited presentations

Inoue A. "Maternal epigenetic inheritance by Polycomb repressive complexes" EMBO meeting CellBio Virtual 2021 (USA/Online) December 2021

Inoue A. "Developmental regulation by maternal inheritance of histone modifications" Research Seminar at Institute for Molecular and Cellular Regulation (Gunma, Japan) November 2021

Inoue A. "Maternal epigenetic inheritance by Polycomb repressive complexes" KALAS International Symposium 2021 (Korea/Online) July 2021

Inoue A. "Transgenerational inheritance of histone modifications in mammals" The 1380th Biology Seminar at the University of Tokyo (Tokyo/Online) May 2021.

Inoue A. "Maternal epigenetic inheritance by Polycomb repressive complexes" The 10th symposium of Smart Aging Research Center at Tohoku University (Miyagi, Japan/Online) May 2021

Genetic information is transmitted to the offspring and greatly contributes to their phenotype and disease susceptibility. However, it is not the sole determinant. For example, in type 2 diabetes, which is a hereditary disease, genome-wide association studies revealed that genetic variants can account for only ~30% of the heritability. Cohort studies of adopted children demonstrated that the contribution of the growth environment to the heritability of type 2 diabetes is also limited. These facts suggest the existence of yet-to-be-determined heritable mechanisms and suggest that the non-genetic bases of heredity need to be considered as a potential cause of hereditary diseases.

Accumulating evidence has demonstrated that epigenetic memory is also transmitted across generations in various organisms. To reveal non-genetic bases of disease heritability, our lab is studying epigenetic inheritance mechanisms in mammalian embryos. Our specific aims are as follows (Figure): (1) To understand the molecular basis and functions of epigenetic establishment and inheritance during gametogenesis and embryogenesis. (2) To understand the link between epigenetic inheritance and the pre- and peri-conceptional parental environment; (3) To understand how the parental environment can affect disease susceptibility in the offspring. To address these questions, we integrate cutting-edge low-input epigenome analysis technologies, reproductive engineering techniques and various genome/epigenome-modified mouse models. Our study will call for an evaluation of the contribution of epigenetic mechanisms to hereditary diseases and provide a foundation for establishing new approaches for preventive and predictive medicine.

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 Microscope Laboratory managed by Dr. Takaharu Okada, the Genomics Laboratory managed by Drs. Yukihide Momozawa and Jun Seita, 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 experiments in immunology, genome research and disease studies. The Laboratory supports both population and single-cell analysis and has upgraded all FACS Aria instruments, including two Aria Fusions, for multi-color analyses. In addition to FACS instruments, the lab possesses a mass spectrometry-based cytometer, HELIOS, which has the potential to analyze more than 40 markers simultaneously with metal-labeled antibodies.

In 2021, even during the difficult and restricted period due to the new coronavirus pandemic, in total 720 analytical and 1274 sorting experiments with FACS and 39 analyses with HELIOS were performed in the Laboratory.

In the FACS laboratory, specialized staff members offer various services for users of the equipment (cell analyzers and cell sorters): (1) *Technical support and training*: In 2021, the facility offered eight technical courses (four for cell sorting and four for cell analysis). The courses were held at three different levels, Calibur basic (1), Canto II (2) and Aria basic (4). An Aria course was also held in English. A total of 44 researchers participated in these courses in 2021. (2) *Cell sorting operation service*: The Laboratory provides a cell sorting operation service, in which researchers can ask an experienced operator to conduct the sorting experiment. In 2021, we provided 120 such services. Advanced cell sorting techniques, such as single-cell sorting, have also been performed. (3) *Management/ maintenance of FACS instruments*: FACS machines are available for registered users around the clock and reservations are accepted up to one month in advance through an internal website. In addition to the in-house FACS Laboratory staff, engineers from Becton Dickinson visit once a week to provide maintenance and technical support.

Table: Instruments and their usage in the FACS Laboratory (2021)

Instrument types	Model	# of machines	# of users	# of training sessions
FACS cell analyzer	Calibur	3	18	2
	Canto II	2	702	15
FACS cell sorter	Aria IIIu/III/Fusion	6	1274	27
Mass cytometer	Helios (CyTOF)	1	39	0

Microscope Laboratory

The Microscope Laboratory provides equipment for cell and tissue imaging, and coordinates technical support. There are 6 fluorescence microscopes and 1 scanning electron microscope available to researchers at IMS.

(1) Inverted Leica SP8 system equipped with hybrid detectors and the LIGHTNING super-resolution image extraction module.
(2) 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. One of the two Ti:Sa lasers is connected to an optical parametric oscillator (OPO) that enables two-photon imaging by long wavelength excitation.
(3) Inverted Leica SP5 system with hybrid detectors.
(4) Inverted Nikon N-SIM/N-STORM super-resolution microscope for dual color imaging.
(5) GE Healthcare DeltaVision Elite system.
(6) Keyence BZ-X700 all-in-one fluorescence microscope.
(7) Hitachi field emission scanning electron microscope (FE-SEM) Regulus8240.

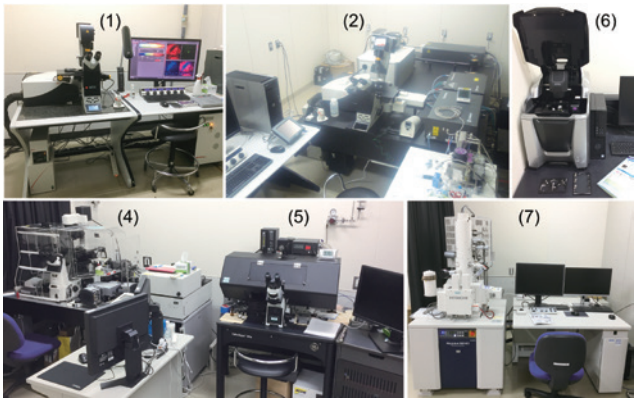


Figure: Leica SP8 confocal microscope (1), Leica SP8 multiphoton microscope (2), Nikon N-SIM/N-STORM super-resolution microscope (4), GE Healthcare DeltaVision Elite system (5), Keyence BZ-X700 microscope (6), and Hitachi Regulus8420 FE-SEM (7)

Genome Platform and related activities

The Genome Platform was launched in September 2020. It supports each laboratory in the Center in library preparation, sequencing and data analysis related to sequencing and plays a central role when large projects are adopted. In FY2021, we installed a 10xGenomincs Chromium Controller to perform single-cell RNA-seq analysis. We also support library preparation for this analysis. As for the sequencer, we have newly introduced the NextSeq2000, which is a middle output (40~330GB) sequencer. In addition, we also support the registration of data in public databases.

This year, we are working on 40 projects. The achievements in library preparation and sequencing are shown in Table 1. A key contribution is that we analyzed gene expression in mouse primitive endoderm stem cells using the single-cell sequencing method in collaboration with the Laboratory for Developmental Genetics. Based on the results, we were able to clarify the establishment of primitive endoderm stem cells and gain knowledge that will lead to understanding the mechanisms for primitive endoderm specification (Ohinata Y *et al.,* Science. 2022). In addition, we are developing an off-target analysis method for gene editing with the RIKEN Program for Drug Discovery and Medical Technology Platforms (DMP).

This platform enables users in IMS to quickly obtain DNA libraries, sequence data and analysis data at a reasonable cost, thus empowering their research. We expect that the intramural interactions fostered by the Genome Platform among the Divisions of Human Immunology, Disease Systems Biology, Cancer Immunology, and Genome Medicine will greatly enhance IMS research activities.

Table1: Central services provided by the Genome Platform in 2021

Library Preparation	# of Samples
whole genome sequence	1,781
targeted sequence	37,347
bulk RNA-seq	104
Ig-seq	34
SMART-Seq	1,344
RamDA-seq	480
ssCAGE	140
10x 3′GE	28

Table2: Next-generation DNA sequencing

Illumina Sequencer	# of Runs
NovaSeq6000	87
HiSeq2500	74
MiSeq	141
PacBio Sequel	**# of Runs**
Sequel I	4
Sequel II	112

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 isolation bio-bubble rooms. The former is used by several IMS research groups, in particular the mucosal immunologists, and the latter is for "humanized mice". In addition, a new SPF animal facility was completed in 2019. The new facility has 32 vinyl isolators and two Individually Vented Cage systems (IVCs) (Figure) and has the capacity to breed 1,500 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 (frozen embryos and sperm) for 944 lines. We also maintain relatively large colonies of several commonly used strains, such as *Rag1* KO and Cre deleters, and provide them to users on demand. We have also provided technical assistance to generate knockout and transgenic mice (163 lines). In addition, we have created 17 lines of germ-free mice. We maintain flexibility so that we can provide space for new experiments in the animal facility, e.g., behavioral testing of germ-free mice.

We have generated genetically modified mice to improve the efficiency of transplantation of human hematopoietic stem cells into NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (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 such transgenic and knock-in mice with confirmed expression of human genes on a C57BL/6 background and have backcrossed them onto the NSG mouse background using the speed-congenic method.

Figure: Vinyl isolators and two types of Individually Vented Cage systems in the new SPF animal facility

Metabolomics (Lipidomics)

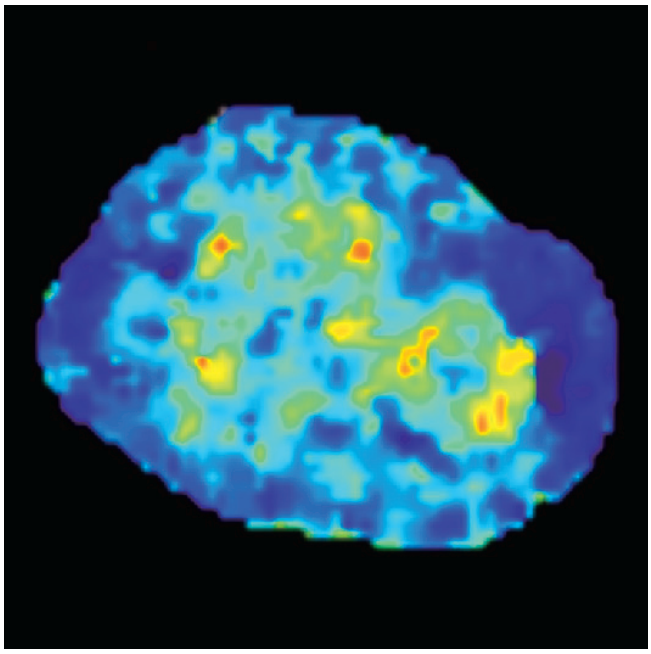
Lipids are extremely diverse molecules and such diversity creates complex biological systems, with lipids acting as key components of cellular membranes, as signaling molecules and as energy-storage molecules. In general, the dysregulation of lipid metabolism is associated with human diseases and, hence, the precise determination of lipids is quite important for understanding their functions in physiology and disease, and for discovering novel bioactive lipids that may have therapeutic benefits.

A powerful method for analyzing lipid metabolites is liquid chromatography-tandem mass spectrometry (LC-MS/MS). The Metabolomics Laboratory is equipped with an AQUITY UPLC (Waters) coupled with a TripleTOF 6600, a QTRAP 6500/5500/4500 (Sciex) and a GC-MS TQ8030 (Shimadzu) to cover untargeted and wide-targeted metabolomics. Also, in 2022, we will introduce timsTOF flex MALDI-2 (Bruker) for MS imaging (spatial lipidomics) analyses. This state-of-the-art lipidomics platform together with our original informatics technologies are powerful tools for elucidating complex lipid structures and visualizing metabolic networks globally and unbiasedly and provide an opportunity for data-driven hypotheses to make the connection between lipid metabolism and biological phenotypes.

Figure: LC-MS/MS-based Advanced Lipidomics Platform

Part 3

Research Projects



COVID-19 projects in IMS

Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has continued as a pandemic. The sudden emergence and spread of SARS-CoV-2 poses a threat to global health and the economy. Although current COVID-19 vaccines have shown efficacy in many countries, the development of strategies to prevent the spread of the virus is still needed. At present, several groups in IMS develop tools for COVID-19 diagnosis, others work on the development of prevention and therapeutic strategies. For diagnostics and biomarkers, Momozawa's group, in collaboration with Tokyo Medical and Dental University, Hiroshima University, and their affiliated hospitals, finished whole-genome sequencing and target sequencing to identify genetic loci associated with individual differences in COVID-19 disease severity. They started to examine the associated genes already reported and perform association analysis to identify new genetic loci. They also deposited our whole-genome sequencing data in a global consortium called the COVID Human Genetic Effort. For COVID-19 treatment, many groups are working on Cov-2 projects. Among them, several groups are collaborating with RIKEN Drug Discovery and Medical Platforms (DMP). T follicular helper (TFH) cells in the germinal center are helper T cells supporting the production of high-affinity antibodies by B cells. Miyauchi's group previously reported that both TFH and the IL-4 cytokine derived from TFH cells have an important role for induction of broad-spectrum virus-neutralizing antibodies during influenza virus infection. So far, they have succeeded in generating antibodies that recognize multiple mutant CoV-2 strains by immunizing TFH-activated mice with CoV-2 antigens. Saito's group is working on the establishment of neutralizing antibodies that target TMPRESS2 instead of the viral spike protein. TMPRESS2 is a host cell membrane protease critical for SARS-CoV-2 viral entry into human cells. Indeed, they established TMPRSS2 mAbs and verified that these mAbs inhibit the infection of human cells by any type of SARS-CoV2 variant. Fukuyama's group already isolated several therapeutic human mAbs from COVID-19 patients in collaboration with Keio University. They have patented these antibodies, which are capable of neutralizing several variants of concern (VOC) strains of SARS-CoV-2. Fujii's group has recently identified a promising QYI peptide for CTL induction against SARS-CoV-2. Since they had previously established the concept of the artificial adjuvant vector cell (aAVC) system against cancer, they established SARS-CoV-2-derived antigen-expressing aAVC (aAVC-Cov-2). In preclinical studies they obtained evidence for anti-viral cytotoxic T cell induction as well as anti-SARS-CoV-2 Ab in vaccinated animals. Recent advances as IMS projects in the fields of diagnostics, treatment and vaccine development for SARS-CoV-2 infection are summarized in Table.

Table: COVID-19-related research conducted at IMS

Teams	Titles
Hidehiro Fukuyama (Lab. for Lymphocyte Differentiation)	Development of COVID-19 antibody drug
Kazuhiko Yamamoto (Laboratory for Autoimmune Diseases)	Development of a method for healthy immune profiling of people recovering from SARS-CoV-2 infection
Yasushi Okazaki (Lab. for Comprehensive Genomic Analysis)	Genome analysis of SARS-CoV-2
Yukihide Momozawa (Lab for Genotyping Development)	Genome analysis to identify genes and genome loci associated with individual differences in susceptibility to COVID-19 infection
Hidehiro Fukuyama (Lab. For Lymphocyte Differentiation)	Isolation of new coronavirus detection antibody and development of on-site rapid virus detection kit
Kosuke Miyauchi (Lab. for Cytokine Regulation)	Construction of a system to isolate a human monoclonal neutralizing antibody against SARS-CoV-2. (with DMP)
Kazuyo Moro (Lab. for Innate Immune Systems)	Development of a new treatment for severe cases of COVID-19
Kenya Honda (Lab. for Gut Homeostasis)	Understanding host-gut microbiota interactions to develop a therapeutic/preventive strategy toward SARS-CoV-2 infection
Hiroshi Ohno (Lab. for Intestinal Ecosystem)	Screening of drug candidate compounds for COVID-19 in large databases using scalable similarity searches
Kengo Usui (Genetic Diagnosis Technology Unit)	Development of diagnostic methods using SmartAmp technology (with PMI)
Takashi Saito (Lab. for Cell Signaling)	Development of monoclonal Ab for TMPRSS (with DMP)
Shin-Ichiro Fujii (Lab. for Immunotherapy)	Development of aAVC-CoV-2 (with DMP)
Chikashi Terao (Lab. for Statistical and Translational Genetics)	Elucidating the relationship between somatic cell mosaicism and the risk of COVID-19 infection
Fumihiko Ishikawa (Lab. for Human Disease Models)	Mechanism of COVID-19 Severity Caused by Mutant Viruses in the Post-Vaccination Period
Shin-Ichiro Fujii (Lab. for Immunotherapy)	Identification of cross-reactive epitopes of SARS-CoV-2 to seasonal coronaviruses

The Human Cell Regulatory Atlas

Determining the relevance of cell-types and -states to diseases is one of the fundamental goals of the Human Cell Regulatory Atlas project in IMS. Using 5'end single-cell RNA-seq technology, which captures the transcription start sites, the project aims to measure the activities of transcribed cis-regulatory elements (tCRE), including promoters and enhancers, in various cell-types and -states across multiple human organs. This project is made possible through an extensive collaborative network involving RIKEN and medical institutions across Japan for sample collection. We generated a tCRE atlas of 436,910 cells from 23 tissues, defining over 100 distinct cell-subtypes (Figure). This tCRE atlas will enable us to interrogate the cell-type specific circuits of gene regulation and assess their contribution to disease predisposition through integration with genetic data. One of our goals is to identify critical cell types and cellular processes for various diseases. The Human Cell Regulatory Atlas in IMS will facilitate discovery of new biology and, at the same time, build a comprehensive integrative database to power the next generation artificial intelligence to solve health and medical needs that we are facing in our lifetime.

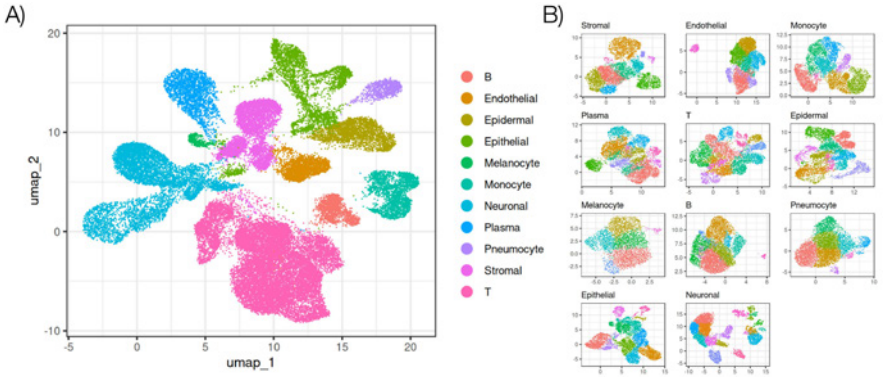


Figure:
A tCRE atlas from 23 human tissues. A) A single-cell UMAP consists of 436,910 cells, divided into 11 major cell-types. B) Sub-clustering of the 11 major cell-types, defining over 100 distinct cell-subtypes.

FANTOM

The FANTOM consortium was established in the year 2000 to elucidate the function of the mammalian genome. The 6th phase of the FANTOM project aims at creating a comprehensive catalog of functional lncRNAs. We created a lncRNA knockdown data set on cell growth and morphology using real-time imaging and CAGE deep sequencing to reveal molecular pathways associated with each lncRNA in human primary fibroblast cells (Ramilowski *et al. Genome Research* 2020).

FANTOM6 also found that the majority of lncRNAs reside in the nucleus and 35% of lncRNAs are chromatin-bound. In order to analyze RNA-chromatin interactions, we developed a new technology named RNA And DNA Interacting Complexes Ligated and sequenced (RADICL-seq) that precisely maps genome-wide RNA-chromatin interactions in intact nuclei (Bonetti *et al. Nature Communications* 2020). Interactome analysis by RADICL-seq revealed distinct genome occupancy patterns for specific classes of transcripts in each cell type. Interestingly, we found that most interactions in *cis* were from the intronic regions of protein-coding transcripts whereas interactions in *trans* were from the exonic regions of non-coding transcripts, which indicates the possibility that the intronic regions of protein-coding transcripts play a regulatory role in gene expression.

After the study on fibroblast cells, the project will proceed with knockdown analysis of lncRNAs in iPS cells to understand the lncRNA properties in stem cells.

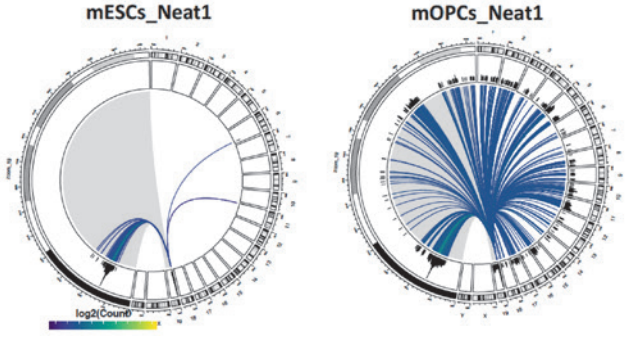


Figure:
Cell-type-specific RNA-chromatin interaction patterns. Circos plots depicting genomic interactions of the Neat1 lncRNA in mouse embryonic stem cells (mESCs) and mouse oligodendrocyte progenitor cells (mOPCs).

Human genome analysis

In 2015, the Japanese government set rare hereditary diseases, cancer, dementia, infection, and pharmacogenomics as priority areas for the implementation of genomic information for actual medical practice. To accomplish this goal, a combined analysis of germline variants with other information including somatic variations, gene expression profiles and environmental factors would be key.

IMS has analyzed various diseases and phenotypes by genome-wide association studies and/or targeted- and whole-genome sequencing-based association studies, including cancer (Momozawa & Nakagawa), pharmacogenomics (Mushiroda), bone and joint diseases (Ikegawa), diabetes mellitus (Horikoshi), cardiovascular diseases (Ito), autoimmune diseases (Yamamoto K), genome immunobiology (Parish), functional genetics (Ishigaki), and integrated analysis of all data and phenotypes (Terao). In addition, we began to extract information about somatic variations from DNA microarray data, which had previously been used only to call germline variants. Further, to better understand disease biology, we integrated our results with knowledge of non-coding regions and single-cell sequencing approaches obtained by laboratories of the FANTOM and Human Cell Atlas projects. Finally, we have established collaborations with large Japanese cohorts including BioBank Japan (BBJ), Tohoku Medical Megabank and domestic and international universities.

A key finding in 2021 was the discovery of a new hereditary bone disease, called "Ikegawa type craniotubular dysplasia", and the identification of its causative gene, *TMEM53*, encoding a nuclear envelope protein. By comprehensive exome sequence analyses followed by *in vitro* and *in vivo* functional studies using gene knock-down and knock-out techniques, we elucidated the novel function of TMEM53 as a gatekeeper of the BMP/TGF-β signals controlling bone development and showed that bi-allelic loss of function of TMEM53 causes the disease.

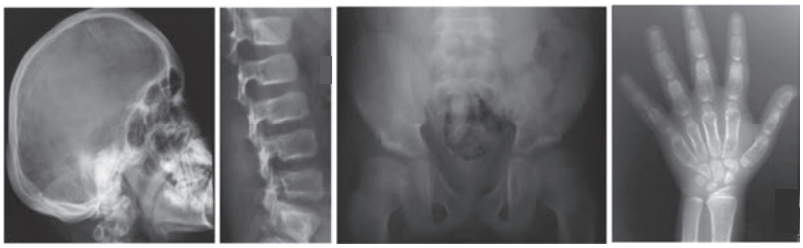


Figure: Phenotype of the new disease, Ikegawa type craniotubular dysplasia
The patients present characteristic radiographic features, including sclerosis of the skull and dysplasias of the spine and metaphyses of the long and short tubular bones.

SEAPharm for establishment of stratified medicine in Asia

In 2012, RIKEN 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 joining the team in 2014, Vietnam in 2016, Nepal, Laos and the Philippines in 2017, and Brunei and Myanmar in 2018. The aims of the collaboration are to identify genomic biomarkers associated with adverse drug reactions, such as severe cutaneous adverse drug reactions (cADRs), including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and hepatic injury, to provide technical assistance and training of young researchers from the SEAPharm member countries, and to hold international seminars and workshops.

In 2018, SEAPharm started a new project involving next-generation sequencing (NGS) of DNA samples to clarify the genetic diversity of 100 pharmacokinetics-related genes in individuals from 9 countries of Southeast Asia, the Middle East and Southern Europe. RIKEN IMS is responsible for the targeted sequencing using a PKSeq panel developed by RIKEN and reported substantial genetic variations in drug-metabolizing enzyme and drug transporter genes among Asian populations. These findings can account for interethnic variabilities in drug response phenotypes and are leading to the acceleration of further pharmacogenomic investigations and the implementation of genotype-guided drug therapies in clinical practice.

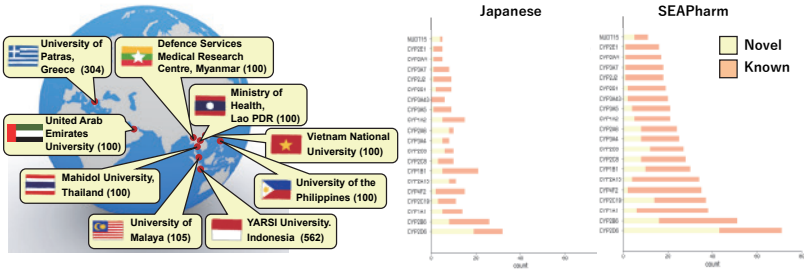


Figure: SEAPharm pharmacogenomic variation project
We genotyped 1,571 genomic DNA samples collected by nine collaborative institutes (the number in parentheses in each country indicates the sample size) using the PKseq panel that targets the coding regions of 62 drug-metabolizing enzymes and 37 drug transporter genes. We were able to identify many previously unreported variants in 20 clinically important drug-metabolizing enzyme genes in the Japanese and SEAPharm populations.

eQTL project: Integration of genetic information into immune functions

Many disease susceptibility variants have been identified by genome wide association study (GWAS). Germline genetic variations provide us with evidence into the causal relationship of an observed phenomenon and its pathogenesis. In this regard, the majority of GWAS risk variants have been reported to locate in the non-coding regions on the chromosome and function as an expression-quantitative trait locus (e-QTL), regulating the expression levels of genes. Therefore, by integrating genomic information, qualitative and quantitative analyses of transcriptomes together with cell-specific epigenomes, we will better understand the causal pathogenic components of immune cells in various immune-mediated diseases.

We are now setting up a system to identify various subtypes of leukocytes from peripheral blood mononuclear cells (PBMC) of heathy individuals. We expect to obtain the utmost unbiased relationship between genotypes and gene expression from healthy donors. Cell separation is performed by fluorescence-activated cell sorting into about 30 different subsets. Cells are then analyzed in the steady state or in further stimulated conditions, such as with combinations of cytokines and cell surface receptor agonists to capture the dynamic responses of gene regulation. Firstly, genotyping as well as RNA-seq are performed. With this data, we will obtain eQTL as well as splicing QTL information. Cap analysis of gene expression (CAGE), assay for transposase-accessible chromatin using sequencing (ATAC-seq) and several histone mark analyses for each subset are powerful tools to be used for identifying the causal relationship between genetic variation and gene expression.

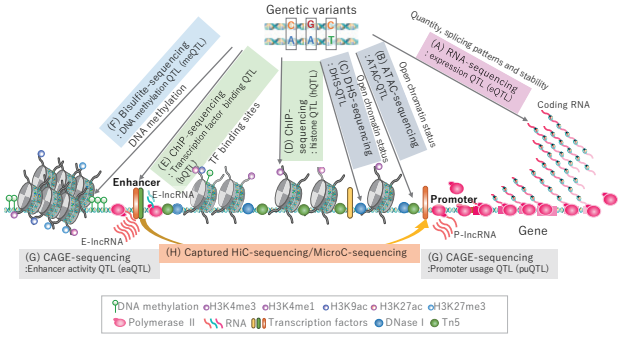


Figure: Integration of genetic information into immune functions: The eQTL project

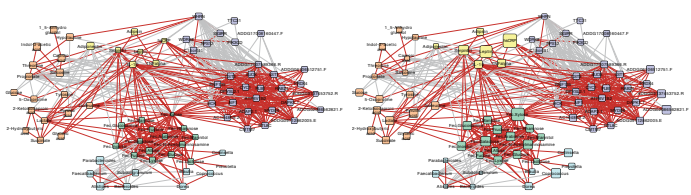
Search for gut microbiota-associated biomarkers involved in the pathogenesis of Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2D) is increasing rapidly, both in Japan and worldwide, with changes in lifestyle and social environment. It is estimated that about 1 in 5 Japanese suffer from diabetic or prediabetic (medically defined as glucose intolerance) conditions. Thus, prevention of T2D and control of its progression are important and urgent needs - medically, socially, as well as economically. We therefore set this issue as one of the center projects to try to identify gut microbial T2D-predictive biomarkers or factors. In collaboration with the University of Tokyo Hospital, we recruited volunteers taking a complete medical checkup with the following criteria: 1) no obesity or glucose intolerance (control), 2) obesity (BMI ≥ 25) and 3) glucose intolerance (fasting blood glucose ≥ 110 mg/dl or HbA1c ≥ 6.0%). In addition to the thorough clinical examination data from the medical checkup, the following were collected in RIKEN: fecal metagenomic and metabolomic data, plasma metabolomic data, and CAGE-based RNAseq data of peripheral blood mononuclear cells (PBMCs). We also collected nutritional and physical activity data using a brief self-administered diet history questionnaire (BDHQ) and accelerometry, respectively.

We found that insulin resistance and metabolic syndrome were significantly associated with fecal monosaccharides and sugar derivatives. We further revealed that these fecal monosaccharides and sugar derivatives strongly associated with host inflammatory gene expression in PBMCs and with plasma cytokine levels (Figure). Representative microbes associated with insulin resistance showed distinct carbohydrate metabolism and host metabolic phenotypes. These results are now submitted for publication.

Figure: Network formation of various parameters in insulin-sensitive and -resistant individuals

Correlations of fecal microbiota (light blue), fecal metabolites (green), plasma metabolites (light brown), plasma inflammatory cytokines (yellow), and PBMC gene expression (light purple) in insulin-sensitive (left) and -resistant (right) individuals are shown. The size of each symbol represents the amount/numbers of the parameters. Red and gray lines reflect positive and negative correlations, respectively, and the thickness of the lines indicates the strength of correlation. Fecal saccharides show a strong correlation with plasma inflammatory cytokines and inflammatory/metabolic genes in PBMCs and these parameters are higher in insulin-resistant individuals compared to insulin-sensitive individuals.



Data-Driven Research for Atopic Dermatitis

Atopic dermatitis (AD) is a heterogeneous and multifactorial disorder. Although it has been suggested that an individual approach to each patient is crucial for understanding AD, suitable methods to do so have not been established yet. The purpose of this study, therefore, is to establish a method for disease clustering into sub-groups and to develop novel predictive treatment algorithms in each sub-group. To achieve this, we perform integrated analysis of genome and transcriptome data and multimodal clinical information from AD patients.

In line with the above concept, we collected a large number of high-quality clinical samples in collaboration with Keio University Hospital and established an integrated data analysis and repository infrastructure called the Medical Data Integration Assistant (MeDIA), which also enables us to conduct integrated studies with animal models. Analysis on cross-tissue ligand-receptor interactions by using transcriptomic analysis suggested augmented skin-PBMC crosstalk in AD patients compared to healthy subjects. We built a regression model that predicts clinical phenotypes of AD with transcriptome modules generated by applying weighted gene co-expression network analysis on RNA-seq data of skin and PBMC. We identified differential immunological signatures associated with two distinctive skin manifestations of AD. Longitudinal tissue transcriptomic data analysis of patients treated with molecularly-targeted therapies also revealed characteristics of each patient's genetic profile that were associated with differences in treatment response.

Our approach will not only pave the way toward realization of personalized preventive medicine for AD, but also for development of new technologies in data-driven medical research, and therefore will have a considerable impact on society.

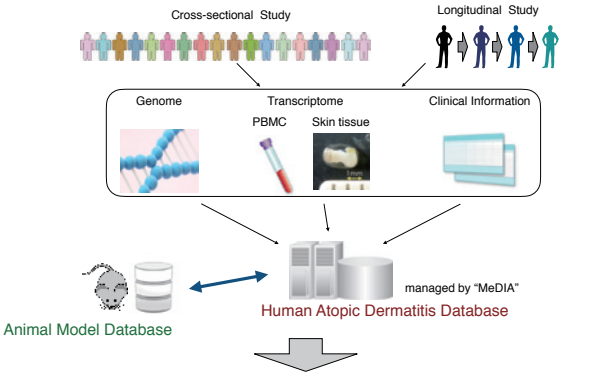


Figure: Study workflow: Data driven research for Atopic Dermatitis

iPS Project

Induced pluripotent stem (iPS) cells possess tremendous therapeutic potential in many areas, including regenerative medicine and immune therapy. On a collaborative basis with individual IMS research laboratories, the core facility for iPS research is aiming to put cancer immunotherapy with iPS-derived NKT cells into clinical use.

The facility has operated an IMS Cell Manufacturing Unit (CMU) to produce iPS-derived human invariant NKT (Vα24⁺iNKT) cells under GMP (Good Manufacturing Practice)/ GCTP (Good Gene, Cellular, and Tissue-based Products Manufacturing Practice) guidelines. The safety of these iPS-Vα24⁺iNKT cells was confirmed by preclinical studies. The facility had finished PMDA (Pharmaceuticals and Medical Devices Agency) consultation for clinical trials of iPS-Vα24⁺iNKT cell-mediated head and neck cancer immunotherapy and has been conducting the clinical trial. The facility is also preparing another clinical trial on combination therapy with iPS-Vα24⁺iNKT cells and dendritic cells for head and neck cancer, and it will be started in the next year.

Currently, to maximize the efficacy of iPS-Vα24⁺iNKT cells, activation with dendritic cells is mandatory and it might complicate the treatment. However, if direct activation of iPS-Vα24⁺iNKT cells can be accomplished, treatment with fully activated iPS-Vα24⁺iNKT cells will be easily achieved. To implement this direct activation, the facility focused on Chimeric Antigen Receptor (CAR) introduction into iPS-Vα24⁺iNKT cells. Among several CARs, the facility chose CD19 and CD25 CARs to treat leukemia and has now been conducting a proof of concept study.

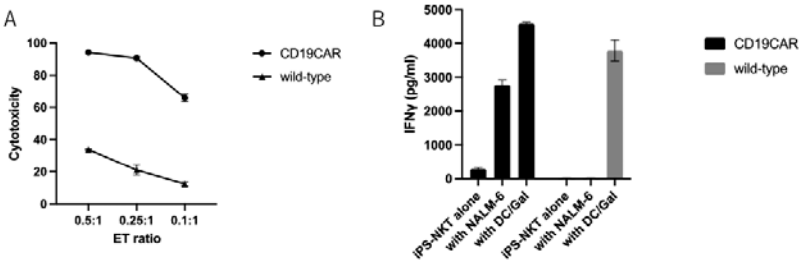


Figure: Anti-tumor effects of CD19CAR iPS-NKT
A) Direct cytotoxicity of CD19CAR iPS-NKT and wild-type iPS-NKT toward the CD19⁺ NALM-6 leukemia cell line. B) IFN-γ production by CD19CAR iPS-NKT and wild-type iPS-NKT cocultured with NALM-6 ± α-galactosylceramide-pulsed dendritic cells (DC/Gal).

Humanized mouse

In the Humanized Mice project, we have reconstituted mice with normal human hematopoiesis and immunity to study the differentiation and function of immature human stem and progenitor cells. We have also recapitulated patients' leukemic status in immune-deficient mice using patient samples. In each patient, there must be specific reasons why immune surveillance has failed. Nevertheless, the mystery of suppressed human immunity by leukemic cells has yet to be addressed.

In this project, our first step has been to functionally identify normal and malignant human stem cells via xenogeneic transplantation. Then we perform multi-omics to determine the distinction between the two stem cell types. This approach has enabled us to find therapeutic molecules expressed by malignant stem cells but not by normal hematopoietic stem cells. During the last few years, we have identified therapeutic targets in different cellular organelles. Humanized mice reconstituted with normal hematopoiesis and immunity are used to assess potential adverse effects of new treatment modalities, while leukemia xenografts are essential in evaluating the treatment efficacy in multiple organs. We are currently trying to maximize the therapeutic impact on aggressive types of myeloid and lymphocytic leukemia by inhibiting two different molecules in different locations of patient malignant cells. Through understanding the genetic and biological heterogeneity of hematologic malignancies, we hope to translate curative precision medicine into the clinic in the future.

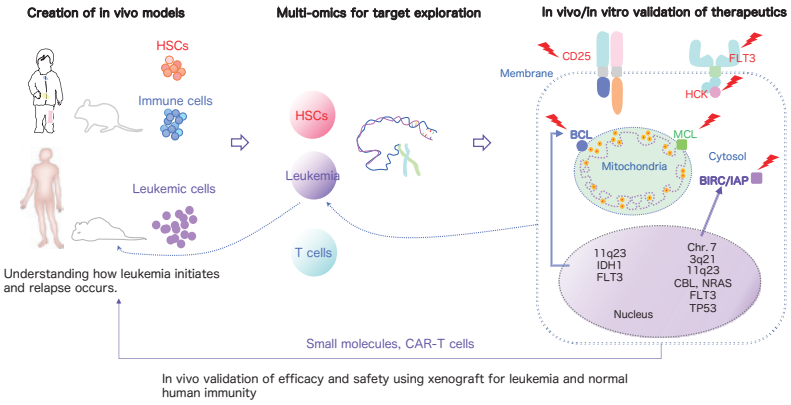
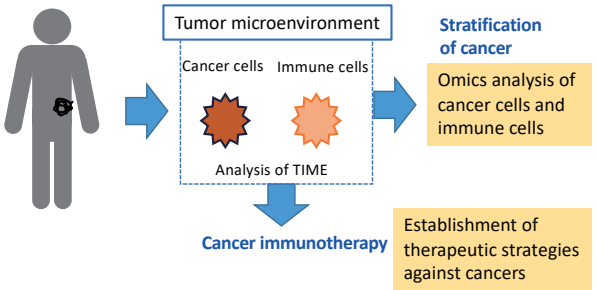


Figure:
We have created an *in vivo* system for human immunity and leukemia. Using this system, we have identified cells responsible for disease initiation and relapse, which were then subjected to mutational and gene expression profiling for target exploration. As therapeutic targets, we have discovered mutated proteins, over-expressed proteins, key survival molecules in the membranes of different organelles, non-mutated vulnerabilities in mitochondria or cytosol. Distinct therapeutic targets in different patients appear to be determined by the leukemogenic events that have occurred in the nucleus. We hope that this step-wise effort, from the creation of *in vivo* systems to the development of therapeutics, will be applied not only to leukemias, but also to non-leukemic hematologic tumors and even solid tumors.

Cancer Immunology

The immune system recognizes tumor cells and can mediate antigen-specific tumor rejection under certain conditions, however the tumors often evade the immune network. To accomplish this escape, tumors may mediate immunosuppression through various soluble and cellular mechanisms. Understanding the role of the immune system in the tumor microenvironment (TME) will lead to a variety of specific approaches designed to initiate or enhance antitumor immunity (Figure). The groups in cancer immunology are using both murine models and human clinical samples from a variety of cancers to find crucial molecules for therapy. Tsunoda's group (Lab for Medical Science Mathematics) proposed a novel method of scientific discovery from omics data with deep learning. After conducting multi-omics analyses of colorectal and renal cancers, they discovered novel immunological classifications and association with poor prognoses. Nakagawa's group (Lab for Cancer Genomics) analyzed whole genome and RNAseq data from biopsy specimens of esophageal cancers before chemotherapy and demonstrated that a subfraction of immune cells and copy number alterations were associated with the response to chemotherapy. Ishikawa's group (Lab for Human Disease Models) published a new precision medicine approach targeting non-mutated survival molecules in AML. To strengthen the therapeutic effect of these small molecules, they set out to engineer T cells that could target the AML cells. Considering that there is little expression of CD25 in normal HSPCs, they chose it as the first target. Using NSG xenograft mice, they found nice therapeutic efficacy of CD25-targeted CAR-T cells against AML, especially in presence of CXCR4. Fujii's group (Lab for Immunotherapy) promoted their aAVC project for cancer. NY-ESO-1 is an esophageal squamous cell carcinoma antigen and WT1 is a Wilms' tumor antigen. They established an NY-ESO-1-expressing aAVC (aAVC-NY-ESO-1) and showed immunological and anti-tumor effect using NOG mice in a preclinical study. For the aAVC-WT1, they started a Phase II clinical study as multicenter joint research. In addition, Koseki's group (Lab for Developmental Genetics) has started an iPS-NKT cell clinical trial for head and neck cancer. These TR projects have been supported by an interaction between IMS and the Drug Discovery and Medical Technology Platforms (DMP).



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 discoveries within the institute. 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 nine 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, the Drug Discovery Antibody Platform Unit. In 2021, IMS now has six collaborative programs with DMP: Artificial adjuvant vector cells (Shin-ichiro Fujii), Cancer therapy with iPS-derived NKT cells (Haruhiko Koseki), Drugs for allergic diseases (Masato Kubo), Neutralizing mAb for HBV infection (Daiki Miki) and Therapeutic mAb for IBD (Takashi Saito). The investigator-initiated Phase I clinical trial of the Artificial adjuvant vector cell project for cancer therapy has been completed and the Phase II study was then started. In addition, another phase I study, an iPS-NKT cell clinical trial for head and neck cancer, has started.

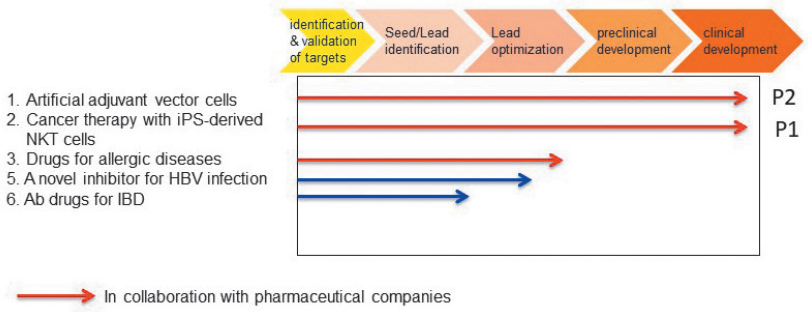
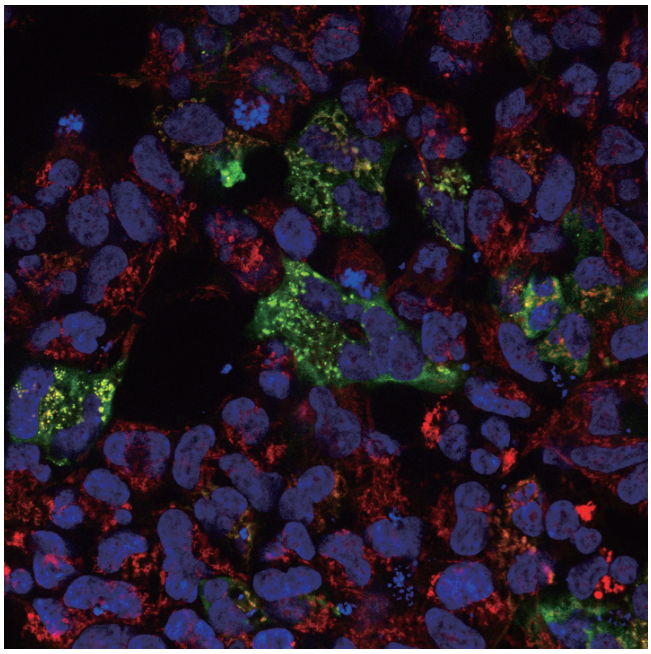


Figure: Collaboration between IMS and DMP for the development of innovative new biologics, pharmaceuticals and medical technologies

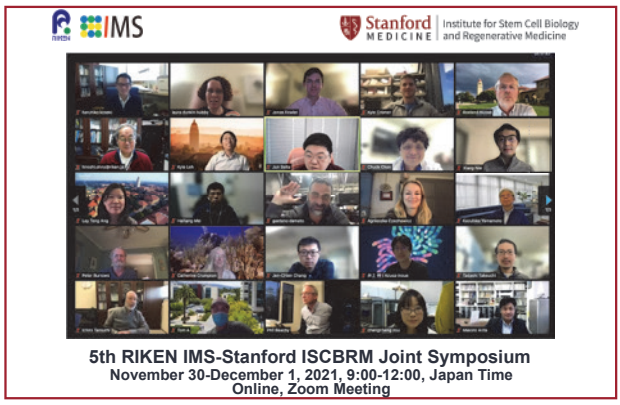
Part 4

Events



RIKEN IMS-Stanford ISCBRM Joint Symposium

In 2021, we held the 5th joint symposium with the Stanford Institute for Stem Cell Biology and Regenerative Medicine (ISCBRM). As the COVID-19 pandemic continues, the symposium was held on Zoom for 2 days, 3 hours each day. This year, the symposium was directed by Prof. Kyle Loh of Stanford, and a wide variety of speakers were invited, such as from full-professors to Ph.D students, and from developmental biology to clinical applications. Topics included immunology, hematology, skin biology, lung regeneration, lipidomics, microbiomes, and stem cell biology. From Stanford ISCBRM; Roel Nusse, Jonas Fowler, Lay Teng Ang, Agnieszka Czechowicz, Hiro Nakauchi, Massimo Nichane, Gaetano D'Amato, and, of course, Irving L. Weissman gave talks. At the last minute, Irv invited Dan Liu to his slot to present his breaking results on human fetal brain stem and progenitor cells. From RIKEN IMS; Hailing Mei, Hiroshi Ohno, Xiang Nie, Makoto Arita, Chengcheng Zou, Takaharu Okada, and Yasuhiro Murakawa presented their latest results. About 70 participants in total from both sides joined each day (Photo), and we had very active and productive discussions after each talk despite the distance and time-zone difference. We missed the social events like in previous years, but we could take a deep dive into the joy of biology. In 2022, the next joint symposium will be hosted by RIKEN IMS. We hope we can have an in-person meeting in Yokohama.



RIKEN-Chiba University-Luxembourg Scientific Symposium

–How to boost research collaborations through open data exchange–

A scientific symposium between RIKEN IMS, Chiba University, the Luxembourg Centre for Systems Biomedicine (LCSB) of the University of Luxembourg, and the Luxembourg Institute of Health (LIH) was held on December 15th, 2021. Annual symposiums have been held since 2014 between RIKEN and Luxembourgian partners as part of a large collaborative framework which features the RIKEN Outpost Laboratory, located on the University of Luxembourg campus (LCSB). This outpost serves as a research network hub in which scientists from RIKEN and partners in Japan undertake collaborative research in Luxembourg with Luxembourgian partners.

This year's symposium focused on accelerating collaboration through open data exchanges. Presenters in Luxembourg focused on platform development, including detection of novel omics layers (exposomes, post-translational modifications) and data standardization of their community-wide systems biology databases. RIKEN and Chiba University scientists presented on large-scale data integration and mathematical modeling in the context of disease systems biology. The meeting was attended by over 100 people with a hybrid format in Japan (on-site and online) and fully online in Luxembourg with funding from JSPS in Japan and FNR in Luxembourg.

The symposium also featured keynote speaker Dr. Claudia Traidl-Hoffmann who spoke on the impact of planetary health and climate change on human diseases. We also welcomed the new LCSB Director Dr. Michael Heneka, and opening remarks were made by Dr. Ulf Nehrbass (CEO of the Luxembourg Institute of Health) and closing remarks by Dr. Haruhiko Koseki (Deputy Director of RIKEN IMS). The symposium resulted in active scientific discussions and boosted collaborative momentum between these partners. The meeting was closed on a highly positive note with plans for further exchange of scientists between these two countries.

RIKEN IMS Joint Human and Mouse Cell Atlas Meeting

RIKEN IMS hosted a joint online meeting focusing on single cell biology research using human and mouse tissues. Single cell biology is bringing about a big impact on the life sciences and healthcare. International research collaboration projects on single cell biology, such as the Human Cell Atlas, are being conducted worldwide. Since 2019, RIKEN IMS has built the Single Cell Medical Network engaging with medical institutions across Japan to collect and process human clinical samples to elucidate the mechanisms of health and disease using the 5'-based single-cell genomics strategy. RIKEN IMS has also contributed to the all-RIKEN research project, integrated life science research to challenge super aging society, by building the mouse aging cell atlas.

During the meeting, we had three talk sessions: "Building the Single Cell Medical Network in Japan" led by Jay W. Shin, "Update on the Mouse Aging Atlas" led by Aki Minoda, "Single-cell data integration: multi-omics, multi-sample and multi-species" led by Chung-Chau Hon, and a panel discussion about "the current and future applications of single cell genomics in improving medicine and life" by outstanding researchers at IMS. Young researchers and representatives from inside and outside of RIKEN IMS presented their cutting-edge research projects followed by active discussion.

Meeting
Thursday, October 29th, 2021, 10:00 AM JST – 5:00 PM JST
Online Zoom

Attendees
95 attendees

Activities planned for the collaboration with the Karolinska Institute and Science for Life Laboratory (SciLifeLab) in Sweden in 2022

The COVID-19 related travel restrictions since 2020 have not allowed us to conduct the planned joint activities in 2021 with the Karolinska Institute (KI) and Science for Life Laboratory (SciLifeLab) in Sweden.

For the year 2022 and onwards, we plan to annually conduct the RIKEN-KI-SciLifeLab Symposium series, one year in Yokohama and the next year in Stockholm. The topics of the coming symposia might be aligned with the new Data Driven Life Sciences (DDLS) project hosted at SciLfeLab.

The RIKEN-KI Joint Doctoral Course is planned to be held in conjunction with the Symposium to give young researchers participating in the doctoral course the opportunity to also participate in the symposium.

We are very much looking forward to continuing the successful joint events with our partners in Sweden.

KI facade Campus Solna (Photo provided by KI Mediabank)

Adjunct Professorship Programs

IMS collaborates with and accepts graduate students from 8 domestic university graduate schools. There are now a total of 32 adjunct professors/associate professors at IMS (Table), and 65 students who had studied at IMS in 2021. On May 22, 2021, 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	Kazuyo Moro (Professor) Takashi Saito (Visiting Professor) Takashi Tanaka (Visiting Professor) Shiro Ikegawa (Visiting Professor)
Graduate School of Medicine, Chiba University	Haruhiko Koseki (Professor) Hiroshi Ohno (Visiting Professor) Ichiro Taniuchi (Visiting Professor) Shin-ichiro Fujii (Visiting Professor) Fumihiko Ishikawa (Visiting Professor)
Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University	Ichiro Taniuchi (Visiting Professor)
Graduate School of Medicine, Yokohama City University	Shiro Ikegawa (Visiting Professor) Hidewaki Nakagawa (Visiting Professor) Taisei Mushiroda (Visiting Professor) Yukihide Momozawa (Visiting Professor) Kaoru Ito (Visiting Professor) Momoko Horikoshi (Visiting Professor)
Graduate School of Medical Life Science, Yokohama City University	Hiroshi Ohno (Visiting Professor) Makoto Arita (Visiting Professor) Takaharu Okada (Visiting Professor) Taishin Akiyama (Visiting Professor) Yukihide Momozawa (Visiting Professor) Hidehiro Fukuyama (Visiting Associate Professor) Takahiro Suzuki (Visiting Associate Professor)
Research Institute of Biological Sciences, Tokyo University of Science	Masato Kubo (Professor) Takashi Saito (Visiting Professor)
Graduate School of Medicine, Keio University	Masayuki Amagai (Professor) Kenya Honda (Professor) Haruhiko Koseki (Visiting Professor) Takaharu Okada (Visiting Professor) Ichiro Taniuchi (Visiting Professor) Sidonia Fagarasan (Visiting Professor)
Graduate School of Science, Tokyo Metropolitan University	Azusa Inoue (Visiting Associate Professor)

Yokohama Campus Open Day Report

To prevent the spread of COVID-19, the open day was conducted in an online format, as in the previous year. The contents provided to the public were roughly divided into two types: live broadcasts on YouTube and Zoom, and videos that viewers could watch themselves on the Web. There were five events in total from IMS.

Total number of visitors on the website (between 10/9-10/31):3601.

Table: Program of online events at RIKEN Yokohama Open Campus on Oct. 9th, 2021 10:00-17:00

Teams	Event title
Laboratory for Cardiovascular Genomics and Informatics	Alcohol patch tests to determine whether you have high or low alcohol tolerance.
Laboratory for Developmental Genetics	Let's look together into the forefront of research on health and disease!
Laboratory for Cellular Epigenomics	Why did YOU come to RIKEN?
Laboratory for Genotyping Development	Realization of personalized medicine
Research Cordination Office	VR Lab Tour! for RIKEN Center for Integrative Medical Sciences

IMS Crosstalk

IMS Crosstalk is a monthly seminar started in January 2020 aiming to boost "crosstalk" between the researchers at IMS. Since a new RIKEN Center for Integrative Medical Sciences (IMS) was formed through the merger of the previous IMS and the Division of Genomic Technologies (DGT), IMS became more comprehensive while centering on genomics and immunology. As such, interactions and collaborations between the researchers have become more relevant to achieving our mission at IMS, which is to clarify the pathogenic mechanisms underlying human diseases and to translate this knowledge into novel therapies for the benefit of society.

In general, the term "crosstalk" means a situation in which a communications system is picking up the "wrong signals". However, in the biological field, crosstalk is not the "wrong signals" but rather is vital for biological phenomenon including, for example, "crosstalk between signaling pathways" and "crosstalk in lymphocyte activation". Similarly, in the IMS Crosstalks, "crosstalk" indicates the scientific interactions and mutual stimulation of the researchers.

To achieve this purpose, speakers are expected to introduce their laboratory activities focusing on a coherent topic with a broad introduction and general perspectives without excessive details of the data. Additionally, a facilitator is assigned for each topic to promote the discussion, which is uncommon for our usual internal seminars. Importantly, we have ongoing collaborations within IMS, which has already established crosstalks between laboratories with different backgrounds. Therefore, researchers from multiple laboratories can make a joint presentation to further promote their collaborations and to stimulate other researchers.

We have had a total of 24 topics by 25 laboratories including 1 joint presentation in 2021. We believe that the crosstalks will play a useful role in promoting the IMS mission.

Figure: IMS Crosstalks 2021

From Table: Focus on a coherent topic with a broad introduction and general perspectives without excessive details of the data.

Date	Talk 1 [12:00~12:30]		Talk 2 [12:30~13:00]		theme (s)
	speaker (s)	facilitator	speaker (s)	facilitator	
2021/1/22	Takaharu Okada	*M. Amagai*	Shin-ichiro Fujii	*F. Ishikawa*	immunology/homeostasis
2021/2/19	Piero Carninci **[12:00~]**			*N. Parrish*	genomics
2021/3/19	Katsuyuki Yugi & Tatsuhiko Tsunoda **[12:00~13:00]**			*C. Terao*	system biology
2021/4/16	Harukazu Suzuki **[12:00~]**			*T. Kasukawa*	genomics
2021/5/21	Nicholas Parrish	*C. Terao*	Chung-Chau Hon	*J. Shin*	genomics/genetics
2021/6/18	Taisei Mushiroda	*Y. Momozawa*	Chikashi Terao	*S. Ikegawa*	genetics
2021/7/16	Hideyuki Yoshida	*I. Taniuchi*	Azusa Inoue	*N. Parrish*	epigenetics
2021/9/17	Takashi Saito	*M. Kubo*	Masato Kubo	*T. Saito*	genetics
2021/10/15	Takashi Tanaka	*T. Akiyama*	Kiyokazu Kakugawa (On behalf of Hilde Cheroutre)	*I. Taniuchi*	immunology
2021/11/19	Takeya Kasukawa	*Chung Chau, HON*	Michiel de Hoon	*A. Minoda*	genomics/bioinformatics
2021/12/17	Aki Minoda	*H. Ohno*	Kazuhiko Yamamoto	*Chung Chau, HON*	genetics/genomics

Researcher Seminar 2021

The IMS Researcher Seminar series was held once every month with the aim of promoting scientific discussions among young researchers at IMS, to introduce research activities conducted in the IMS laboratories, to improve the presentation skills of young researchers at IMS and to prepare researchers for presenting outside of RIKEN. At the seminars, research scientists, postdocs, research associates and graduate students presented their work. They received questions from the audience and comments from the chair at the end of their talk, and intriguing ideas were exchanged.

Table: Resercher Seminars Jan-Dec 2021

Date	Chair	Speakers	Laboratory	Position
Jan. 15	Yasuhiro Murakawa	Osamu Masui	Laboratory for Developmental Genetics	Research Scientist
		Tommy Walter Terooatea	Epigenome Technology Exploration Unit	Postdoctoral Researcher
Feb. 19	Takeya Kasukawa	Kazuki Okumura	Laboratory for Transcriptional Regulation	Research Scientist
		Artem Lysenko	Laboratory for Medical Science Mathematics	Research Scientist
Mar. 19	Takashi Tanaka	Jafar Sharif	Laboratory for Developmental Genetics	Senior Research Scientist
		Akiko Oguchi	RIKEN-IFOM Joint Laboratory for Cancer Genomics	Junior Research Associate
Apr. 16	Takashi Saito	Rie Koide	Genome Immunobiology RIKEN Hakubi Research Team	Postdoctoral Researcher
		Tsuyoshi Kiniwa	Laboratory for Innate Immune Systems	Special Postdoctoral Researcher
May 21	Michiel De Hoon	Pauline Robbe	Laboratory for Transcriptome Technology	Postdoctoral Researcher
		Yuuri Yasuoka	Laboratory for Comprehensive Genomic Analysis	Research Scientist
Jun. 18	Kazuyo Moro	Mari Hashimoto	Laboratory for Human Disease Models	Special Postdoctoral Researcher
		Hailiang Mei	YCI Laboratory for Metabolic Epigenetics	Postdoctoral Researcher
Jul. 16	Ichiro Taniuchi	Takuma Misawa	Laboratory for Immune Cell Systems	Research Scientist
		Tadashi Takeuchi	Laboratory for Intestinal Ecosystem	Student Trainee
Sep. 17	Jun Seita	Mayumi Kusunose	Office of the Center Director	Senior Technical Scientist
		Baihao Zhang	Laboratory for Mucosal Immunity	Special Postdoctoral Researcher
Oct. 15	Masato Kubo	Nobuko Akiyama	Laboratory for Immunogenetics	Senior Scientist
		Steve Heaton	Genome Immunobiology RIKEN Hakubi Research Team	Research Part-time Worker II
Nov. 19	Momoko Horikoshi	Shohei Kojima	Genome Immunobiology RIKEN Hakubi Research Team	Postdoctoral Researcher
		Shota Sasagawa	Laboratory for Cancer Genomics	Postdoctoral Researcher
Dec. 17	Chikashi Terao	Kazuaki Matsumoto	Transcriptional Regulation	Student Trainee
		Callum Parr	Laboratory for Advanced Genomics Circuit	Research Scientist

Award Winners 2021

Name of the awardee	Name of the award	Date of the announcement
Makoto Arita, Team Leader, Laboratory for Metabolomics	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, Awards for Science and Technology, Research Category	Apr 2021
Hiroshi Ohno, Team Leader, Laboratory for Intestinal Ecosystem	Mochida Memorial Research Award	Nov 2021
Kazuyo Moro, Team Leader, Laboratory for Innate Immune Systems	The 4th Shimadzu Incentive Award	Dec 2021
Chikashi Terao, Team Leader, Laboratory for Statistical and Translational Genetics	The Japan Research Foundation for Healthy Aging, Young Investigator Imura Award	Dec 2021
Kaoru Ito, Team Leader, Laboratory for Cardiovascular Genomics and Informatics	Communications Biology Reviewer of the Month, Springer Nature	Feb 2021
Hiroshi Ohno[1], Eiji Miyauchi[2], Naoko Sato[3] Team Leader[1], Senior Scientist[2], Senior Research Scientist[3], Laboratory for Intestinal Ecosystem	RIKEN Eihou award	Mar 2021
Long Guo, Senior Scientist, Laboratory for Bone and Joint Diseases	ECTS East-meets-West Award, The European Calcified Tissue Society	May 2021
Eiji Miyauchi, Senior Scientist, Laboratory for Intestinal Ecosystem	Research Incentive Award, The Intestinal Microbiology Society	Jun 2021
Koya Fukunaga, Research Scientist, Laboratory for Pharmacogenomics	President's Award, The 29th Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics	Sep 2021
Wang Zheng, Research Scientist, Laboratory for Bone and Joint Diseases	Excellent Presentation Award, The 39th Annual Meeting of the Japanese Society for Bone and Mineral Research	Oct 2021
Tsuyoshi Kiniwa, Special Postdoctoral Researcher, Laboratory for Innate Immune Systems	Best Presentation Award (Silver prize), The 85th Annual Meeting of the Japanese Society of Interferon & Cytokine Research	May 2021
Chisayo Kozuka, Special Postdoctoral Researcher, YCI Laboratory for Metabolic Epigenetics	Research Grant, The 32th Annual Meeting of the Study Group of Molcular Diabetology	Dec 2021
Hiroaki Masuoka, Postdoctoral Researcher, Laboratory for Microbiome Sciences	The best presented abstract award, Danone Nutricia Research at the Danone Microbiome Symposium in IHMC 2021	Jun 2021
Satoshi Morozumi, Junior Research Associate, Laboratory for Metabolomics	The young scientist presentation award, The 46th Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry	Sep 2021
Haruki Uchino, Junior Research Associate, Laboratory for Metabolomics	The young scientist presentation award, The 46th Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry	Sep 2021
Yuya Sekine, Junior Research Associate, Laboratory for Genotyping Development	JUA Annual Meeting Award (Poster Section), The 109th Annual Meeting of the Japanese Urological Association	Dec 2021
Jing-yi Xue, Junior Research Associate, Laboratory for Bone and Joint Diseases	ECTS East-meets-West Award, The European Calcified Tissue Society	May 2021
Ritsu Nagata, Junior Research Associate, Laboratory for Intestinal Ecosystem	Encouragement Prize, The 58th Japanese Society for Mucosal Immunology	Jul 2021
Yuki Okawa, Student Trainee, Laboratory for Cancer Genomics	Excellent presentation award, The 27th Annual Meeting of Japanese Scociety for Hereditary Tumors	Jun 2021
Kazuhisa Yamazaki, Senior Visiting Scientist, Laboratory for Intestinal Ecosystem	The JSP Award for Distinguished Services, The Japanese Society of Periodontology	May 2021
Masato Akiyama, Visiting Researcher, Laboratory for Statistical and Translational Genetics	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, The Young Scientists' Award	Apr 2021
Hirotaka Ieki, Visiting Scientist, Laboratory for Cardiovascular Genomics and Informatics	Young Investigator Award for Clinical Research, The 85th Annual Scientific Meeting of the Japanese Circulation Society	Mar 2021
Kazuo Miyazawa, Visitng Researcher (JSPS, PD), Laboratory for Cardiovascular Genomics and Informatics	Genomic and Precision Medicine Early Career Investigator Award, American Heart Association (AHA)	Nov 2021
Yasutaka Motomura, Visiting Researcher, Laboratory for Innate Immune Systems	16th JSI Young Investigator Award, Japanese Society for Immunology	Dec 2021
Takashi Taida, Visiting Researcher, Laboratory for Intestinal Ecosystem	Young Investigator Award, Japan Digestive Disease Week 2021	Nov 2021
Takashi Taida, Visiting Researcher, Laboratory for Intestinal Ecosystem	Outstandinng Poster Award, Japan Digestive Disease Week 2021	Nov 2021
Long Guo, Senior Scientist, Laboratory for Bone and Joint Diseases	The Japanese Society for Bone and Mineral Research Travel award (ECTS 2021)	Apr 2021
Zheng Wang, Research Scientist, Laboratory for Bone and Joint Diseases	The Japanese Society for Bone and Mineral Research Travel Award (ANZBMS 2021)	Jun 2021
Jing-yi Xue, Junior Research Associate, Laboratory for Bone and Joint Diseases	The Japanese Society for Bone and Mineral Research Travel award (ECTS 2021)	Apr 2021

RIKEN International Program Associate (IPA)

IMS accepted three 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 2021 were

Yan Jun Lan (ETH Zurich, Switzerland) in the Laboratory for Advanced Genomics Circuit

Shruti Bhagat (Karolinska Institute, Sweden) in the Preventive Medicine and Applied Genomics Unit

Jingjie Chang (Tokyo Medical and Dental University, Japan) in the Laboratory for Transcriptional Regulation

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, 25 JRA students studied in IMS.

Yuki Ariyasu (Laboratory for Metabolomics)
Akiko Oguchi (RIKEN-IFOM Joint Laboratory for Cancer Genomics)
Takahiro Matsunaga (Laboratory for Gut Homeostasis)
Haruki Uchino (Laboratory for Metabolomics)
Umi Tahara (Laboratory for Skin Homeostasis)
Hiroto Horikawa (Laboratory for Gut Homeostasis)
Tomo Kakihara (Laboratory for Microbiome Sciences)
Kentarou Kubota (Laboratory for Innate Immune Systems)
Sayoko Kuroha (Laboratory for Metabolomics)
Jingyi Xue (Laboratory for Bone and Joint Diseases)
Zhengzheng Shi (Laboratory for Intestinal Ecosystem)
Yuya Sekine (Laboratory for Genotyping Development)
Yuki Tanaka (Laboratory for Cellular Function Conversion Technology)
Susumu Toshima (Laboratory for Tissue Dynamics)
Ryo Nakagawa (Laboratory for Human Disease Models)
Kohei Fujiwara (Laboratory for Metabolomics)
Mio Yoshida (Laboratory for Metabolomics)
Nao Tanaka (Laboratory for Statistical and Translational Genetics)
Taichi Akase (Laboratory for Large-Scale Biomedical Data Technology)
Tatsuya Ishikawa (Laboratory for Immune Homeostasis)
Raku Son (RIKEN-IFOM Joint Laboratory for Cancer Genomics)
Yuichiro Tanaka (Laboratory for Intestinal Ecosystem)
Ritsu Nagata (Laboratory for Intestinal Ecosystem)
Naoto Fujioka (Laboratory for Innate Immune Systems)
Satoshi Morozumi (Laboratory for Metabolomics)

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, 13 postdocs conducted their research at IMS through the SPDR program.

Rei Nakano (Laboratory for Cellular Function Conversion Technology)
Tsuyoshi Kiniwa (Laboratory for Innate Immune Systems)
Chisayo Kozuka (Laboratory for Developmental Genetics)
Sotaro Ochiai (Laboratory for Tissue Dynamics)
Juan Ortiz Quinonez (Laboratory for Advanced Genomics Circuit)
Mari Hashimoto (Laboratory for Human Disease Models)
Takaharu Sasaki (Laboratory for Intestinal Ecosystem)
Baihao Zhang (Laboratory for Mucosal Immunity)
Youxian Li (Laboratory for Gut Homeostasis)
Hideki Terajima (Laboratory for Gut Homeostasis)
Ryota Teramoto (Laboratory for Comprehensive Genomic Analysis)
Steven Matthew Heaton (Genome Immunobiology RIKEN Hakubi Research Team)
Ayako Matsuyama (Laboratory for Tissue Dynamics)

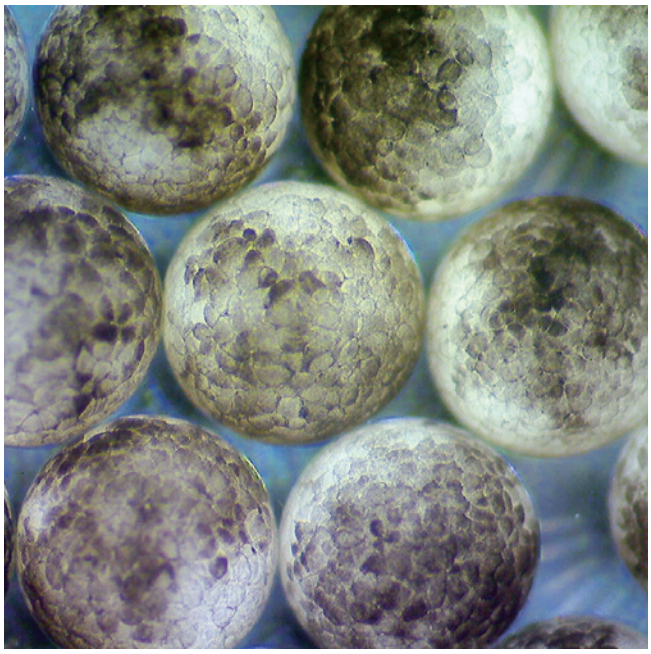
Guest Lectures 2021

Table: Guest Lectures Jan-Dec 2021

Date	Speaker	Affiliation	Country	Title
Feb. 26	Dr. Juan Betancur	Chugai Pharmaceutical Co., Ltd.	Japan	The leap from academia to industry
Feb. 26	Dr. Jordan Ramilowski	Medical Research Center, Yokohama City University	Japan	From RIKEN to a University in Japan: how to smoothly make the transition and what to expect
Apr. 20	Dr. Shilpa Garg	University of Copenhagen	Denmark	Efficient, high-resolution bioinformatic approaches for integrative sequencing analysis of complex diseases?
May 27	Dr. Shigehiro Kuraku, Dr. Mitsutaka Kadota	National Institute of Genetics, RIKEN Center for Biosystems Dynamics Research	Japan	Controlling sample/library preparation of Hi-C for the acquisition of high-quality data for genome scaffolding and epigenomic analysis
May 27	Dr. Pelin Sahlén	KTH Royal Institute of Technology & SciLifeLab	Sweden	HiCap: a novel tool for functional association
May 27	Dr. Taro Tsujimura	Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University	Japan	Modulating enhancer regulation: mechanisms and applications
May 27	Dr. Tomohiko Tamura	Yokohama City University Graduate School of Medicine	Japan	Chromatin regulation by transcription factors in myeloid cell development
May 28	Dr. Etsuo A. Susaki	Juntendo University Graduate School of Medicine	Japan	CUBIC-HistoVIsion: a versatile three-dimensional whole-organ/body staining and imaging
Jun. 2	Prof. Juha Kere	Karolinska Institute	Sweden	Genomic medicine – future or fallacy?
Jun. 9	Dr. Tetsuya Yamamoto	Institute for Chemical Reaction Design and Discovery, Hokkaido University	Japan	Soft matter physics of transcription regulation
Jun. 15	Dr. Shimon Tashiro	Tohoku University	Japan	Amendment of "Research Ethics Guidelines"
Jun. 16	Dr. Yuuki Obata	The Francis Crick Institute	UK	Gut instincts: How microbes talk to neurons
Jun. 22	Mr. Yodai Takei	California Institute of Technology	USA	Integrated Spatial Genomics Reveals Organizational Principles of Single-Cell Nuclear Architecture
Jul. 2	Dr. Hirotsugu Ishizu	Keio University School of Medicine	Japan	Regulatory non-coding RNA arising from retrotransposons
Jul. 8	Dr. Kei-ichiro Ishiguro	Institute of Molecular Embryology and Genetics, Kumamoto University	Japan	Insights on meiotic cell cycle in mammalian germ cells
Jul. 20	Dr. Fan-Yan Wei	Institute of Development, Aging and Cancer, Tohoku University	Japan	RNA epitranscriptome and extracellular signaling
Jul. 27	Dr. Noriko Toyama-Sorimachi	Research Institute, National Center for Global Health and Medicine	Japan	Lysosomal amino acid transporters as promising therapeutic targets of inflammatory diseases
Aug. 19	Prof. Haruko Takeyama	Waseda University	Japan	Towards understanding the mechanisms of expression regulation by spatial and regional omics analysis from micro-punched tissues
Aug. 26	Dr. Keiji Kuba	Akita University Graduate School of Medicine	Japan	Regulation of mRNA deadenylation/metabolism in response to heart failure stress
Sep. 3	Dr. Po-Ru Loh	Harvard Medical School	USA	Protein-altering variants with large phenotypic effects: rare SNPs, common VNTRs, and rare CNVs
Sep. 7	Dr. Hiro-oki Iwakawa	Institute for Quantitative Biosciences, The University of Tokyo	Japan	The mechanisms of the secondary siRNA biogenesis in plants
Sep. 15	Dr. Akihito Harada	Division of Transcriptomics, Medical Institute of Bioregulation, Kyushu university	Japan	Epigenomic lineage tracing toward the understanding acquisition of tissue-specific gene expression profile
Sep. 30	Dr. Koshi Imami	Kyoto University	Japan	Nascent proteome matters
Oct. 5	Dr. Michael Wilson	University of Toronto	Canada	From mammals to fish and back again: comparative epigenomic insights into cardiovascular gene regulation
Oct. 20	Dr. Pei-I Tsai	University of California San Francisco	USA	Culling Unfit mtDNA in the Adult Rejuvenates Wellbeing in Mitochondria Disease
Oct. 27	Dr. Toshiro Hara	Massachusetts General Hospital/Broad Institute	USA	Observing and harnessing cellular plasticity in glioblastoma
Nov. 2	Dr. Yasuhiko Minokoshi	National Institute for Physiological Sciences	Japan	Hypothalamic regulation of carbohydrate and lipid intake and utilization in peripheral tissues
Nov. 10	Dr. Kazuhiro Nakamura	Nagoya University Graduate School of Medicine	Japan	Brain circuit mechanisms for sympathetic responses to environmental stressors
Dec. 22	Dr. Masaki Miyazaki	Institute for Frontier Life and Medical Sciences, Kyoto University	Japan	Lineage-specific transcription factors, Regulome, and the gene expression signature in adaptive immune cells. – To B(T) or not to B(T). That is the question –

Part 5

Data and Statistics



Publications 2021

Table: IMS Publications from January to December, 2021

Journal	Impact Factor (2020)	Number of papers
Nat Biotechnol	54.9	1
Nat Med	53.4	2
Nature	50.0	10
Science	47.7	2
Cell	41.6	5
Cancer Discov	39.4	1
Nat Genet	38.3	5
Immunity	31.7	2
Cell Metab	27.3	1
Nat Immunol	25.6	4
Diabetes Care	19.1	1
Ann Rheum Dis	19.1	4
Sci Transl Med	18.0	1
Mol Cell	18.0	1
Sci Immunol	17.7	1
Trends Immunol	16.7	2
Nat Commun	14.9	14
J Clin Invest	14.8	1
J Exp Med	14.3	7
Sci Adv	14.1	2
J Immunother Cancer	13.8	1
Genome Biol	13.6	1
Allergy	13.1	1
Cancer Res	12.7	1
Dev Cell	12.3	2
Brief Bioinform	11.6	1
Leukemia	11.5	1
Proc Natl Acad Sci U S A	11.2	5
Genome Med	11.1	1
Am J Hum Genet	11.0	2
Arthritis Rheumatol	11.0	2
J Allergy Clin Immunol	10.8	3
Others		198
Total		**286**

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Budget, Personnel and Patents

IMS Budget FY2021

IMS Budget FY2021	JPY Million
Government funding for operations	3,560
External competitive funding	2,450
Total	**6,010**

Patents

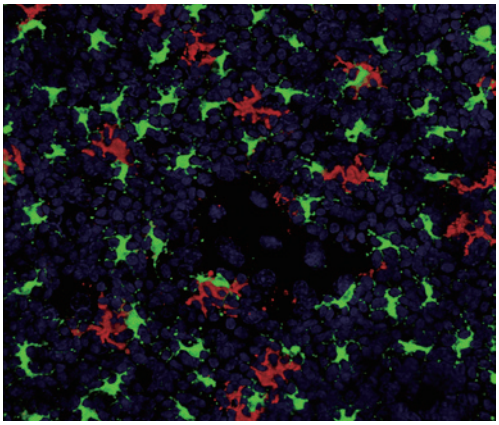
There were 12 patents registered from January to December 2021.

Patents	Total	International patents	Domestic patents (Japan)
2021	12	9	3

Personnel FY2021

Category	Number
Director	1
Deputy Director	2
Senior Advisor	2
Team Leader	37
OCD Director	1
Coordinator	3
Deputy Team Leader	6
Senior Scientist	24
Senior Research Scientist	3
Research Scientist	56
Postdoctoral Researcher	26
Special Fixed Term Contract Researcher	1
Special Postdoctoral Researcher	12
Research Fellow	15
Research Associate	7
Senior Technical Scientist	3
Technical Scientist	20
Expert Technician	17
Technical Staff I	64
Technical Staff II	44
International Program Associate	3
Junior Research Associate	25
Student Trainee	118
Research Administrator	5
Research Administrative Support Staff	5
Assistant	26
Part-time Staff	42
Senior Visiting Scientist	25
Visiting Scientist	196
Visiting Technical Scientist	13
Visiting Researcher	10
Temporary Staffing	15
Research Consultant	5
Consultant	2
Special Temporary Employee	2
Total	**836**

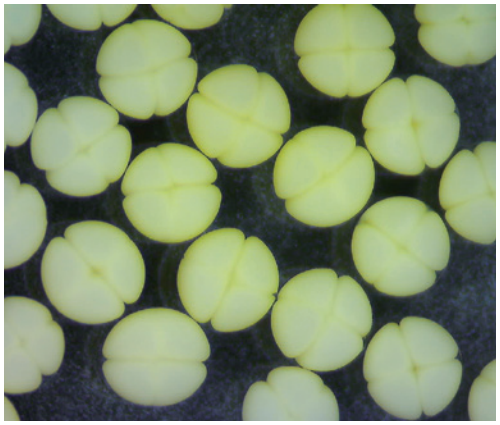
Original Photos of the Cover and Front Pages



Cover

Immune cell network of mouse skin. MHCII^{+} Langerhans cells (green), CD2^{+} Innate lymphoid cells (red), and DAPI (blue).

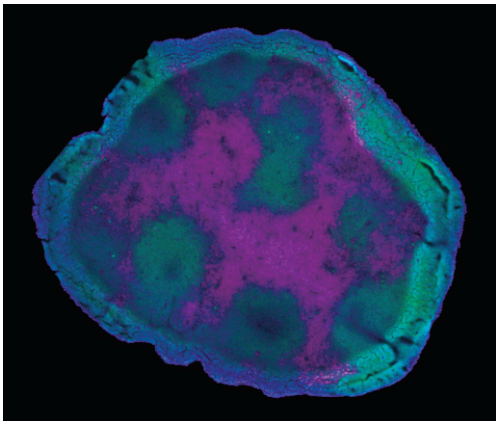
Credit to Dr. Tetsuro Kobayashi
Laboratory for Innate Immune Systems



Front page of Part 1

Albino Xenopus laevis embryos, which are useful for fluorescent imaging analysis by introducing exogenous genes.

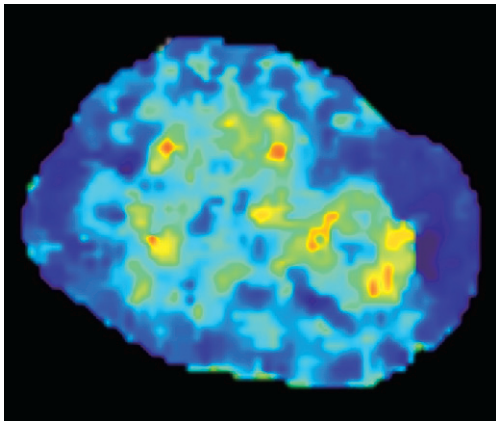
Credit to Dr. Yuuri Yasuoka
Laboratory for Comprehensive Genomic Analysis



Front page of Part 2

Immunohistochemical analyses showing B cell follicles in green and T cell area in magenta of an activated popliteal lymph node in a WT mouse 7 days post-immunization.

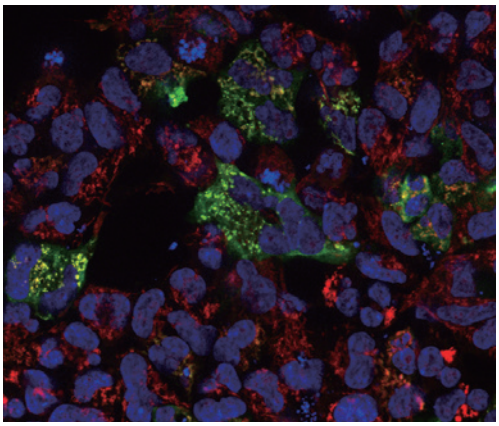
Credit to Dr. Yuki Sugiura & Sidonia Fagarasan
Keio University & IMS Laboratory for Mucosal Immunity



Front page of Part 3

Imaging mass spectrometry of GABA of activated popliteal lymph node in a WT mouse 7 days post-immunization. Note the high signal of GABA detected in B cells follicles.

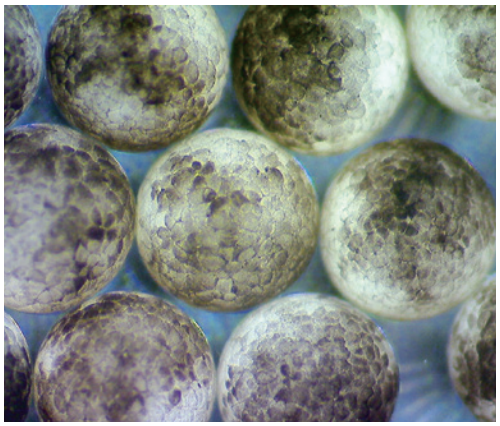
Credit to Dr. Yuki Sugiura & Sidonia Fagarasan
Keio University & IMS Laboratory for Mucosal Immunity



Front page of Part 4

Mitochondrial-targeting Cas9 protein (green) is well localized in mitochondria (red) but not nuclei (blue) of HEK293 cells transfected with Cas9 expressing vectors.

Credit to Dr. Yuuri Yasuoka
Laboratory for Comprehensive Genomic Analysis

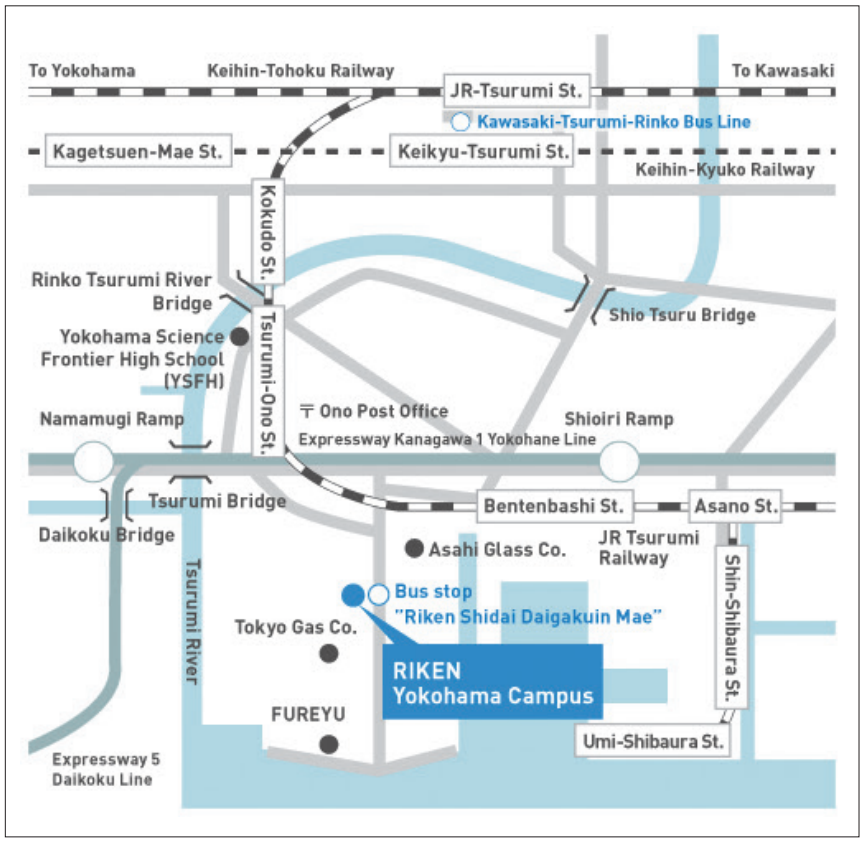


Front page of Part 5

Xenopus tropicalis embryos at morula stage, which have developed synchronously after *in vitro* fertilization.

Credit to Dr. Yuuri Yasuoka
Laboratory for Comprehensive Genomic Analysis

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.

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.

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