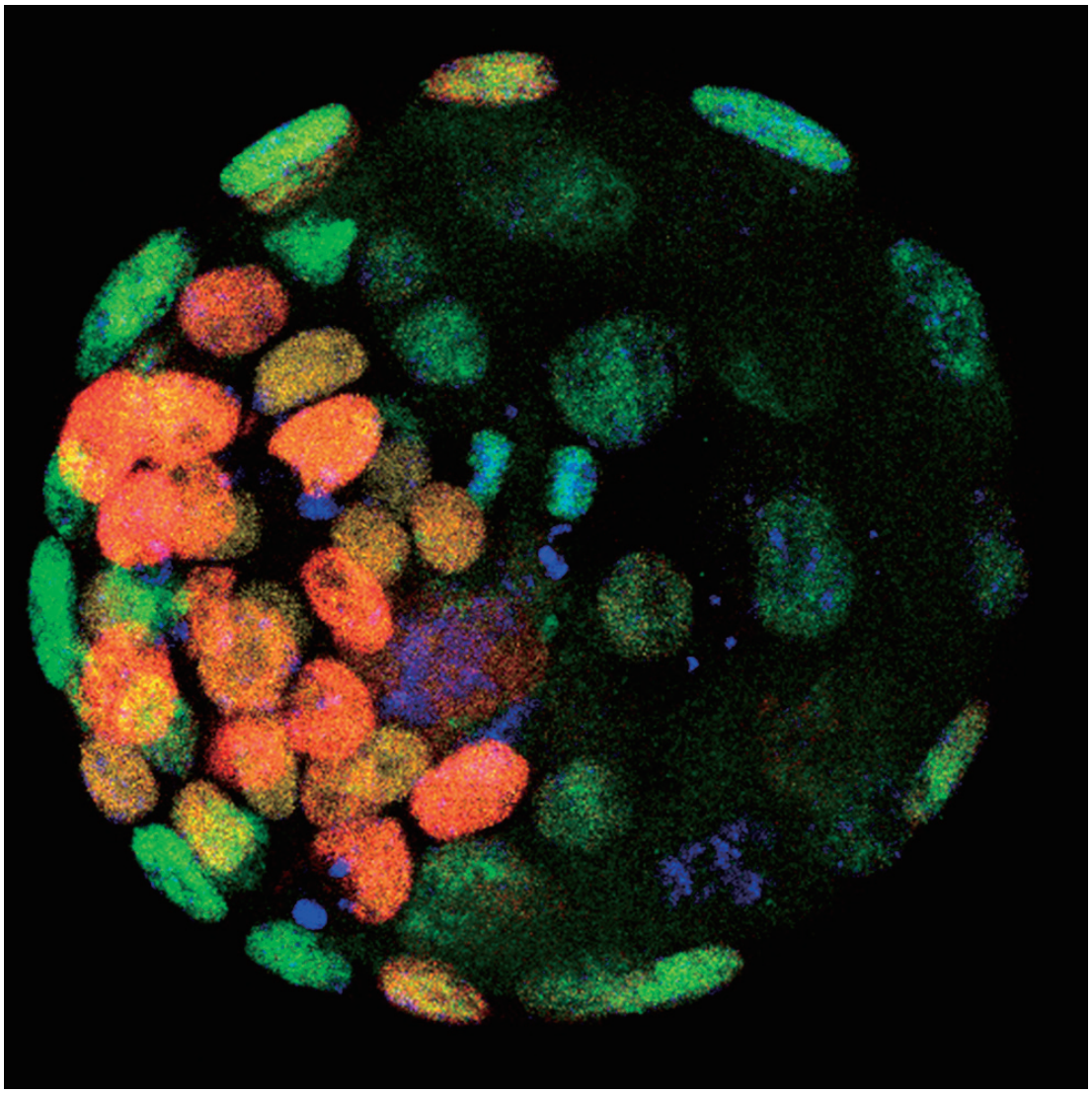
RIKEN IMS Annual Report 2020

RIKEN Center for Integrative Medical Sciences



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YCI Laboratory for Next-Generation Proteomics: **Yibo Wu**
YCI Laboratory for Metabolic Epigenetics: **Azusa Inoue**

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Director's Report

Greetings. I have had the challenging task beginning in April 2020 of following in the exceptional footsteps of the previous director, Dr. Tadashi Yamamoto, who successfully guided our center and its predecessor from 2015 through 2019. My tenure began at a difficult time for our institute and the world as COVID-19 was spreading explosively in many countries and a state of emergency was about to be declared in Japan.

Particularly for an institute that strives to internationalize Japanese research, the social distancing mandates and travel restrictions imposed for pandemic control have hindered our work. For example, more than a hundred foreign researchers who had planned to work at RIKEN were unable to enter Japan. Social distancing also prevented the usual training for new employees. When the restrictions in Japan were at their greatest extent, wet lab experimental research stopped on all projects except those directly related to COVID-19. Dry-lab research (mainly informatics) could continue and wet-lab research has gradually resumed.

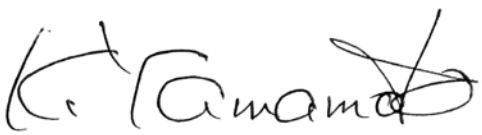
The heart of RIKEN IMS is interdisciplinary research involving integration and collaboration among immunology, genomics, and transcriptomics. The laboratories conducting our research in these three fields are largely housed in separate buildings on our Yokohama campus. Integration of our research in immunology, genomics, and transcriptomics was already underway before my tenure as director began, but the pandemic restrictions have slowed our progress. For example, seminars lost the benefits of in-person interactions as they were forced to go online. On the other hand, we are actively promoting the regular IMS seminar and the online format now used for this and other symposiums makes it easier to invite speakers from overseas.

The pandemic has forced our international conferences and other collaborations to be cancelled, postponed or held online. An international summer school and symposium jointly hosted with Tsinghua University (China) has been postponed. Cancelled was the International Symposium on Immunology co-sponsored by IMS with the Japanese Society of Immunology. In 2020, we held an online symposium co-organized with Stanford University (US) that focused on development/differentiation and another co-organized with Karolinska Institute/SciLifeLab (Sweden) that focused on artificial intelligence.

Among the many excellent papers published by IMS scientists in 2020 was one by Dr. Ohno's team on the relationship between gut bacteria and immunity in a mouse model of multiple sclerosis. They found that one gut bacterium that induces Th17 cells (related to inflammation) and another that induces proliferation of antigen-specific T cells may work synergistically to activate antigen-specific T cells in the central nervous system. With research in humans, this major insight may facilitate development of preventative or therapeutic treatments of multiple sclerosis or other autoimmune diseases.

To address the pandemic crisis, we are implementing more than 10 COVID-19-related extensions of existing research projects, including Dr. Fujii's project that is described later in this annual report. In addition, we are improving our biosafety level 3 facilities not only to handle SARS-CoV-2, but also to be used for research on future infectious diseases, such as new influenza strains.

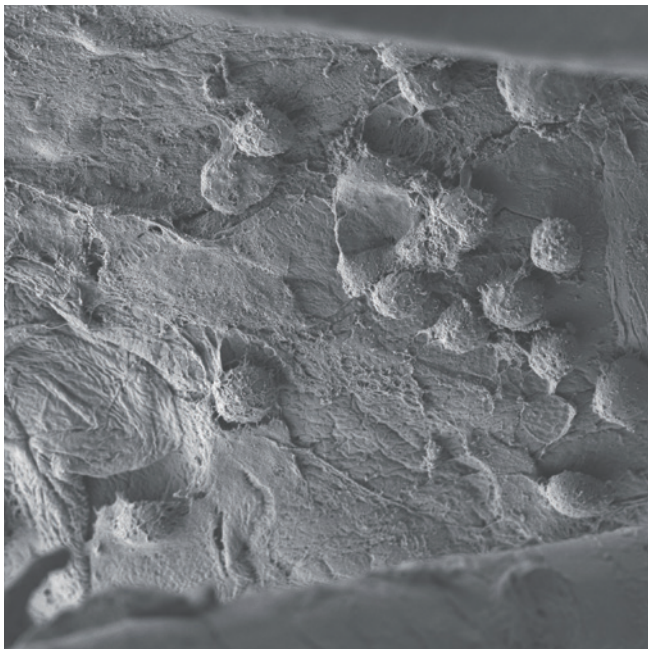
As vaccination against COVID-19 ramps up and the pandemic hopefully subsides, the future of RIKEN IMS is difficult, but we are working hard to succeed. Innovative results are anticipated from the increases we are facilitating in the crosstalk between experimental wet lab research and dry lab informatics research. Another promising initiative is the use of more human-derived samples in immunology research to supplement the IMS's world-class expertise with animal models; the in-house development of new methodological approaches is expected to accelerate this work. We hope that our improvements to the RIKEN IMS research environment will encourage even more of the best young researchers from around the world to gather here and innovate with us.



Kazuhiko Yamamoto
Director
RIKEN Center for Integrative Medical Sciences

Part 1

Research Highlights



All the articles in this part were written by ELSS, Inc.

Signals from two gut bacteria trick the body into attacking its own central nervous system

Hiroshi Ohno

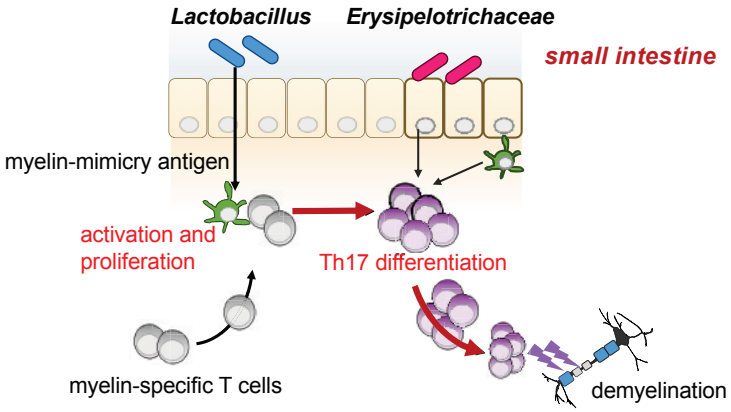


Figure:
Two gut bacteria trigger the autoimmune reaction characteristic of MS.
One species presents a myelin-mimic antigen that induces T cell proliferation and the other acts as an adjuvant to promote differentiation into inflammatory Th17 cells.

In the autoimmune disease multiple sclerosis (MS), the immune system attacks the myelin sheath insulating the central nervous system. Damage to the myelin sheath disrupts nerve signaling, impairing communication between the brain and the body. Patients with MS experience numbness, slurred speech, and difficulty walking, among other symptoms. Although the gut microbiome has been linked to MS, how microbes in the gut affect the central nervous system remained a mystery. Using a mouse model of MS, researchers led by Hiroshi Ohno at the RIKEN Center for Integrative Medical Sciences revealed an important clue: two species of gut bacteria trick the immune system into attacking myelin.

As described in their recent publication in *Nature*, the researchers began by investigating connections between gut bacteria and disease symptoms, using antibiotics to selectively remove gut bacteria. They noticed that mice treated with ampicillin showed weaker symptoms and less demyelination. The mice were only protected against demyelination when they were fed ampicillin, not when it was injected, so it was clear that an intestinal microbe was exacerbating disease progression.

Microbiome analysis of these mice revealed that ampicillin almost completely deleted one microbe in particular: a novel species called OTU0002 of family Erysipelotrichaceae. To test their hypothesis that this microbe was causing the autoimmune reaction, the team orally administered germ-free mice with OTU0002 alone. These mice still showed demyelination, but their symptoms were milder than those of the original mice, indicating that more than one microbe must be involved.

Applying a clue from another study– that proteins of some gut bacteria mimic host proteins, thereby triggering autoreactive T cells to proliferate– the team used in silico analysis to search the mouse gut microbiome for myelin-mimic proteins. They found them in *Lactobacillus reuteri*. When mice were infected with both *L. reuteri* and OTU0002, they showed full-strength symptoms. Further research showed that *L. reuteri* induces myelin-specific T cells to proliferate. OTU0002 then stimulates these T cells to differentiate into inflammatory Th17 cells, leading to devastating demyelination.

This is the first time that a mechanism like this– where two commensal bacteria synergistically trigger a host autoimmune reaction– has been reported. Next steps include determining how the Th17 cells migrate from the intestine to infiltrate and attack the central nervous system and investigating whether a similar mechanism occurs in humans. Dr. Ohno says "Our findings could be extended to the pathogenesis of other autoimmune diseases, including rheumatoid arthritis, type 1 diabetes mellitus, and systemic lupus erythematosus, and even to diseases such as obesity and type 2 diabetes mellitus– wherever systemic chronic inflammation is involved in the pathogenesis."

Original paper:
Miyauchi E, Kim S-W, Suda W, Kawasumi M, Onawa S, Taguchi-Atarashi N, Morita H, Taylor T D, Hattori M, Ohno H. Gut microbes act in concert to exacerbate inflammation in spinal cords. ***Nature*** 585, 102-106 (2020)

A quantum leap for lipidomics research

Makoto Arita

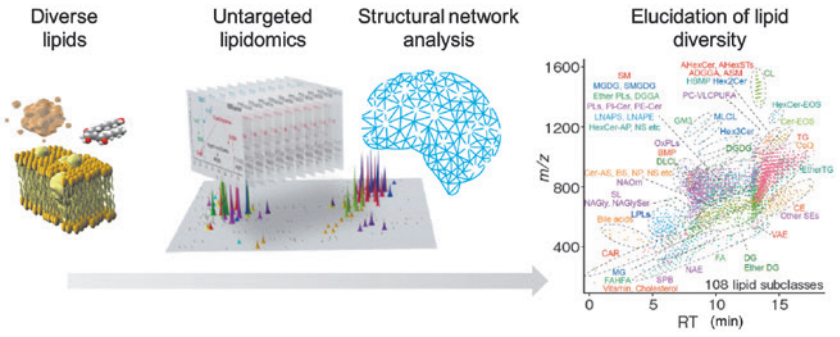


Figure:
An untargeted lipidomics platform to elucidate lipid diversity in organisms. We developed an untargeted lipidomics platform that analyzes and integrates mass spectrometry (MS) data from various techniques to accurately differentiate lipids. The platform, which builds upon standardized lipidomics procedures, harmonizes data obtained from different laboratories. The results of a recent study show a diversity of lipid structures that is far greater than previously realized.

Lipids are ubiquitous. Their hydrophobicity makes them "sticky" and difficult to analyze, which hinders our ability to characterize the totality of lipids (lipidome) in any organism. The team led by Makoto Arita in RIKEN Center for Medical Sciences characterize the lipidomes of living organisms by developing mass spectrometry (MS) techniques that differentiate lipids. Little were realized how diverse lipid structures would be. Initially, they expected the number in the thousands. However, they were extremely diverse ; there exist more than 45,000 different lipids. This diversity facilitates the creation of complex biological systems, in which lipids act as key components of cellular membranes, signaling molecules, energy-storage molecules, and substrates.

In lipidomics (and metabolomics) analysis, liquid chromatography tandem mass spectrometry (MS/MS) and ion mobility spectrometry (IMS) are the gold-standard techniques; they provide thousands of molecular ions per specimen. A major challenge has been to develop a computational platform that *1*) does not target specific molecules but measures all relevant molecules (i.e., is untargeted) and *2*) integrates the obtained raw MS data to accurately determine molecular structures. Such a platform would allow us to semi-quantitatively elucidate complex biological systems from their lipidome (and metabolome) profiles.

They recently established such a platform, packaged in Mass Spectrometry-Data Independent AnaLysis software version 4 (MS-DIAL 4; http://prime.psc.riken.jp/), which untangles lipid mass spectral fragmentations to create an atlas of lipids with an estimated false discovery rate of 1% to 2%. They tested it by analyzing 1,056 biological samples from various sources (human blood, mouse tissues, other mammalian cells, microbiota, algae, and plants) and derived a catalog of 8,051 different lipid species. They used those experimental data to create a comprehensive database of retention time and collision cross section based on machine learning, and MS/MS for 581,047 lipid structures, publishing the product as a "lipidome atlas".

MS-DIAL 4 appropriately represents the structures of 117 lipid subclasses–twice that of previous versions–based on fragment evidence for annotations at the levels of lipid class, molecular species, and acyl chain positional isomers. Importantly, MS-DIAL 4 complies with the lipidomics standards initiative: its classifications follow standardized definitions, structures are represented by an international shorthand notation system, and lipidomics results can be exported in a common data format. Thus, their platform enhances standardized lipidomics procedures and harmonizes data across laboratories, and is poised to accelerate lipidomics research.

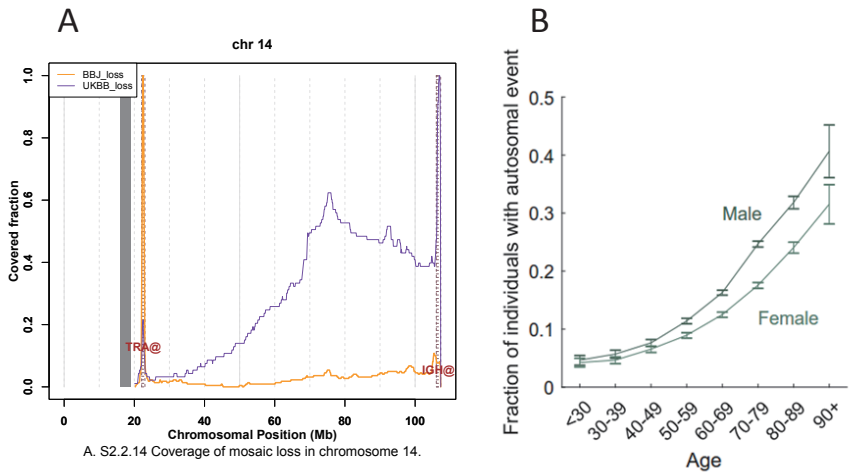
Dysregulated lipid metabolism is associated with disease (e.g., obesity, atherosclerosis, diabetes). The ability to accurately distinguish lipid species will transform our understanding of lipid functions and facilitate discovery of bioactive lipids with therapeutic benefits. In the near future, visualization technologies, such as imaging MS, will revolutionize our understanding of the relationships among lipids.

By integrating MS-DIAL 4 with other omics and imaging MS data, it is expected to uncover previously unknown lipid pathways and accelerate the development of biomarkers, drugs, and clinical applications.

Original paper:
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-J, Koelmel J, Cajka T, Fiehn O, Saito K, Arita M & Arita M*.
A lipidome atlas in MS-DIAL 4. ***Nature Biotechnology*** 38, 1159-1163 (2020)

The impact of mosaic chromosomal alterations on oncogenesis and the aging process: the first findings outside Europe

Chikashi Terao



A. S2.2.14 Coverage of mosaic loss in chromosome 14.

Figure:
A. Frequencies of loss events at chromosome 14 in Japanese and British. T-cell receptor region (TRA@): T cell lineage (common in Japanese); B-cell receptor region (IGH@): B cell lineage (common in British).
B. The prevalence of mosaic chromosomal alterations (mCAs) detected from blood-derived DNA genotyping stratified by age and sex (%) in Japanese.

Criticisms of genetic studies for common phenotypes include their lack of clinical application and poor representation of non-European populations.

Consider leukemia. Japanese have a tenfold higher incidence of adult T cell leukemia but a fivefold lower incidence of chronic lymphocytic leukemia (B cell malignancy) compared to Europeans. Why?

Chikashi Terao of the RIKEN Center for Integrative Medical Sciences and his colleagues have a new explanation.

The team produced a genomic landscape of clonal hematopoiesis with chromosomal alterations (mCAs) for Japanese and British populations using data from two biomedical databases: the BioBank Japan (BBJ) and the UK BioBank (UKBB). They found that Japanese and British had markedly different rates of hematopoietic clones arising from B and T cell lineages: mosaic deletions at the TRA locus (i.e. clonal expansion in the T cell lineage) were common in Japanese but rare in British, whereas those at the IGH locus (i.e. clonal expansion in the B cell lineage) were common in British but rare in Japanese (Figure A). The differences were detectable in those without blood cancers.

The prevalence of mCAs increased with advancing age, and 35% of Japanese in their 90s were mCA carriers. "Our findings suggest that mCAs in hematopoietic clones are inevitable in the elderly", says Terao (Figure B). mCA was significantly associated with a 10% increase in overall mortality and a 4.7-fold increase in leukemia mortality compared with non-carriers. Further, chromosome 17 CN-LOH showed an over 80-fold increase in leukemia mortality. They also found inherited variants strongly associated with mCAs (odds ratio up to 91).

"This was the first large-scale analysis of mCAs ever in Asia. We captured mosaicism, an interplay between inherited genetic variations and acquired mutations at the chromosome level, not the single-base level, hence the substantially larger effect sizes. This helps improve individual risk prediction."

The finding on mosaicism has recently been included in a new publication, in which the UK, USA, Finland and Japan were compared.

"This signifies the importance of genetic studies conducted in diverse populations", says Terao.

Original paper:
Terao C, Suzuki A, Momozawa Y, Akiyama M, Ishigaki K, Yamamoto K, Matsuda K, Murakami Y, McCarroll S A, Kubo M, Loh P R, & Kamatani Y. Chromosomal alterations among age-related haematopoietic clones in Japan. ***Nature*** 584, 130-135 (2020)

Learning how cancer cells evade group 2 innate lymphoid cells

Shigeo Koyasu

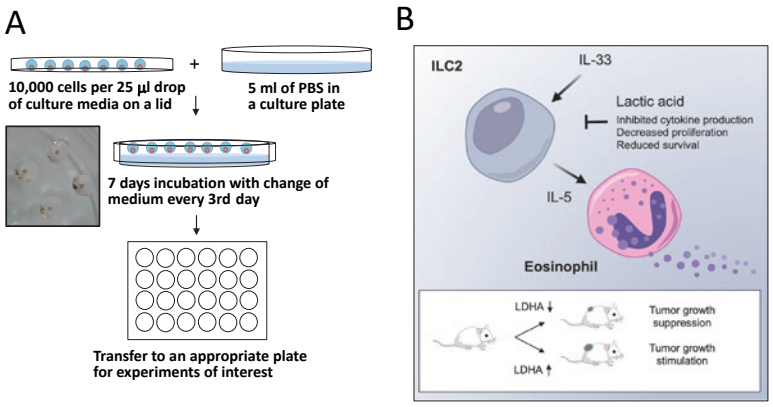


Figure: A novel spheroid culture revealed how certain cancer cells suppress ILC2s
A. The underside of the lid of a petri dish was dotted with droplets of culture medium, onto which melanoma cells were plated. The lid was then placed on a culture plate containing phosphate-buffered saline (PBS) to prevent the droplets from drying out. The photo represents melanoma spheroids on day 7.
B. Activation of ILC2s by interleukin 33 (IL-33) induces the release of cytokines such as interleukin 5 (IL-5), which in turn induces eosinophils to release cytotoxic proteins such as major basic protein (MBP). Co-culture experiments with ILC2 revealed that certain cancer cells, such as those in malignant melanomas, counter this assault via the IL-33-ILC2–eosinophil axis by producing lactic acid, which suppresses ILC2 function. Abbreviation: LDHA, lactic acid dehydrogenase.

Group 2 innate lymphoid cells (ILC2s) are abundant in non-lymphoid tissues, including skin, where they mediate allergic reactions and protect against infection by parasitic worms (helminths). When epithelial cells contact a helminth, they release interleukin 33 (IL-33), which triggers ILC2s to mass-produce IL-5 and promote inflammation via eosinophils to prevent infection.

ILC2s use similar tactics to suppress the growth of epithelial solid tumors. However, in solid tumors, ILC2s comprise only a small proportion of the immune cells present. This situation suggests that cancer cells evade surveillance and attenuation by ILC2s.

To understand how cancer cells are able to slip past ILC2s, Marek Wagner, a cancer biologist and a member of Koyasu's research team, examined the relationship between ILC2s and the environment of melanoma tumors. First, he confirmed that injecting mice with IL-33 causes eosinophils to infiltrate melanomas and thus suppresses their growth. Wagner also realized that the more malignant the cancer, the lower the ILC2 levels in the tissues around the melanomas.

Next, Koyasu and Wagner devised an ingenious system (Figure, A) to investigate how cancer cells suppress ILC2 function and survival. To address this question, the researchers considered two features of carbohydrate metabolism in cancer cells: high levels of glucose transporters and lactate dehydrogenase (LDHA) during proliferation, and the metabolism of glucose into lactate, which causes lactate to accumulate around the cancer and acidify its microenvironment.

To test whether lactate accumulation reduces ILC2 activity *in vivo*, the team used RNA interference and generated malignant cells expressing low levels of LDHA. As expected, ILC2s and eosinophils infiltrated cancer tissues and suppressed the growth of these low-LDHA-expressing cells more than typical cancer cells (Figure, B).

Finally, to corroborate their findings in humans, the team analyzed gene expression data from melanoma patients. As in mice, both IL-33 and eosinophil levels were elevated in humans with malignant melanomas; patient survival rates were high for cases with high ILC2 activity; and ILC2 and eosinophil levels were inversely related to lactate production. However, when the researchers then looked at lung cancers or pancreatic cancers, survival rates were unrelated to IL-33 and eosinophil levels.

Overall, the team's results suggest that the growth of melanoma tumors depends on the balance between the mutually suppressive activities of ILC2s and cancer cells. They also underscore the view that ILC2 function depends on numerous factors, including the tissue environment and type of cancer. Why? As Dr. Koyasu puts it, "ILCs are generally tissue-resident cells. It may be that they change their character depending on the tools a tissue has to maintain its structure and repel invaders."

Original paper and reference:
Wagner M, Ealey KN, Tetsu H, Kiniwa T, Motomura Y, Moro K, Koyasu S. Tumour-derived lactic acid contributes to the paucity of intratumoural ILC2s. ***Cell Rep*** 30, 2743-2757 (2020) https://doi.org/10.1016/j.celrep.2020.01.103

Wagner M, Koyasu S. A 3D Skin melanoma spheroid-based model to assess tumor-immune cell interactions. ***Bio Protoc*** 10, e3839 (2020) https://doi.org/10.21769/BioProtoc.3839

A curious chemokine, CCL5, paradoxically suppresses the action of immune cells, but RUNX-mediated repression of CCL5 augments immune responses in cancer

Wooseok Seo and Ichiro Taniuchi

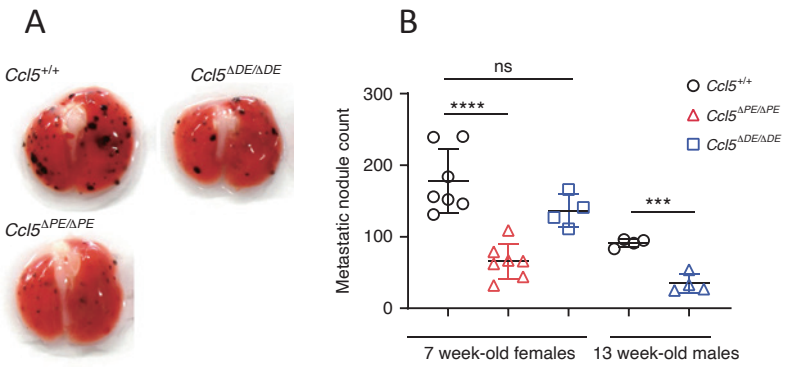


Figure:
A. Macroscopic images of lungs 14 days after melanoma cells were injected into Ccl5$^{+/+}$, Ccl5$^{\Delta PE/\Delta PE}$ and Ccl5$^{\Delta DE/\Delta DE}$ mice.
B. The number of metastatic nodules per mouse (Mean ± SD; ***p < 0.001; ****p < 0.0001; ns non-significant).

During the analysis of Runx mutant mouse strains, Wooseok Seo of the RIKEN Center for Integrative Medical Sciences and his colleagues were intrigued by the unique properties of a chemokine, C-C Motif Chemokine Ligand 5 (CCL5). Chemokines are fundamental regulators of immune cell migration between the bloodstream and tissues; thus, regulating expression of chemokines could augment immune responses in disorders such as cancer. CCL5, also known as RANTES, is a small protein secreted by cells that regulate the immune system. Despite its crucial roles in inflammation and cancer immunity, how exactly CCL5 modulates immune responses is not well-understood.

Furthermore, CCL5 is produced by both cancer cells (cancer-secreted CCL5) and the immune system (host-derived CCL5), but the latter has been shown to have rather contradictory functions during tumorigenesis and metastasis: it may work as either pro-cancer or anti-cancer depending on circumstances.

The RIKEN team addressed these issues by identifying two enhancers and demonstrated that expression of *Ccl5* (the gene that encodes CCL5) is regulated by each of these enhancers during inflammation and homeostasis phases, respectively, and that these enhancers are further regulated by RUNX/CBFβ complexes and the SATBI chromatin organizer. A proximal enhancer (located near *Ccl5*), when combined with transcription factors, increases the production of CCL5 during the steady state. A distal enhancer further away from *Ccl5* induces CCL5 expression in activated immune cells. The team also discovered that the transcription factor RUNX suppresses the function of these two enhancers.

Even more striking was the role CCL5 plays in cancer progression. The team injected melanoma cells into normal mice and knockout mice lacking either the proximal enhancer or the distal enhancer.

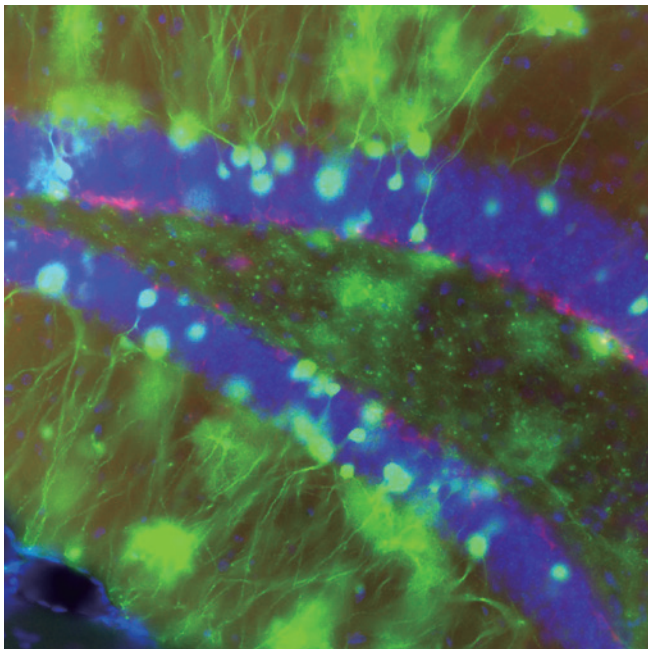
After 14 days, the mice without the proximal enhancer had fewer and smaller tumor cell clusters in their lungs than normal mice (Figure), suggesting that CCL5 produced via the proximal enhancer encouraged tumor cell growth. By contrast, increased CCL5 expression through RUNX3 mutation was associated with more metastatic tumors in the lung. "This indicates that high levels of CCL5 are not good for tumor immunity", stated principal investigator Ichiro Taniuchi.

"The identification of functional enhancers such as these should facilitate the understanding of how other potential regulators might work to activate *Ccl5*. If we can somehow remove CCL5 from our system, we may actually strengthen our immune system. This could move CCL5 one step closer towards to being an actionable immunotherapeutic target", says Seo.

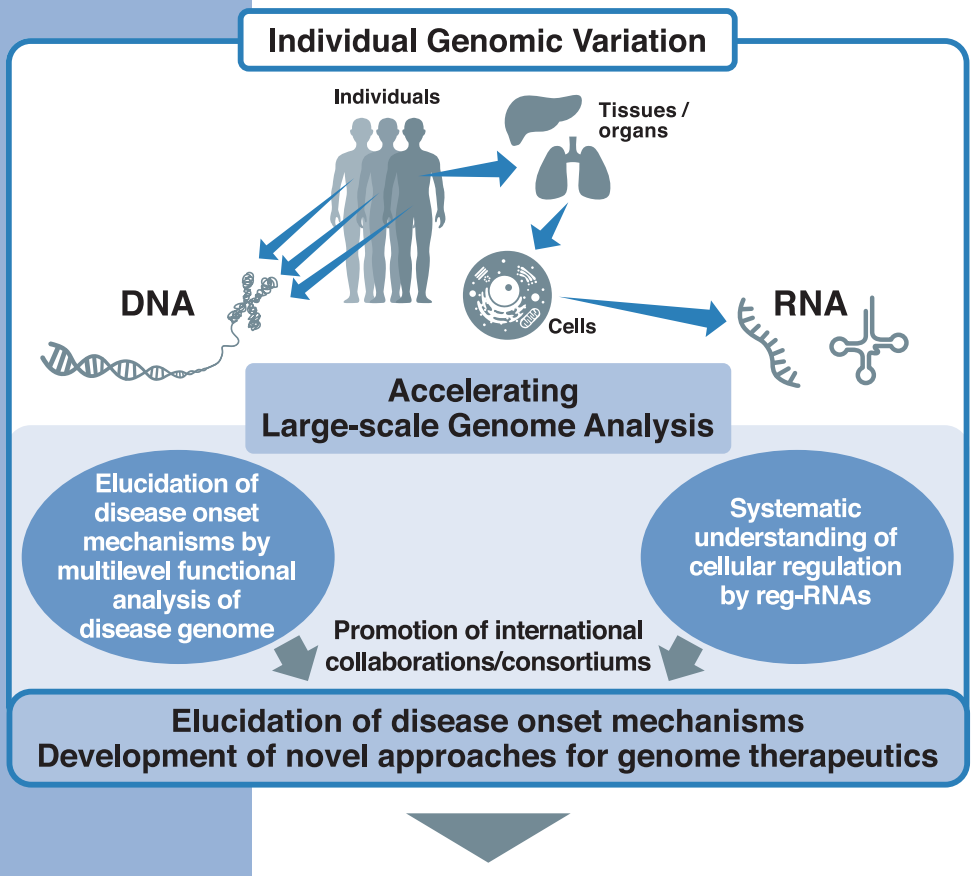
Original paper:
Seo W, Shimizu K, Kojo S, Okeke A, Kohwi-Shigematsu T, Fujii S & Taniuchi I. Runx-mediated regulation of CCL5 via antagonizing two enhancers influences immune cell function and anti-tumor immunity. ***Nature Communications*** 11, 1562 (2020)

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

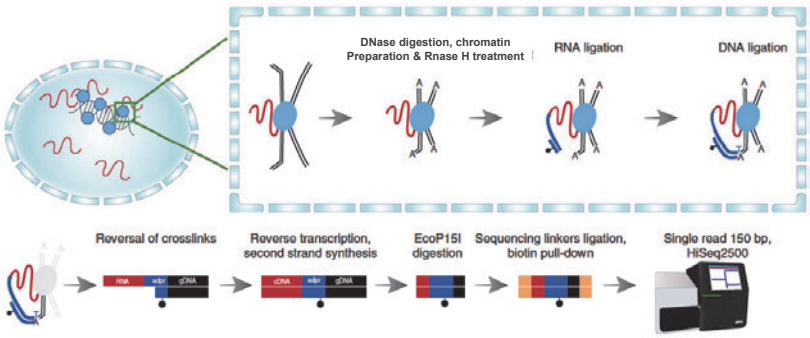


Figure: Schematic representation of the RADICL-seq protocol

Top: Series of enzymatic reactions occurring in fixed nuclei after partial lysis of the nuclear membrane. The adduct formed by genomic DNA (black), RNA (red), and proteins (blue circles) is subjected to controlled DNase I digestion and chromatin preparation. After RNase H digestion, an adapter (dark blue) containing an internally biotinylated residue (black dot) bridges the RNA and DNA molecules that lie in close proximity. Bottom: Series of enzymatic reactions performed in solution. After reversal of crosslinks, the RNA–DNA chimera is converted into a fully double-stranded DNA (dsDNA) molecule and digested by the EcoP15I enzyme to a designated length (adpr, adapter). After ligation of the sequencing linkers (yellow) and biotin pull-down, the library is subjected to PCR amplification and high-throughput sequencing.

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)

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, … Baillie JK, 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, 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

Carninci P. "Fine regulations of cellular networks: novel approaches using antisense lncRNAs" The Human Cell Atlas Developmental Asia and Australia Clinical and rare disease applications seminar (United Kingdom/Online) December 2020

Carninci P. "Decoding human genome: an international effort spanning two decades" The 2020 ENCODE Research Applications and Users Meeting (ENCODE 2020) (Spain/Online) October 2020

Carninci P. "Analytical technologies for bulk and single cell genomics" The 5th IABS Cell Therapy Conference (Tokyo, Japan) February 2020

Carninci P. "Lessons from large consortia: collaborations, internationalization, career paths" EMBO Expert meeting, How do elite programs for early carrer researchers work? (Tokyo, Japan) February 2020

Carninci P. "Surprises from studies of mammalian transcriptome" The 1st CIBoG Retreat (The 12th NAGOYA Global Retreat) (Nagoya, Japan) February 2020

Our laboratory focuses on developing technologies and exploring the regulatory functions of lncRNAs. Many of lncRNAs reside in the cell nucleus and interact with the genomic DNA in the mammalian genome. In order to analyze the 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. Interactome analysis by RADICL-seq revealed distinct genome occupancy patterns for specific classes of transcripts in each cell type. Interestingly, 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. RADICL-seq is expected to be widely used in FANTOM6 and other projects as a valuable technique for understanding the roles of lncRNAs from a spatial perspective.

FANTOM6 aims at creating a comprehensive catalogue of functional lncRNAs. As a pilot project, we targeted 285 lncRNAs for a systematic knockdown analysis in human primary fibroblast cells to explore their functions. From this analysis we created the largest-to-date publicly available (https://fantom.gsc.riken.jp/zenbu/reports/#FANTOM6) lncRNA knockdown data set on cell growth and morphology using realtime imaging and CAGE deep sequencing to reveal molecular pathways associated with each lncRNA. We found that nearly 30% of the lncRNAs affect cell growth, morphology and migration.

A functional lncRNA, named SINEUP, is known to upregulate the production of a specific protein. We discovered the mechanism by which interaction of SINEUP with a pair of RNA binding proteins, PTBP1 and HNRNPK, allows SINEUP to be transported from the cell nucleus to the cytoplasm, making it possible for it to act on its target mRNA for protein translation. Understanding the mechanisms of functional lncRNAs is an important first step for applications of lncRNAs to improve human health.

Laboratory for
Cellular Function Conversion Technology

Team Leader: Harukazu Suzuki

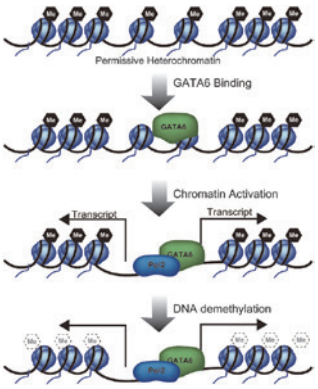


Figure: A schematic illustration of a model for the interrelation between GATA6-mediated DNA demethylation and chromatin status
GATA6 first binds to GATA6 binding motifs in permissive heterochromatin sites, then opens and activates the chromatin at the binding sites and finally completes the DNA demethylation.

Recent Major Publications

Suzuki T, Furuhata E, Maeda S, Kishima M, Miyajima Y, Tanaka Y, Lim J, Nishimura H, Nakanishi Y, Shojima A, Suzuki H. Master Transcription Factors Regulate the DNA Methylation Landscape During Hepatocyte Differentiation. ***bioRxiv*** https://www.biorxiv.org/content/10.1101/2020.12.16.423165v1 (2020)

Nakano R, Kitanaka T, Namba S, Kitanaka N, Sato M, Shibukawa Y, Masuhiro Y, Kano K, Matsumoto T, Sugiya H. All-trans retinoic acid induces reprogramming of canine dedifferentiated cells into neuron-like cells. ***Plos One*** 15, e0229892 (2020)

Pervolarakis N, Nguyen QH, Williams J, Gong Y, Gutierrez G, Sun P, Jhutty D, Zheng GXY, Nemec CM, Dai X, Watanabe K, Kessenbrock K. Integrated Single-Cell Transcriptomics and Chromatin Accessibility Analysis Reveals Regulators of Mammary Epithelial Cell Identity. ***Cell Rep*** 33, 108273 (2020)

DNA methylation is a fundamental epigenetic modification to regulate mammalian gene expression, where each type of cell creates a specific methylation profile during its differentiation. Hepatocyte differentiation is well characterized at the transcriptome level, although epigenetic controls during the differentiation process have yet to be investigated. We analyzed omics data, including DNA methylation profiles, during hepatocyte differentiation. We found GATA6 as a key transcription factor (TF) for its binding motif-dependent chromatin activation and DNA demethylation in definitive endoderm differentiation, an initial step in hepatocyte lineage commitment.

We developed a bioinformatics pipeline to identify TFs involved in DNA demethylation. We found that specific TF families are involved in DNA demethylation in a cell lineage-specific manner. We validated more than 30 TFs possessing DNA demethylation induction activity, suggesting that many TFs are involved in regulation of cell type-specific DNA methylation profiles. Furthermore, we succeeded in obtaining insight into TF-mediated binding site-specific DNA demethylation, where more than two TF families, sharing similar but distinct binding motifs, are expressed in different cell lineages and/or different differentiation stages, creating distinct DNA methylation profiles of terminally differentiated cells.

We have begun to explore the role and outcomes of disordered DNA demethylation. We generated two iPS cell lines with either a RUNX1 knock-out or a RUNX1 mutation without DNA demethylation ability to understand the role of RUNX1-mediated DNA demethylation in hematopoiesis. Further, we found that deficiency of Ten-Eleven Translocation-2 (TET2), known as a DNA demethylation factor, results in aberrant megakaryocyte-erythroid progenitor (MEP) cells. We are exploring the link between the MEP abnormality and pathogenesis of myeloproliferative neoplasms.

In studies of the epithelial-to-mesenchymal transition (EMT) process, we identified previously unrecognized cell states within a subpopulation of mammary epithelial cells using single-cell ATAC-seq and RNA-seq. Further, we successfully identified candidate epigenome regulators during EMT using a large-scale CRISPR-Cas9 screen. In drug-induced cell reprogramming studies, we found that transplantation of reprogrammed neuron-like cells improved brain functions, such as locomotor function and memory function, in a chronic ischemic stroke mouse model.

Laboratory for Genome Information Analysis

Team Leader: Chung-Chau Hon

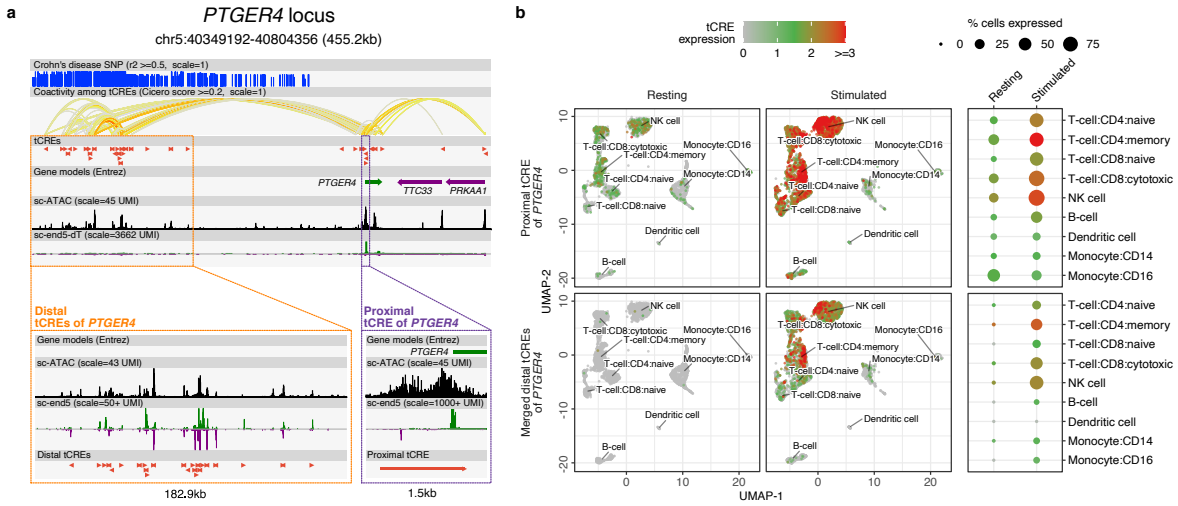


Figure: Detection of tCRE activity in single cells at a Crohn's disease risk locus

a) Genetic association signal, sc-5end signal and chromatin accessibility at the Prostaglandin E2 receptor 4 (*PTERG4*) locus. The *PTERG4* locus is located in proximity to the linkage disequilibrium (LD) blocks associated with Crohn's disease. We found a cluster of distal tCREs within these LD blocks that overlap with multiple disease-associated variants and are linked by co-activity (yellow arcs) to the proximal *PTERG4* tCRE.

b) Expression of proximal and distal *PTERG4* tCREs in single cells of resting and stimulated peripheral blood mononuclear cells. The expression of both proximal and distal *PTERG4* tCREs is enriched in NK cells and T-cells and is activated upon stimulation, in keeping with the known pivotal roles of T-cells in autoimmune disorders.

Recent Major Publications

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. ***Nature Communications*** 12, 925 (2021)

Hon CC and Carninci P. Expanded ENCODE delivers invaluable genomic encyclopedia. ***Nature*** 583, 685-686 (2020)

Van der Wijst MGP, De Vries DH, Groot HE, Trynka G, Hon CC, Bonder MJ, Stegle O, Nawijn MC, Idaghdour Y, Van der Harst P, Ye CJ, Powell J, Theis FJ, Mahfouz A, Heinig M, Franke L. Single-cell eQTLGen Consortium: a personalized understanding of disease. ***eLife*** 9;9:e52155 (2020)

Invited presentations

Hon CC. "Building a better map to navigate through the genetic landscape of diseases" 84th Annual Scientific Meeting of the Japanese Circulation Society (Online) August 2020

Our mission is to understand the molecular basis of genetic predisposition to human disease by functional annotation of disease-associated variants and interrogation of their functions by functional genomics. Expression of genes specifying cell-types and states is primarily controlled by the activities of their cognate cis-regulatory elements (CREs), mostly promoters and enhancers, which are highly enriched in trait-associated variants. Therefore, understanding the cell-type/state-specific activities of CREs and their target gene expression not only helps to decipher the principles of gene regulation, but also the cell-type/state-specific contexts of genetic predisposition. Profiling of CREs in single cells allows us to gain a high resolution understanding of these principles. We demonstrated that single-cell RNA-5'end-sequencing (sc-5end-seq) methods can detect transcribed CREs (tCREs), enabling the quantification of promoter and enhancer activities in single cells. We found that sc-5end-seq with either random or oligo(dT) priming could detect enhancer RNAs at a surprisingly similar sensitivity. To identify genuine tCREs, we implemented a workflow that effectively eliminates false positives from sc-5end-seq data. Comparing tCRE to accessible CRE (aCRE, based on chromatin accessibility) in stimulated immune cells, we found that while both tCREs and aCREs recovered the cell-type specific CREs and stimulation-induced transcription factor activities, tCREs are more accurate in linking enhancers to promoters, more sensitive in detection of alternative promoters and more enriched in disease heritability. Our data highlights an extra dimension of sc-5end data for studying gene regulation.

Laboratory for

Applied Computational Genomics

Team Leader: Michiel de Hoon

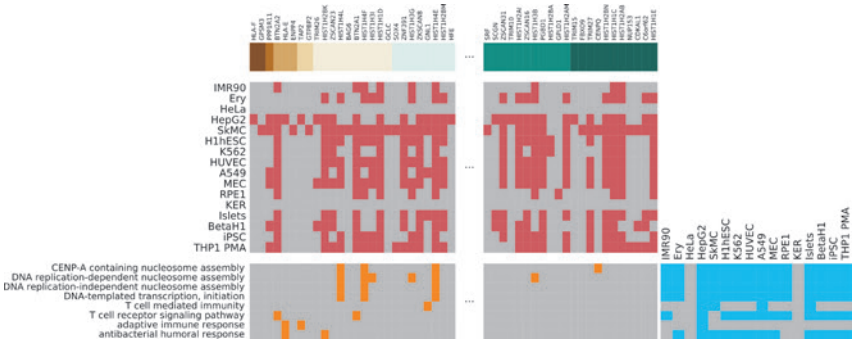


Figure:
Example of the functional annotation generated for non-coding RNA ENSG00000272462, including associated protein-coding genes, their expression correlation with the non-coding RNA and gene ontology categories assigned to this non-coding RNA in each cell type.

Recent Major Publications

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)

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

Agrawal S. "The biological role of long noncoding RNAs in human cells inferred from chromatin conformation data" The ENCODE Research Applications and Users Meeting (Barcelona, Spain) October 2020

De Hoon M. "FANTOM: Functional annotation of the mammalian genome" Genomics Winter School, Future Biotech Winter Retreat 2020 (Novosibirsk, Russia) February 2020

Though genes are commonly thought of as stretches of DNA that contain instructions for building a protein, less than 2% of the human genome consists of protein-coding regions, while the remaining 98% is non-coding. Nevertheless, 76% of the human genome is transcribed, typically in a highly cell type-dependent fashion, generating a wide variety of non-coding RNAs. As most genetic variants associated with disease are located in such non-coding regions, understanding the biological role of non-coding RNAs is of fundamental importance. However, 95% of non-coding genes currently do not have a functional annotation.

Non-coding RNAs localized to the cell nucleus are particularly interesting as they may be involved in transcriptional control of nearby genes. We analyzed sequencing data from Hi-C, a chromosome conformation capture protocol that detects pairs of DNA segments in close physical proximity, to understand the 3D structure of the human genome in the nucleus across 18 cell types. This allowed us to assign cell type-specific functional categories to most non-coding RNAs based on their physical association with functionally annotated protein-coding genes. We found that non-coding RNAs typically have specialized functions in specific cells initially, but during evolution expand their functionality to more general biological roles in multiple cell types. All functional annotations of non-coding RNAs generated by our analysis are provided to the community through ZENBU-Reports, a scientific visualization system for genome-wide data.

Next, we focus our attention to understanding the mechanism of regulation in the cell nucleus. In a collaborative effort with other RIKEN teams, we are currently developing experimental methods to visualize the 3D structure of chromatin in the nucleus using electron microscopy and to identify specific regulatory DNA sites and RNA molecules in these images.

Laboratory for

Large-Scale Biomedical Data Technology

Team Leader: Takeya Kasukawa

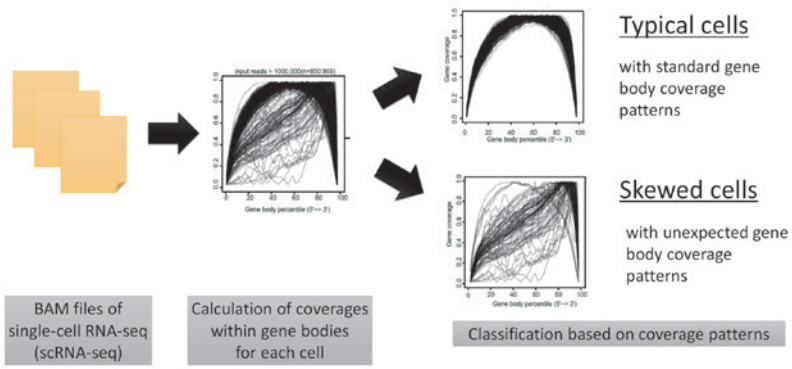


Figure: SkewC method to identify skewed cells in single-cell RNA-seq data
The method can identify skewed cells, which affect downstream analyses, including clustering and differentially expressed genes in single-cell RNA-seq datasets.

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 are working on several 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" (http://single-cell.clst.riken.jp/). Our new QC method (SkewC) can identify "skewed cells," that can 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 transcription start sites (refTSS: http://reftss.clst.riken.jp/), which can be used as a reference point to integrate many types of transcriptome and epigenome data to enable the study of transcriptional regulation. Our team is also working on data coordination in the FANTOM6 project, in which we aim to identify functions of lncRNAs (http://fantom.gsc.riken.jp/6/). We are also working on studies targeting several diseases: a transcriptome analysis of human blood samples from aged patients with frailty phenotype; and a 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 are working to provide and support the information infrastructure for several IMS laboratories. We are also investigating infrastructure and regulatory requirements for handling human-derived sequence data in on-premises and cloud environments in a collaboration with other IMS laboratories.

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 (2020)

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, 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. "Data coordination for large-scale transcriptome data production in FANTOM and single-cell projects" RIKEN Hackathon FY2019 Open Symposium (Kobe, Japan) February 2020

Laboratory for

Advanced Genomics Circuit

Team Leader: **Jay W. Shin**

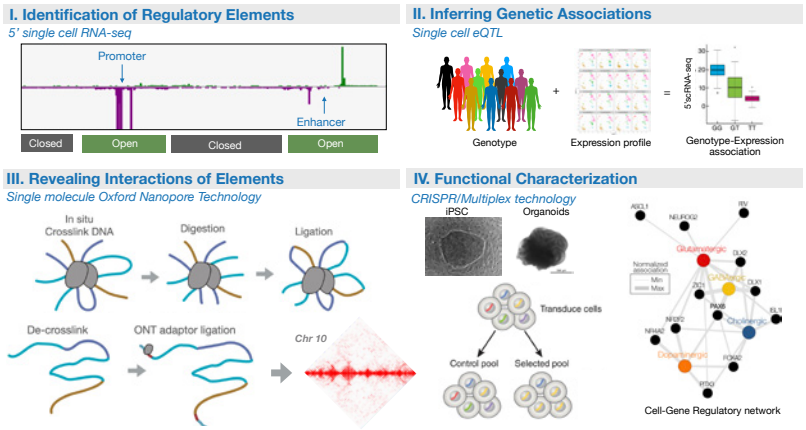


Figure: Unraveling the gene regulatory elements of the human genome

I) First we identify regulatory elements using single cell 5'RNA-seq, which reveals promoter and enhancer RNAs. II) We use a multiplex strategy to profile human PMBCs from a large cohort and associate their genotype with gene expression, III) We explore chromatin interactions of regulatory elements by using a third generation DNA sequencer and IV) functionally characterize these interactions with gene perturbation strategies including CRISPR.

Recent Major Publications

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, HCA STWG, Regev A, Heyn. Building a high-quality Human Cell Atlas. ***Nat Biotechnol*** 39, 149-153 (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, 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 Non-Coding RNAs via Molecular Phenotyping. ***Genome Res*** 30, 1060-1072 (2020)

Invited presentations

Shin JW. "Building the Human Gene Regulatory Atlas" Cold Spring Harbor Asia (Suzhou, China/Online) October 2020

Shin JW. "Human Cell Atlas Asia" The 4th Human Cell Atlas Asia Annual Meeting (Zhejiang, China/Online) October 2020

Shin JW. "The Human Cell Atlas" Single Cell Genomics in Biomedical Science Symposium (Melbourne, Australia/Online) September 2020

Shin JW. "Building the Human Gene Regulatory Atlas" 10X User Group Meeting in Asia Pacific (Online) May 2020

Shin JW. "Building Communities Around Single Cell Genomics" The 1st Human Single Cell Atlas Meeting Seoul National University (Seoul, South Korea) January 2020

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 to correctly identify the causality of genetic disorders, to rectify cellular malfunctions, reengineer cells, and possibly 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.

Genetic Diagnosis Technology Unit Nucleic Acid Diagnosis System Developing Unit

Unit Leader: Kengo Usui

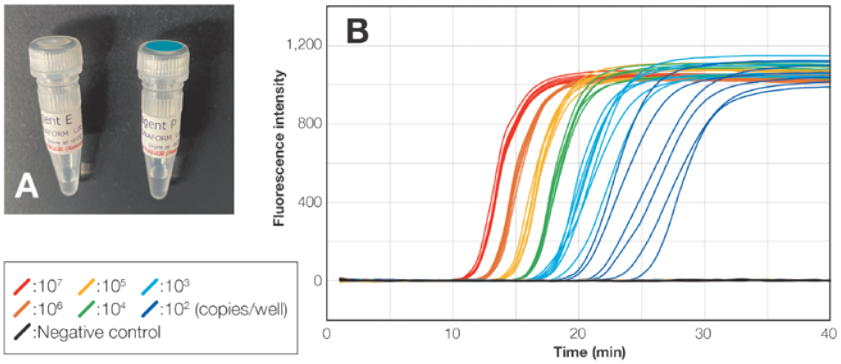


Figure: SmartAmp SARS-CoV-2 detection kit
A, Reagents of the detection kit. The kit consists of only two reagents, Reagent E (left tube, includes enzyme, dNTP and reaction buffer) and Reagent P (right tube, includes primer mix with fluorescence oligonucleotide, Eprimer™).
B, Amplification results using the detection kit. The SARS-CoV-2 virus in the isolated sample is rapidly identified, within 30 min, by increasing fluorescence intensity.

We developed an original nucleic acid amplification technology, "SmartAmp", which is an isothermal rapid DNA amplification technology. In 2020, the worldwide pandemic caused by the novel coronavirus, SARS-CoV-2 resulted in a dramatic change in our lifestyle. To contribute to resolving this once-in-a-century epidemic situation, we concentrated almost all of our efforts in this year to develop a SARS-CoV-2 rapid detection kit* using the SmartAmp method.

On 17 January 2020, the first novel SARS-CoV-2 genome sequence (strain Wuhan-Hu-1, NC_045512.2) was deposited in NCBI/GenBank, and we immediately began to construct a SARS-CoV-2-specific SmartAmp primer set by selecting the Orf1b-Nsp15 region as a target of the primers. We evaluated the primer set with authentic genomic RNA isolated from a passenger on the Diamond Princess cruise ship (provided by the Kanagawa Prefectural Institute of Public Health). Ultimately, our newly-developed SmartAmp SARS-CoV-2 detection kit was approved as a IVD (*in vitro* diagnosis) kit on 17 August 2020. The detection kit is shipped from a collaborating company "K.K. DNAFORM". The kit was used not only at hospitals in Kanagawa, but also by several Japanese sport federations and associations (All Japan Judo Federation, Japan Professional Bowling Association, and Japan Football Association, etc.) for SARS-CoV-2 testing.

The SARS-CoV-2 detection kit can be used with our pretreatment method, "SmartExtract", which is able to isolate viral RNA from nasopharyngeal swabs or saliva. Currently, a collaborating company has provided several devices based on our systems that allow for the initial RNA isolation all the way to identification of the virus. Thus, the convenience of the testing kit has been improved considerably. Additionally, we have developed a prototype of a "Direct-SmartAmp method", which does not require the RNA-isolation step, i.e., the sample can be directly mixed in the testing reagent.

*The development was performed in collaboration with the RIKEN Preventive Medicine & Diagnosis Innovation Program (PMI), Kanagawa Prefecture and K.K. DNAFORM.

Recent Major Publications

Soma T and Usui K. Nucleic acid amplification test for SARS-CoV-2 in Japan. ***Clin Immunol & Allergol*** 75, 101-108 (2021)

Hanami T, Tanabe T, Hanashi T, Yamaguchi M, Nakata H, Mitani Y, Kimura Y, Soma T, Usui K, Isobe M, Ogawa T, Itoh M, Hayashizaki Y and Kondo S. Scanning single-molecule counting system for Eprobe with highly simple and effective approach. ***Plos One*** 15, e0243319 (2020)

Tokumoto S, Miyata Y, Usui K, Deviatiiarov R, Ohkawa T, Kondratieva S, Shagimardanova E, Gusev O, Cornette R, Itoh M, Hayashizaki Y and Kikawada T. Development of a Tet-On Inducible Expression System for the Anhydrobiotic Cell Line, Pv11. ***Insects*** 11, 781 (2020)

Laboratory for
Cellular Epigenomics

Team Leader: **Aki Minoda**

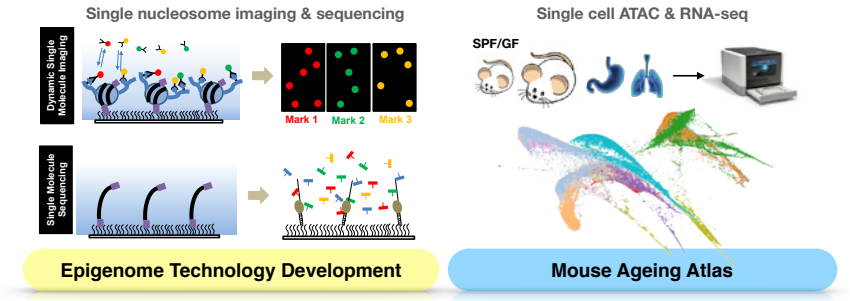


Figure: Summary of research activities
Novel epigenome technology development and application of single-cell genomics in various models.

Recent Major Publications

Hashimoto K, Kouno T, Ikawa T, Hayatsu N, Miyajima Y, Yabukami H, Terooatea T, Sasaki T, Suzuki T, Valentine M, Pascarella G, Okazaki Y, Suzuki H, Shin JW, Minoda A, Taniuchi I, Okano H, Arai Y, Hirose N, Carninci P. Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians. ***Proc Natl Acad Sci U S A*** 116, 24242-24251 (2019)

Watanabe K, Liu Y, Noguchi S, Murray M, Chang JC, Kishima M, Nishimura H, Hashimoto K, Minoda A, Suzuki H. OVOL2 induces mesenchymal-to-epithelial transition in fibroblasts and enhances cell-state reprogramming towards epithelial lineages. ***Sci Rep*** 9, 6490 (2019)

Invited presentations

Minoda A. "Chromatin state analysis at the single molecule resolution" The Molecular Biology Society of Japan (Japan/Online) December 2020

Minoda A. "Single cell analyses of ageing tissues with and without microbiota" 10x Genomics APAC User Group Meeting (Online) November 2020

Minoda A. "Two sides of the same 'COVID-19' coin" The impact of the COVID- 19 crisis on women in science: Challenges and solutions (Online) September 2020

Minoda A. "Providing the way to a successful single cell multi-omics analyses" Dolomite Bio-Illumina Webinar (Online) September 2020

Minoda A. "Mouse Ageing Atlas" The 1st International Symposium on Human InformatiX (Kyoto, Japan) February 2020

Our lab aims to determine epigenomic and transcriptomic changes in a comprehensive manner in various models by either developing our own technology or 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. Our current major focus is ageing, which is thought to underlie the pathogenesis of many diseases.

Enabling genomic mapping of multiple histone modifications at single-nucleosome resolution

To increase epigenomic resolution, we are developing a method to determine multiple targets (histone modifications) at the single-nucleosome level. We first carry out single-molecule imaging using fluorophore-labeled antibodies that are specific for different histone marks. Proteins are removed after imaging of the antibodies, so that the genomic DNA that was wrapped around the nucleosomes is left at the same position in the flowcell and can be sequenced by single molecule sequencing (Figure). By applying such technology to various models, we believe that we will be able to gain better epigenomic insights into various biological questions.

Construction of a Mouse Ageing Atlas with single-cell genomics

We are generating single-cell genomics (scATAC-seq and 5' scRNA-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 (metagenomics, 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.

Understanding the secrets of plant stem cells by applying single-cell genomics (RNA- and ATAC-seq) to plant tissues

We are carrying out scRNA-seq on meristem tissues, which are enriched with stem cells, from various plant models in an attempt to gain insights into the mechanism of plant stem cell maintenance, which is thought to be the foundation for plant longevity and regenerative ability.

Laboratory for Comprehensive Genomic Analysis

Team Leader: Yasushi Okazaki

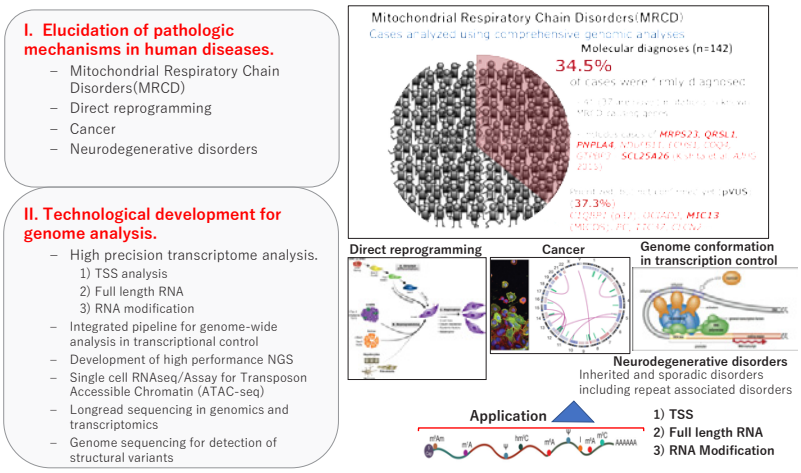


Figure: Future plans

We will continue to apply omics and functional analyses to mitochondrial and neurological diseases, direct reprogramming and other themes in molecular medicine and biology. In addition, we will continue technological development for genomic and transcriptomic analysis. 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 technological development of single-cell technologies, we will apply these technologies to various unsolved important questions in molecular medicine.

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, Hoon MD, 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)

Yatsuka Y, Kishita Y, Formosa L, Shimura M, Nozaki F, Fujii T, Nitta KR, Ohtake A, Murayama K, Ryan M, Okazaki Y. A homozygous variant in NDUFA8 is associated with developmental delay, microcephaly, and epilepsy due to mitochondrial complex I deficiency. ***Clin Genet*** 98, 155-165 (2020)

Ramilowski J, Yip CW, Agrawal S, Chang JC, Ciani Y, Kulakovskiy I, Mendez M, Ooi J, Ouyang J, Parkinson N, Petri A, Roos L, Severin J, Yasuzawa K, Abugessaisa I, Akalin A, Antonov I, Arner E, Bonetti A, Bono H, Borsari B, Brombacher F, Cannistraci C, Cardenas R, Cardon M, Chang H, Ducoli J, 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 E, Itoh M, Kaczkowski B, Kanhere A, Kawabata E, Kawaji H, Kawashima T, Kelly T, Kojima, *et al*. Functional Annotation of Human Long Non-Coding RNAs via Molecular Phenotyping. ***Genome Res*** 30, 1060-1072 (2020)

Invited presentations

Okazaki Y. "Overcoming The Challenges In WGS For UNdiagnosed Diseases"The NextGen Omics Series UK (London, UK) November 2020

Yasuoka Y. "Repeated subfunctionalization of Brachyury genes for notochord development" 22nd Annual Meeting of the Society of Evolutionary Studies (Japan/Online) September 2020

Yasuoka Y, Matsumoto M, Yagi K, Okazaki Y. "Evolutionary origin and ancestral roles of the reprogramming factor GLIS1" 91st Annual Meeting of Zoological Society of Japan (Japan/Online) September 2020

From FY2016 to FY2017, we provided research support for RIKEN researchers and researchers outside of RIKEN, such as cross-disciplinary projects within RIKEN and national projects, such as the Genome Network Analysis Support facility (GeNAS). Beginning in FY 2018, the Laboratory for Comprehensive Genomic Analysis (CGA) was formed. CGA not only took over some of the missions for research and development from GeNAS, but also began to develop its own distinct research activities. For this purpose, in FY2020, we continued to enhance our own research activities, as well as collaborative activities for other laboratories.

The CGA laboratory conducts omics analyses to elucidate the pathophysiology of human diseases, especially in the field of functional genomics. 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 are focusing our research activities on identifying and characterizing novel causative genes for human hereditary disorders and to discover new potential drug targets for realization of personalized medicine. As our primary targets, we are now working on genome and transcriptome analyses of mitochondrial and neurological diseases. Starting in FY2020, as a part of a collaborative project aiming to identify causative variants in "rare diseases undiagnosed by exome sequencing", we are analyzing such challenging clinical cases with our genome/transcriptome technologies. In addition to managing our own research projects, we continue to contribute to research projects led by other RIKEN (IMS and non-IMS) teams and outside laboratories in Japan, through the "IMS genome platform". For example, we provide our special genome and transcriptome analysis technologies, such as Cap Analysis of Gene Expression (CAGE) and bulk, as well as single-cell RNAseq and single-cell ATAC-seq utilizing various next generation sequencers. Some of these essential technologies have been developed by us and we will continuously strive to create and improve such technologies. In addition, we have introduced long-read sequencers from Oxford Nanopore Technologies, which can read up to hundreds of kilobases per DNA molecule. As well, we are also utilizing the PacBio Sequel 2 long-read sequencer. We not only utilize these sequencers/technologies, but also develop our own target enrichment technology using the long-read sequencers. We are working with several dozen collaborators utilizing these technologies. For example, we are now analyzing taste receptor and neural cells with single-cell technologies. Finally, we also are clarifying molecular mechanisms underlying direct reprogramming of fibroblasts into another distinctively differentiated cell type.

Laboratory for

Applied Regulatory Genomics Network Analysis

Team Leader: **Erik Arner**

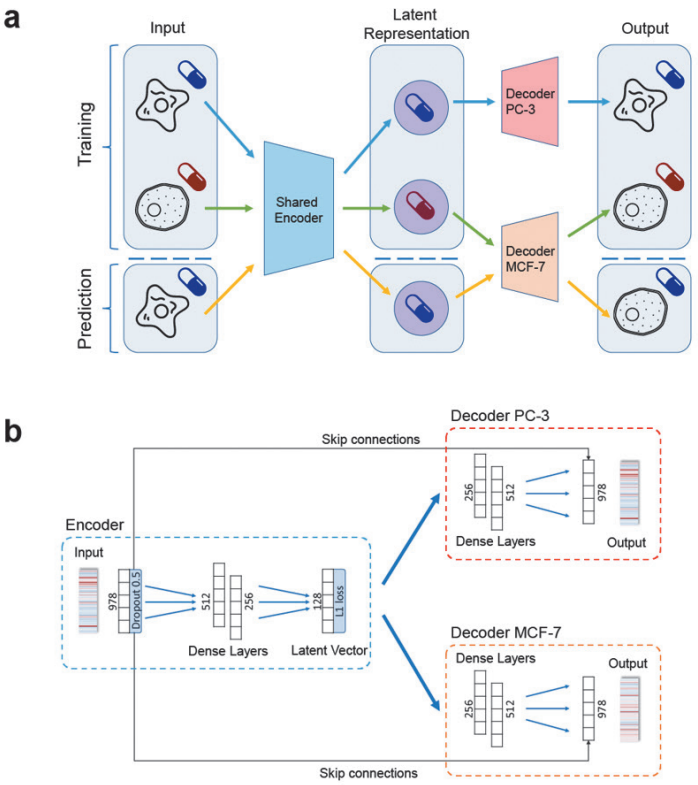


Figure: Recent publications from the lab
(a) Transcriptional profiles are encoded by a shared encoder that captures the drug response in a cell neutral way. The latent representations of the drug responses are decoded by cell specific decoders, which reconstruct the original input in a cell type-specific way. After the shared encoder and decoders are trained, the response to a drug in one cell type can be predicted by using the drug's response in another cell type. (b) Architecture of the encoder and the decoders used in DeepCellState.

Recent Major Publications

Umarov R, Arner E. DeepCellState: an autoencoder-based framework for prediction of cell type specific transcriptional states induced by drug treatment. ***bioRxiv*** (2021)
doi: https://doi.org/10.1101/2020.12.14.422792

Napolitano F, Rapakoulia T, Annunziata P, Hasegawa A, Cardon M, Napolitano S, Vaccaro L, Iuliano A, Wanderlingh LG, Kasukawa T, Medina DL, Cacchiarelli D, Gao X, Di Bernardo D, Arner E. Automatic identification of small molecules that promote cell conversion and reprogramming. ***bioRxiv*** (2020)
doi: https://doi.org/10.1101/2020.04.01.021089

Kwon AT, Mohri K, Takizawa S, Arakawa T, Takahashi M, Kaczkowski B, Furuno M, Suzuki H, Tagami S, Mukai H, Arner E. Efficient Development of Platform Cell Lines Using CRISPR-Cas9 and Transcriptomics Analysis. ***bioRxiv*** (2020)
doi: https://doi.org/10.1101/2020.09.16.299248

Invited presentations

Arner E. "Studies of dynamic enhancer usage – from system-wide studies to disease loci" International Institute of Molecular and Cell Biology in Warsaw Online Seminar, (Warsaw, Poland/Online) October 2020

Based on genome-wide technologies developed at the Center, with emphasis on recent technological advances such as single-cell transcriptome analysis, enhancer expression analysis and RNA-chromatin interaction profiling, we analyze gene regulation with a focus on clinical and medical applications. This includes exploration of the transcriptional effects of regulatory molecules at the cellular level and profiling of clinical samples in order to identify regulatory networks perturbed in disease states. In a recently completed project, we developed a data mining method for identifying small molecules that, alone or in combination, can facilitate cell conversion. In another study, we developed DeepCellState, a deep learning framework for accurate prediction of the transcriptional response to drug treatment in one cell type based on the response in another cell type. Using CRISPR technology and transcriptome profiling, we also developed platform cell lines useful for development of novel antibody-drug conjugates. Ongoing projects include: single cell transcriptome analysis of cancer cells after treatment with pharmaceuticals that act at the epigenomic level; profiling of enhancer/promoter activity and RNA-chromatin interactions in primary acute myeloid leukemia cells; and developing deep learning methods for genome-wide prediction of regulatory elements in mammalian genomes.

Preventive Medicine and Applied Genomics Unit

Unit Leader: Hideya Kawaji

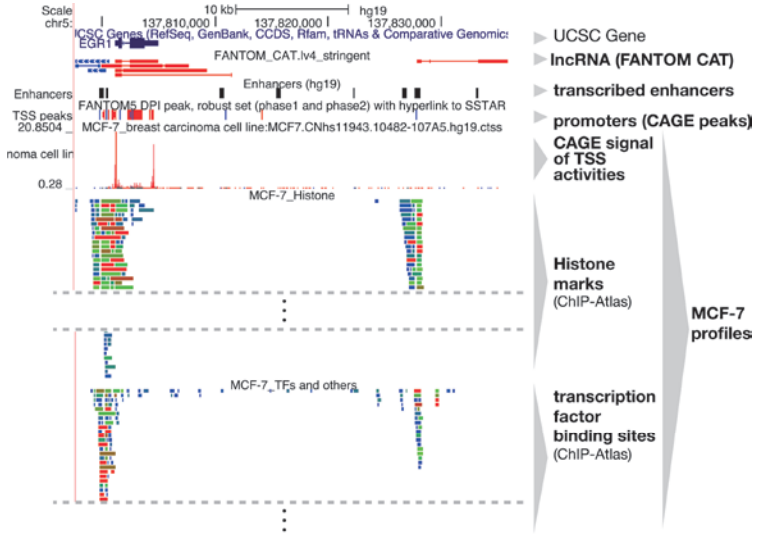


Figure: Integrated view of transcriptome and epigenetic marks provided by the FANTOM5 web resource

Genomic view of the *EGR-1* locus with the UCSC Genome browser database, which includes data provided by FANTOM5 and ChIP-Atlas (The figure is from Nucleic Acids Res. D752-758, 2019).

Recent Major Publications

Hirabayashi S, Bhagat S, Matsuki Y, Takegami Y, Uehata T, Kanemaru A, Itoh M, Shirakawa K, Takaori-Kondo A, Takeuchi O, Carninci P, Katayama S, Hayashizaki Y, Kere J, Kawaji H, Murakawa Y. NET-CAGE characterizes the dynamics and topology of human transcribed cis-regulatory elements. ***Nat Genet*** 51, 1369-1379 (2019)

Ohashi F, Miyagawa S, Yasuda S, Miura T, Kuroda T, Itoh M, Kawaji H, Ito E, Yoshida S, Saito A, Sameshima T, Kawai J, Sawa Y, Sato Y. CXCL4/PF4 is a predictive biomarker of cardiac differentiation potential of human induced pluripotent stem cells. ***Sci Rep*** 9, 4638 (2019)

Lizio M, Abugessaisa I, Noguchi S, Kondo A, Hasegawa A, Hon CC, de Hoon M, Severin J, Oki S, Hayashizaki Y, Carninci P, Kasukawa T, Kawaji H. Update of the FANTOM web resource: expansion to provide additional transcriptome atlases. ***Nucleic Acids Res*** 47, D752-D758 (2019)

Invited presentations

Kawaji H, "Genomics resource of non human primates: recent updates and future perspective", the 43rd annual meeting of the molecular biology society of Japan (Online) December 2020

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, and 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 developments to assist in such translational as well as basic science research.

Our collaborative research with RIKEN PMI led to the identification of biomarkers for iPSC induction to cardiac muscle (Ohashi F *et al.* Scientific Reports, 2019), a diagnostic biomarker of myeloproliferative neoplasms (Morishita S *et al.* Cancer Sci., 2020), and a prognostic biomarker of cholangiocarcinoma (Takahashi T *et al.*, Eur J Surg Oncol., 2020). We also contributed to the field of oligonucleotide therapeutics in an assessment of off-target effects of gapmer antisense oligos (Yoshida *et al.* Genes to Cells, 2019). In collaborations within RIKEN IMS, we integrated epigenetic data into the FANTOM5 resource, the largest database of *cis*-regulatory regions based on transcriptome profiles, to assist translational researchers as well as basic scientists focusing on *cis*-regulatory regions (Lizio *et al.* Nucleic Acids Res. 2019; Figure) and contributed to its update (Abugessaisa I *et al.* Nucleic Acids Res. 2020). We further succeeded in discovering a substantial number of novel enhancers by development of a method called NET-CAGE, which can capture even RNAs that are subject to rapid degradation, such as enhancer RNAs (Hirabayashi *et al.* Nature Genet. 2019).

RIKEN-IFOM Joint Laboratory for Cancer Genomics

Team Leader: Yasuhiro Murakawa

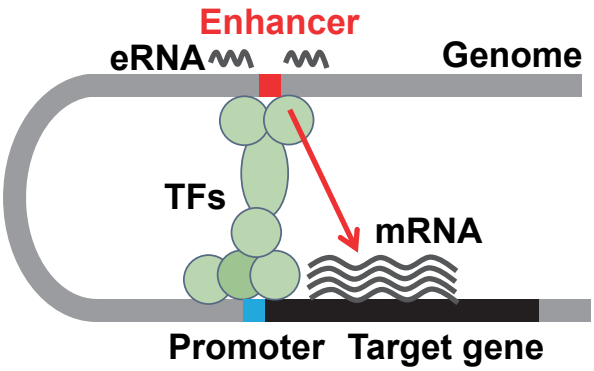


Figure: Enhancer-mediated gene regulation
Enhancers are small segments of distal *cis*-regulatory DNA elements that significantly enhance the expression of target genes and play key roles in the establishment of cell type-specific function and identity.

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.

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 paradigms that cannot be otherwise solved.

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

Recent Major Publications

Matsui H, Shirakawa K, Konishi Y, Hirabayashi S, Sarca AD, Fukuda H, Nomura R, Stanford E, Horisawa Y, Kazuma Y, Matsumoto T, Yamazaki H, Murakawa Y, Battivelli E, Verdin E, Koyanagi Y, Takaori-Kondo A. CAGE-seq reveals that HIV-1 latent infection does not trigger unique cellular responses in a Jurkat T cell model. ***J Virol*** 02394-20 (2021) online ahead

Ooki A, Onodera S, Saito A, Oguchi A, Murakawa Y, Sakamoto T, Sueishi K, Nishii Y, Azuma T. CAGE-seq analysis of osteoblast derived from cleidocranial dysplasia human induced pluripotent stem cells. ***Bone*** 141, 115582 (2020)

Takahashi T, Kawaji H, Murakawa Y, Hayashizaki Y, Murakami T, Yabushita Y, Homma Y, Kumamoto T, Matsuyama R. Significance of HMGA2 expression as independent poor prognostic marker in perihilar and distal cholangiocarcinoma resected with curative intent. Endo I. ***Eur J Surg Oncol*** 47, 394-400 (2020)

Invited presentations

Murakawa Y. "A new genomic and computational approach to study human genomic enhancers and its association with diseases" International Symposium of CCII (Kyoto, Japan) January 2021

Laboratory for Genotyping Development

Team Leader: Yukihide Momozawa

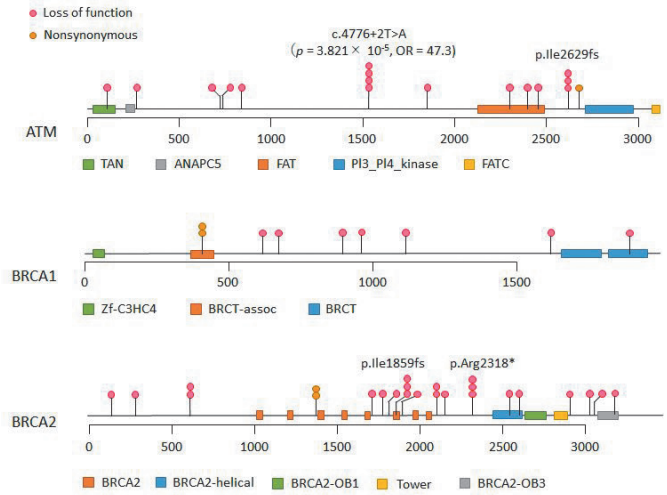


Figure: Location and number of pathogenic variants in ATM, BRCA1 and BRCA2 associated with pancreatic cancer

We found that pathogenic variants in ATM, BRCA1 and BRCA2 were significantly associated with pancreatic cancer in 1,005 cases and 23,705 controls. Locations of pathogenic variants found in patients and Pfam domains are shown with lollipop structures, with the variant type indicated by color. Pink and yellow circles indicate loss of function and nonsynonymous variants, respectively. The x-axis indicates the number of amino acid residues.

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 develop methods and databases useful for personalized medicine. Our laboratory has functioned as a research hub for large-scale genomic analyses, collaborating with domestic and international universities, research institutes, and companies.

Our laboratory published 20 papers in 2020. The main achievements this year are:

- Pancreatic cancer is devastating because of its high mortality rate, and 5-10% of pancreatic cancer is considered to be caused by only one pathogenic variant. Identifying a pathogenic variant would assist with the early detection of at-risk individuals and would be beneficial for selecting a drug treatment. We sequenced 27 cancer-predisposing genes in 1,005 pancreatic cancer patients and 23,705 controls to identify BRCA1, BRCA2, and ATM associated with pancreatic cancer. Among them, 3.4% of patients with pathogenic variants in BRCA1 and BRCA2 may respond to treatments with a PARP inhibitor, which was newly covered by health insurance in Japan in Dec 2020.
- The domestic dog represents an ideal model for identifying susceptibility genes, many of which are shared with humans. We performed a genome-wide association study in 40 clinically important measurements in 472 healthy dogs from eight breeds in five European countries. We identified three experimental wide significant associations. A single SNP was responsible for a larger proportion of the phenotype variance (6.8-78.4%) than humans. These findings illustrate the utility of canine GWAS to reveal the genetic contribution to individual differences in clinically important measurements.

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

Recent Major Publications

Fujita M, Liu X, Iwasaki Y, Terao C, Mizukami K, Kawakami E, Takata S, Inai C, Aoi T, Mizukoshi M, Maejima K, Hirata M, Murakami Y, Kamatani Y, Kubo M, Akagi K, Matsuda K, Nakagawa H, Momozawa Y. Population-based Screening for Hereditary Colorectal Cancer Variants in Japan. ***Clin Gastroenterol Hepatol*** doi: 10.1016/j.cgh.2020.12.007 (Epub 2020)

Mizukami K, Iwasaki Y, Kawakami E, Hirata M, Kamatani Y, Matsuda K, Endo M, Sugano K, Yoshida T, Murakami Y, Nakagawa H, Spurdle AB, Momozawa Y. Genetic characterization of pancreatic cancer patients and prediction of carrier status of germline pathogenic variants in cancer-predisposing genes. ***EBioMedicine*** 60, 103033 (2020)

Momozawa Y, Merveille AC, Battaille G, Wiberg M, Koch J, Willesen JL, Proschowsky HF, Gouni V, Chetboul V, Tiret L, Fredholm M, Seppälä EH, Lohi H, Georges M, Lequarré AS. Genome wide association study of 40 clinical measurements in eight dog breeds. ***Sci Rep*** 10, 6520 (2020)

Invited presentations

Momozawa Y. "Canine genome wide association study" National Institute of Genetics Meeting (Online) December 2020

Momozawa Y. "A large-scale genetic analysis of prostate cancer" The 6th Annual Meeting of the Japan Society of Urologic Oncology (Kyoto, Japan) October 2020

Momozawa Y. "The perspective of genome medicine in (breast) cancer based on Biobank Japan data" The 58th Annual Meeting of Japan Society of Clinical Oncology (Kyoto, Japan) October 2020

Momozawa Y. "Large-sale genomic analysis in hereditary (colorectal) cancer" The 79th Annual Meeting of Japanese Cancer Association (Hiroshima, Japan) October 2020

Momozawa Y. "Role of rare variants in the genetics of complex diseases in humans" National Institute of Genetics Meeting (Mishima, Japan) February 2020

Laboratory for

Statistical and Translational Genetics

Team Leader: **Chikashi Terao**

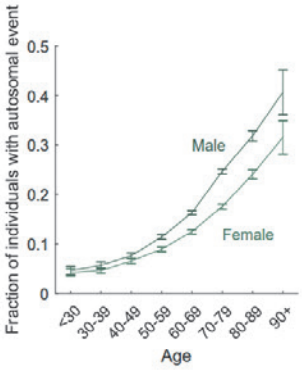


Figure: Mosaic chromosomal alterations (mCAs) and precancerous cell expansion are inevitable when humans become old

Recent Major Publications

Terao C*, Suzuki A, Momozawa Y, Akiyama M, Ishigaki K, Yamamoto K, Matsuda K, Murakami Y, McCarroll SA, Kubo M, Loh PR, Kamatani Y. Chromosomal alterations among age-related hematopoietic clones in Japan. ***Nature*** 584, 130-135 (2020)

Koyama S, Ito K, Terao C, Akiyama M, Horikoshi M, Momozawa Y, Matsunaga H, Ieki H, Ozaki K, Onouchi Y, Takahashi A, Nomura S, Morita H, Akazawa H, Kim C, Seo JS, Higasa K, Iwasaki M, Yamaji T, Sawada N, Tsugane S, Koyama T, Ikezaki H, Takashima N, Tanaka K, Arisawa K, Kuriki K, Naito M, Wakai K, Suna S, Sakata Y, Sato H, Hori M, Sakata Y, Matsuda K, Murakami Y, Aburatani H, Kubo M, Matsuda F, Kamatani Y, Komuro I. Population-specific and trans-ancestry genome-wide analyses identify distinct and shared genetic risk loci for coronary artery disease. ***Nat Genet*** 52, 1169-1177 (2020)

Amariuta T, Ishigaki K, 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

Terao C. "Genetic analyses and translational studies for rheumatoid diseases" Symposium, The 65th Annual Meeting of the Japan Society of Human Genetics (Japan/Online) November 2020

Terao C. "Genetic data informs pathophysiology, treatment targets and risk of disease onset in rheumatic diseases" The 7th JCR Basic Research Conference (Tokyo, Japan) October 2020

Terao C. "Genetics of cardiovascular diseases and clinical application of genetic findings" The 4th JCS Council Forum on Basic CardioVascular Research (Nagoya, Japan) September 2020

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) August 2020

Terao C. "Construction and application of database for rheumatic diseases under the viewpoint of physician scientist" The 10th Rheumatology Education Inter League REIL (Osaka, Japan) January 2020

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.

Dozens of genome-wide association studies in our laboratory have identified thousands of susceptibility loci for multiple complex traits. Most of these accomplishments were viewed in the field as the largest ever human genetic analyses for non-Europeans (available at http://jenger.riken.jp/en/). Our analyses have clarified the similarity and differences between populations with regard to genetics of the traits. We performed downstream analyses to understand the biological background of traits and found that trait-relevant cell types can be linked by applying integrative analysis of genetic and epigenetic data. In 2020, we successfully identified somatic chromosomal alterations in autosomes (mosaic chromosomal alterations, mCAs) from DNA microarray data. We identified a surprisingly high number of mCAs (more than 33,000) in 180,000 participants. We observed that more subjects carried mCAs according to their age and more than 40% of subjects in their 90s were positive for mCAs, strongly indicating that mCAs are inevitable when we become aged (Figure). We also found that mCAs in the T-cell receptor (TCR) were more frequently observed, but ones in the B-cell receptor (BCR), were less frequently observed in Japanese than UK populations. Since chronic lymphocytic leukemia (CLL), a type of of B-cell leukemia, is common in Europeans but rare in Japanese and T-cell leukemia is common in Japanese but rare in Europeans, the differences in genes harbored in mCAs could foreshadow epidemiological differences in hematopoietic malignancies between Japan and Europe. Furthermore, three different mutational precursors of CLL (including trisomy 12, loss of chromosomes 13q, and 13q copy-neutral loss of heterozygosity) were between two and six times less common among Japanese individuals. This suggests that the Japanese and European populations differ in selective pressures on clones long before the development of clinically apparent CLL. We identified six previously undescribed loci at which inherited variants predispose to mosaic chromosomal alterations that duplicate or remove the inherited risk alleles, including large-effect rare variants at *NBN, MRE11* and *CTU2* (odds ratio, 28–91).

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, and to enable the future Genomic Medicine for East Asians. The second is to employ deep learning techniques to predict the biological consequences of trait-relevant variants. Genetic evidence, in conjunction with epigenome, transcriptome, and other cellular multi-omics data, may lead to the discovery of novel biological principles. This project will proceed more efficiently as a result of collaborations with other IMS laboratories.

Laboratory for Pharmacogenomics

Team Leader: Taisei Mushiroda

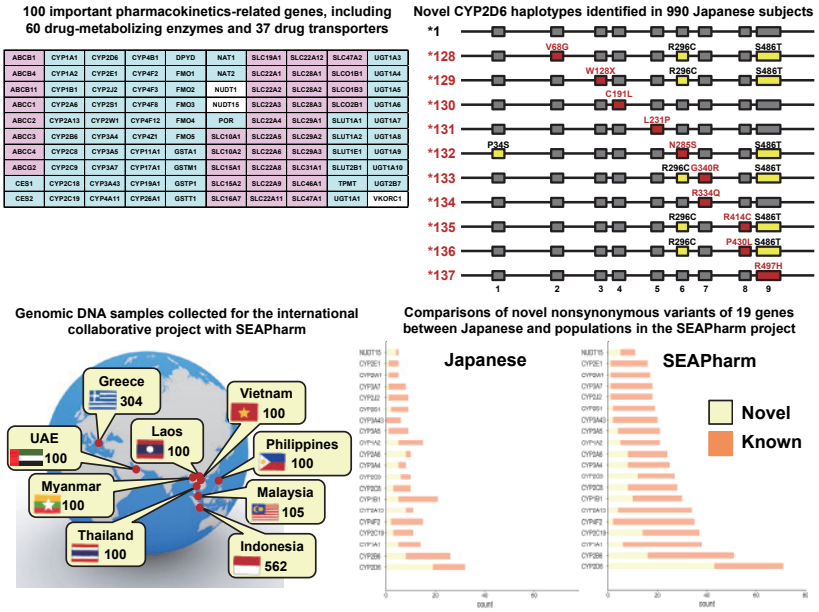


Figure: Targeted next generation sequencing panel, PKseq, for resequencing of 100 genes related to pharmacokinetics

Pharmacokinetic (PK) variabilities in intestinal absorption, hepatic drug metabolism, biliary and renal excretions 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. It targets the coding regions of 37 drug transporters, 30 cytochrome P450 isoforms, 10 UDP-glucuronosyltransferases, 5 flavin-containing monooxygenases, 4 glutathione S-transferases, 4 sulfotransferases, and 10 other genes.

Recent Major Publications

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)

Nakamura R, Ozeki T, Hirayama N, Sekine A, Yamashita T, Mashimo Y, Mizukawa Y, Shiohara T, Watanabe H, Sueki H, Ogawa K, Asada H, Kaniwa N, Tsukagoshi E, Matsunaga K, Niihara H, Yamaguchi Y, Aihara M, Mushiroda T, Saito Y, Morita E. Association of HLA-A*11:01 with Sulfonamide-Related Severe Cutaneous Adverse Reactions in Japanese Patients. ***J Invest Dermatol*** 140, 1659-1662 (2020)

Tamura K, Imamura CK, Takano T, Saji S, Yamanaka T, Yonemori K, Takahashi M, Tsurutani J, Nishimura R, Sato K, Kitani A, Ueno NT, Mushiroda T, Kubo M, Fujiwara Y, Tanigawara Y. CYP2D6 Genotype-Guided Tamoxifen Dosing in Hormone Receptor-Positive Metastatic Breast Cancer (TARGET-1): A Randomized, Open-Label, Phase II Study. ***J Clin Oncol*** 38, 558-566 (2020)

Invited presentations

Mushiroda T. "A recent update of pharmacogenomics" The 41st Annual Scientific Meeting of the Japanese Society of Clinical Pharmacology and Therapeutics (Fukuoka, Japan) December 2020

Hikino K. "PGx informed precision medicine in pediatrics" The 41st Annual Scientific Meeting of the Japanese Society of Clinical Pharmacology and Therapeutics (Fukuoka, Japan) December 2020

Mushiroda T. "Targeted NGS panel for developing infrastructure to support pharmacogenomics research" Bio Asia Pacific 2020 (Bangkok, Thailand) October 2020

Fukunaga K. "Bioinformatics analysis tools using in SEAPharm research 100 pharmacogenes PKSeq panel" Bio Asia Pacific 2020 (Bangkok, Thailand) October 2020

Hikino K. "HLA-B*51:01 and CYP2C9*3 are risk factors for phenytoin-induced eruption in the Japanese population: analysis of data from the Biobank Japan Project" The 5th International Stevens-Johnson Syndrome Symposium (Kyoto, Japan) February 2020

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, UGT1A1, NUDT15 and BRCA1/2 tests, to predict drug responses prior to drug administration. We conduct genomic analyses for the identification of PGx biomarkers useful for predicting drug responses.

A newly-developed 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 determination of haplotypes of CYP2D6, a very important drug-metabolizing enzyme 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. In addition, we clarified genetic diversity in the 100 genes by sequencing 1,571 genomic DNA samples of individuals from nine countries in Southeast Asia, Sothern Asia, Middle East, and Southern Europe, in collaboration with the South East Asian Pharmacogenomics Research Network (SEAPharm). These results indicate that PKseq will be useful not only for the identification of all the variants of PK-related genes associated with drug responses, but also for clinical sequencing to achieve genotype-guided drug therapies.

Laboratory for
Bone and Joint Diseases

Team Leader: **Shiro Ikegawa**

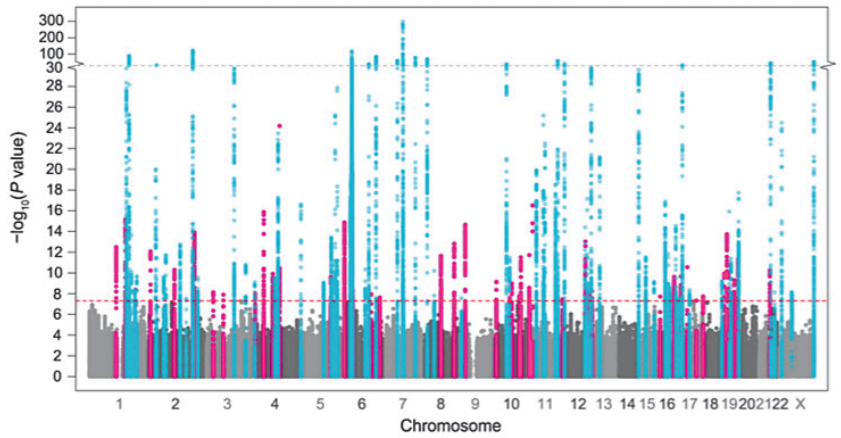


Figure:
GWAS meta-analysis results from SLE East Asians including 13,377 cases and 194,993 controls. Known and novel loci are represented in light blue and pink, respectively. The red dashed line: the genome-wide association significance threshold of $p=5\times10^{-8}$. The grey dashed line: $p=10^{-30}$.

Recent Major Publications

Yin X, Kim K, Suetsugu H, Bang SY, Wen L, Koido M, Ha E, Liu L, Sakamoto Y, Jo S, Leng RX, Otomo N, Laurynenka V, Kwon YC, Sheng Y, Sugano N, Hwang MY, Li W, Mukai M, Yoon K, Cai M, Ishigaki K, Chung WT, Huang H, Takahashi D, Lee SS, Wang M, Karino K, Shim SC, Zheng X, Miyamura T, Kang YM, Ye D, Nakamura J, . . . , 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*** doi: 10.1136/annrheumdis-2020-219209 (Epub 2020)

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, . . . , Yamamoto K, Murakami Y, Nakamura Y, Raychaudhuri S, Inazawa J, Yamauchi T, Kadowaki T, Kubo M, Kamatani Y. Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases. ***Nat Genet*** 52, 669-679 (2020)

Matsuda M, Yamanaka Y, Uemura M, Osawa M, Megumu K, Saito MK, Nagahashi A, Nishio M, Guo L, Ikegawa S, Sakurai S, Kihara S, Maurissen TL, Nakamura M, Matsumoto T, Yoshitomi H, Ikeya M, Kawakami N, Yamamoto T, Woltjen K, Ebisuya M, Toguchida J, Alev C. Recapitulating the human segmentation clock with pluripotent stem cells. ***Nature*** 580, 124-129 (2020)

Invited presentations

Ikegawa S. "Identification of Novel Disease Genes and Re-classification of Dysosteosclerosis" The 65th Annual Meeting of the Japan Society of Human Genetics (English Symposium) (Nagoya, Japan/Online) November 2020

Ikegawa S. "Genomic Study of Rare Diseases of Skeleton" India -Japan Webinar on "Rare Genetic Disorders" by Embassy of India (Tokyo, Japan/Online) October 2020

Ikegawa S. "Genomic Study of Posterior Longitudinal Ligament of the Spine– Past, present and future" The 35th Annual Meeting of the Japan Orthopedic Society Basic Research Meeting) (Tokyo, Japan/Online) October 2020

Ikegawa S. "Genomic Medicine in Orthopedics– Past, present and future)" The 135th Annual Meeting of the Middle Japan Orthopedic Society (Shimane, Japan/Online) October 2020

Ikegawa S. "Differential Diagnosis of Skeletal Dysplasias and Genetic Skeletal Disorders" Virtual APAC MPS Summit 2020 (Taipei/Tokyo/Sydney/Seattle/Online) September 2020

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 (AIS), OPLL, and ANF are in progress, and we have succeeded in the identification of several 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 engaging 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).

Through the analyses of phenotypes and diseases genes, we consider the molecular mechanisms of bone and joint formation and the pathogenesis of common bone and joint diseases, as well as 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.

Laboratory for

Genomics of Diabetes and Metabolism

Team Leader: **Momoko Horikoshi**

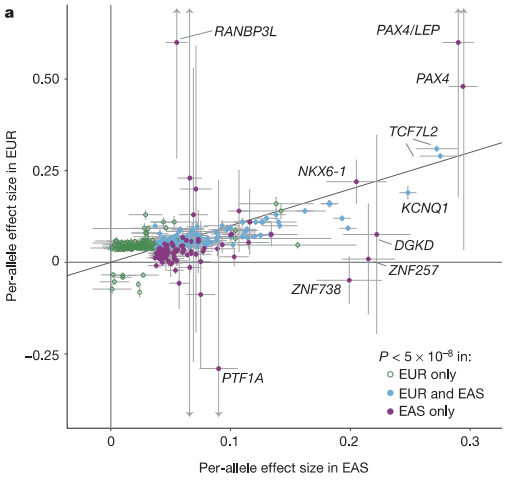


Figure: Effect size comparison of lead variants identified in the East Asin T2D GWAS BMI-unadjusted meta-analysis and previous European T2D GWAS meta-analysis

For 332 unique lead variants identified from the BMI-unadjusted meta-analyses, per-allele effect sizes from the European meta-analysis (y axis) were plotted against per-allele effect sizes from our East Asian meta-analysis (x axis). Maximal effective sample sizes were N_{eff} = 211,793 for East Asians and N_{eff} = 231,436 for Europeans.

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 investigating 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 (publication 3). 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 (publication 1). We identified 301 distinct association signals at 183 loci. Previously undescribed associations included signals in or near genes and a microRNA cluster that affect 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. Association studies in diverse populations demonstrated the benefit of identifying additional loci and elucidating disease-associated genes, biology and pathways.

Recent Major Publications

Spracklen C.N.*, Horikoshi M*, Kim Y.J.*, 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)

Warrington NM*, Beaumont RN*, Horikoshi M*, Day FR*, Helgeland Ø*, Laurin C, Bacelis J, Peng S, Hao K, Feenstra B, Wood AR, Mahajan A, Tyrrell J, Robertson NR, Rayner NW, Qiao Z, Moen GH, Vaudel M, Marsit CJ, Chen J, Nodzenski M, Schnurr TM, Zafarmand MH, Bradfield JP, Grarup N, Kooijman MN, Li-Gao R, Geller F, Ahluwalia TS, Paternoster L, Rueedi R, Huikari V, Hottenga JJ, Lyytikäinen LP, Cavadino A, Metrustry S, Cousminer DL, Wu Y, Thiering E, Wang CA, Have CT, Vilor-Tejedor N, Joshi PK, Painter JN, Ntalla I, Myhre R, Pitkänen N, van Leeuwen EM, Joro R, Lagou V *et al*. Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. ***Nat Genet*** 51, 804-814 (2019)

Suzuki K, Akiyama M, Ishigaki K, Kanai M, Hosoe J, Shojima N, Hozawa A, Kadota A, Kuriki K, Naito M, Tanno K, Ishigaki Y, Hirata M, Matsuda K, Iwata N, Ikeda M, Sawada N, Yamaji T, Iwasaki M, Ikegawa S, Maeda S, Murakami Y, Wakai K, Tsugane S, Sasaki M, Yamamoto M, Okada Y, Kubo M, Kamatani Y#, Horikoshi M#, Yamauchi T#, Kadowaki T#. Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. ***Nat Genet*** 51, 379-386 (2019)

*These authors jointly contributed to the work.
#Corresponding authors

Laboratory for

Cardiovascular Genomics and Informatics

Team Leader: **Kaoru Ito**

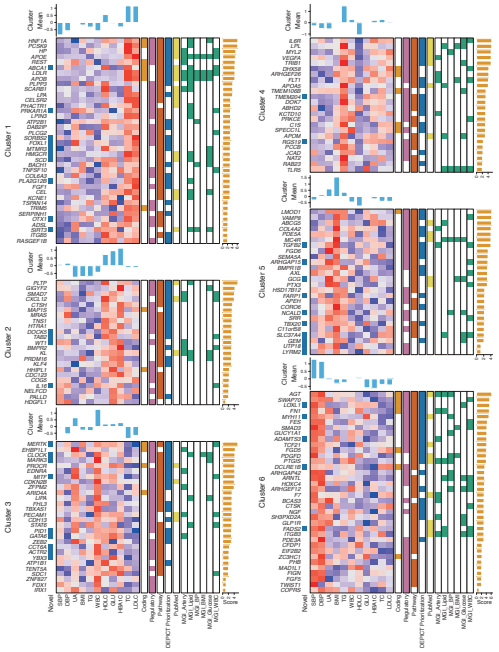


Figure: Functional clustering and causal gene prioritization of 175 genome-wide significant loci for coronary artery disease

We performed trans-ancestry genome-wide analysis for coronary artery disease (CAD). One hundred seventy-five genome-wide significant loci were identified and clustered into six clusters by k-means clustering of Z-scores. Heatmaps show the normalized Z-score of each lead variant for CAD-polygenic risk score associated phenotypes. Red color indicates positive-, and blue color indicates negative- normalized Z-value. Z-values are aligned to CAD risk-increasing alleles. The bar charts on the right of the heatmaps indicate the cluster-mean effect on the phenotypes. Each locus was annotated with the prioritized genes based on the functional evidence that is shown on the upper side of each heatmap. The lowermost bar-charts indicate the total scores for annotated genes. MGI, Mouse Genome Informatics; BP, blood pressure; BMI, body mass index; WBC, white blood cell.

Recent Major Publications

Koyama S, Ito K, Terao C, Akiyama M, Horikoshi M, Momozawa Y, Matsunaga H, Ieki H, Ozaki K, Onouchi Y, Takahashi A, Nomura S, Morita H, Akazawa H, Kim C, Seo JS, Higasa K, Iwasaki M, Yamaji T, Sawada N, Tsugane S, Koyama T, Ikezaki H, Takashima N, Tanaka K, Arisawa K, Kuriki K, Naito M, Wakai K, Suna S, Sakata Y, Sato H, Hori M, Sakata Y, Matsuda K, Murakami Y, Aburatani H, Kubo M, Matsuda F, Kamatani Y, Komuro I. Population-specific and trans-ancestry genome-wide analyses identify distinct and shared genetic risk loci for coronary artery disease. ***Nat Genet*** 10, 5 (2020)

Matsumoto T, Kodera S, Shinohara H, Ieki H, Yamaguchi T, Higashikuni Y, Kiyosue A, Ito K, Ando J, Takimoto E, Akazawa H, Morita H, Komuro I. Diagnosing Heart Failure from Chest X-Ray Images Using Deep Learning. ***Int Heart J*** 61, 781 (2020)

Matsunaga H, Ito K, Akiyama M, Takahashi A, Koyama S, Nomura S, Ieki H, Ozaki K, Onouchi Y, Sakaue S, Suna S, Ogishima S, Yamamoto M, Hozawa A, Satoh M, Sasaki M, Yamaji T, Sawada N, Iwasaki M, Tsugane S, Tanaka K, Arisawa K, Ikezaki H, Takashima N, Naito M, Wakai K, Tanaka H, Sakata Y, Morita H, Sakata Y, Matsuda K, Murakami Y, Akazawa H, Kubo M, Kamatani Y, Komuro I. Transethnic Meta-Analysis of Genome-Wide Association Studies Identifies Three New Loci and Characterizes Population-Specific Differences for Coronary Artery Disease. ***Circ Genom Precis Med*** 13, e002670 (2020)

Invited presentations

Ito K. Cardiomyopathy Learning from Cancer and Cancer Therapy "Pathogenesis of Cardiomyopathy Revealed by Genomic Analysis of CTRCD" The 6th Japan Society for the Study of Cardiomyopathy (Online) August 2020

Ito K. The Japanese Foundation for the Advancement of Science Symposium on Preventing Cardiovascular Diseases in the Future "Risk stratification by polygenic score" The 84th Annual Meeting of the Japanese Circulation Society (Online) July-August 2020

Ito K. Genomic Medicine Update "Genomic research in coronary artery disease" The 84th Annual Meeting of the Japanese Circulation Society (Online) July-August 2020

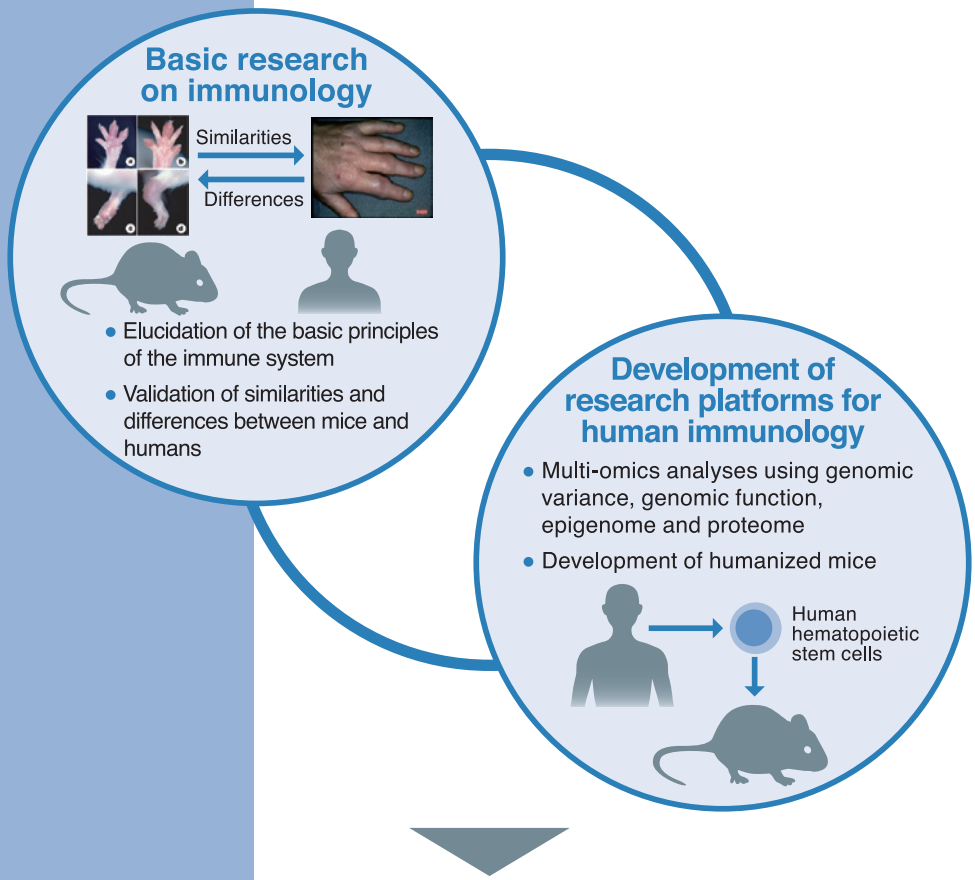
Ito K. Precision Medicine Targeting Atrial Fibrillation and Cardiogenic Embolism "Genome-wide Association Study for Atrial fibrillation and Precision Medicine" The 84th Annual Meeting of the Japanese Circulation Society (Online) July-August 2020

Cardiovascular diseases cause more than 15% of the deaths in the Japanese population and represent more than 20% of the total medical expenses in Japan. Thus, it is important for our society to understand the pathogenesis of these disorders and to uncover new therapeutic targets for their treatment. To achieve these goals, we aim to discover the precise genetic mechanisms underlying those diseases by utilizing leading-edge technologies, such as whole genome sequencing and artificial intelligence. Additionally, we conduct research to push forward the clinical applications of genetic information in the field of cardiovascular medicine.

Our current diseases of interest are coronary artery diseases (CAD), atrial fibrillation (AF), Kawasaki disease (KD), peripheral artery disease (PAD), chronic thromboembolic pulmonary hypertension (CTEPH), cancer therapy-related cardiac dysfunction (CTRCD), and heart failure (HF). We are currently seeking 1) to understand the genetic causes of CAD/AF and the genetic differences between Japanese and European populations; 2) to develop a novel genetic analysis method to solve the "P greater than N" scenario, where the sample size is small, but the number of variants to be analyzed is large; 3) to elucidate the mechanism of CTEPH/CTRCD using human omics data from patients in multiple hospitals; 4) to develop a more sophisticated genetic risk scoring system in the CAD and AF projects by artificial intelligence; and 5) to develop a comprehensive system to prioritize variants of unknown significance using massively parallel *in vitro* assays with artificial intelligence. In the process, we play a central role in the AMED national GRIFIN project for cardiovascular disease.

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

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

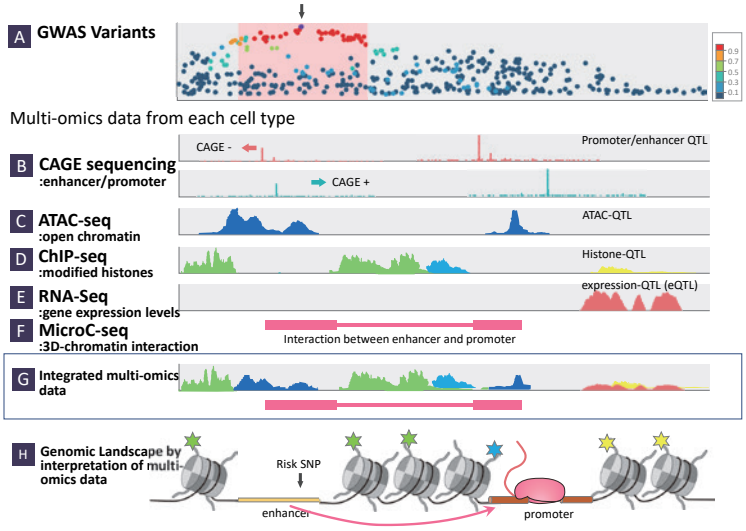


Figure: Functional genetics of autoimmune diseases

Recent Major Publications

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

Terao C, Suzuki A, Momozawa Y, Akiyama M, Ishigaki K, Yamamoto K, Matsuda K, Murakami Yo, McCarroll SA, Kubo M, Loh P, Kamatani Y. Chromosomal alterations among age-related haematopoietic clones in Japan. ***Nature*** 584, 130-135 (2020)

Ishigaki K, Akiyama M, Kanai M, Takahashi A, Kawakami E, Sugishita H, Sakaue S, Matoba N, Siew-Kee L, 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)

Invited presentations

Suzuki A. "Functional analysis of Peptidylarginine deiminases in rheumatoid arthritis model mice" Annual Meeting of American Society of Human Genetics (USA/Online) October 2020

Yamamoto K. "Polygenic diseases, Genome wide association studies and functional genetics" ISPOR Asia Pacific 2020 (Seoul, Korea/Online) September 2020

Yamamoto K. "Integration of genetic information to immune functions" The 12th International Forum on Rheumatoid Arthritis (IFRA 2020) (Beijing, China/Online) September 2020

Autoimmune diseases are thought to arise from the interaction of genetic and environmental factors. Improvements in commercial arrays since 2007 have led to the development of the current form of genome-wide association studies (GWASs). GWASs rely on single-nucleotide polymorphisms (SNPs) and haplotype blocks. The loci associated with disease susceptibility can then be determined by comparing variants represented by tag SNPs between patients and controls and identifying those that are significantly different. Genetic risk factors are essentially causative for specific diseases and traits. However, understanding the biological mechanism of risk from a genetic factor is challenging. Although associations between variants and diseases can be identified, it is difficult to identify the causal variant among multiple variants located on the same haplotype, and experimental validations are needed to determine the functional variants. Furthermore, understanding the biological functions of risk variants is not straightforward, since more than 80% of disease-associated variants are located in non-coding regions of the genome.

The capabilities of next-generation sequencing techniques for analyzing the functions of non-coding regions have advanced dramatically in recent years, enabling the comprehensive analysis of enhancers, promoters, histone modifications, and chromatin structures. Analysis of expression quantitative trait loci (eQTL) has been used to investigate how particular variants lead to different expression levels of a particular gene. QTL are DNA markers on a chromosome that indicate genes involved in a quantitative trait. 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.

We believe that insights gained from these studies will enable us to understand precise causal pathogenic processes and develop better strategies to control autoimmune diseases.

Laboratory for
Cell Signaling

Team Leader: Takashi Saito

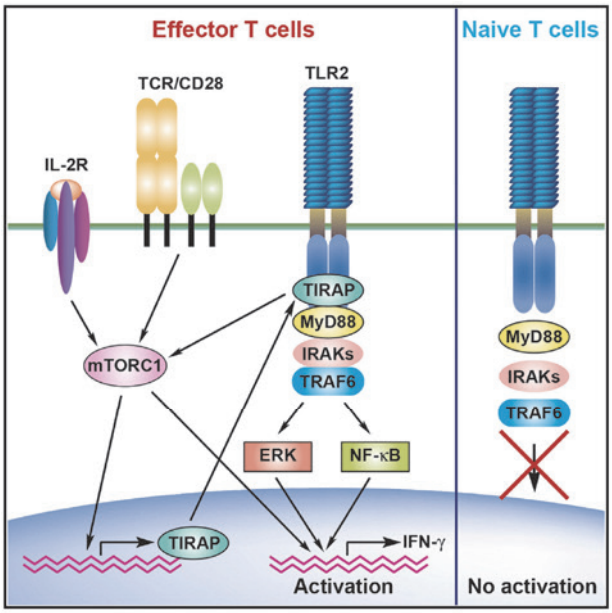


Figure: TLR2-mediated T-cell activation is dependent on TIRAP expression in T cells and regulated by mTORC1 signaling
Effector T cells are activated by TLR2 signals in the absence of TCR signals. TLR2-mediated activation is mediated through TIRAP, whose expression is induced by mTOR signals through TCR or IL-2 signals, whereas naïve T cells are not activated by TLR2 due to the lack of TIRAP expression.

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 and to modulate T cell function/activation to prevent immune diseases such as autoimmunity and allergic inflammation. For this purpose, we have analyzed 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 are analyzing the dynamic regulation of other inhibitory co-stimulation receptors such as LAG3. These inhibitory receptors were colocalized with the TCR-MC to mediate inhibition of T cell activation. Our analyses provide a dynamic view of signal regulation and also define inhibitory mechanisms.

We have analyzed negative regulation of T cell activation, particularly by autoimmune-related 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 signals. We previously found that T cells are activated by co-stimulation of TCR and TLRs. TLR2 in particular activates effector T cells (as Th1) but not naïve T cells without TCR stimulation. We found that naïve T cells failed to respond to TLR2 stimulation due to the defective expression of TIRAP. TIRAP is expressed upon stimulation through mTORC1 activation via TCR or IL-2 signaling (Figure).

Recent Major Publications

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)

Sasaki T, Yajima T, Shimaoka T, Ogawa S, Saito T, Yamaoka K, Takeuchi T, Kubo M. Synergistic effect of IgG4 antibody and CTLs causes tissue inflammation in IgG4-related disease. ***Int Immunol*** 32, 163-174 (2020)

Invited presentations

Saito T. "Regulation of adhesion and activation of T cells at Immune synapse" The 43rd Annual Meeting of Molecular Biology Society of Japan 2020 (Kobe, Japan/Online) December 2020

Saito T. "Regulation of T cell activation and function by innate signaling" OIST Conference (Okinawa, Japan/Online) February 2020

Saito T. "Negative regulation of T cell activation by phosphatases" Seminar at Hokkaido University (Sapporo, Japan/Online) January 2020

Laboratory for Lymphocyte Differentiation

Team Leader: Tomohiro Kurosaki

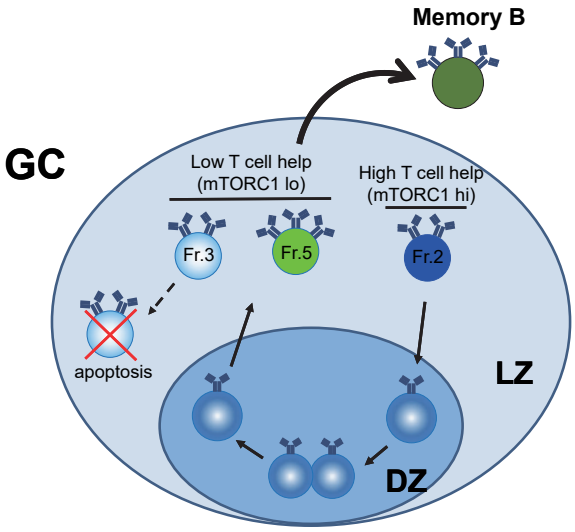


Figure: Fraction 5 GC memory precursor cells acquire increased BCR-mediated survival signals
After returning to the light zone (LZ GC B cells), Fraction 3 and 5 LZ GC B cells receive weak T cell help, resulting in low levels of mTORC1, which is one of the prerequisites for development to memory B cells. However, the mTORC1^{low} state is necessary but not sufficient. In contrast to Fraction 3 cells, Fraction 5 cells begin to upregulate cell-surface BCR and the anti-apoptotic molecule Bcl2. Hence, both weak T cell help and provision of survival signals by BCR and Bcl2 are required for GC B cells to adopt a memory B cell fate.

Memory B cells and long-lived plasma cells (LLPCs) are responsible for effective long-term immunity against pathogens. The majority of these cells responding to T cell-dependent antigens are generated from the germinal center (GC) reaction. Indeed, memory B cells emerge from the GC as recirculating cells and, upon secondary antigen challenge, they are primed to elicit rapid antibody responses.

Immunization with NP hapten leads to the accumulation of the high-affinity Abs in a large proportion of LLPCs. Thus, the LLPC pool is thought to be primarily composed of specificities possessing the highest affinity for the primary antigen. On the other hand, we and others have recently demonstrated that GC B cells that receive only weak T cell help are preferentially recruited into the memory B cell compartment. Since GC B cells receiving weak T cell help are generally thought to undergo apoptosis, our model has raised the question of how precursors of mature memory B cells are prevented from dying and able to differentiate into memory B cells.

To address this question, after identifying a memory-prone population (pro-memory and pre-memory B cells), we have identified key features for these precursor cells to develop into mature memory B cells. We found that pro-memory B cells began to upregulate Bcl2 and cell-surface BCR, contributing to the development of mature memory B cells. Furthermore, we provide evidence that downregulation of Bcl6 in pro-memory B cells could be one of the regulatory mechanisms to increase Bcl2 and BCR. Together, we propose a model in which weak help from T cells together with provision of an increased survival signal are key for GC B cells to adopt a memory B cell fate. In regard to the increased survival signal, stepwise decreases in Bcl6 expression (pro-memory>pre-memory>mature memory B cells) play a key role.

Recent Major Publications

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)

Tanaka S, Ise W, Inoue T, Ito A, Ono C, Shima Y, Sakakibara S, Nakayama M, Fujii K, Miura I, Sharif J, Koseki H, Koni PA, Raman I, Li QZ, Kubo M, Fujiki K, Nakato R, Shirahige K, Araki H, Miura F, Ito T, Kawakami E, Baba Y, Kurosaki T. Tet2 and Tet3 in B cells are required to repress CD86 and prevent autoimmunity. ***Nat Immunol*** 21, 950-961 (2020)

Mesin L, Schiepers A, Ersching J, Barbulescu A, Cavazzoni CB, Angelini A, Okada T, Kurosaki T, Victora GD. Restricted Clonality and Limited Germinal Center Reentry Characterize Memory B Cell Reactivation by Boosting. ***Cell*** 180, 92-106 (2020)

Invited presentations

Kurosaki T. "Function of Tet proteins in B cell tolerance" Seminar at Fudan University (Shanghai, China/Online) December 2020

Kurosaki T. "B cell and antibody responses against SARS-CoV-2 infections" The 48th Annual Meeting of the Japanese Society of Clinical Immunology (Tokyo, Japan/Online) October 2020

Kurosaki T. "Two functional humoral memory compartments and their generation mechanism" B Cell Renaissance: Epigenetics, Regulation and Immunotherapy, Keystone Symposia (Banff, Canada) March 2020

Kurosaki T. "Fate Decision germinal center B Cell" Microbiology Seminar, University of Alabama at Birmingham (Birmingham, USA) February 2020

Kurosaki T. "Function of humoral memory compartments and their generation mechanism" New Horizons in B Cell Biology (Shanghai, China) January 2020

Laboratory for Transcriptional Regulation

Team Leader: Ichiro Taniuchi

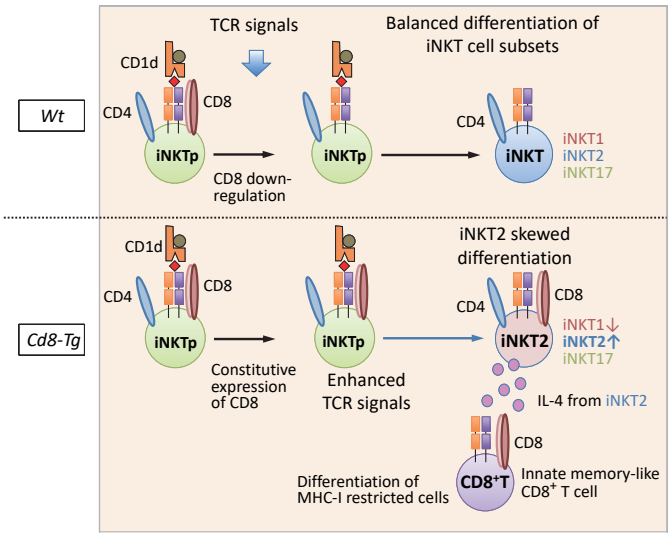


Figure: Constitutive CD8 expression drives innate CD8⁺ T-cell differentiation via induction of iNKT2 cells
Expression of CD8 is downregulated after receiving TCR mediated positive selection signals. Constitutive CD8 expression from a transgene results in enhanced differentiation of innate memory-like CD8⁺ thymocytes in both a cell-intrinsic and -extrinsic manner, the latter being accomplished by an increase in the IL-4 producing iNKT2 subset. These findings shed new light on the relevance of CD8 down-regulation in shaping the balance of iNKT cell subsets by modulating TCR signaling.

Recent Major Publications

Liu M, Kuo F, Capistrano KJ, Kang D, Nixon BG, Shi W, Chou C, Do MH, Stamatiades EG, Gao S, Li S, Chen Y, Hsieh JJ, Hakimi AA, Taniuchi I, Chan TA, Li MO. TGF-β suppresses type 2 immunity to cancer. ***Nature*** 587, 115-120 (2020)

Nomura A, Taniuchi I. The Role of CD8 Downregulation during Thymocyte Differentiation. ***Trends Immunol*** 41, 972-981 (2020)

Seo W, Shimizu K, Kojo S, Okeke A, Kohwi-Shigematsu T, Fujii SI, Taniuchi I. Runx-mediated regulation of CCL5 via antagonizing two enhancers influences immune cell function and anti-tumor immunity. ***Nat Commun*** 11, 1562 (2020)

Invited presentations

Taniuchi I. "Pathogenesis of primary immunodeficiency by miss sense mutation in transcription factors" The 48th Annual Meeting of Japanese Society of Clinical Immunology (Tokyo, Japan/Online) October 2020

The vertebrate immune system consists of two components, innate and adaptive. The adaptive immune system appeared later during evolution, minimally by acquisition of a system for generating pools of lymphocytes with a broad variety of antigen-specificities. Thus, the primary developmental program of T lymphocytes that occurs in the thymus has been shaped 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). My laboratory has been addressing 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, in which expression of the ThPOK transcription factor serves as a key determinant.

CD4 and CD8 glycoproteins serve as co-receptors to assist recognition of peptide antigen presented on class-II and class-I MHC, respectively. While CD8 expression is temporally down-regulated in post-selection thymocyte, CD4 expression is maintained. This difference in the kinetics of expression of the two co-receptors during differentiation of post-selected thymocytes, known as a kinetic signaling model, has been proposed to be a key determinant for the helper- versus cytotoxic-lineage choice. Our current study tested this model by generating a mouse strain in which CD8 is constitutively expressed from a transgene. We found that constitutive CD8 expression drives innate memory-like CD8⁺ T cell differentiation rather than directing MHC-I restricted cells toward the helper-lineage. This enhanced differentiation of innate memory-like CD8⁺ T-cells is caused in part by skewed differentiation of the iNKT2 cell subset secreting IL-4. Collectively, our study reveals a novel significance of CD8 down-regulation in fine tuning a balance of iNKT cell subset differentiation to minimize innate memory-like CD8⁺ T cell differentiation.

Laboratory for
Immune Cell Systems

Team Leader: **Shigeo Koyasu**

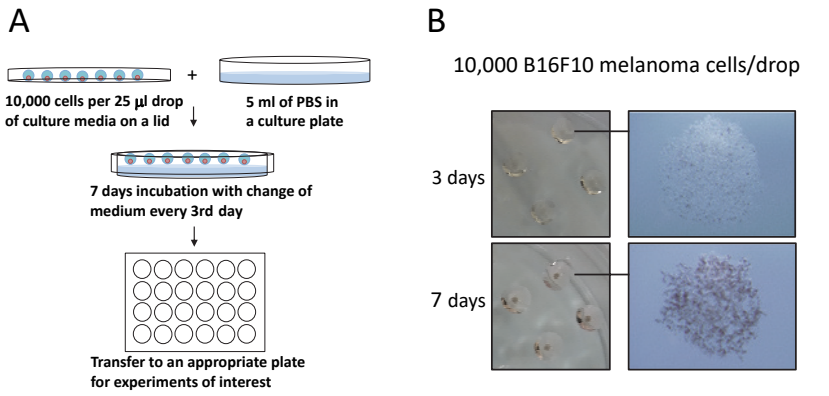


Figure: A simple, fast and easy technique for generating 3D spheroids through the use of a hanging drop culture method
(A) Ten thousand tumor cells per drop of cell culture medium are placed on the lid of a PBS containing dish and incubated for 7 days. Pre-established spheroids are collected and co-cultured indirectly or directly with an immune cell population of interest. (B) Representative images of skin melanoma spheroids. B16F10 melanoma cells (10,000 cells per 25 µl drop of cell culture medium) placed on the lid of a PBS containing dish and incubated for 3 and 7 days.

Recent Major Publications

Wagner M, Ealey KN, Tetsu H, Kiniwa T, Motomura Y, Moro K, Koyasu S. Tumour-derived lactic acid contributes to the paucity of intratumoural ILC2s. ***Cell Rep*** 30, 2743-2757 (2020)

Wagner M, Koyasu S. A 3D Skin melanoma spheroid-based model to assess tumor-immune cell interactions. ***Bio Protoc*** 10, e3839 (2020)

Nakamura R, Yoshizawa A, Moriyasu T, Deloer S, Senba M, Kikuchi M, Koyasu S, Moro K, Hamano S. Group 2 innate lymphoid cells exacerbate amebic liver abscess in mice. ***iScience*** 23, 101544 (2020)

We have been studying the function of group 2 innate lymphoid cells (ILC2), which are 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 and mucosal barriers (i.e., respiratory and intestinal mucosa), and in fat-associated lymphoid clusters (FALCs) in the visceral adipose tissue. Despite intensive studies on the role of ILC2 in allergic disorders, their role in anti-tumor immunity has been obscure and controversial. It is generally believed that type 2 immune responses are beneficial for tumor growth. However, the action of ILC2 on tumor cells seems to be context dependent, and several studies have shown that IL-5-mediated eosinophilia can effectively control the growth of certain tumors such as melanoma. To examine the interaction between a solid tumor and immune cells, tumor cell culture in two-dimensional (2D) monolayers has limitations. In light of the increasingly recognized role of interactions between tumor and immune cells and to better characterize tumor-immune cell interactions, we have established a three-dimensional (3D) tumor organoid system using skin melanoma cells. This is a simple, fast and easy technique for generating 3D spheroids through the use of a hanging drop culture method. Within the hanging drop, cells have no direct contact with substratum and due to gravity accumulate at the free liquid-air interface to form a single spheroid (see Figure). Pre-established spheroids can be co-cultured directly or indirectly with different populations of immune cells. Our method provides a tumor microenvironment that resembles micrometastases and replicates many features of solid tumors. As in the non-proliferating regions of avascular solid tumors, tumor cells within the inner regions of the spheroids usually demonstrate perturbed gene and protein expression, altered metabolism, cell cycle arrest, and necrotic death. Using this method, we have discovered an immunosuppressive activity imposed on ILC2s by tumor cells through the accumulation of lactic acid in the tumor microenvironment.

Laboratory for
Innate Immune Systems

Team Leader: Kazuyo Moro

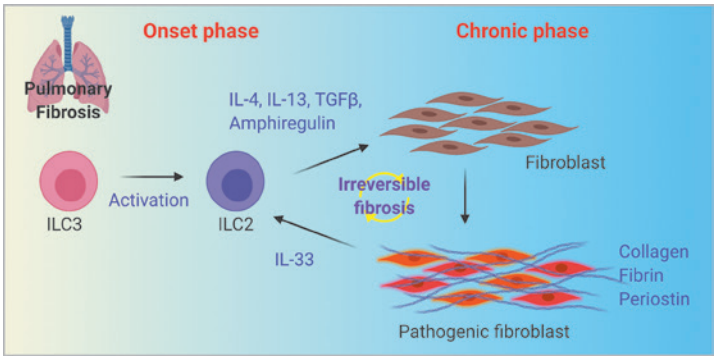


Figure: ILC-mediated pulmonary fibrosis (PF)
ILC3s are necessary for ILC2 activation during the PF-onset phase in *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, and ILC2s directly induced collagen production by fibroblasts during the chronic phase. Enhanced IL-33 production by pathogenic fibroblasts reactivated ILC2s during the chronic phase and caused irreversible fibrosis.

Recent Major Publications

Miyajima Y, Ealey KN, Motomura Y, Mochizuki M, Takeno N, Yanagita M, Economides AN, Nakayama M, Koseki H, Moro K. Effects of BMP7 produced by group 2 innate lymphoid cells on adipogenesis. ***Int Immunol*** 32, 407-419 (2020)

Wagner M, Ealey KN, Tetsu H, Kiniwa T, Motomura Y, Moro K, Koyasu S. Tumor-Derived Lactic Acid Contributes to the Paucity of Intratumoral ILC2s. ***Cell Rep*** 30, 2743-2757.e5 (2020)

Kobayashi T, Ricardo-Gonzalez RR, Moro K. Skin-Resident Innate Lymphoid Cells – Cutaneous Innate Guardians and Regulators. ***Trends Immunol*** 41, 100-112 (2020)

Invited presentations

Moro K. "Mechanism of antigen-independent eosinophilic inflammation by ILC2" The Workshop on Eosinophils in Allergy and Related Diseases 2020 (Japan/Online) November 2020

Moro K. "The role of IL-4 and IL-13 during asthma" The 60th Annual Meeting of The Japanese Respiratory Society (Kobe, Japan/Online) September 2020

Moro K. "The role of ILC2 in pulmonary fibrosis" Japanese Society of Allergology and World Allergy Organization Joint Congress 2020 (Kyoto, Japan/Online) September 2020

Moro K. "ILC2, a potential target for respiratory diseases" The 142nd Meeting of the Japanese Pharmacological Society, Kanto Branch (Chiba, Japan/Online) June 2020

Moro K. "ILC2 induce innate IgE secretion by B1 cells via IL-4 production and regulate allergic susceptibility" The 119th Annual Meeting of the Japanese Society of Dermatological Association (Kyoto, Japan/Online) June 2020

We have investigated the remarkable properties of innate immunity through studying group 2 innate lymphoid cells (ILC2), an innate lymphocyte lineage that we identified ten years ago. 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. Our lab couples *in vitro* and *in vivo* studies with transcriptome and other omics approaches to study the biology of innate immune systems. Our research employs animal disease models, cell biology, single cell sequencing, and imaging.

Bone morphogenic protein 7 (BMP7) possess multiple functions, including regulating adipogenesis. While investigating adipose tissue, we found that ILC2s are abundant in visceral white adipose tissues and that ILC2-derived BMP7 regulates adipocyte differentiation and lipid accumulation. During *in vitro* culture, fat-derived ILC2s promoted differentiation of fibroblast-like precursors into white adipocytes that contain lipid. Fat-derived ILC2s express BMP2 and BMP7 and induced differentiation of mesenchymal stem cell-like precursors into adipocytes. The fat droplet size was larger in BMP7-conditional knock out mice, suggesting a role of ILC2-derived BMP7 in the induction of brown-adipocyte differentiation. Our study highlighted a crucial role of ILC2s in controlling adipogenesis in the steady state via BMP7 production.

Pulmonary fibrosis (PF) is a disease that causes collagen deposition in the lungs. Despite numerous studies on the pathology of PF, the lack of animal models has significantly hampered advancement of our understanding of the disease. We found that 100% of *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, which lack ILC2 and ILC3 suppression mechanisms, spontaneously developed PF (Figure). Through studying of these mice, we have revealed an indispensable contribution of ILC2 and ILC3 in fibrosis formation. Single-cell RNA-sequence analysis indicated that ILC3 activation occurred during the disease-onset phase in *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, which triggered subsequent activation of the ILC2 subpopulation. By dissecting pathogenic mechanisms of disease initiation *in vitro* and *in vivo*, we have delineated complex networking among ILC2, ILC3 and fibroblasts, which will lead us to develop novel therapeutic options that prevent disease progression.

Laboratory for

Immune Homeostasis

Team Leader: Taishin Akiyama

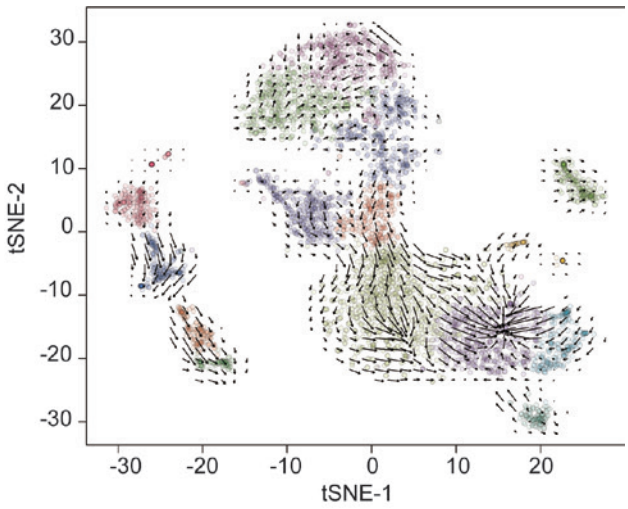


Figure: Single cell RNA-seq analysis reveals heterogeneity and differentiation dynamics of thymic epithelial cells in the adult thymus
RNA velocity analysis of single RNA-seq data reveals the differentiation trajectory of medullary thymic epithelial cells in the adult thymus.

Recent Major Publications

Ito-Kureha T, Miyao T, Nishijima S, Suzuki T, Koizumi SI, Villar-Briones A, Takahashi A, Akiyama N, Morita M, Naguro I, Ishikawa H, Ichijo H, Akiyama T*, Yamamoto T*. The CCR4-NOT deadenylase complex safeguards thymic positive selection by down-regulating aberrant pro-apoptotic gene expression. ***Nat Commun*** 11, 6169 (2020)

Akiyama T*, Horie K, Hinoi E, Hiraiwa M, Kato A, Maekawa Y, Takahashi A, Furukawa S. How does spaceflight affect the acquired immune system? ***NPJ Microgravity*** 6, 14 (2020)

Nakamura T, Hashikawa C, Okabe K, Yokote Y, Chirifu M, Toma-Fukai S, Nakamura N, Matsuo M, Kamikariya M, Okamoto Y, Gohda J, Akiyama T, Semba K, Ikemizu S, Otsuka M, Inoue JI, Yamagata Y*. Structural analysis of TIFA: Insight into TIFA-dependent signal transduction in innate immunity. ***Sci Rep*** 10, 5152 (2020)

The thymus produces a large number of T cells with properly selected repertoires. Medullary thymic epithelial cells (mTECs) are essential for T cell tolerance induction in the thymus. mTECs ectopically express and present thousands of tissue-specific antigens (TSAs) to developing T cells in the thymus, and T cells that recognize TSAs with high affinity undergo apoptosis (negative selection) or are converted into regulatory T cells, which are critical for suppressing the onset of autoimmune diseases.

In various regenerative tissues, transit-amplifying cells are an intermediate population linking stem cells and mature cells. Previous studies suggest that mTECs undergo homeostatic turnover, with a duration of approximately 14 days, implying the presence of transit amplifying cells in the adult thymus. However, the presence of transit-amplifying cells among adult mTECs remains unclear. We performed single cell RNA-seq analysis of TECs to elucidate heterogeneity and differentiation dynamics of mTECs in the adult thymus and identified a candidate cluster for transit amplifying mTECs. We further confirmed the existence of transit amplifying mTECs by cell labeling and fate mapping analyses.

In the thymus, immature thymocytes undergo positive selection that is crucial for generation of a self-MHC-restricted TCR repertoire. However, detailed mechanisms underlying this thymic positive selection remain to be determined. Deadenylation of mRNA poly(A) tails is the rate-limiting step in mRNA translation, but its role in thymic positive selection has not been determined. We have reported that the deadenylase CCR4-NOT complex controls positive selection of thymocytes by suppressing aberrant up-regulation of pro-apoptotic molecules during positive selection.

As disturbance of adult thymic homeostasis provokes immunodeficiency, autoimmunity, thymoma, and other diseases, these studies will aid the development of novel therapeutic strategies against such thymus-related diseases.

Laboratory for
Immune Crosstalk

Team Leader: Hilde Cheroutre

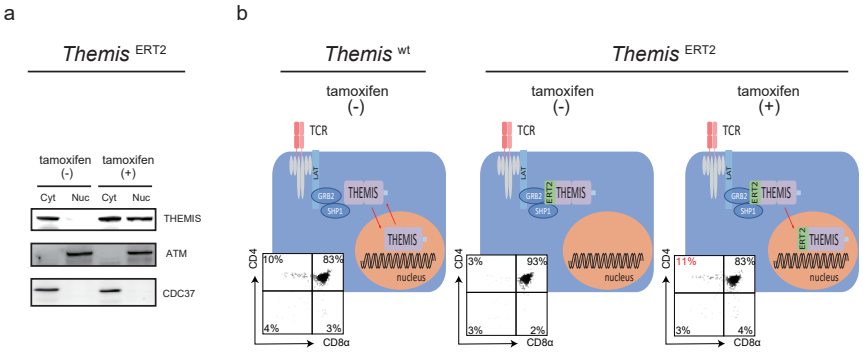


Figure: Dynamic subcellular translocation of THEMIS is necessary for its function in T cells
(a) The THEMIS-ERT2 fusion protein is retained in the cytoplasm at steady state. Administration of Tamoxifen induces translocation of THEMIS from the cytoplasm to the nucleus. Lysate from total thymocytes was separated into the cytoplasmic (Cyt) and nuclear (Nuc) fraction and blotted with anti-THEMIS and anti-ATM (nuclear protein) and anti-CDC37 (cytoplasmic protein) as controls.
(b) Subcellular localization of THEMIS and T cell development in the thymus. THEMIS is present in the cytoplasm and nucleus of normal thymocytes and TCRαβ$^+$ T cells (left panel). Artificial retention of THEMIS-ERT2 in the cytoplasm abrogates T cell development (middle panel), resulting in reduced frequencies of CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ mature thymocytes. Addition of Tamoxifen restores translocation of THEMIS from the cytoplasm to the nucleus and results in normal T cell development (right). Dot profiles show the CD4 and CD8 expression pattern by thymocytes.

Invited presentations

Cheroutre H. "Dietary Antigens build 'De-Fence' at the Mucosal Border of the Intestine" Asociacion Chilena de Immunologia (Santiago, Chile/Online) November 2020

Cheroutre H. "The Cd8 Intergenic Region Controls the Generation of Cytotoxic CD4 Effector Cells" Meeting at La Jolla Institute (La Jolla, USA/Online) June 2020

Cheroutre H. "The Cd8 Intergenic Region Controls the Generation of Cytotoxic CD4 Effector Cells" Seminar at National Institute of Health (Washington, USA/Online) May 2020

We identified THEMIS as an indispensable molecule for T cell development. GWAS linked the *THEMIS* locus with autoimmune diseases such as Celiac Disease, Multiple Sclerosis, Rheumatoid Arthritis, and Atopic Dermatitis. THEMIS functions as an adapter that modulates T cell receptor (TCR) signal strength and THEMIS-deficiency causes impaired conventional TCR αβ T cell development in mice. The mechanisms used by THEMIS to control TCR signaling remain unclear and controversial. Besides the cytoplasm, THEMIS also localizes in the nucleus. Although the functions of cytoplasmic THEMIS have been studied intensively, its nuclear function is poorly elucidated. To address this gap, we generated various THEMIS mouse mutants to manipulate the subcellular localization of THEMIS and examined the consequences of this for T cell development and function.

- nTHEMIS: THEMIS exists exclusively in the nucleus.
- cTHEMIS: THEMIS exists exclusively in the cytoplasm.
- n/cTHEMIS: THEMIS exists both in cytoplasm and nucleus but cannot translocate into the other compartment.
- THEMIS-ERT2: THEMIS is retained in the cytoplasm by ERT2. With tamoxifen treatment, cytoplasmic THEMIS-ERT2 is able to translocate into the nucleus.

All these THEMIS mutant mice showed a deficiency in T cell development similar to *Themis* germline knock-out mice, indicating that THEMIS in the nucleus or cytoplasm is not sufficient for its function. Rather THEMIS needs to be able to translocate between compartments to carry out its normal function. To provide proof-of-concept for this, THEMIS-ERT2 mutant mice were given several doses of Tamoxifen, which caused recovery of conventional T cell development and seeding of the periphery. This result indicated that dynamic movement of THEMIS from cytoplasm to nucleus is indispensable for its function. A follow-up study aims to elucidate the mechanism of THEMIS translocation and to examine its nuclear function and identify nuclear binding partners.

Our overall goal is to dissect the mechanisms employed by THEMIS to modulate TCR signals and control gene expression and ultimately to elucidate the correlation between THEMIS and protective immunity, as well as its role in the T cell-specific inflammatory diseases mentioned above.

Laboratory for

Inflammatory Regulation

Team Leader: Takashi Tanaka

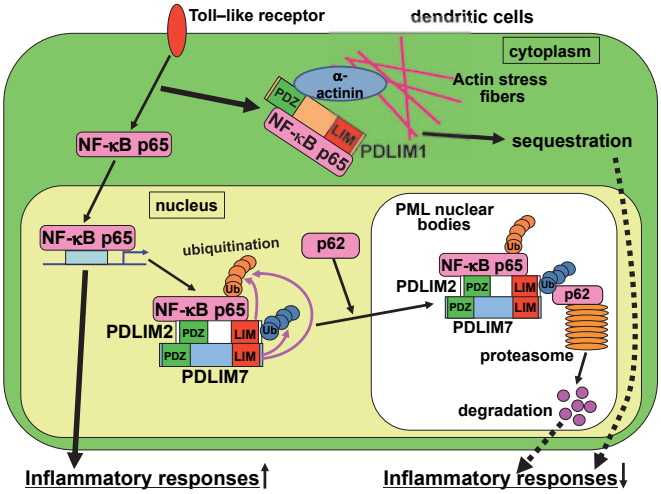


Figure: LIM proteins constitute a new family of negative regulators of NF-κB signaling
PDLIM2 and PDLIM7, ubiquitin E3 ligases for the p65 subunit of NF-κB, form heterodimers and cooperatively promote the delivery of p65 to the proteasome via p62/Sqstm1 for subsequent p65 degradation. By contrast, PDLIM1 sequestrates p65 in the cytoplasm and inhibits its nuclear translocation, thereby suppressing NF-κB signaling. Thus, the LIM protein family negatively regulates inflammatory responses, but individual family members do so by different mechanisms.

Recent Major Publications

Sugimoto-I 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)

Miyazaki R, Saiga H, Kato T, Bakoshi T, Senba R, Shintani A, Suzuki M, Takao K, Sasaki I, Iizuka A, Sugiyama M, Iwami N, Fukuda-O Y, Hemmi H, Tanaka T, Miyake M, Kaisho T, Hoshino K. The mechanism of action of Spi-B in the transcriptional activation of the interferon-α4 gene. ***Biochem Biophys Res Commu*** 525, 477-482 (2020)

The inflammatory response is critical for immune cells to fight invading microbial pathogens. On the other hand, excessive inflammation causes massive damage to the host, indicating that regulatory mechanisms to promptly terminate inflammatory responses are important to prevent immunopathology. Our research goal is to identify a series of key negative regulators of inflammation and to 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. We have recently shown that PDLIM7, another member of the LIM protein family, is also a ubiquitin E3 ligase that inhibits NF-κB-mediated inflammatory responses. PDLIM7 polyubiquitinates p65 and promotes its proteasomal degradation. Moreover, PDLIM7 heterodimerizes with PDLIM2 and promotes synergistic PDLIM2-mediated degradation of p65. Mechanistically, PDLIM7 induces K63-linked ubiquitination of PDLIM2 and then the proteasome/autophagosome cargo protein p62/Sqstm1 binds to both polyubiquitinated PDLIM2 and the proteasome, thereby facilitating the delivery of the NF-κB-PDLIM2 complex to the proteasome and subsequent p65 degradation. Consistently, double knockdown of PDLIM7 and either PDLIM2 or p62/Sqstm1 results in augmented proinflammatory cytokine production compared to control cells or single knockdown cells. These data delineate a new role for PDLIM7 and p62/Sqstm1 in the regulation of NF-κB signaling by bridging a ubiquitin E3 ligase and the proteasome.

We have also reported that PDLIM1, another LIM protein, binds to and sequesters p65 in the cytoplasm, thereby inhibiting NF-κB activation in dendritic cells, whereas PDLIM4 suppresses STAT3 signaling by recruiting PTPBL, a protein tyrosine phosphatase, and facilitating dephosphorylation of STAT3 in $CD4^{+}$T cells. We propose that LIM proteins constitute a new family negatively regulating inflammatory responses through different mechanisms.

Laboratory for
Cytokine Regulation

Team Leader: Masato Kubo

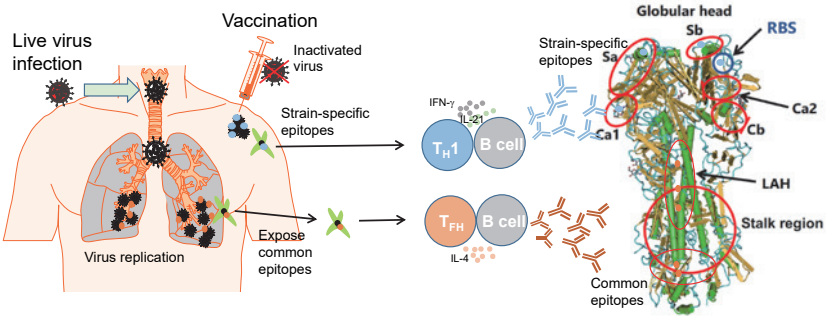


Figure: The breadth of antibody responses induced by influenza vaccination and infection
Vaccination with inactivated viruses induces rapid but narrow IgG responses against influenza HA through T_H1 cell-dependent B cell activation. On the other hand, natural viral infection induces broadly reactive IgG responses through T_{FH} cell-dependent GC B cell activation.

Pandemic infection with the new coronavirus (SARS-CoV-2) that causes respiratory diseases is a very serious public health problem. Influenza viruses are also known to be the causative pathogen of a severe acute respiratory infection. Vaccines are widely accepted as the most effective protective measure against respiratory virus infection. We and others found that the immune responses induced with currently available inactivated vaccines were very different from the immune responses induced by a natural viral infection. In a recent publication in Trends in Immunology, we discuss the germinal center (GC) dependency of the responses and the breadth of antibody responses between vaccines and infection (Figure) (Kubo M *et al.* 2020 Trends in Immunology).

Inactivated vaccines induce effective neutralizing antibodies against the emerged influenza virus, but the response is narrow and limited to the vaccine virus strain. Meanwhile, natural viral infection induces a potentially wider breadth of antibody responses, which can protect against heterotypic virus infection. The broadly neutralizing antibody (bnAb) responses in the natural infection largely depends on T_{FH} cell-dependent GC responses, because the lack of Bcl-6 expression in T or B cells diminishes the production of bnAbs. On the other hand, the neutralizing antibodies induced by the inactivated vaccines are independent of the GC response. We discuss recent advances concerning the contribution of T_{FH} cells and GC responses to the generation of bnAbs that recognize common epitopes shared by heterotypic influenza viruses. This information may shed light on strategies for the development of a universal vaccine capable of establishing protection beyond the barrier of virus mutation and strain differences.

Recent Major Publications

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)

Kubo M. Diurnal Rhythmicity Programs of Microbiota and Transcriptional Oscillation of Circadian Regulator, NFIL3. ***Front Immunol*** 11, 552188 (2020)

Kubo M, Miyauchi K. Breadth of Antibody Responses during Influenza Virus Infection and Vaccination. ***Trends Immunol*** 41, 394-405 (2020)

Invited presentations

Kubo M. "Role of IL4/IL13 in the pathogenesis of atopic dermatitis" The 50th Annual Meeting of the Japanese Society for Cutaneous Immunology and Allergy (Kochi, Japan/Online) December 2020

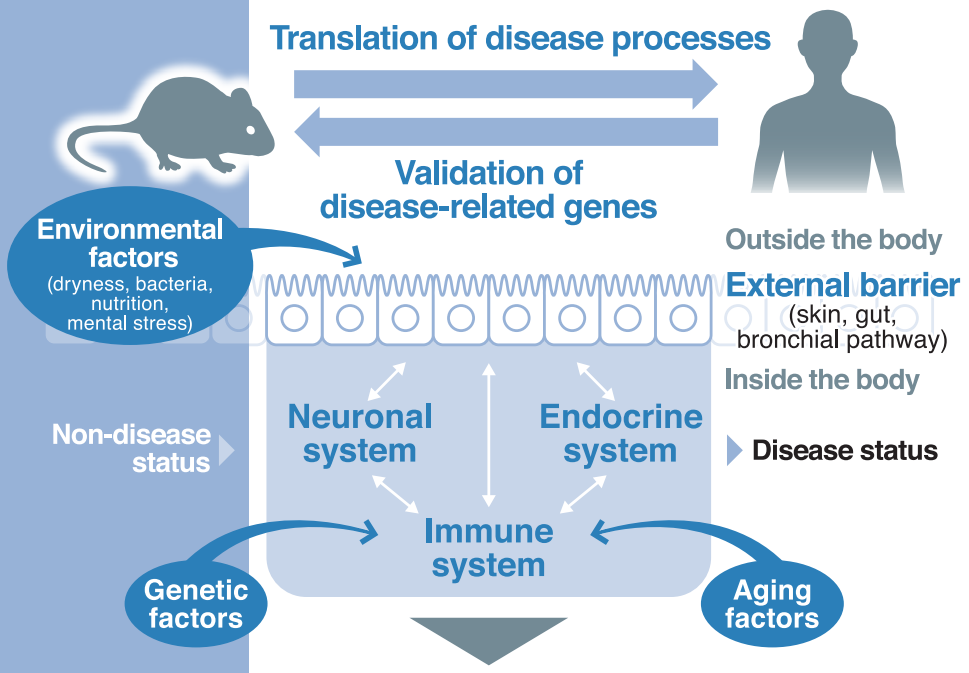
Kubo M. "IL-4 and IL-13–their roles in Type 2 inflammation in allergic diseases" 1st Anniversary Commemorative Lecture of Dupixent Launch (Tokyo, Japan/Online) September 2020

Kubo M. "The Role of Type 2 Cytokines in Allergic Inflammation" Science Exchange Meeting in Kagoshima 2020 (Kagoshima, Japan/Online) June 2020

Kubo M. "Skin homeostasis and atopic dermatitis" 7th International Postgraduate Conference on Pharmaceutical Sciences (iPoPS2020) (Noda, Japan) February 2020

Kubo M. "The Role of Type 2 Cytokines in Allergic Inflammation" Sanofi lecture series on Severe Asthma (Tokyo, Japan) February 2020

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**

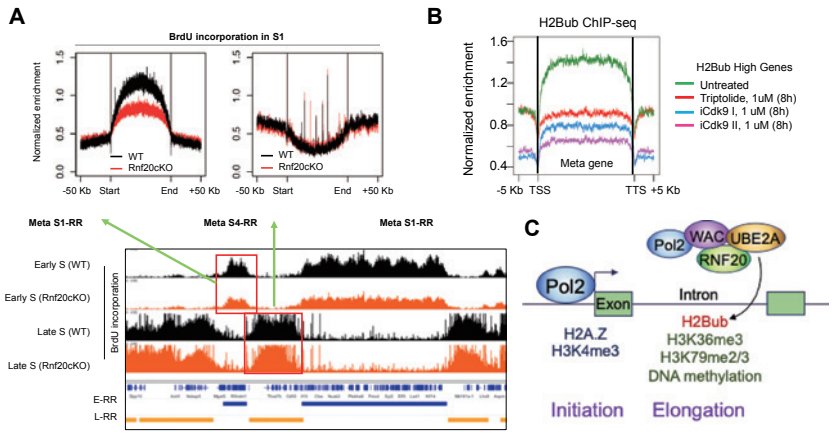


Figure: Regulation of mammalian replication timing by H2BK120ub

A, The bottom panel shows a screenshot of genomic regions that are undergoing DNA replication in early or late S-phase. The y-axis shows the level of DNA replication measured by BrdU incorporation. The top panel shows that the early replicating regions are affected by the loss of RNF20, and thereby H2BK120ub, while the late replicating regions are not. B, H2BK120ub is deposited over the gene body in WT cells (green). However, suppression of Pol2 activity by the selective Pol2 inhibitor triptolide (red), or inhibition of the CDK9 kinase that is essential for Pol2 elongation (blue and pink, two types of inhibitors), leads to loss of H2BK120ub. C, Working model showing Pol2-dependent recruitment of RNF20 and downstream H2BK120ub deposition in the gene body.

Recent Major Publications

Eto H, Kishi Y, Yakushiji-Kaminatsui N, Sugishita H, Utsunomiya S, Koseki H, Gotoh Y. The Polycomb group protein Ring1 regulates dorsoventral patterning of the mouse telencephalon. ***Nat Commun*** 11, 5709 (2020)

Healy E, Mucha M, Glancy E, Fitzpatrick DJ, Conway E, Neikes HK, Monger C, Van Mierlo G, Baltissen MP, Koseki Y, Vermeulen M, Koseki H, Bracken AP*. PRC2.1 and PRC2.2 Synergize to Coordinate H3K27 Trimethylation. ***Mol Cell*** 76, 437-452.e6 (2019)

Fursova NA, Blackledge NP, Nakayama M, Ito S, Koseki Y, Farcas AM, King HW, Koseki H, Klose RJ. Synergy between Variant PRC1 Complexes Defines Polycomb-Mediated Gene Repression. ***Mol Cell*** 74, 1-17 (2019)

Invited presentations

Koseki H. "Polycomb in haematipoietic differentiation" The Chromatin and Epigenetics biweekly virtual seminar series, Tsinghua University (Online) December 2020

Koseki H. "Daten als Basis der medizinischen Forschung für eine personalisierte Medizin: Das Mikrobiom und das Metabolom, die Treiber von entzündlichen Erkrankungen und Krebsleiden" KOOPERATIONSVERANSTALTUNG BADEN-WÜRTTEMBERG – KANAGAWA (Japan/Online) November 2020

Our laboratory, the Developmental Genetics Laboratory, focuses on the role of epigenetic regulation in organ development. Our recent research focuses on DNA replication of the mammalian genome. The mammalian genome is approximately 1000-times larger than the typical prokaryotic genome. To facilitate efficient replication of mammalian DNA, some regions of the genome replicate early while others replicate late in S-phase, a process that is known as replication timing. We have found that mono-ubiquitination of histone H2B lysine 120 (H2BK120ub) plays a role in regulating replication timing in mammalian cells (Figure 1A). RNF20, an E3 ubiquitin ligase, interacts with RNA polymerase II (RNAP2) during transcriptional elongation and deposits H2BK120ub (Figure 1B, 1C). H2BK120ub-enriched regions replicate early in S-phase. By regulating DNA replication, H2BK120ub may thus play a role in organ development and in regulation of immune functions.

A part of our laboratory is committed to the maintenance of a high-standard mouse facility in IMS. Through this Core Animal Facility, our group generates knock-out and transgenic animals for the various research laboratories in IMS. We also provide germ-free, gnotobiotic and humanized mice to IMS researchers.

We are also engaged in the generation of human iPSC-derived NKT (iPS-NKT) cells, with the goal of future clinical use of these cells in cancer therapy. We have started a Phase I clinical study to evaluate the safety of iPS-NKT cells in October 2020. The Core Facility for iPSC Research is developing a protocol with shorter cell culture times and higher efficiency of NKT induction and production from human iPSCs. This will help to facilitate a robust supply of NKT cells for more patients in the future. This research is mainly supported by the Agency for Medical Research and Development (AMED).

Laboratory for
Intestinal Ecosystem

Team Leader: Hiroshi Ohno

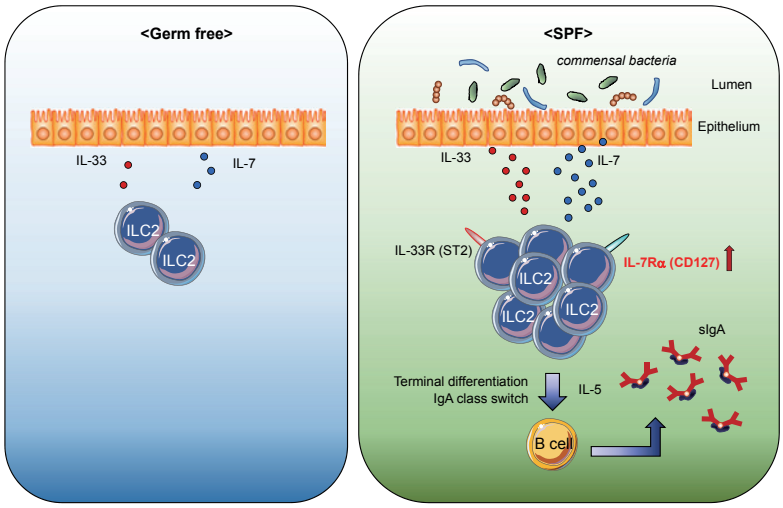


Figure: Stomach ILC2s respond to commensal bacteria
In germ-free (GF) mice (*left*), there are few ILC2s. Under GF conditions, IL-7 expression in the stomach tissue is hardly detectable and IL-7 receptor expression on stomach ILC2s is also lower than that under SPF conditions. By contrast, the expression of IL-33 in the stomach tissue is similar and that of the IL-33 receptor on the stomach ILC2s is slightly decreased in GF compared to SPF conditions. Under SPF conditions (*right*), stimulation by commensal bacteria upregulates the expression of IL-7 and IL-33 in the stomach tissue as well as that of IL-7 receptors on stomach ILC2s. This leads to an increase in the number of stomach ILC2s and their IL-5 secretion, which then promotes IgA production from plasma B cells (modified from Ohno H and Satoh-Takayama N. ***Exp Mol Med*** 52: 1377-1382 (2020)).

Recent Major Publications

Miyauchi E, Kim S-W, Suda W, Kawasumi M, Onawa S, Taguchi-Atarashi N, Morita M, Taylor TD, Hattori M, Ohno H. Gut microbes act in concert to exacerbate inflammation in spinal cords. ***Nature*** 585, 102-106 (2020)

Shimokawa C, Kato T, Takeuchi T, Ohshima N, Furuki T, Ohtsu Y, Suzue K, Imai T, Obi S, Olia A, Izumi T, Sakurai M, Arakawa H, Ohno H, Hisaeda H. CD8$^+$ regulatory T cells are critical in prevention of autoimmune-mediated diabetes. ***Nat Commun.*** 11, 1922 (2020)

Satoh-Takayama N, Kato T, Motomura Y, Kageyama T, Taguchi-Atarashi N, Kinoshita-Daitoku R, Kuroda E, Di Santo JP, Mimuro H, Moro K, Ohno H. Bacteria-Induced Group 2 Innate Lymphoid Cells in the Stomach Provide Immune Protection through Induction of IgA. ***Immunity*** 52, 635-649.e4 (2020)

Invited presentations

Ohno H. "Autoimmune diseases and gut microbiota" Fudan University Immunology Seminar (China/Online) December 2020

Ohno H. "Gut microbiota and autoimmune diseases" The 29th Symposium on Intestinal Flora (Tokyo, Japan) November 2020

Ohno H. "The role of gut microbiome in obesity and glucose intolerance" 2020 International Congress on Obesity and Metabolic Syndrome. Korean Society for the Study of Obesity (South Korea/Online) September 2020

Ohno H. "Birth cohort-based analysis of pediatric atopic dermatitis and gut microbiota" Educational lecture, The 119th Annual Meeting of the Japanese Dermatological Association (Online) June 2020

Ohno H. "Gut microbiota and autoimmune diseases" The 4th Chiba Probiotics Academic Seminar (Chiba, Japan) February 2020

Enormous numbers of commensal bacteria, the gut microbiota, reside in our intestines. Our lab has been studying the molecular mechanisms of host-gut microbiota interaction.

We do not unconditionally accept those microorganisms. Instead, the intestinal immune system senses the kinds and quantity of bacteria in the gut lumen and tries to contain them. To this end, our gut equips M cells, specialized intestinal epithelial cells, for recognition and uptake of luminal bacteria to initiate intestinal immune responses. Our lab has been elucidated the molecular mechanisms of M-cell differentiation and function.

Host-gut microbiota interactions deeply impact our physiology and pathology. We are studying this by applying an integrated omics approach, where cyclopedic analyses at different layers of organismal activities are combined, including (meta)genomics, epigenomics, (meta)transcriptomics and metabolomics. In the past year, we have published two papers reporting the role of the gut microbiota in autoimmune diseases. Multiple sclerosis (MS) is an autoimmune demyelinating disease in the central nervous system. By employing murine experimental autoimmune encephalomyelitis (EAE) as a model of MS, we have shown that two commensal bacteria in the small intestine act together to exacerbate EAE pathogenesis. We have also reported that *Ruminococcus* species in the gut can suppress the pathogenesis of autoimmune type 1 diabetes mellitus by increasing immunosuppressive CD8$^+$ regulatory T cells.

The stomach has been almost a no man's land in terms of immunology research, especially the innate lymphoid cells (ILCs). We have shown that ILC2s are dominant among ILCs, are dependent on stomach microbiota for their presence and are important for containing *Helicobacter pylori* infection (Figure).

In addition, we are studying the impact of the gut microbiota and its metabolites on the gut immune system and the host metabolic activities, and also on diseases such as pediatric allergic diseases, type 2 diabetes and autoimmune diseases.

Laboratory for Integrative Genomics

Team Leader: **Jun Seita**

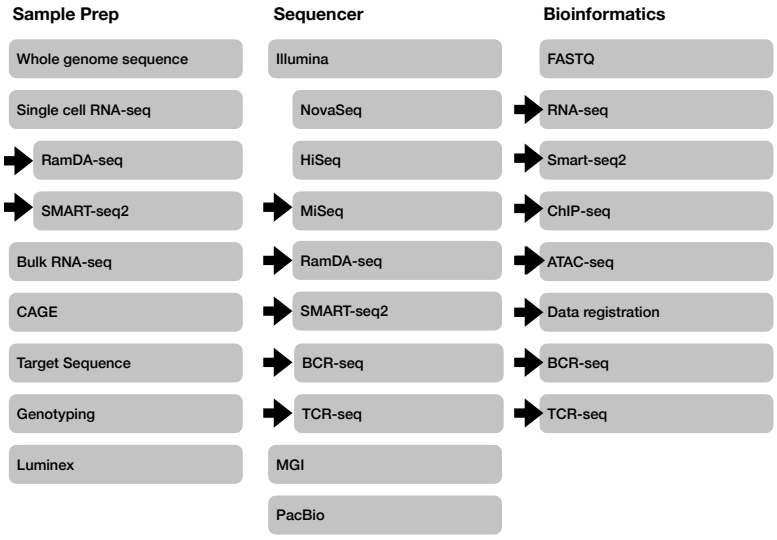


Figure: Solutions provided by the IMS Genome Platform
Various technologies and methodologies are available to all IMS researchers via the IMS Genome Platform (as of Feb 2021). The laboratory for Integrative Genomics provides single-cell RNA-seq technologies and T cell and B cell antigen receptor-related assays.

In 2020, the laboratory for Integrative Genomics went through two major transitions: change in leadership and change in the framework for core-laboratory function. At the end of March 2020, Dr. Ohara retired from his team leader position. From the beginning of the Research Center for Allergy and Immunology (RCAI) in 2001, Dr. Ohara has been leading this laboratory for almost 20 years and been building the fundamental structure of the lab, where the lab members have been exploring original research as well as providing core laboratory functions to the entire institute. On Oct 1st, Dr. Jun Seita assumed the team leader position and a new era has begun.

After several mergers, IMS is now much larger than the original RCAI, and an efficient structure to provide core-laboratory functions had to be different. In 2020, the Center introduced a "Platform", framework for core-laboratory functions and we have participated in the "Genome Platform" launched at the end of September. As shown in the Figure, the "Genome Platform" provides technologies and methodologies around DNA and RNA, and we mainly provide various solutions for single cell RNA seq.

Genomics technology and methodology are currently evolving even faster than before, especially towards single-cell resolution. We will keep pursuing cutting-edge approaches in genomics, implementing new technologies and methodologies, and providing such solutions to all IMS researchers via the Genome Platform.

Recent Major Publications

Okada M, Shimizu K, Iyoda T, Ueda S, Shinga J, Mochizuki Y, Watanabe T, Ohara O, Fujii SI. PD-L1 Expression Affects Neoantigen Presentation. ***iScience*** 23, 101238 (2020)

Nishizono H, Darwish M, Endo TA, Uno K, Abe H, Yasuda R. Glycine receptor α4 subunit facilitates the early embryonic development in mice. ***Reproduction*** 159, 41-48 (2020)

Yazaki J. Novel Protein-oligonucleotide Conjugation Method Involving a High-affinity Capture HaloTag. ***Bio Protoc*** 10, e3759 (2020)

Invited presentations

Seita J. "Toward data-driven medical sciences" Educational Lecture at the 5th Annual Meeting of the Society of Internet of Medical Things (Virtual) December 2020

Seita J. "Deep Learning for Medicine" Symposium at the 50th Annual Meeting of the Japanese Society for Cutaneous Immunology and Allergy (Kochi, Japan) December 2020

Seita J. "Now and future of AI in Medicine" Keynote Lecture at the 61st Annual Meeting of Japanese College of Angiology (Virtual) October 2020

Laboratory for
Mucosal Immunity

Team Leader: Sidonia Fagarasan

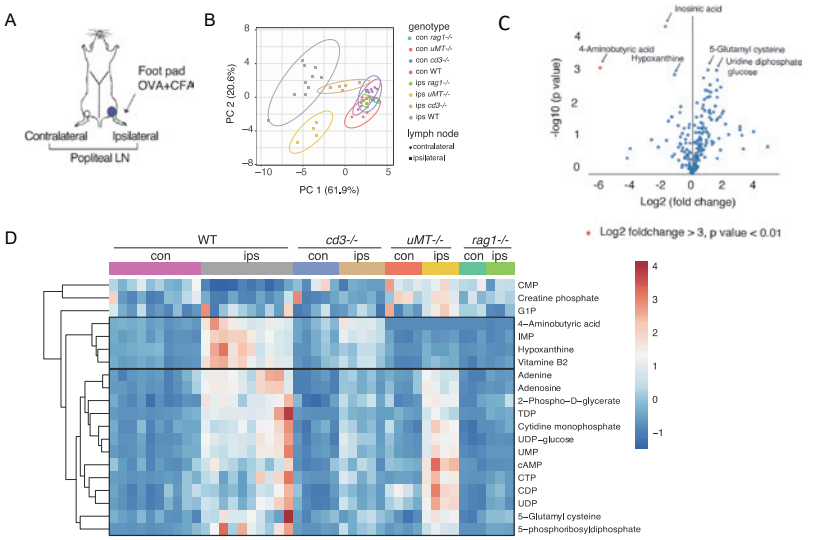


Figure: Metabolic remodeling and B cell-dependent GABA (4-Aminobutyric acid) production in draining lymph nodes upon immunization

A. Footpad immunization scheme; B. Principal component analyses of metabolites extracted from contralateral LN and ipsilateral LN C. Volcano plot and D. Heatmap showing metabolites that differed significantly between contralateral LN and ipsilateral LN.

Recent Major Publications

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)

Guzman-Bautista ER, Suzuki K, Asami S, Fagarasan S. Bacteria-immune cells dialog and the homeostasis of the systems. ***Curr Opin Immunol*** 66, 82-89 (2020)

Hatae R, Chamoto K, Kim YH, Sonomura K, Taneishi K, Kawaguchi S, Yoshida H, Ozasa H, Sakamori Y, Akrami M, Fagarasan S, Masuda I, Okuno Y, Matsuda F, Hirai T, Honjo H. Combination of host immune metabolic biomarkers for the PD-1 blockade cancer immunotherapy. ***JCI Insight***, 5 (2020)

Invited presentations

Fagarasan S. "Shaping of microbial landscape and systemic biochemistry by adaptive immune system" The 9th NIF Winter School on Advanced Immunology (Awaji, Japan) January 2020

Fagarasan S. "The biochemical dialog between major physiological systems mediated by immune cells" MBSJ 2020 (Online) December 2020

Immunometabolism: mapping pathways and identifying metabolites with immune regulatory function

The evolving field of immunoregulation studies how the activity of lymphocytes is shaped by their local environment via a variety of receptor interactions with soluble and cell-bound proteins. However, small metabolites derived from immune cell metabolism are likely present in both intracellular and extracellular milieu *in vivo*, many of which may have signaling potential that we have yet to understand. We hypothesized that water-soluble metabolites provide environmental cues mediating interactions between immune cells to impact on their function. We generated contrasting samples representing immune cell homeostasis and activation using a classic footpad immunization with ovalbumin (OVA) protein emulsified in complete Freund's adjuvant (CFA). We then performed comprehensive metabolome profiling of the ipsilateral (activated, or draining) and contralateral (resting, or non-draining) popliteal lymph nodes (LNs) in WT and immunodeficient animals lacking T cells (*cd3e*$^{-/-}$), B cells (*muMt*$^{-/-}$) or all mature T and B cells (*rag1*$^{-/-}$). We identified the metabolite and neurotransmitter GABA (4-Aminobutyric acid) as a candidate signaling molecule synthesized in a B cell-dependent manner (Figure) and confirmed that GABA is produced and secreted predominantly by activated B cells and plasma cells. We recently found that B cell deficient mice (uMT mice) have enhanced anti-tumor responses (Akrami *et al.*, PNAS, 2020). We tested whether B cell-derived GABA may be limiting cytotoxic T cell activity and indeed found enhanced anti-tumor responses in mice with B cell-specific inactivation of the GABA generating enzyme GAD67. Mechanistically we demonstrate that B cell-derived GABA promotes monocyte differentiation into anti-inflammatory macrophages secreting IL-10 and inhibiting CD8$^+$ T cell killer function. Unraveling the differing metabolic requirements of specific immune subsets has particular relevance in cancer, as a growing number of therapies in development target the metabolic enzymes active in highly-proliferative tumor cells. Understanding the divergent metabolic pathways utilized in B cells, T cells and macrophages 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

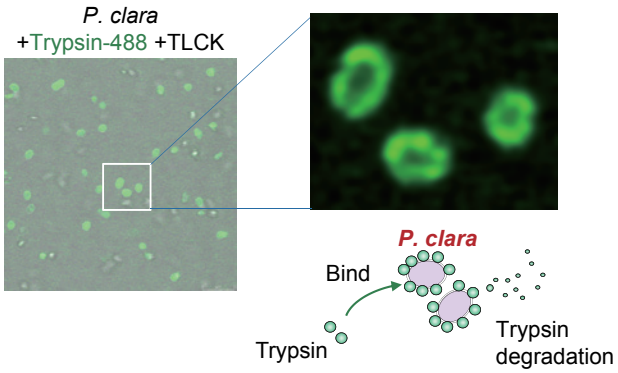


Figure: Trypsin accumulates on the surface of *P. clara*

P. clara was incubated with Alexa488-labelled trypsin in the presence of the trypsin inhibitor TLCK *in vitro*. Trypsin was found to accumulate on the surface of *P. clara* within minutes.

Recent Major Publications

Tuganbaev T, Mor U, Bashiardes S, Liwinski T, Nobs SP, Leshem A, Dori-Bachash M, Thaiss CA, Pinker EY, Ratiner K, Adlung L, Federici S, Kleimeyer C, Moresi C, Yamada T, Cohen Y, Zhang X, Massalha H, Massasa E, Kuperman Y, Koni PA, Harmelin A, Gao N, Itzkovitz S, Honda K, Shapiro H, Elinav E. Diet Diurnally Regulates Small Intestinal Microbiome-Epithelial-Immune Homeostasis and Enteritis. ***Cell*** 182, 1441-1459.e21 (2020)

Nagayama M, Yano T, Atarashi K, Tanoue T, Sekiya M, Kobayashi Y, Sakamoto H, Miura K, Sunada K, Kawaguchi T, Morita S, Sugita K, Narushima S, Barnich N, Isayama J, Kiridooshi Y, Shiota A, Suda W, Hattori M, Yamamoto H, Honda K*. TH1 cell-inducing *Escherichia coli* strain identified from the small intestinal mucosa of patients with Crohn's disease. ***Gut Microbes*** 12, 1788898 (2020)

Finlay BB, Goldszmid R, Honda K, Trinchieri G, Wargo J, Zitvogel L. Can we harness the microbiota to enhance the efficacy of cancer immunotherapy? ***Nat Rev Immunol*** 20, 522-528 (2020)

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, and T_H1 cells. We have also identified 11 bacterial strains that induce IFNγ-producing $CD8^+$ T cells in the gut and enhance the therapeutic efficacy of immune checkpoint inhibitors in mouse syngeneic tumor models. 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 trypsin-degrading bacteria from healthy human feces. Although trypsin is indispensable for the host as one of the digestive enzymes, it must be strictly regulated to avoid host injury due to its strong protease activity. Fecal proteome analysis revealed that trypsin is abundant in the colon of germ-free (GF) mice, but markedly reduced in SPF mouse colons, suggesting that commensal intestinal bacteria play a role in controlling trypsin levels. Furthermore, high trypsin activity was observed in fecal samples from both humans with inflammatory bowel disease (IBD) and IL-10-deficient mice with colitis, indicating that if trypsin remains proteolytically active in the large intestine, it is associated with disturbance of gut homeostasis. This year, we have successfully identified *Paraprevotella clara* and *Paraprevotella xylaniphila* from the microbiome of healthy human donors as potent trypsin-degrading commensals. Mechanistically, multiple lines of evidence suggest that trypsin specifically binds to the surface of *Paraprevotella* spp. through a mechanism involving type IX secretion system-dependent polysaccharide-binding molecules, and that the binding promotes trypsin autolysis. We also found that intestinal colonization with *Paraprevotella* spp. and consequent trypsin reduction prevented degradation of secretory IgA. Moreover, *Paraprevotella* spp. prevented lethal infection by murine hepatitis virus, which is a mouse coronavirus that requires proteolytic cleavage of the spike protein for its entry into host cells. Therefore, a microbiome-based approach targeting excessive proteases may represent a therapeutic strategy for inflammatory and infectious diseases.

Laboratory for
Skin Homeostasis

Team Leader: **Masayuki Amagai**

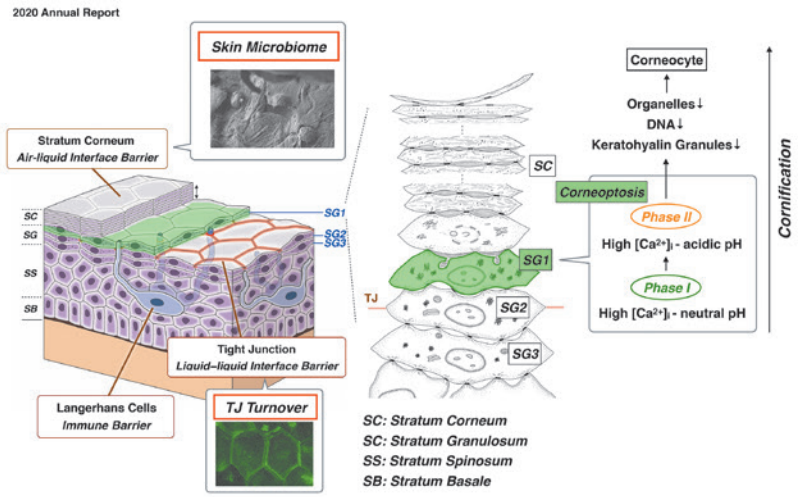


Figure: Comprehensive analysis of skin barrier homeostasis
Our team is trying to clarify the mechanisms of skin barrier homeostasis by focusing on the stratum corneum (SC), tight junction (TJ), and SG1 cells. We established a live imaging system, especially focusing on a unique SG1 cell death pathway termed 'corneoptosis'. By using an optimized plasmid injection method to study the cornification process in mice, we found that corneoptosis occurs in two sequential phases (phase I and II). We also study host-microbe interactions on skin.

Skin is the place where immunity meets external antigens. Cutaneous sensitization is now considered to be the initial key step in many allergic disorders, not only atopic dermatitis (AD), but also asthma, food allergy, and anaphylaxis. Skin harbors several barriers to prevent easy penetration of external antigens into the body. However, the exact molecular mechanisms by which the skin barriers form and are maintained are largely unknown.

Epidermis, the outermost component of the skin, is composed of keratinized stratified squamous epithelia and consists of the stratum basale, stratum spinosum, stratum granulosum (SG) and stratum corneum (SC), from bottom to top (Figure). Our group has been focusing on the SC as an air-liquid barrier and the tight junction (TJ) as a liquid-liquid barrier formed between SG2 cells, among many other skin barriers. There is a fundamental biophysical paradox regarding the function of the epidermis, namely, how it can maintain the barrier, yet still constantly replace and shed cells.

Our group is trying to clarify how epidermal barrier homeostasis in maintained under normal conditions and how impaired barrier function occurs and affects microenvironments of the skin in various disease conditions. 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 begins with an irreversible sustained elevation of intracellular Ca^{2+}, followed by rapid acidification. We demonstrate via intravital imaging procedures that SG1 cells die after prolonged (~60 min) intracellular Ca^{2+} elevation (phase I). Next, irreversibly sustained rapid intracellular acidification is induced (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. 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 severe allergic diseases.

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)

Atsugi T, Yokouchi M, Hirano T, Hirabayashi A, Nagai T, Ohyama M, Abe T, Kaneko M, Zouboulis CC, Amagai M, Kubo A. Holocrine Secretion Occurs outside the Tight Junction Barrier in Multicellular Glands: Lessons from Claudin-1-Deficient Mice. ***J Invest Dermatol*** 140, 298-308. e5 (2020)

Someya T, Amagai M. Toward a new generation of smart skins. ***Nat Biotechnol*** 37, 382-388 (2019)

Invited presentations

Amagai M. "Cracking the codes of autoimmune disease, pemphigus." the 46th annual meeting of Taiwanese Dermatological Association (Kaohsiung, Taiwan/Online) November 2020

Amagai M. "Solving skin barrier homeostatic mechanisms by *in vivo* imaging." UCSF Dermatology Grand Rounds (San Francisco, USA/Online) November 2020

Amagai M. "Homeostatic mechanisms of skin barrier and their disruption in skin inflammation." JSA/WAO Joint Congress 2020 (Kyoto/Online) September 2020

Laboratory for
Tissue Dynamics

Team Leader: Takaharu Okada

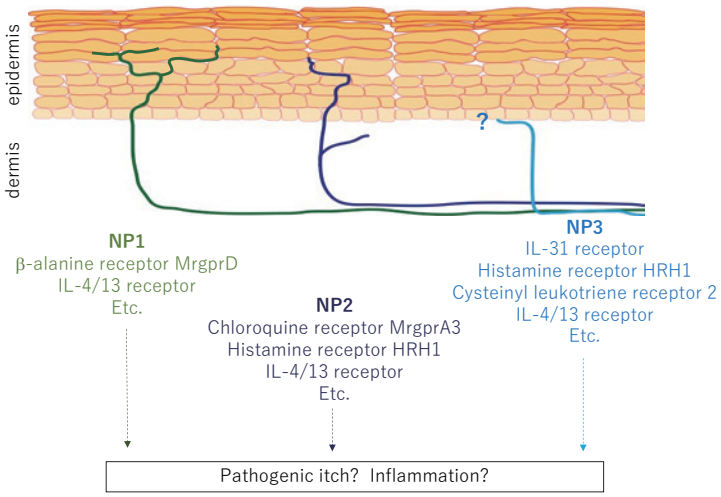


Figure: Subsets of skin sensory nerves differentially express itch-associated receptors
Previous single-cell RNA-sequencing analysis has suggested that sensory neurons expressing itch-associated receptor genes in the mouse dorsal root ganglion can be broadly divided into three subsets, NP1, NP2 and NP3 (Usoskin *et al.*, Nat Neurosci 2015). The NP1 subset innervates the epidermis and most of their nerve endings are thought to be located in the epidermis (Zylka *et al.*, Neuron 2005; Olson *et al.*, Elife 2017). The NP2 subset has also been reported to innervate the epidermis, though it is not clear if the majority of their endings are located in the epidermis (Han *et al.*, Nat Neurosci 2013). It is not known whether NP3 innervates the epidermis.

Recent Major Publications

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)

Mesin L, Schiepers A, Ersching J, Barbulescu A, Cavazzoni CB, Angelini A, Okada T, Kurosaki T, Victora GD. Restricted clonality and limited germinal center reentry characterize memory B cell reactivation by boosting. ***Cell*** 180, 92-106. e11 (2020)

Takahashi S, Ishida A, Kubo A, Kawasaki H, Ochiai S, Nakayama M, Koseki H, Amagai M, Okada T. Homeostatic pruning and activity of epidermal nerves are dysregulated in barrier-impaired skin during chronic itch development. ***Sci Rep*** 9, 8625 (2019)

Invited presentations

Okada T. "Imaging analysis of epidermal sensory nerves and keratinocyte tight junctions" The 58th Annual Meeting of the Biophysical Society of Japan, Symposium 2S-7 (Online) September 2020

Okada T. "Dynamic homeostasis of epidermal sensory nerves and its breakdown caused by barrier dysfunction" The 119th Annual Meeting of the Japanese Dermatological Association, Sponsored Symposium 3 (Online) June 2020

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, which express distinct sets of the itch-associated receptor genes (Figure). 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. In addition, we are performing 3D imaging analyses of the itch nerve subsets in the skin and have found that the three subsets innervate different anatomical regions of the skin. Furthermore, this innervation pattern becomes altered in the atopic dermatitis models. We are also investigating gene expression changes in the sensory neurons in atopic dermatitis models by single-cell RNA-sequencing analyses. As a result, we have found that multiple genes potentially responsible for the altered innervation pattern are upregulated or downregulated in the subsets of itch neurons. We will continue functional analysis of the itch nerve subsets based on these findings.

Laboratory for Metabolomics

Team Leader: Makoto Arita

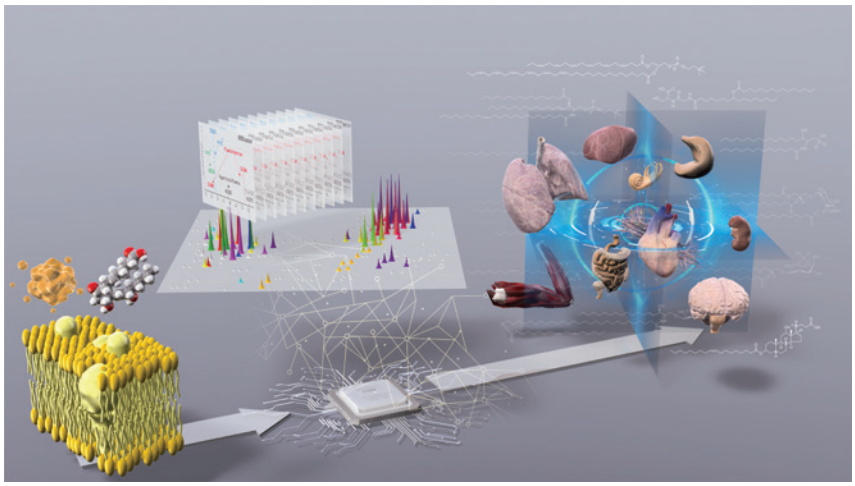


Figure: Untargeted lipidomics to reveal the connection between lipid metabolism and tissue homeostasis

Lipids are extremely diverse molecules, but their diversity, structure, and exact function are not well understood. Precise determination of each molecular species of lipid is a prerequisite for understanding their functions in physiology and disease, and for discovering novel, bioactive lipid-derived mediators that may have therapeutic benefits. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a powerful method for analyzing lipid metabolites, providing insight into the structure and function of endogenous lipid metabolites that regulate inflammation and tissue homeostasis.

We established an untargeted 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 that includes their mass spectrum properties such as retention time, collision cross section and mass fragmentation pattern to correctly characterize lipids, and this has been published as a "lipidome atlas". This untargeted lipidomics platform is a powerful technology for visualizing lipid networks globally. It also provides an opportunity for data-driven hypothesis generation to make the connection between lipid metabolism and biological phenotypes. We applied this system to understand the gut microbiota-associated unique lipid structures and their complex metabolic networks in mice.

Recent Major Publications

Yasuda S, Okahashi N, Tsugawa H, Ogata Y, Ikeda K, Suda W, Arai H, Hattori M, Arita M. Elucidation of gut microbiota-associated lipids using LC-MS/MS and 16S rRNA sequence analyses. ***iScience*** 23, 101841 (2020)

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)

Ogawa M, Ishihara T, Isobe Y, Kato T, Kuba K, Imai Y, Uchino Y, Tsubota K, Arita M. Eosinophils promote corneal wound healing via the 12/15-lipoxygenase pathway. ***FASEB J*** 34, 12492-12501 (2020)

Invited presentations

Arita M. "Biology of LipoQuality: Advanced lipidomics technology and its application in biology" The 43rd Annual Meeting of the Molecular Biology Society of Japan (Online) December 2020

Arita M. "Advanced lipidomics technology and its application in biology" The 45th Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry (Online) September 2020

Arita M. "Linking lipid metabolism and inflammatory disorders" The 93rd Annual Meeting of the Japanese Biochemical Society (Online) September 2020

Arita M. "Biology of LipoQuality: Omega-3 fatty acid cascade that controls inflammation and tissue homeostasis" Science Webinar (Online) May 2020

Laboratory for
Microbiome Sciences

Team Leader: Hiroshi Ohno

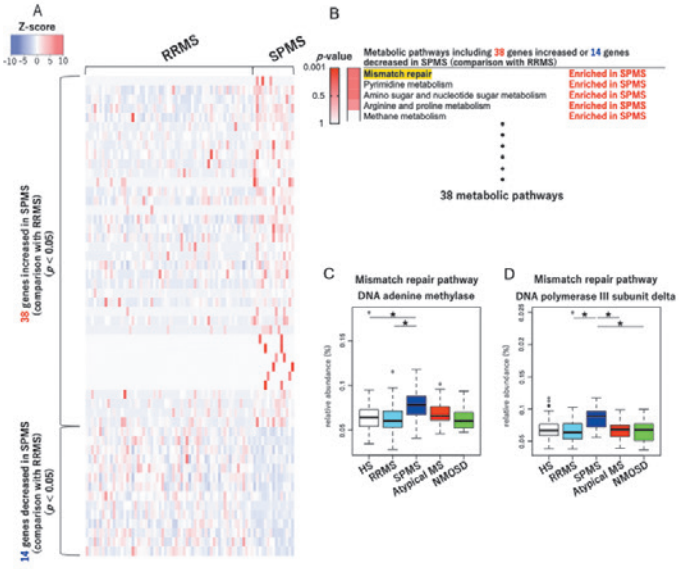


Figure: Microbial function based on metagenomic data (comparison between relapsing-remitting MS patients and secondary progressive MS patients)

(A) 38 genes were increased and 14 were decreased in patients with secondary progressive MS when compared with healthy subjects. (B) We ranked the metabolic pathways including these genes in descending order of the degree of increase or decrease between the two groups. (C, D) Among the genes included in the mismatch repair pathway, the relative abundances of two genes, DNA adenine methylase and DNA polymerase III delta subunit, were increased in secondary progressive MS patients when compared to relapsing-remitting MS patients. HS, Healthy Subjects; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; NMOSD, Neuromyelitis Optica Spectrum Disorder. *p <0.05 (Wilcoxon rank sum test). PNAS, 117:22402-22412, (Takewaki D, Suda W† *et al*. ***Proc Natl Acad Sci U S A.*** 117, 22402-22412 (2020) †Corresponding author)

Recent Major Publications

Takewaki D, Suda W†, Sato W, Takayasu L, Kumar N, Kimura K, Kaga N, Mizuno T, Miyake S, Hattori M, Yamamura T†. Alterations of the gut ecological and functional microenvironment in different stages of multiple sclerosis. ***Proc Natl Acad Sci U S A*** 117, 22402-22412 (2020)

Masuoka H, Suda W†, Tomitsuka E, Shindo C, Takayasu L, Horwood P, Greenhill AR, Hattori M, Umezaki M, Hirayama K. The influences of low protein diet on the intestinal microbiota of mice. ***Sci Rep*** 10, 17077 (2020)

Miyauchi E, Kim SW, Suda W, Kawasumi M, Onawa S, Taguchi-Atarashi N, Morita H, Taylor TD, Hattori M, Ohno H†. Gut microorganisms act together to exacerbate inflammation in spinal cords. ***Nature*** 585, 102-106 (2020)

Invited presentations

Suda W. "Recent advance in human gut microbiome study" International Workshop on Eukaryotic Microbiome (Tokyo, Japan) March 2020

The Laboratory for Microbiome Sciences has been engaged in the study of 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.

Our team has published 19 papers in FY2020. In one representative paper, we revealed for the first time the ecological and functional differences in the gut microbiome between patients with relapsing-remitting multiple sclerosis (RRMS) and secondary progressive multiple sclerosis (SPMS) (PNAS, 117:22402-22412, 2020). Whole metagenomic analysis of RRMS and SPMS samples revealed that the gut microbiome of SPMS patients has an enhancement in oxidative stress and microbial DNA repair, which suggests a possible association between gut-derived oxidative stress and chronic neuroinflammation (Figure). These breakthrough results could lead to the development of innovative treatments for progressive MS.

Currently we are working on a longitudinal metagenomic analysis of the mouse gut microbiome from birth to death. Despite a shared genetic background, we observed diverse phenotypes of hosts and related transitions of the gut microbiomes. We also found a strong association between lifespan and temporal dynamics of the abundance of several microbes. Most of these microbes were "life-core" microbes that are observed at most periods throughout the life of the mouse. This study implies the possibility of a deep association between lifespan and the temporal dynamics of the abundance of "life-core" microbes. Understanding the relationship between microbial dynamics and the host could lead to future control of human health based on gut microbiome time-course monitoring.

Laboratory for

Integrated Cellular Systems

Team Leader: **Katsuyuki Yugi**

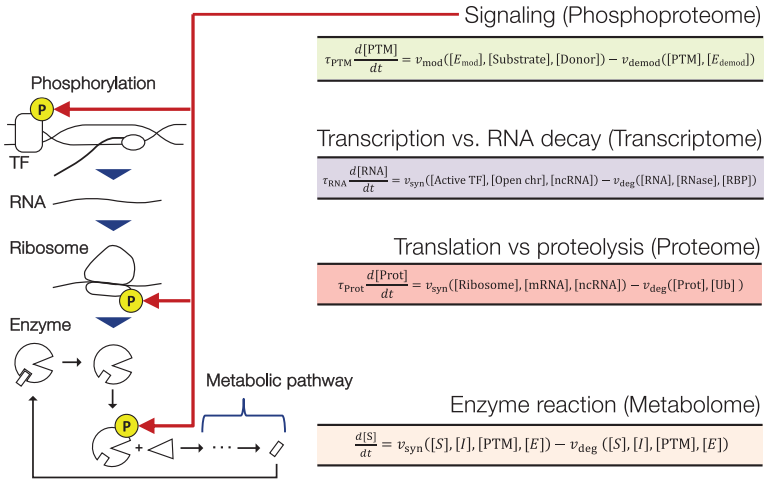


Figure: Differential equation representation of a trans-omic network
We integrate multiple omic data, postulating a dynamic picture of cellular processes driven by reaction kinetics. Each reaction rate (terms represented by '*v*') is a function of the number of molecules that belong to the same or different 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).

Recent Major Publications

Kokaji T, Hatano A, Ito Y, Yugi K, Eto M, Morita K, Ohno S, Fujii M, Hironaka KI, Egami R, Terakawa A, Tsuchiyal 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)

Hoshino D, Kawata K, Kunida K, Hatano A, Yugi K, Wada T, Fujii M, Sano T, Ito Y, Furuichi Y, Manabe Y, Suzuki Y, Fujii NL, Soga T, Kuroda S. Trans-omic Analysis Reveals ROS-dependent pentose phosphate pathway activation after high-frequency electrical stimulation in C2C12 myotubes. ***iScience*** 23, 101558 (2020)

Ohno S, Quek LE, Krycer JR, Yugi K, Hirayama A, Ikeda S, Shoji F, Suzuki K, Soga T, James DE, Kuroda S. Kinetic trans-omic analysis reveals key regulatory mechanisms for insulin-regulated glucose metabolism in adipocytes. ***iScience*** 23, 101479 (2020)

Invited presentations

Yugi K. "Trans-omics: Integration of multiple omic data on the basis of reaction kinetics" Informatics in Biology, Medicine and Pharmacology 2020 (Online) September 2020

Yugi K. "Unwritten tips and secrets of trans-omics: experimental designs, and inconspicuous technologies" Technical seminar for young researchers of the MEXT Grant-in-Aid for Scientific Research on Innovative Areas "Transomic Analysis of Metabolic Adaptation" (Online) August 2020

Yugi K. "Development of next-generation trans-omics technologies for the characterization of psychiatric disorders" Annual Meeting of the MEXT Grant-in-Aid for Scientific Research on Innovative Areas "Constructive understanding of multi-scale dynamism of neuropsychiatric disorders" 2020 (Online) July 2020

Yugi K. "Reconstruction of insulin signal flow from phosphoproteome and metabolome data" KI–RIKEN Joint International Doctoral Course 2020 "Bioinformatics analysis of gene regulation in omics data and its applications to medical problems" (Stockholm, Sweden) March 2020

Metabolism is a biological process involved in various diseases, not only metabolic diseases such as obesity and diabetes, but also autoimmune diseases, psychiatric diseases, 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). 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.

Drug Discovery Antibody Platform Unit

Unit Leader: Takashi Saito

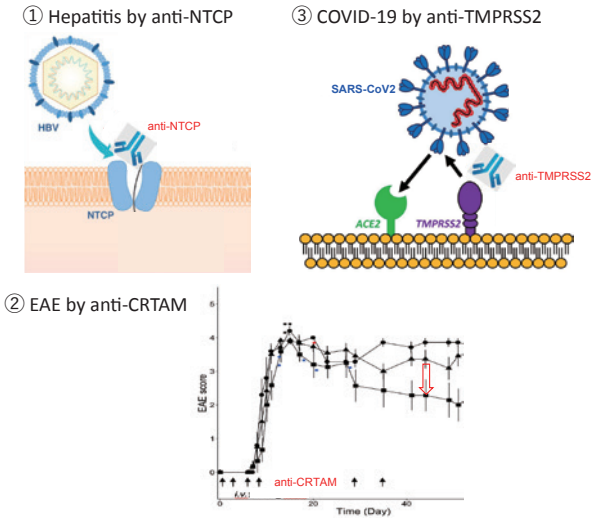


Figure: Development of monoclonal antibodies for therapeutic use targeting various diseases
Monoclonal Abs have been developed to modulate/inhibit the diseases of viral hepatitis ①, SARS-CoV2 infection ②, Multiple sclerosis mouse model (experimental autoimmune encephalomyelitis) ③, and cancer.

Recent Major Publications

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)

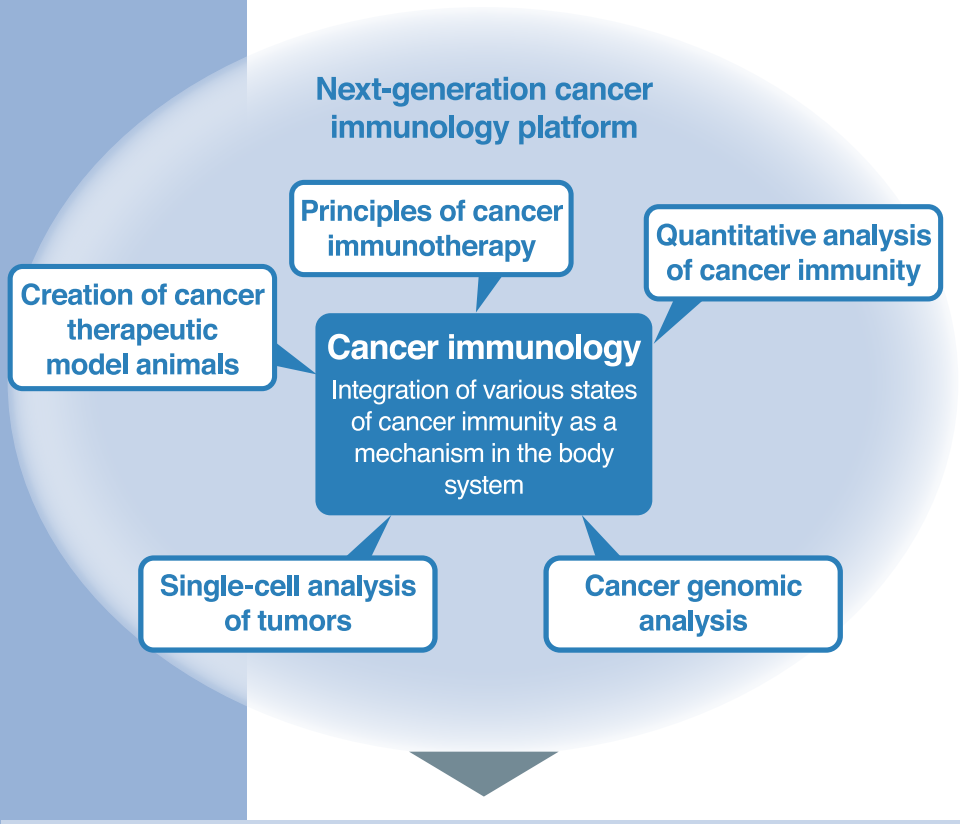
Nakano T, Ochiai S, Suzuki S, Yamaide F, Morita Y, Inoue Y, Arima T, Kojima H, Suzuki H, Nagai K, Morishita N, Hata A, Shozu M, Suzuki Y, Taniguchi M, Takemori T, Kohno Y, Shimojo N. Breastfeeding promotes egg white sensitization in early infancy. ***Pediatr Allergy Immunol*** 31, 315-318 (2020)

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.

1) We have developed a mAb against a human receptor of hepatitis B virus (HBV), NTCP, in the last few years. Because HBV binds via the preS1-domain of the viral L protein to the apical sodium-acid dependent bile acid transporter (NTCP), NTCP could be a key target for the development of anti-HBV agents. Indeed, the established mAb inhibits the entry of HBV into human liver cells *in vitro* and further inhibits *in vivo* infection in a system using human-liver chimeric mice. Human chimeric NTCP mAbs are being developed for further clinical applications. 2) 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 the induction of IBD, inflammatory bowel diseases, as well as late-phase induction of EAE, experimental autoimmune encephalomyelitis. Several established mAbs were tested for their ability to modulate these diseases in mice. Indeed, preliminary results showed a significant inhibition of late-phase of EAE induction by administration of a CRTAM mAb, suggesting potential therapeutic use of mAbs. 3) More recently, we have tried to establish 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. However these small molecules also have some side-effects, therefore the mAbs against TMPRSS2 may have more specific inhibitory activity and possibly modulate COVID-19 (Figure). Furthermore, we are also trying to generate mAbs specific for various cancers.

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
Immunogenetics

Team Leader: Tadashi Yamamoto

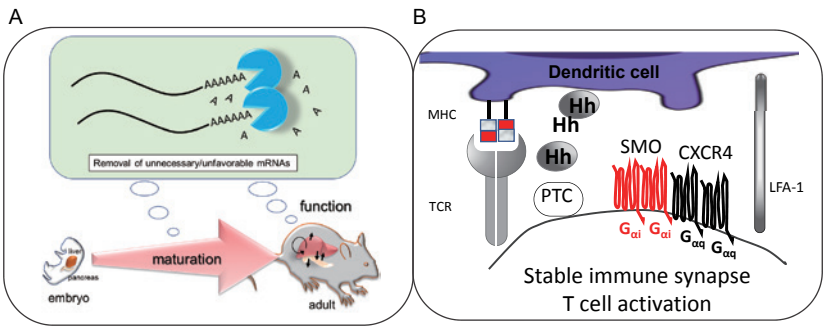


Figure: Research projects in the laboratory
A. A role for mRNA deadenylation in tissue development and function.
The CCR4-NOT complex promotes mRNA decay by shortening the polyA tails of mRNAs. Reduction of unnecessary mRNAs such as those encoding immature state- or cell death-related molecules ensures proper tissue development and function.
B. Smoothened competes with CXCR4 for $G_{\alpha i}$ coupling to fortify the immune synapse and regulate T cell activation.
The Hedgehog signaling pathway is active at the immune synapse between a naïve T call and a DC, where the signal transducer Smoothened (SMO) co-localizes with CXCR4 and forms heterodimers. SMO has a higher affinity for $G_{\alpha i}$ and, by depriving the available pool of $G_{\alpha i}$ proteins, it forces switching of CXCR4 signaling from $G_{\alpha i}$ to $G_{9/11}$. This is the mechanism that allows naïve T cells to settle on the surface of a DC and become unresponsive to migratory cues, thus leading to the formation of a stable immune synapse.

Recent Major Publications

Ito-Kureha T, Miyao T, Nishijima S, Suzuki T, Kozumi S-I, Villar-Brioness A, Takahashi A, Akinama N, Morita M, Nagura I, Ishikawa H, Ichijo H, Akinama T, Yamamoto T. The CCR4-NOT deadenylase complex safeguards thymic positive selection by down-regulating aberrant pro-apoptotic gene expression. ***Nat Commun*** 11, 6169 (2020)

Mostafa D, Yanagiya A, Georgiadou E, Wu Y, Stylianides T, Rutter GA, Suzuki T, Yamamoto T. Loss of β-cell identity and diabetic phenotype in mice caused by disruption of CNOT3-dependent mRNA deadenylation. ***Commun Biol*** 3, 476 (2020)

Takahashi A, Suzuki T, Soeda S, Takaoka S, Kobori S, Yamaguchi T, Mohamed H, Yanagiya A, Abe T, Shigeta M, Furuta Y, Kuba K, and Yamamoto T. The CCR4-NOT complex maintains liver homeostasis through mRNA deadenylation. ***Life Sci Alliance*** 3, e201900494 (2020)

Invited presentations

Yamamoto T. "The CCR4-NOT complex maintains liver homeostasis through mRNA deadenylation" The 43rd Annual Meeting of the Molecular Biology Society of Japan (Online) December 2020

1) Control of mRNA stability is one of the essential post-transcriptional mechanisms to regulate gene expression levels. We have found that mRNA deadenylase, an enzyme that triggers mRNA degradation by shortening the polyA tail, ensures normal development and function in tissues such as liver, adipocytes, pancreatic β-cells and T-cells (Figure A). The mRNA deadenylase targets different mRNAs depending on the cell type and developmental stage. While the CCR4-NOT complex regulates the level of mRNAs encoding transcription factors, cell cycle regulators and DNA damage response-related proteins in liver, the complex regulates progenitor cell markers and β cell-disallowed genes in pancreatic β-cells. When function of the CCR4-NOT complex is disrupted, those genes are abnormally increased, resulting in altered tissue function and disease phenotypes such as hepatitis and diabetes. These data suggest that the CCR4-NOT complex contributes to tissue homeostasis by modulating the transcriptome through mRNA deadenylation. We have been investigating molecular mechanism by which the mRNA deadenylase recognizes target mRNAs depending on their context. The mRNA deadenylase also influences transcriptional activity of various genes. Our long-term goal is to understand how transcription and mRNA decay coordinate proper mRNA levels for tissue homeostasis.

2) The morphogen Hedgehog (Hh) is responsible for patterning tissues during metazoan development. Hh pathway components localize to the immune synapse in a dynamic way and our results suggest involvement of Hedgehog in sustainable signaling at the immune synapse. We aim to understand how Hedgehog signaling at the immune synapse interacts with other T cell signaling pathways and how they are affected in cancer and autoimmunity (Figure B).

Laboratory for Medical Science Mathematics

Team Leader: Tatsuhiko Tsunoda

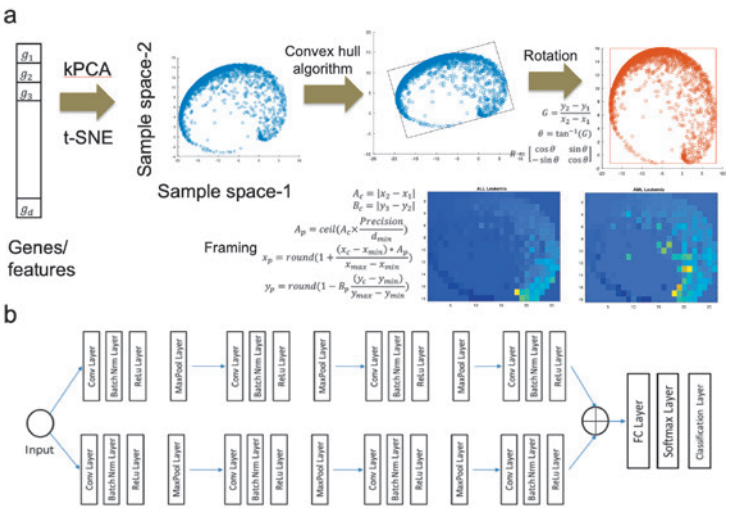


Figure: DeepInsight – deep learning for 'omic data analysis
(a) Pipeline. Transformation from feature vector to image pixels. (b) Parallel convolutional neural network (CNN) architecture used in DeepInsight.

Effective utilization of rapidly developing 'omic profiling technologies and, in particular, the introduction of personalized/precision/preventive medicine have recently become major goals of medical research. This paradigm shift requires moving away from traditional approaches that do not adequately consider the individual characteristics of each patient. Our laboratory develops strategies to address these challenges by bringing the 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 types of anti-cancer immune responses, using molecular profiles and then we clarify 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 past and current 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 and application 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) Developing AI and deep learning technologies for image and 'omic data analyses.

Recent Major Publications

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)

Choobdar S, Ahsen ME, Crawford J, Tomasoni M, Fang T, Lamparter D, *et al*., Assessment of network module identification across complex diseases. ***Nature Methods*** 16, 843-852 (2019)

Sharma A*, Vans E, Shigemizu D, Boroevich KA, Tsunoda T*. DeepInsight: a methodology to transform a non-image data to an image for convolution neural network architecture. ***Scientific Reports*** 9, 11399 (2019)

Invited presentations

Sharma A. "CNN to non-image data by DeepInsight method" MATLAB EXPO (Japan/Online) September 2020

Tsunoda T. "Personalized Cancer Medicine with Heterogeneity and Immunological Analysis." CREST International Symposium on Big Data Application (Tokyo, Japan) January 2020

Laboratory for
Cancer Genomics

Team Leader: Hidewaki Nakagawa

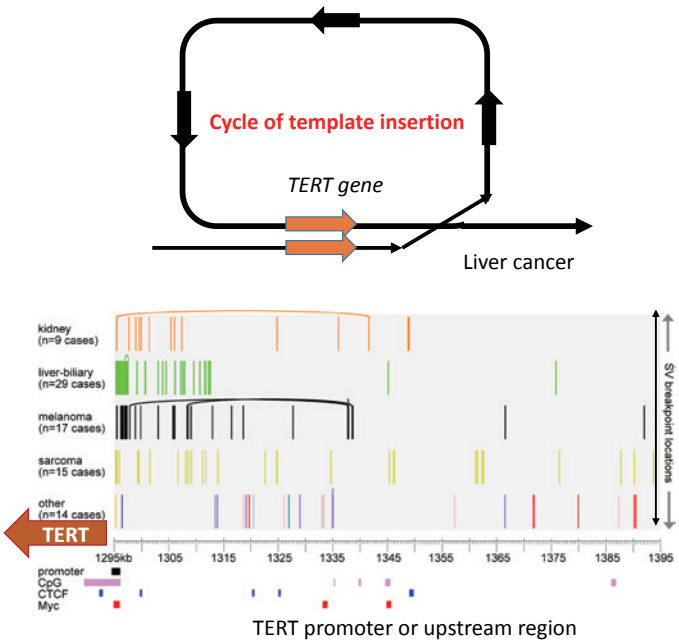


Figure: Diverse structural variants (SVs) of the *TERT* gene that were found in a pan-cancer whole genome sequencing (PCAWG) project
Cycles of "template insertion" of the *TERT* gene were identified in liver cancers (upper). Diverse SVs in the promoter or upstream region of the *TERT* gene were frequently observed in kidney cancers, hepatobiliary cancers, melanomas, and sarcomas (lower). SV breakpoint locations within the region ~100kb upstream of *TERT*. The curved line connects two breakpoints common to the same SV.

Recent Major Publications

Kawasaki K, Toshimitsu K, Matano M, Fujita M, Fujii M, Togasaki K, Ebisudani T, Shimokawa M, Takano A, Takahasi S, Ohta Y, Nanki K, Igarashi R, Ishimaru K, Shida H, Sukawa Y, Saito Y, Sasagawa S, Lee H, Ha K, Fukunaga K, Tanabe M, Ishihara S, Hamamoto Y, Yasuda H, Sekine S, Kudo A, Kitagawa Y, Kanai T, Nakagawa H, and Sato T. An organoid biobank of rare human neuroendocrine neoplasms enables genotype-phenotype mapping. ***Cell*** 183, 1420-35 (2020)

Fujimoto A*, Fujita M, Maejima K, Hasegawa T, Nakano K, Oku-Sasaki A, Wong J, Shiraishi Y, Miyano S, Imoto S, Akagi T, and Nakagawa H*. Comprehensive analysis of indels in whole-genome microsatellite regions and microsatellite instability across 21 cancer types. ***Genome Res*** 30, 334-346 (2020)

The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. ***Nature*** 578, 82-93 (2020)

Invited presentations

Nakagawa H. "GWAS and polygenic risk score (PRS) for prostate cancer" The 64th Annual meeting of the Japan Society of Human Genomic (Nagoya, Japan) November 2020

Nakagawa H. "Whole genome and immuno-genomic analysis of liver cancer" The 79th Annual meeting of the Japanese Cancer Association (Hiroshima, Japan) October 2020

Nakagawa H. "Cancer Whole Genome Sequencing for Genomic Medicine" Annual meeting of Japan Society of Urologic Oncology (Kyoto, Japan) October 2020

Nakagawa H. "Cancer Whole-Genome Sequencing" Annual meeting of The Japanese Society for Hereditary Cancer (Osaka, Japan) August 2020

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 discovery of genomic biomarkers and personalized cancer medicine.

Laboratory for Immunotherapy

Team Leader: Shin-ichiro Fujii

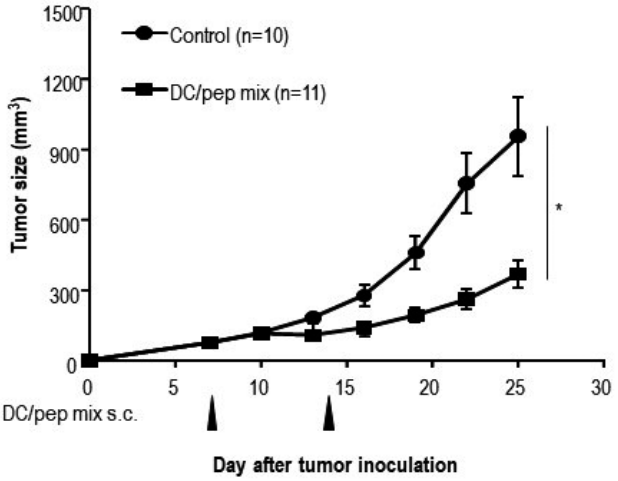


Figure: Therapeutic efficacy of DCs pulsed with multiple neoantigen epitopes
We screened neoantigen epitopes in murine colorectal cancer MC38 cells and identified three H2-Kb restricted, somatically-mutated immunogenic neoantigens. MC38 cells were administered to mice s.c. One week later, the mice were treated with neoantigen-pulsed DCs twice at a one-week interval. Immunotherapy with neoantigen peptide-pulsed DCs resulted in decreased tumor growth in the MC38 tumor-bearing mice.

Recent Major Publications

Okada M, Shimizu K, Iyoda T, Ueda S, Shinga, Mochizuki Y, Watanabe T, Ohara O, Fujii S. PD-11 expression affects neoantigen presentation. ***iScience*** 23, 101238 (2020)

Seo W, Shimizu K, Kojo S, Okeke A, Kohwi-Shigematsu T, Fujii S, Taniuchi I. Runx-mediated regulation of CCL5 via antagonizing two enhancers influences immune cell function and anti-tumor immunity. ***Nat Commun***. 11, 1562 (2020)

Shimizu K, Iyoda T, Yamasaki S, Kadowaki N, Tojo A, Fujii S. NK and NKT Cell-Mediated Immune Surveillance Against Hematological Malignancies. ***Cancers (Basel)*** 12, 817 (2020)

Invited presentations

Fujii S. "Development of artificial adjuvant vector cells (aAVC) inducing both innate and adaptive immunity" The 41st Annual Scientific Meeting of the Japanese Society of Clinical Pharmacology and Therapeutics (Fukuoka, Japan) December 2020

Fujii S. "Immune cells in tumor microenvironment" The 82nd Annual Meeting of the Japanese Society of Hematology (Japan/Online) October 2020

Fujii S. "Anti-cancer therapeutic cellular drug, artificial adjuvant vector cells by targeting *in vivo* dendritic cells" The 79th Annual Meeting of the Japanese Cancer Association (Hiroshima, Japan) October 2020

Fujii S. "Development of anti-cancer therapeutic cellular drug as *in vivo* DC targeting therapy artificial adjuvant vector cells (aAVC)" The 12th Annual Meeting of Japanese Scoiety of Immunotherapy for Hematological Disorders (Osaka, Japan) September 2020

Fujii S. "Tumor-Immune network and recent progress in Cancer Immunotherapy" The 106th General Meeting of the Japanese Society of Gastroenterology (Hiroshima, Japan) August 2020

We have worked to harness the innate and adaptive immune systems, resulting in the induction of immunological memory against cancer. To this end, we have performed both basic immunology and translational studies. We have focused on the role of dendritic cells (DCs) *in vivo*, and in particular on two ongoing projects in cancer immunology and infectious disease in the current year. First, we developed an immunotherapeutic strategy using tumor-derived neo-antigens. We identified three H2-K^b-restricted, somatically-mutated epitopes as immunogenic neoantigens and showed their efficacy using DCs (Figure). Second, we extended the artificial adjuvant vector cells (aAVC) system to COVID-19. We have been developing SARS-CoV-2-derived antigen-expressing aAVC (aAVC-Cov-2). We will examine a proof of concept in anti-viral cytotoxic T cell induction as well as anti-SARS-CoV-2 Ab production in vaccinated animals. We have been working on two translational research projects. We finished the phase II NKT cell therapy for NSCLC patients trial in collaboration with the National Hospital Organization (NHO) and found that the NKT and NK cell responses in treated patients were stronger than those in untreated patients. In another study, we have established an investigator-initiated phase I clinical trial for relapsed and refractory acute myelogenous leukemia using aAVC-expressing Wilms Tumor (WT1) antigen (aAVC-WT1) therapy. This trial has been conducted at the Department of Hematology/Oncology, the Institute of Medical Science, the University of Tokyo in a collaboration started in 2017. We have been engaged in the immunological analyses of the aAVC-WT1 treated patients. We are now preparing for the phase II clinical trial.

Laboratory for
Human Disease Models

Team Leader: **Fumihiko Ishikawa**

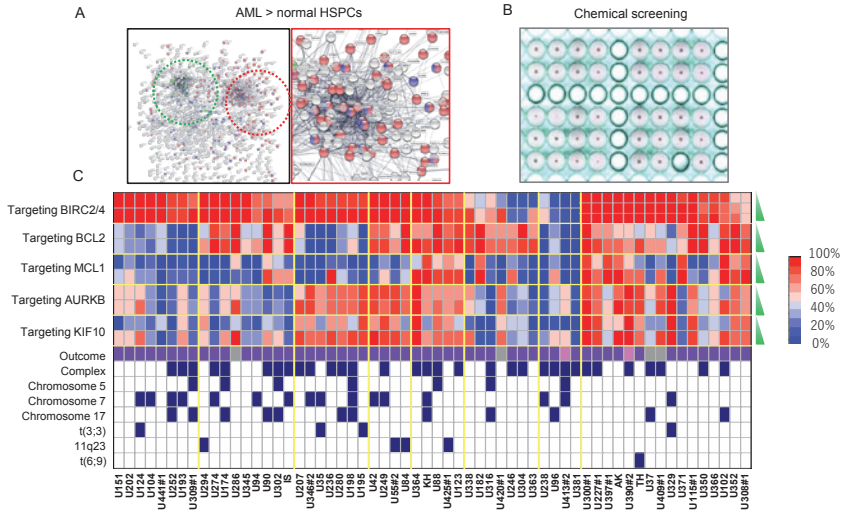


Figure: Identification of patient leukemia-specific vulnerabilities

(A) Interaction among genes upregulated in AML compared with normal HSPCs is shown (left). Genes in the red circle are related with cellular survival. Interaction among the cell survival/cell death related genes is shown in the right panel. (B) *In vitro* chemical screening was setup using 36 compounds targeting molecules that were chosen from RNAseq results. (C) Viability of leukemic cells from 60 AML cases after treatment with five compounds identified in (B) is presented as a heat map. Purple columns in the outcome row indicate that the patients were deceased, gray (unknown) and pink (alive). Seven poor prognostic chromosomal aberrations are shown at the bottom.

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. ***Nature 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

Hashimoto M. "Integrative transcriptomic and chemical screening identifies patient-specific vulnerabilities in poor-prognosis AML" Japan Society of Hematology Annual Meeting 2020 (Kyoto, Japan) October 2020

Ishikawa F. "Identification of vulnerabilities in genetically-complex AML" Australian Blood Club by Australian Society of Hematology (Online) October 2020.

Ishikawa F. "Creating Treatment Strategies for Poor Prognosis Acute Myeloid Leukemia" Cancer Short Course at The Jackson Laboratory (Online) September 2020

Ishikawa F. "Creating Treatment Strategies for Poor Prognosis Acute Myeloid Leukemia" Immuno-oncology workshop at National Cancer Institute (Online) July 2020

In 2017, through single cell DNA sequencing and xenogeneic transplantation, we identified mutated FLT3 as a critical molecule directly associated with *in vivo* leukemia initiation. Further, imbalance between anti-apoptotic and pro-apoptotic molecules at the inner mitochondrial membrane causes treatment resistance against FLT3 inhibition. Since the FLT3 mutation is detected in 20% of patients with acute myeloid leukemia (AML), we set out to identify therapeutic targets in the remaining 80% of AML patients. To this end, we integrated multiple methods such as DNA sequencing, RNA sequencing, and chemical screening using leukemia initiating cells derived from poor prognosis patients. From bioinformatic analyses of transcriptome data, we found that several pathways such as anti-apoptosis, cell cycle, and immune regulation were enriched in AML initiating cells as compared with normal hematopoietic stem/progenitor cells (HSPCs). With chemical screening using 36 compounds, we further identified five compounds targeting BIRC2/4, BCL2, MCL1, AURKB, and KIF10 that could effectively eliminate patient AML cells. Using two compounds to which patient-derived AML cells showed the highest sensitivity, we confirmed that potent therapeutic efficacy could be achieved in a patient-derived xenograft model. Finally, we aimed to connect chromosomal aberrations and somatic mutations with drug sensitivity/resistance in individual patients. This study might provide potentially curative precision medicine for AML patients who are not cured with current medical therapeutics.

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**

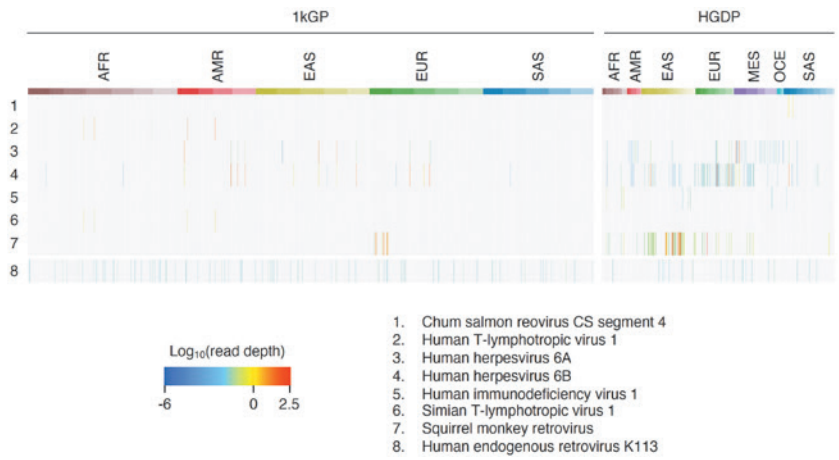


Figure: Virus-derived variation in 3,332 diverse human genomes
We analyzed 3,332 whole genome sequencing datasets for sequences derived from viruses. The heatmap shows read depth of seven viruses with abundant reads in at least one dataset, as well as variation in reads mapping to a HERV. The column colors represent the human populations present in two databases, the 1,000 Genomes Project (1kGP) and the Human Genome Diversity Project (HGDP). AFR: African; AMR: American; EAS: East Asian; EUR: European; SAS: South Asian.

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. Human herpesvirus 6 (HHV-6) infection was recently proposed to be influenced by piRNAs (Liu S *et al.*, *Cell*. 2018). 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 *et al.*, *bioRxiv*. 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

Sakashita A, Maezawa S, Takahashi K, Alavattam KG, Yukawa M, Hu YC, Kojima S, Parrish NF, Barski A, Pavlicev M, Namekawa SH. Endogenous retroviruses drive species-specific germline transcriptomes in mammals. ***Nat Struct Mol Biol*** 27, 967-977 (2020)

Liu X, Kosugi S, Koide R, Kawamura Y, Ito J, Miura H, Matoba N, Matsuzaki M, Fujita M, Kamada AJ, Nakagawa H, Tamiya G, Matsuda K, Murakami Y, Kubo M, Sato K, Momozawa Y, Ohashi J, Terao C, Yoshikawa T, Parrish NF, Kamatani Y. Endogenization and excision of human herpesvirus 6 in human genomes. ***PLoS Genet*** 16, e1008915 (2020)

Ophinni Y, Palatini U, Hayashi Y, Parrish NF. piRNA-Guided CRISPR-like Immunity in Eukaryotes. ***Trends Immunol*** 40, 998-1010 (2019)

Invited presentations

Parrish NF. "Immunity Induced by Endogenous Viral Elements" The 24th Annual Meeting of the Japanese Society for Vaccinology (Aichi, Japan/Online) December 2020

Parrish NF. "Virus-derived structural variation in human genomes: new phenotypes from old viruses" The 43rd Annual Meeting of the Molecular Biology Society of Japan (Online) December 2020

Parrish NF. "Human genome plasticity via horizontal gene transfer from human herpesvirus 6" Society for Molecular Biology and Evolution Annual Meeting (Online) June 2020

YCI Laboratory for

Immunological Transcriptomics

Young Chief Investigator: **Hideyuki Yoshida**

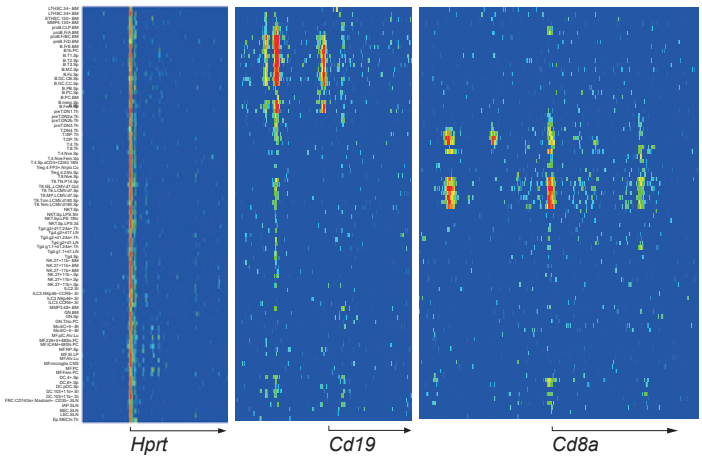


Figure: Cell type-specific structure of chromatin
"Open" and "closed" chromatin in various immune cells was examined by ATAC-seq and is shown by a color gradient (open=red, closed=blue). Since the transcriptional regulatory elements are often found in "open" regions, profiling of open chromatin promotes a clearer understanding of the mechanisms of gene regulation. *Hprt*, housekeeping gene; *Cd19*, B cell-specific gene; *Cd8a*, cytotoxic T cell-specific gene. The base of the arrow under each gene label indicates the transcription start site.

Recent Major Publications

Tenno M, Wong AYW, Ikegaya M, Miyauchi E, Seo W, See P, Kato T, Taida T, Oishi-Ohno M, Ohno H, Yoshida H, Ginhoux F, Taniuchi I. Essential functions of Runx/Cbfβ in gut conventional dendritic cells for priming Roryt^{+} T cells. ***Life Sci Alliance*** 3, e201900441 (2020)

Gal-Oz ST, Maier B, Yoshida H, Seddu K, Elbaz N, Czysz C, Zuk O, Stranger BE, Ner-Gaon H, Shay T. ImmGen report: sexual dimorphism in the immune system transcriptome. ***Nat Commun*** 10, 4295 (2019)

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. The cis-Regulatory Atlas of the Mouse Immune System. ***Cell*** 176, 897-912 (2019)

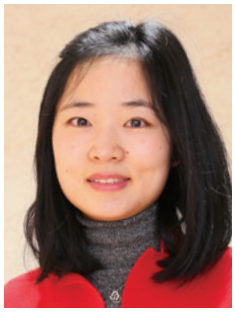
Gene regulation is one of the most elemental mechanisms governing cell functions and biological processes, including immune cells and the immune system, and has been studied in many contexts. Recent advances in epigenome and transcriptome profiling, which take advantage of next-generation sequencing (NGS), enable us to investigate gene regulation in an unprecedented manner and, hence, the uncharted mechanisms in biology and immunology are now becoming approachable. 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. Transcriptomic approaches can be applied to various studies in immunological settings and we have been engaged in 1) a focused subject and 2) a data-driven project for a systematic approach.

1) Focused subject: gene regulation in immune tolerance.

Negative selection of self-reactive T cells occurs in the thymus and is one of the most essential mechanisms to achieve immune tolerance. Thymic medullary epithelial cells (TECs) express peripheral tissue antigens (PTAs), whose expression was expected to be restricted to specific peripheral tissues. Developing T cells that strongly react with a PTA are eliminated by apoptosis. Since the disrupted expression of PTAs results in severe autoimmune disorders, understanding the mechanisms controlling their expression is important to understand the pathogenesis of autoimmune diseases and to develop new treatments. We are analyzing gene expression in TECs by single-cell RNA-seq to examine in detail their gene regulation as well as to define TEC heterogeneity, and then validating these findings by employing mouse models.

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

Bioinformatics has greatly impacted gene regulation research 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**

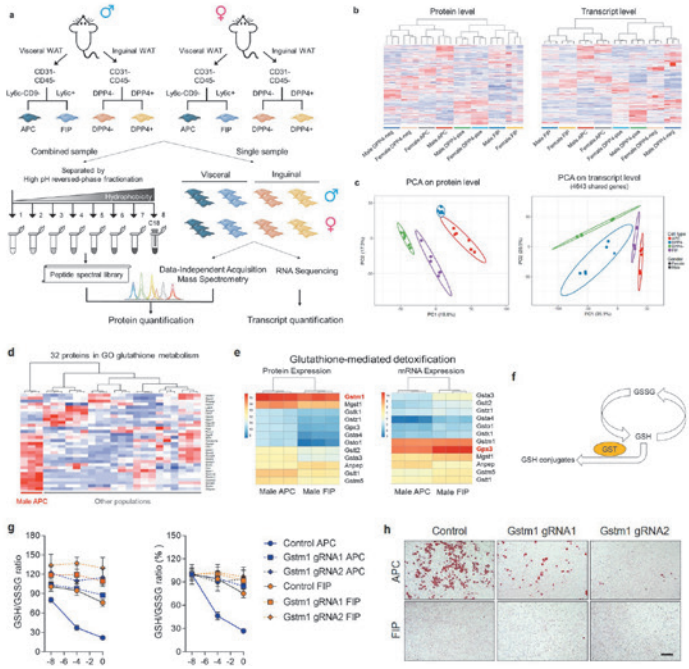


Figure: A multilayered omics analysis reveals sex- and depot-dependent adipose stromal cell heterogeneity

(a) An integrated transcriptomic and proteomic approach to dissect molecular signatures of adipose stromal cell populations. (b) Gene expression at the protein and transcript levels across all 24 samples. Data were normalized to total abundance in each sample and z-score transformed. Red represents a z-score larger than 0 and blue represents a z-score smaller than 0. (c) Principal component analysis showing sample group separation based on proteomics (left) and transcriptomics data (right). (d) Expression of 32 proteins involved in glutathione metabolism clearly discriminated male gWAT APCs from all the other populations. (e) Expression of genes involved in glutathione-mediated detoxification between male gWAT APCs and FIPs at protein (left) and transcript (right) levels. (f) Glutathione exists in reduced (GSH) and oxidized (GSSG) forms, with the ratio of GSH:GSSG indicative of cellular redox status and oxidative stress. Glutathione-S-transferases (GSTs) catalyze the conjugation of GSH to xenobiotic substrates and regulate cellular GSH:GSSG ratios. (g) GSH:GSSG ratios in cell lysates from primary FIPs and APCs transduced with the indicated CRISPR lentivirus. Day 0 denotes the time when samples were harvested for cell differentiation analysis by Oil Red O staining. N = 4. Data are shown as the mean ± s.e.m. * $p < 0.05$ between APC and FIP by two-way ANOVA. (h) Representative bright field images of Oil Red O-stained cultures of differentiated primary FIPs and APCs transduced with the indicated CRISPR lentivirus. Scale bar = 200 uM.

Recent Major Publications

Bo Shan, Clive S. Barker, Mengle Shao, Qianbin Zhang, Rana K. Gupta, Yibo Wu. Multilayered omics reveal Sex- and depot-dependent adipose progenitor cell heterogeneity. ***Cell Metabolism*** (In revision)

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)

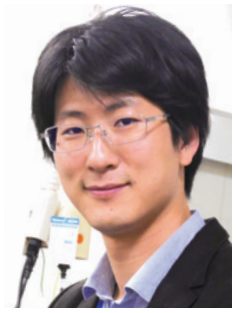
Mostafa D, Yanagiya A, Georgiadou E, Wu E, Stylianides T, Rutter A G, Suzuki T, Yamamoto T. Loss of β-cell identity and diabetic phenotype in mice caused by disruption of CNOT3-dependent mRNA deadenylation. ***Commun Biol*** 3, 476 (2020)

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). Upon energy excess, WAT expands through two primary mechanisms: hyperplasia, which involves differentiation of adipocyte precursors, and hypertrophy, which is due to the enlargement of existing adipocytes. We have developed two research efforts centered around these mechanisms, one focuses on the molecular and functional heterogeneity of adipocyte progenitor populations, and the other on the intercellular communication of adipocytes, precursors and immune cells in the WAT.

To investigate sex- and depot (i.e. visceral or subcutaneous WAT)-dependent adipocyte progenitor cell heterogeneity, we performed a multilayered omics analysis for eight adipose stromal cell populations and quantified 4870 proteins and 15477 transcripts. The data are freely accessible as a resource at "Pread Profiler". Both proteomic and transcriptomic data clearly separated the eight cell populations. However, gene expression at the protein level appeared to better reflect the functional heterogeneity of the different cell populations.

Proteomic data revealed functional pathways that could discriminate cell populations. For example, expression of 32 proteins involved in glutathione metabolism distinguished male gonadal WAT (gWAT) adipocyte precursor cells (APCs) from all the other populations. Ingenuity pathway analysis (IPA) revealed that glutathione-mediated detoxification was enhanced in male gWAT APCs compared to fibro-inflammatory precursors (FIPs). Notably, GSTM1 was the most abundant at the protein level, while Gpx3 had the highest abundance at the transcript level. We then conducted CRISPR-Cas9 gene editing to inactivate *Gstm1* in isolated APCs and FIPs. The differentiation-linked decrease in the GSH:GSSG ratio was almost completely blocked in APCs expression either of the two gRNAs, and *Gstm1*-deficiency also reduced the adipogenic potential of APCs. Interestingly, *Gpx3* was functionally dispensable for the maintenance of cellular redox status and differentiation of APCs.

Together, this multilayered omics analysis provides unprecedented insights into adipose stromal cell heterogeneity.



YCI Laboratory for

Metabolic Epigenetics

Young Chief Investigator: **Azusa Inoue**

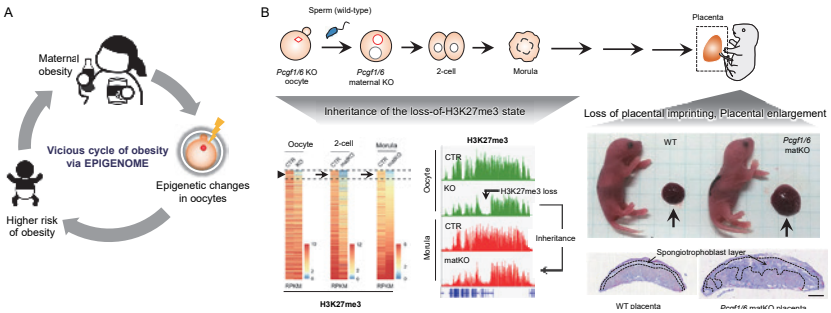


Figure: Aims and research tools of the Metabolic Epigenetics YCI Lab
(A) Illustration of a hypothetical model showing maternal inheritance of metabolic disorders via the oocyte epigenome. (B) Evidence that a region-specific loss of H3K27me3 state in oocytes is irreversibly inherited by embryos. This causes loss of imprinting in the placenta and placental enlargement.

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. ***Nature Genetics*** 53, 539-550 (2021)

Chen Z, Yin Q, Inoue A, Zhang C, Zhang Y. Allelic H3K27me3 to allelic DNA methylation switch maintains noncanonical imprinting in extraembryonic cells. ***Sci Adv*** 5, eaay7246 (2019)

Shishikura K, Kuroha S, Matsueda S, Iseki H, Matsui T, Inoue A, Arita M. Acyl-CoA synthetase 6 regulates long-chain polyunsaturated fatty acid composition of membrane phospholipids in spermatids and supports normal spermatogenic processes in mice. ***FASEB J*** 33, 14194-203 (2019)

Invited presentations

Inoue A. "Maternal epigenetic inheritance by PRC1-PRC2 crosstalk" The RIKEN-Helmholtz Zentrum Munchen joint epigenetics seminar (Online) October 2020

Inoue A. "Maternal epigenetic inheritance ~perspectives from animal cloning~" The 113th Society for Reproduction and Development meeting (Online) September 2020

Inoue A. "Variant PRC1-mediated H2A mono-ubiquitination ensures maternal inheritance of H3K27me3" The 93rd annual meeting of the Japanese biochemical society (Online) September 2020

Inoue A. "Variant PRC1-mediated H2A mono-ubiquitination ensures maternal inheritance of H3K27me3" Research seminar at Gregor Mendel Institute of Molecular Plant Biology GmbH (Vienna, Austria/Online) May 2020

Inoue A. "Intergenerational epigenetic inheritance in mammals" Lecture at CiRA retreat 2019 (Shiga, Japan) February 2020

Obesity is a growing social problem in the modern world and the obese population has been increasing worldwide. Since obesity is associated with an increased risk of various diseases, including cancer, infertility, heart diseases, and type 2 diabetes (T2D), and greatly impacts national healthcare costs, development of preventive medicine and treatment for metabolic syndromes has been long awaited. Recently, intergenerational heritability of T2D has received much attention. Genetic variants and mutations are estimated to account for <30% of T2D heritability, suggesting the existence of a non-genetic inheritance mechanism. Studies in animal models have suggested that gametes, at least in part, mediate the inheritance. While significant progress has recently been made in understanding the mechanisms of sperm-mediated paternal inheritance, almost nothing is known about the mechanisms of oocyte-mediated maternal inheritance.

Our lab is studying how maternal metabolic disorders are inherited by the next generation via epigenetic mechanisms. Our specific aims are as follows: (1) To understand the molecular basis and functions of maternal epigenetic inheritance (Figure); (2) To understand whether and how maternal metabolic disorders could alter the epigenomes of oocytes, early embryos and offspring. We integrate low-input epigenome analysis technologies and reproductive engineering techniques to address these questions. Our studies will not only reveal the mechanisms of intergenerational epigenetic inheritance in mammals but also provide a foundation for establishing new approaches to prevent inheritance of metabolic disorders.

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 2020, even during the difficult and restricted period due to COVID-19, 510 analytical and 925 sorting experiments with FACS and 5 analyses with HELIOS were performed in the Laboratory.

In the FACS laboratory, a specialized staff member offers various services for users of the equipment (cell analyzers and cell sorters): (1) *Technical support and training*: In 2020, 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 42 researchers participated in these courses in 2020. (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 2020, 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 (2020)

Instrument types	Model	# of machines	# of users	# of training sessions
FACS cell analyzer	Calibur	4	5	1
	Canto II	2	505	8
FACS cell sorter	Aria IIIu/III/Fusion	7	925	35
Mass cytometer	CyTOF2	1	5	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), which 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.

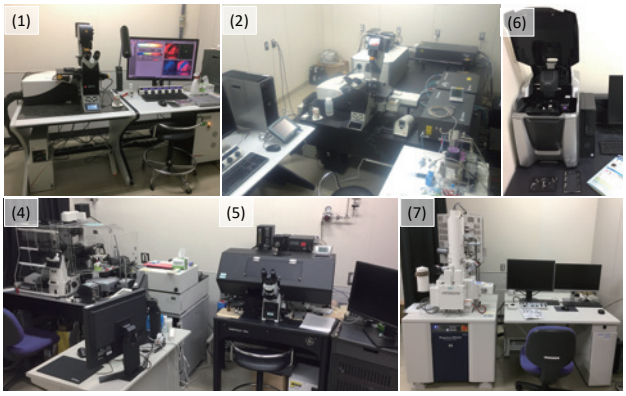


Figure: Leica SP8 confocal microscope (1), Leica SP8 multiphoton microscope (2), Nikon N-SIM/N-STORM super-resolution microscope (4), GE Healthcare DeltaVision Elite system (5), Keyence BZ-X700 microscope (6), and Hitachi Regulus8420 FE-SEM (7)

Genome Platform and related activities

The Sequence Platform that originally provided next-generation sequencing services to IMS researchers evolved to become the Genome Platform 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. For library preparation, we support whole-genome sequencing, targeted sequencing, CAGE, single-cell RNA-seq, and bulk RNA-seq. For sequencing, we support NovaSeq6000, HiSeq2500, MiSeq, and PacBio Sequel runs. We plan to introduce DNBSEQ-T7, DNBSEQ-G400 and NextSeq2000 after April 2021. Data analysis is available for single-cell RNA-seq, bulk RNA-seq, Chip-Seq, and ATAC-seq.

This year, we are working on 43 projects. The achievements in library preparation and sequencing are shown in Table 1. In a large project, we are conducting whole-genome sequencing analysis of 4,000 individuals for a dementia cohort study with the collaboration of Kyushu University. In addition, we analyzed somatic mutations in acute myeloid leukemia (AML) patients using the target sequence method in collaboration with the Laboratory for Human Disease Models. Based on these results, we were able to clarify the pathogenesis of AML resistance to treatment and gain knowledge that will lead to personalized medicine (Nat Cancer).

This platform enables users in IMS to obtain DNA libraries, sequence data and analysis data quickly and 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 2020

Library Preparation	# of Samples
whole genome sequence	4,000
target sequence	20,896
bulk RNA-seq	32
SMART-Seq	2,880
RamDA-seq	2,400
ssCAGE	76

Table2: Next-generation DNA sequencing

Illumina Sequencer	# of Runs
NovaSeq6000	107
HiSeq2500	70
MiSeq	118
PacBio Sequel	**# of Runs**
Sequel I	80
Sequel II	232
MGI DNBSEQ	**# of Runs**
MGISEQ2000	110

Animal Facility

We continue to maintain over 50,000 mice in the specific-pathogen-free (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 are used by several IMS research groups, in particular the mucosal immunologists, and the latter are for "humanized mice". Last year, a new SPF animal facility was completed and we have begun its management. The new facility has 32 vinyl isolators and 2 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 748 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 (124 lines). In addition, we have created 18 lines of germ-free mice. We maintain flexibility so that we can provide space in the animal facility for new experiments, e.g., behavioral testing for 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 the 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: Two types of Individually Vented Cage systems in new SPF animal facility

Part 3

Research Projects

COVID-19 projects in IMS

Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has recently become a pandemic. The sudden emergence and spread of SARS-CoV-2 poses a threat to global health and the economy over the world. Although a new vaccine for the SARS-CoV-2 may be available in many countries in the near future, the development of strategies to prevent the virus's spread is still needed. At present, several groups in IMS are developing tools for COVID-19 diagnosis, prevention and therapy. For the development of diagnostics and biomarkers, Usui's group already constructed a SARS-CoV-2 detection kit based on the original isothermal amplification technology "SmartAmp method". The usefulness of this kit was evaluated with viral genomic RNA of actual SARS-CoV-2 isolated from passengers of the Diamond Princess cruise ship, which was quarantined in Yokohama. The kit was approved by the Pharmaceuticals and Medical Devices Agency in August 2020. Momozawa's group is working to discover biomarkers based on the idea that individual differences in immunity-related genes may affect the severity of clinical phenotypes of infection. The identification of such individual differences could reveal the mechanisms of COVID-19 that lead to various clinical outcomes. They participate in a global consortium called the COVID Human Genetic Effort and perform whole genome and targeted sequencing in patients.

For COVID-19 treatment, many groups are working on SARS-CoV-2 projects. Among them, several groups are collaborating with RIKEN Drug Discovery and Medical Platforms (DMP). Miyauchi's group previously reported studies of neutralizing antibodies against influenza viruses, in which live influenza virus infection induced broad-spectrum neutralizing antibodies in a T_{FH} cell-dependent mechanism. They have applied this knowledge to the generation of monoclonal antibodies (mAb) against SARS-CoV-2. So far, they established many hybridoma clones producing anti-CoV-2-S antibody, which were generated from lymph node B cells after co-administration of the SARS-CoV-2-spike (S) protein and live influenza viruses to mice. Saito's group is working on a different type of neutralizing antibody, one for TMPRSS2. TMPRSS2 is critical for viral entry based on studies showing that small molecule inhibitors of TMPRSS2 inhibit SARS-CoV-2 infection. However, these inhibitors have some side effects and his group thinks that TMPRSS2 mAb may more specifically inhibit the infection. As a Drug Discovery Antibody Platform Unit, they have tried to establish human TMPRSS2 mAb to inhibit SARS-CoV2 infection. Fukuyama's group already isolated several therapeutic human mAbs from COVID-19 patients in collaboration with Keio University. In addition, they showed a vaccine adjuvant effect of vitamin D3 that may lead to a safer COVID-19 vaccine by regulating the vitamin D3 pathway. Fujii's group previously established the concept of the artificial adjuvant vector cell (aAVC) system against cancer and have now extended it to COVID-19. They established SARS-CoV-2-derived antigen-expressing aAVC (aAVC-Cov-2), and will perform a proof of concept study 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
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-Cov2 (with DMP)

The Human Gene Regulatory Atlas

Precise determination of our health status and risk for disease is one of the fundamental missions of the Human Gene Regulatory Atlas project in IMS. Based on single-cell genomic technologies, the Human Gene Regulatory Atlas aims to define gene-to-cell networks across multiple human organs and delineate their regulatory processes that encompass healthy and disease states. The 5'-focused single-cell RNA-seq technology will reveal gene regulatory elements, including promoter and enhancer activities, and decode *cis*-regulatory programs to evaluate the risks for genetic disorders. The Human Gene Regulatory Atlas is made possible through an extensive collaborative network involving RIKEN and medical institutions across Japan. The Human Gene Regulatory Atlas in IMS facilitates discovery of new biology and, at the same time, builds a comprehensive integrative database to power the next generation artificial intelligence to solve health and medical needs that we are facing in our lifetime.

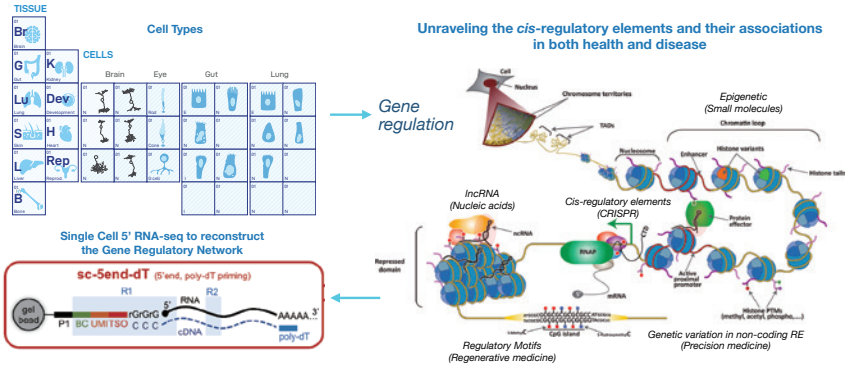


Figure: The Human Gene Regulatory Atlas
Beyond the 'periodic table' of the human cell types, the Human Gene Regulatory Atlas aims to elucidate the regulatory processes of individual cells in both health and diseased states. The project relies on single cell 5' RNA seq and other genomics technologies to decode the regulatory elements of the human genome, including non-coding regions. The rich dataset will serve as the foundation to build gene- and cell-regulatory atlases required for next generation precision medicine, drug development and cell-based therapeutics.

FANTOM

FANTOM is an international consortium established in the year 2000 aiming at exploring the regulatory functions of the mammalian genome. The 6th phase of the FANTOM project published the world's largest knockdown dataset of long noncoding RNAs (lncRNAs) as its pilot data (Ramilowski *et al. Genome Research* 2020). Previous FANTOM projects and other study groups revealed that lncRNAs constitute the majority of transcripts in the human genome, but little is known about their functions, although several of them have been found to play important roles in human health. The FANTOM5 project identified 19,175 potentially functional lncRNAs in the human genome (Hon *et al. Nature* 2017). In order to gain a better understanding about lncRNA functions, FANTOM6 established an automated high-throughput platform for cell culture to systematically knockdown 285 lncRNAs in human primary fibroblasts using the antisense LNA-modified GapmeR antisense oligonucleotide (ASO) technology. The effects of the suppression of lncRNA expression were examined by real-time imaging of cell growth and morphology. The transcriptomic responses in the knockdown analyzed by Capped Analysis of Gene Expression (CAGE) provided deeper insights, with molecular phenotypes associated with the lncRNA functions. Collectively, over 30% of the tested lncRNAs showed the effects of the knockdown on cell growth and morphology. All data are publicly available at https://fantom.gsc.riken.jp/zenbu/reports/#FANTOM6.

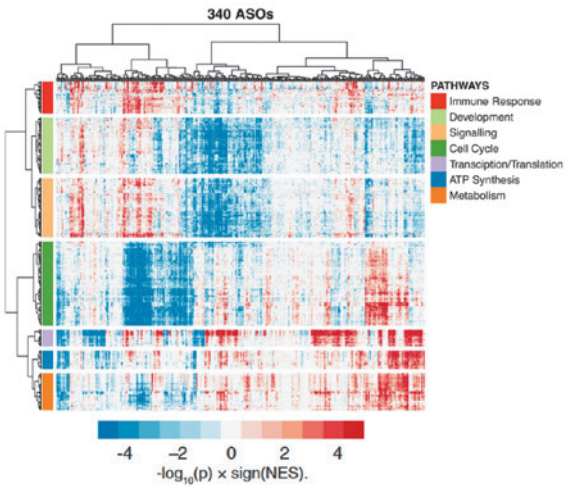


Figure: Enriched biological pathways across 340 ASOs
Scale indicates Gene Set Enrichment Analysis (GSEA) enrichment value calculated as -log10 (p) x sign (NES).

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 combination 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 (Horikoshi), cardiovascular diseases (Ito), autoimmune diseases (Yamamoto K), and integrated analysis of all data and phenotypes (Terao). In addition, we began to extract information of 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 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 2020 was elucidating the basics underlying mosaic chromosomal alterations (mCA), clonal hematopoiesis with somatic chromosomal alterations. We identified 33,250 mCAs in 179,417 BBJ participants and found that mCA seems inevitable with advancing age. Key differences in the genomic locations of mCA between Japanese and Europeans could help predict the relative rates of B-cell and T-cell leukemia between the populations.

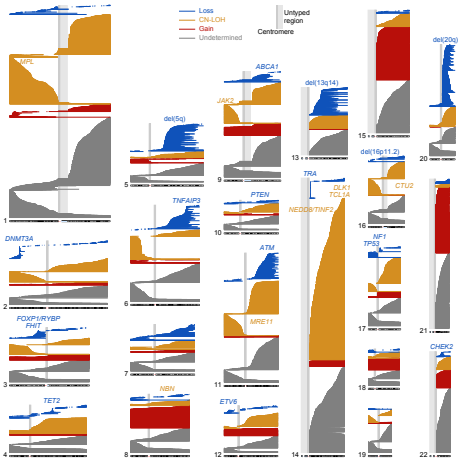


Figure: Genomic locations of 33,250 autosomal mCAs detected in 27,910 unique BBJ participants

mCA events are plotted as blue, orange and red horizontal lines, according to their mCA classes. Events with undetermined copy numbers are plotted in grey. Commonly deleted regions are labelled in blue; loci associated with Copy Neutral - Loss of Heterzygosity (CN-LOH) mutations in *cis* are labelled in orange.

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 (ADRs), 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.

Recently, SEAPharm has started a new project involving next-generation sequencing (NGS) of about 2,000 genomic DNA samples from 12 countries to clarify the genetic diversity of 100 pharmacokinetics-related genes in individuals from Southeast Asia, Sothern Asia, 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 inter-ethnic variabilities in drug response phenotypes, and are leading to acceleration of further pharmacogenomic investigations and genotype-guided drug therapies in clinical practice.

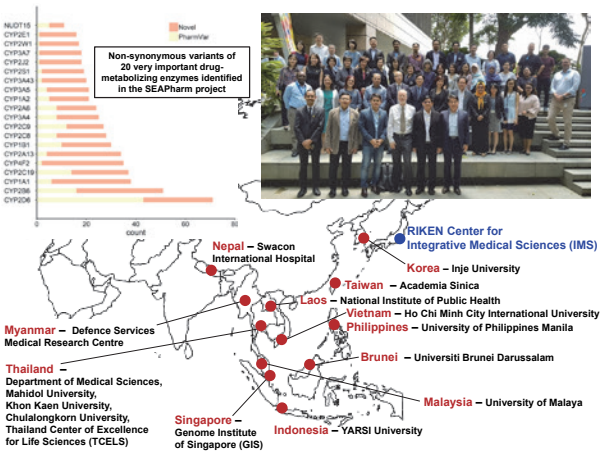


Figure: Members of the South East Asian Pharmacogenomics Research Network (SEAPharm)

Please visit https://www.facebook.com/SEAPHARM/

International Cancer Genome Consortium (ICGC) and PCAWG project

The ICGC was established in 2007 and concluded its mission to define the genomes of 25,000 primary untreated cancers (the 25K Initiative) in 2018. The ICGC solved numerous data governance, ethical and logistical challenges to make global genomic data sharing for cancer a reality, providing the international community with comprehensive genomic data for many cancer types. As the second ICGC initiative, the ICGC launched a "Pan-Cancer" Whole Genome project (PCAWG) in 2014, in which WGS data together with RNA-Seq of 2834 samples were analyzed in uniform pipelines within the same computational environment and cloud computing. RIKEN has been contributing to this project as a member of a technical working group and as PI/researchers in working groups for several projects. In February 2020, PCAWG published more than 20 papers in Nature and Nature sister journals and wrapped up. The RIKEN group has been focusing on mitochondria genome (mtDNA) mutations in cancer WGS data and found instances of somatic transfer of mitochondrial DNA into the cancer nuclear genome. They also observed excessive accumulation of high-allele-frequency truncating mutations in mtDNA, specifically in kidney cancers.

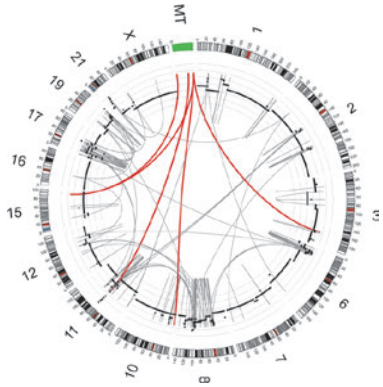


Figure:
A Circos plot representing somatic mtDNA nuclear transfer events in a bladder cancer genome. Human chromosomes and mtDNA (MT) are shown in the outer layer. Chromosomal rearrangements are shown as gray curves and mtDNA nuclear transfers are represented by red curves.

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.

IMS is now setting up a system to identify various subtypes of leukocytes from peripheral blood mononuclear cells (PBMC) of heathy individuals. The aim of this project is 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.

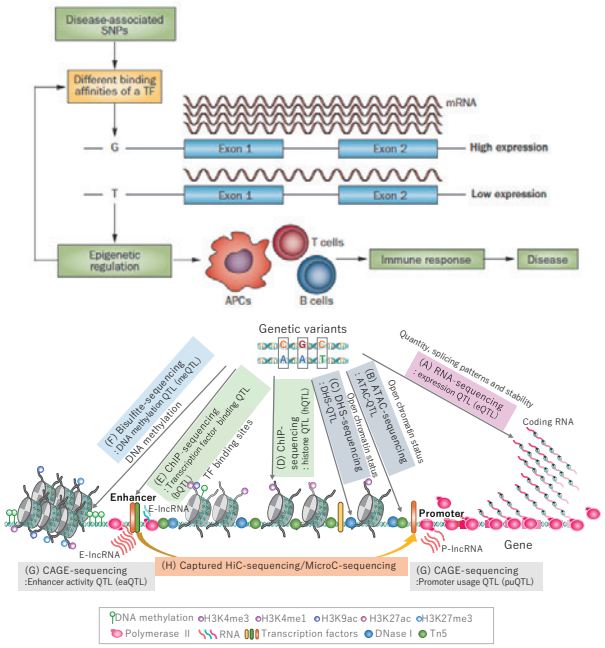


Figure: Integration of genetic information into immune functions: The eQTL project

Search for new biomarkers involved in the pathogenesis of Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2D) is a highly prevalent metabolic disease both in Japan and worldwide. It is estimated that about 1 in 5 Japanese suffer from diabetic or prediabetic (medically defined as glucose intolerance) conditions. Thus, prevention of T2D is an urgent need - medically, socially as well as economically. As a center project, various teams in IMS have been engaged in identifying gut microbial T2D-preventive biomarkers or factors involved in the pathogenesis of T2D. To this end, they have been collaborating with the University of Tokyo Hospital to recruit volunteers with the following criteria among those taking a complete medical checkup: 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 as part of the medical checkup, collected in RIKEN were: fecal and saliva metagenomic and metabolomic data, plasma and urine metabolomic data, CAGE-based RNAseq data of peripheral blood mononuclear cells, and whole genome sequencing data (Figure). Also nutritional and physical activity data have been collected using a brief self-administered diet history questionnaire (BDHQ) and accelerometry, respectively.

They found that insulin resistance and metabolic syndrome were significantly associated with fecal monosaccharides and sugar derivatives. Furthermore, these fecal monosaccharides and sugar derivatives strongly associated with host inflammatory gene expression and cytokine levels. Representative microbes associated with insulin resistance showed distinct carbohydrate metabolism and host metabolic phenotypes. These results are now submitted for publication.

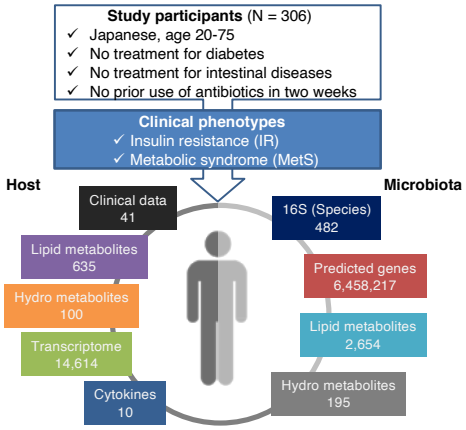


Figure: Schematic summary of this project

Medical Sciences Innovation Hub Program (MIH)

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 have not been established yet. The purpose of this study, therefore, is to establish a method for disease clustering into subgroups 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 keeping with the above approach, IMS-MIH collaborative research project has been collecting large numbers of high-quality clinical samples in collaboration with Keio University Hospital and have established an integrated data analysis and repository infrastructure called Medical Data Integration Assistant (MeDIA). They have acquired more than 1000 transcriptome datasets [mRNA-seq of skin tissue and peripheral blood mononuclear cells (PBMC)] and are performing data analysis by using supervised and unsupervised machine learning with other annotating data such as clinical data and serum cytokine profiles. Notably, they revealed that PBMC transcriptome analysis classified the disease phenotypes of AD patients into five clusters characterized by different blood cells. In addition, skin transcriptome analysis addresses the issue of characterizing a population of patients responding to treatment with biologics. The project also advances the verification of therapeutic molecular targets and understanding of pathological conditions by guiding the analytical findings in humans to animal model research.

The project team is highly focused on the integration of technology and knowledge possessed by experts in various fields, and is also working on collaborations with various companies and open science, with an awareness of social implementation. Their approach will not only pave the way toward realization of personalized medicine for AD, but also for development of new technologies in data-driven medical research, and therefore will have a considerable impact on society.

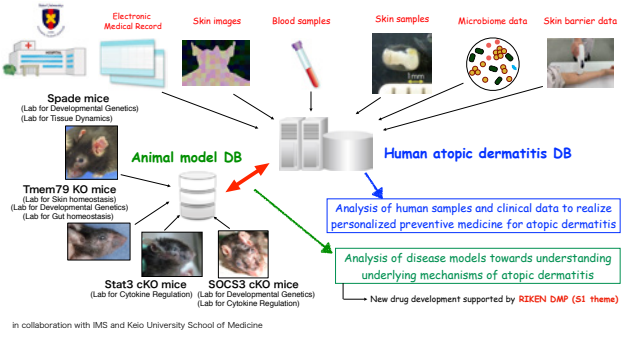


Figure: Study workflow: Data driven research for Atopic Dermatitis

Humanized mouse

In the past decade, DNA sequencing revealed that patients with malignant diseases carry multiple somatic mutations in a patient-specific manner. The genetic complexity and heterogeneity in cancer/leukemia patients complicates understanding of disease initiation and relapse as well as drug development for human malignancies. In our acute myeloid leukemia (AML) research, we took a multiomics approach to find therapeutic targets in AML initiating cells as compared with normal hematopoietic stem and progenitor cells. By combining genetic and chemical screening, we found five critical vulnerabilities in AML: BIRC2/4, BCL2, MCL1, AURKB, and KIF10. In the presence of multiple somatic mutations and chromosomal abnormalities in patient AML cells, BIRC2/4 inhibition resulted in efficient killing of leukemic cells *in vitro*. Furthermore, we took advantage of patient-derived xenografts to recapitulate patient leukemic status in mice followed by *in vivo* treatment experiments. By using two molecular targeting drugs to which individual patients showed the highest sensitivity, we confirmed profound therapeutic efficacy, as evidenced by complete elimination of patient-derived leukemic cells in the bone marrow and spleen as well as recovery of normal hematopoietic cells. Integrative analyses of somatic mutational profiling, gene expression profiling, transcription factor binding to promoter regions of target genes, and drug sensitivity experiments has enabled us to identify the optimal compounds for each patient. We hope to bring this precision medicine into clinical practice in the future.

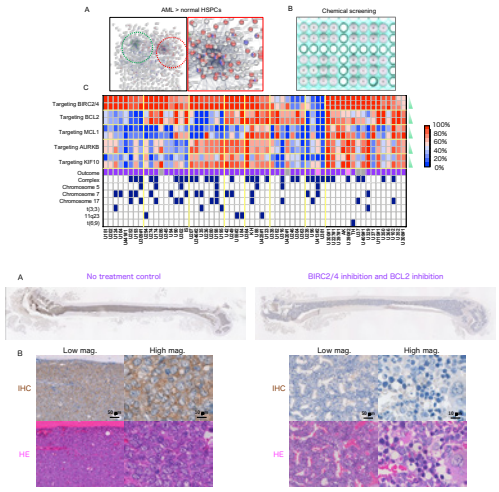


Figure:
A. a whole mount bone marrow section after immunohistochemical staining with anti-human CD45 antibody. Left: no treatment control, Right: treatment with AZD5582 and ABT199.
B. Lower and higher magnification images of immunohistochemical staining with anti-hCD45 antibody and HE staining. Upper: IHC CD45, Lower: HE staining.

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 has finished PMDA (Pharmaceuticals and Medical Devices Agency) consultation for the clinical trial of iPS-Vα24⁺iNKT cell-mediated head and neck cancer immunotherapy and that is currently in progress.

To assess the safety of clinical trials using allogeneic transplantation, the facility also needed to establish a cell tracking system to enable tracing of the transplanted cells in the patient. To accomplish this, the facility focused this year on AkaBLI, an all-engineered bioluminescence *in vivo* imaging system, and they generated human iPSCs containing the *Akaluc* gene by using the CRISPR/Cas9 system. Akaluc-expressing human iPS cells were further differentiated into iPS-Vα24⁺iNKT cells and then injected intravenously into human cytokine knock-in NSG mice. By using the IVIS imaging system, strong bioluminescence signals derived from the transplanted cells were clearly observed in the recipient mice (Figure). These results suggest that Akaluc-expressing iPS-Vα24⁺iNKT cells will be useful to determine residence time of transplanted cells in the patient and to establish future therapeutic planning.

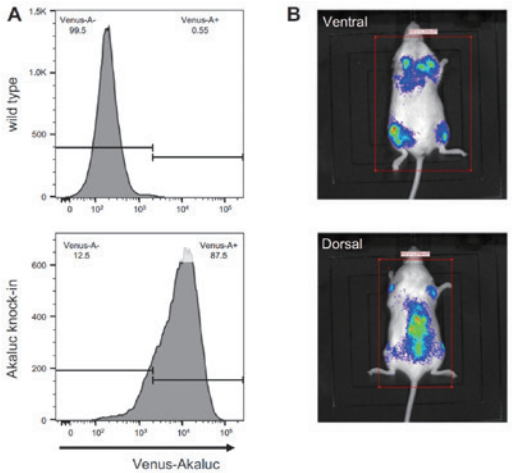
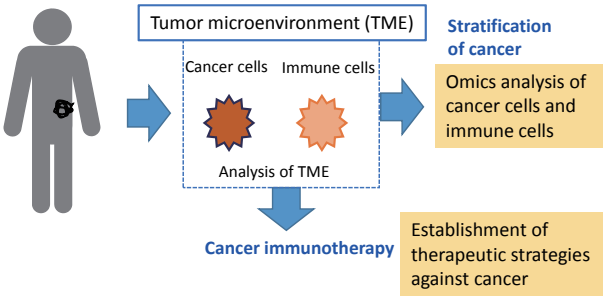


Figure: Analysis of Akaluc-expressing iPS-Vα24⁺iNKT cells in the mouse
(A) Flow cytometry analysis of Venus-Akaluc expressing cells by comparing wild type iPS-Vα24⁺iNKT cells and Venus-Akaluc knock-in iPS-Vα24⁺iNKT cells. (B) Bioluminescence images of mice 1 month after intravenous injection of Venus-Akaluc expressing iPS-Vα24⁺iNKT cells. Pictures were kindly provided by Dr. Shin-ichiro Fujii in the Laboratory for Immunotherapy.

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) brought the ideas and methods from mathematics and computational sciences into play. They demonstrated the results of immunogenomic analysis and provided a new classification of gastric cancer. This group also established a new method of quantifying multicellular colonization in tumor metastasis using NGS data. Nakagawa's group (Lab for Cancer Genomics) analyzed genome and RNA sequence data from esophageal cancers and showed that the immune response in the TME was significantly corelated with the chemotherapy response. They also used RNA sequencing data to search for candidate drugs that could modulate the immune microenvironment. Ishikawa's group (Lab for Human Disease Models) has identified acute myeloid leukemia (AML)-initiating cells by performing xenogeneic transplantation followed by DNA and RNA sequencing. By integrating the results, they found critical molecules in individual patients and linked the vulnerabilities with AML initiating genetic events. Fujii's group (Lab for Immunotherapy) identified immunogenic neoantigens and confirmed that DCs pulsed with these peptides elicited antitumor CTL responses. As part of the effort to develop translational research (TR) applications, Koseki's group (Lab for Developmental Genetics) has started an iPS-NKT cell clinical trial for head and neck cancer. Fujii's group has recently done an investigator-initiated Phase I clinical trial of aAVC-WT1 therapy against AML and is preparing for a phase II study. These TR projects have been supported by the RIKEN 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 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 2020, 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 inflammatory bowel diseases (Takashi Saito). An investigator-initiated Phase I clinical trial of the Artificial adjuvant vector cell project for cancer therapy has just been completed.

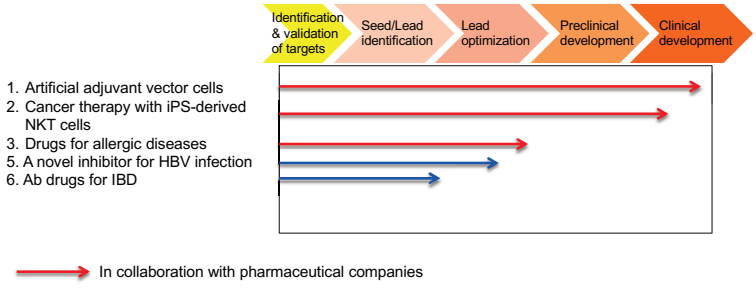


Figure: Collaboration between IMS and DMP for the development of innovative new pharmaceuticals and medical technologies

RIKEN International Program Associate (IPA)

IMS accepted five 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 2020 were
Jiahui Ma (Peking University, China) in the Laboratory for Genome Information Analysis
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
Jack Thomas Flanagan (The University of Liverpool, UK) in the Laboratory for Genomics of Diabetes and Metabolisms
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, 22 JRA students studied in IMS.

Yuki Ariyasu (Laboratory for Metabolomics)
Shintaro Ono (Laboratory for Integrative Genomics)
Nao Otomo (Laboratory for Bone and Joint Diseases)
Akiko Oguchi (RIKEN-IFOM Joint Laboratory for Cancer Genomics)
Takahiro Matsunaga (Laboratory for Gut Homeostasis)
Haruki Uchino (Laboratory for Metabolomics)
Zhujun Wang (Laboratory for Gut Homeostasis)
Hiroyuki Suetsugu (Laboratory for Bone and Joint Diseases)
Tahara Umi (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)

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

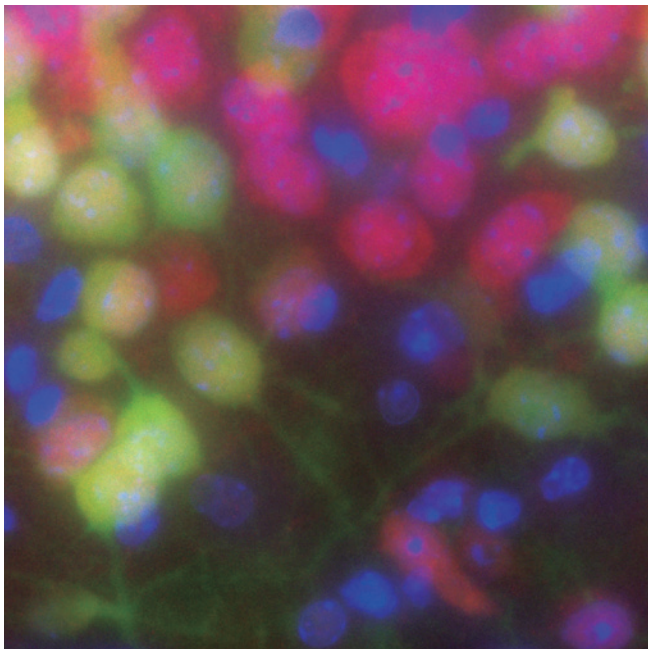
Callum Parr (Laboratory for Advanced Genomics Circuit)
Rei Nakano (Laboratory for Cellular Function Conversion Technology)
Tsuyoshi Kiniwa (Laboratory for Innate Immune Systems)
Sotaro Ochiai (Laboratory for Tissue Dynamics)
Chisayo Kozuka (Laboratory for Developmental Genetics)
Juan Ortiz Quinonez (Laboratory 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)

Award Winners 2020

Name of the awardee	Name of the award	Date of the announcement
Kenya Honda, Team Leader, Laboratory for Gut Homeostasis	The 2020 Carlos J. Finlay UNESCO Prize for Microbiology	Nov 2020
Shiro Ikegawa, Team Leader, Laboratory for Bone and Joint Diseases	The 72nd Public Health Culture Award, Ministry of Health, Labour and Welfare	Dec 2020
Chikashi Terao, Team Leader, Laboratory for Statistical and Translational Genetics	Japanese Society for Immunology Young Investigator Award	Dec 2020
Kazuhiko Yamamoto, Team Leader, Laboratory for Autoimmune Diseases	The European League Against Rheumatism (EULAR) Honorary Membership Award	Jun 2020
Chikashi Terao, Team Leader, Laboratory for Statistical and Translational Genetics	RIKEN Eihou award	Mar 2020
Katsuyuki Yugi, Team Leader, Laboratory for Integrated Cellular Systems	The 9th Mishima Kaiun Memorial Foundation Academic Award	Jul 2020
Guo Long, Research Scientist, Laboratory for Bone and Joint Diseases	Research Encouragement Award, The Japanese Society for Bone and Mineral Research	Jun 2020
Guo Long, Research Scientist, Laboratory for Bone and Joint Diseases	Encouragement Award, The Japan Society of Human Genetics	Aug 2020
Guo Long, Research Scientist, Laboratory for Bone and Joint Diseases	Excellent Presentation Award, The 38th Annual Meeting of the Japanese Society for Bone and Mineral Research	Oct 2020
Chi Wai Yip, Research scientist, Laboratory for Advanced Genomic Circuit	RIKEN Ohbu Research Incentive Aaward	Mar 2020
Ari Itoh, Research Scientist, Laboratory for Human Disease Models	Best Presentation Award, The 48th Japanese Society for Immunology	Feb 2020
Sonoko Takahashi, Postdoctoral Researcher, Laboratory for Tissue Dynamics	The 21st Maruho Research Award	Dec 2020
Rei Nakano, SPDR, Laboratory for Cellular Function Conversion Technology	Veterinary Science Young Investigator Awards, The Japanese Society of Veterinary Science	Sep 2020
Jen-Chien Chang, Postdoctoral Researcher, Laboratory for Cellular Epigenomics	Life Epigenetics Imputation Award	Jun 2020
Tommy Terooatea, Postdoctoral Researcher, Laboratory for Cellular Epigenomics	Keystone Symposia Scholarship	May 2020
Sayoko Kuroha, Junior Research Associate, Laboratory for Metabolomics	Young Scientist Award, The 93rd Annual Meeting of the Japanese Biochemical Society	Sep 2020
Hidenori Aoki, Student Trainee, Laboratory for Metabolomics	Young Scientist Award, The 93rd Annual Meeting of the Japanese Biochemical Society	Sep 2020

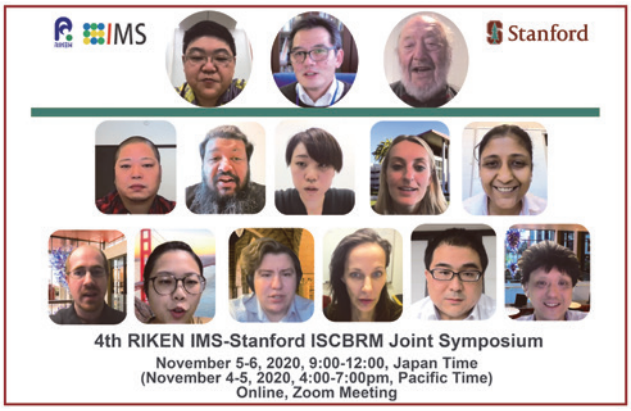
Part 4

Events



RIKEN IMS-Stanford ISCBRM Joint Symposium

RIKEN IMS and the Stanford Institute of Stem Cell Biology and Regenerative Medicine (ISCBRM) have been collaborating since 2017. This year, we had the 4th joint symposium, which was originally planned to be held at the RIKEN Yokohama campus. However, because of the COVID-19 pandemic, the meeting was rescheduled and the venue was moved to online. Due to the time-zone difference between Japan and California, the duration of the meeting was much shorter than usual, just 3 hours a day. Despite such limitations, it turned out that we enjoyed deep and productive discussions throughout the symposium. Since the number of presentation slots was limited, speakers were selected from among the young investigators (Photo), and this might have been the key for active discussion. From RIKEN IMS; Kazuki Okuyama, Natsuko Otaki, Saumya Agrawal, Yan Jun Lan, and Long Guo presented their latest projects. From Stanford ISCBRM; Rahul Sinha, Agnieszka Czechowiez, Aaron Newman, Carolyn Dundes, Gerlinde Wernig, and Charles Chan gave talks on their cutting-edge research. Topics included immunology, hematology, organoids, brain development, bioinformatics, and bone biology. About 70 participants in total from both sides joined each day,



and very active discussion occurred after each talk over the Pacific! Although we missed the social events like in previous years, it was quite productive to know that we could enjoy exchanging scientific ideas over the web-meeting. The next joint symposium will take place in 2021, hopefully on the Stanford Campus.

RIKEN-McGill Symposiums

Since 2016, RIKEN IMS and the McGill University Faculty of Medicine have established close interactions to pursue research in the areas of genomics, immunology, and cancer across a broad range of diseases. The first comprehensive cooperation agreements/MOUs between the two institutes were even earlier, in July 2010. Starting from the first symposium, which was held May 2017 at McGill University, Montreal, Canada, we had two other symposia - in February 2018 at Yokohama, Japan and in November 2019 at Montreal. These past symposia encompassed the research areas of genome biology, including human genome medicine, immunology and infectious disease and cancers. The fourth symposium was planned for 6th-7th April 2020 at Yokohama; however, it was postponed because of the world-wide pandemic of SARS-CoV-2 from March 2020. Meanwhile, we were fortunate to obtain financial support from RIKEN and AMED for the RIKEN IMS-McGill partnership, so we decided to organize a virtual on-line symposium on 26th-27th January 2021 to exchange information and to discuss how we will advance this international collaborative activity. Despite the 14 hour time difference between the two sites, the 4th on-line symposium had 200 registrants and 120-140 participants on average in each session. On the second day, two PhD students presented their outstanding work and two junior PIs jointly presented their research plan funded by AMED. This was followed by a panel discussion session about how the two organizations will support this international activity at both laboratory and institutional level, and how we will stimulate human exchange.

The 7th RIKEN-KI-SciLifeLab Symposium: Biomedical Data for Artificial Intelligence

Hosted online this year, the 7th RIKEN-KI-SciLifeLab Symposium was held on November 2nd, 9-11 CET, 17-19 JST. The specific topic for the 2020 symposium was "The role of AI in the future direction of Life Sciences research".

This symposium series is organized between RIKEN in Japan, and the Karolinska Institute (KI) and SciLifeLab in Sweden. The symposia alternate between RIKEN and SciLifeLab/KI, and the 2020 symposium was the seventh one. The overall goals of the symposia are a) to identify common scientific interests, b) to identify complementary skills and technologies for collaborations, and c) to encourage the exchange of PhD students and postdocs between RIKEN and SciLifeLab/KI. Several collaborations between groups at KI, SciLifeLab and RIKEN started based on first contacts during one of the symposia.

The 2-hours long online symposium started with greetings and comments from the three directors, Professors Kazuhiko Yamamoto (RIKEN IMS), Anders Gustafsson (KI) and Olli Kallioniemi (SciLifeLab) about the direction of future research in the respective institutions. A keynote presentation was given by Prof. Sabine Koch (KI) about AI in medicine and health. Professor Magnus Boman (KTH, KI) presented a White Paper based on results from the 2019 symposium. Young researchers and representatives from RIKEN IMS, KI and SciLifeLab reported about ongoing AI-related research activities. The symposium was then summarized by Professors Carl Johan Sundberg (KI) and Piero Carninci (RIKEN IMS).

Meeting
Monday, November 2nd, 2020, 9:00-11:00 CET, 17:00-19:00 JST
Online Zoom

Attendees
140 attendees

Adjunct Professorship Programs

IMS collaborates with and accepts graduate students from 8 domestic university graduate schools. There are now a total of 35 adjunct professors/associate professors in IMS (Table), and 56 students who had studied at IMS in 2020. On July 11th and October 10th, IMS held briefing sessions 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) Takashi Saito (Visiting 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) Piero Carninci (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) Shigeo Koyasu (Visiting 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)

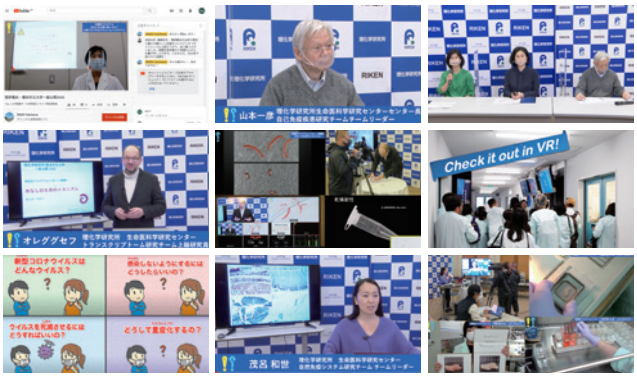
RIKEN Yokohama Campus Open Day 2020

Due to the situation with COVID-19, RIKEN Yokohama Campus Open Day was held only virtually for the first time. The Yokohama campus was the first campus to move online from other RIKEN campuses (Wako, Kobe, Osaka, etc.). There were four live events and two videos from IMS.

Total number of visitors on the website from Oct. 10th to 13th was 1796.

Table: Program of online events at RIKEN Yokohama Open Campus on Oct. 10th, 2020 10:00-17:00

Title	Teams
How to make a specimen? Let's watch a preparation in real time!	Laboratory for Innate Immune Systems
Briefing session on joint graduate school programs	Lab for Immune Homeostasis Lab for Pharmacogenomics Lab for Developmental Genetics Lab for Cardiovascular Genomics and Informatics Lab for Cellular Epigenomics
Do you have high or low alcohol tolerance? Let's find out with an alcohol patch test!	Laboratory for Pharmacogenomics/ Laboratory for Autoimmune Diseases
The mechanisms of the Sleeping Chironomid (Lecture in Japanese)	Laboratory for Transcriptome Technology
Video: Proper knowledge about the new coronavirus so you can stay safe!	Genetic Diagnostic Technology Development Unit
VR: Virtual Lab tours	Center Director Office



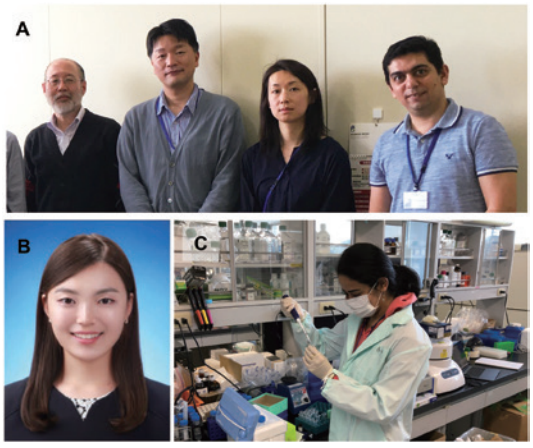
IMS Internship Program

The IMS Internship Program was launched in April 2018 with the aim of hosting young distinguished international scientists to carry out research activities in IMS laboratories. The program benefits both the young researchers, by giving them the chance to experience working in our world-class laboratories, and RIKEN, by promoting IMS activities to up-and-coming young scientists from around the world. The program is open to Master's and Ph.D. students, medical students at a grade equivalent to a Master's student in Japan, and young postdocs who received their Ph.D. within the last three years. Calls for applications occur twice per year for internships (up to three months) that take place in winter and summer. For each application period, 2-3 candidates are selected on a competitive basis, and successful candidates receive financial support that essentially covers all of their travel and living expenses. The program has been viewed as a great success.

During January-March 2020, we hosted three students as follows:
Mehdi Emam (McGill University, Canada) in the Laboratory for Transcriptional Regulation, on "The study of roles of ThPOK and Runx1 in macrophages differentiation"
Gi-song Kim (Yonsei Medical School, Korea) in the Laboratory for Advanced Genomics Circuit, on "Exploring 5'-based Single Cell RNA-seq data in developmental human brain organoids"
Anushka Khasnobish (Okayama University, Japan) in the Laboratory for Microbiome Sciences, on "Training for human microbiome analysis using NGS datasets"

Interns for 2020 summer had already been selected, but they were not able to visit Japan within the year 2020 due to the spread of COVID-19. They are still in their home countries, hoping to perform their internships at IMS in 2021.

Photo: (A) Dr. Emam (far right) and Dr. Taniuchi (far left); (B) Ms. Kim; (C) Ms. Khasnobish



IMS Crosstalk

IMS Crosstalk is a monthly seminar started in January 2020 aiming to boost "crosstalk" between the researchers in 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 achieve our mission in 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 the 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 have already established crosstalk 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 21 topics by 25 laboratories including 4 joint presentations so far and have another 18 topics scheduled in 2021. We believe that the crosstalk will play a useful role to promote the IMS mission.

Figure: IMS Crosstalks 2020

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	
2020/01/17	Hiroshi Ohno	*S.Fagarasan*	Toshitada Takemori	*T.Saito*	immunology
2020/02/21	Momoko Horikoshi & Kaoru Ito	*C.Terao*	Shiro Ikegawa	*Y.Momozawa*	genetics
2020/03/19	Kengo Usui	*CC.Hon*	Osamu Ohara	*H. Koseki*	genomic technologies
2020/05/29	Kenya Honda	*I.Taniuchi*	Wataru Suda	*K. Honda*	microbiome
2020/06/26	Makoto Arita	*Y. Murakawa*	Yibo Wu	*S. Fagarasan*	proteomics and metabolomics
2020/07/17	Aki Minoda & Kazuyo Moro	*S. Fagarasan*	Taishin Akiyama	*H. Yoshida*	immunology / genomics
2020/09/18	Haruhiko Koseki	*I. Taniuchi*	Ichiro Taniuchi	*H. Yoshida*	epigenetics
2020/11/13	Chung-Chau Hon & Yasuhiro Murakawa **[12:00~13:00]**			*J. Shin*	genomics
2020/11/27	Hidewaki Nakagawa & Yukihide Momozawa	*K. ito*	Yasushi Okazaki	*T. Kasukawa*	genomics
2020/12/18	Sidonia Fagarasan	*I. Taniuchi*	Tomohiro Kurosaki	*T.Okada*	immunology

Researcher Seminars 2020

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

Table: Resercher Seminars Jan-Dec 2020

Date	Chair	Name of the Presenter	Laboratory	Position
Apr. 17	Takaharu Okada	Keiichiro Shiraga	Laboratory for Skin Homeostasis	Visiting Scientist
		Pauline Robbe	Laboratory for Transcriptome Technology	Visiting Researcher
May 29	Aki Minoda	Shohei Kojima	Genome Immunobiology RIKEN Hakubi lab	Postdoctoral Researcher
		Naoko Toki	Laboratory for Transcriptome Technology	Part-timer
Jun. 26	Kaoru Ito	Yosuke Isobe	Laboratory for Metabolomics	Visiting Scientist
		Keiko Hikino	Laboratory for Pharmacogenomics	Research Associate
Jul. 17	Hiroshi Ohno	Takashi Kondo	Laboratory for Developmental Genetics	Senior Research Scientist
		Yuuri Yasuoka	Laboratory for Comprehensive Genomic Analysis	Research Scientist
Sep. 18	Nicholas Parrish	Yixin Dong	Laboratory for Developmental Genetics	Research Scientist
		Koya Fukunaga	Laboratory for Pharmacogenomics	Research Scientist
Oct. 23	Chung-chau Hon	Naoko Satoh	Laboratory for Intestinal Ecosystem	Senior Research Scientist
		Giovanni Pascarella	Laboratory for Transcriptome Technology	Research Scientist
Nov. 27	Kengo Usui	Shinsuke Ito	Laboratory for Developmental Genetics	Research Scientist
		Takeshi Ozeki	Laboratory for Pharmacogenomics	Research Scientist
Dec. 18	Ichiro Taniuchi	Ari Itoh	Laboratory for Human Disease Models	Research Scientist
		Saumya Agrawal	Laboratory for Applied Computational Genomics	Research Scientist

Guest Lectures 2020

Table: Guest Lectures Jan-Dec, 2020

Date	Speaker	Affiliation	Country	Title
Jan. 9	Dr. Mareike Albert	Center for Regenerative Therapies,Technische Universität Dresden	Germany	Gene regulatory mechanisms of neocortex development and evolution
Jan. 16	Dr. Bérénice Benayoun	University of Southern California	USA	Genomic regulation of vertebrate aging
Jan. 27	Dr. Anthony J. Covarrubias	Buck Institute	USA	Mechanistic insight on how aging-related inflammation impacts NAD metabolism
Jan. 30	Dr. Rune Linding	Humboldt-Universität zu Berlin	Germany	Deep hidden physics modeling of cell signaling Networks
Jan. 30	Dr. Edda Klipp	Institute of Biology, Humboldt-Universität zu Berlin	Germany	Mathematical modeling of metabolism in cancer and physical constraints for cell growth
Feb. 4	Dr. Atsushi Kumanogoh	Osaka University Graduate School of Medicine	Japan	Involvement of semaphorins in coupling immune and metabolic systems
Feb. 7	Dr. Koshi Imami	Kyoto University/JST Presto	Japan	Proteomic technologies to dissect the translational regulation through ribosomes
Feb. 13	Dr. Soichi Ogishima	Tohoku Medical Megabank Organization, Tohoku University	Japan	The responsible sharing of genomic and phenotypic data for research and development of genomic medicine
Feb. 20	Dr. Barbara E. Bierer	Harvard Medical School	USA	SMART IRB: single IRB review of multi-site research and other changes in human participant research in the US
Feb. 21	Dr. Reiko Kuroda	Institute of Science and Technology Research, Chubu University	Japan	Snail coiling: CRISPR editing of a single gene turns righties into lefties
Mar. 2	Dr. Doan Duy Hai Tran	Institute of Biochemistry, Hannover Medical School	Germany	Transcription/export complex (TREX), elongation rate and mRNA 3'end processing
May 18	Dr. Yasutsugu Suzuki	Pasteur Institute	Japan	Non-retroviral endogenous viral element limits cognate virus replication in *Aedes aegypti* ovaries
Aug. 25	Dr. Tomoya Kitajima	RIKEN Center for Biosystems Dynamics Research	Japan	Why are eggs so error prone?
Sep. 1	Dr. Shun-ichi Sekine	RIKEN Center for Biosystems Dynamics Research	Japan	Cryo-EM sheds light on chromatin transcription by RNA polymerase II
Oct. 7	Dr. Irving Weissman	Institute for Stem-Cell Biology and Regenerative Medicine, Stanford University School of Medicine	USA	Normal and neoplastic stem cells
Oct. 7	Dr. Minoru Takasato	RIKEN Center for Biosystems Dynamics Research	Japan	Generating 3-dimensional organs of the urinary tract using human iPS cells
Oct. 30	Mr. Daijiro Sakurai	RIKEN Career Support Office	Japan	Taking a new step on your career path: Practical tips and support seminar
Oct. 30	Dr. Shimpei Gotoh	Graduate School of Medicine, Kyoto University	Japan	Applications of human pluripotent stem cells to lung research
Nov. 20	Dr. Kenichi Masuda	RIKEN Cluster for Science, Technology and Innovation Hub	Japan	Re-consider a job based on experiences in academia
Nov. 24	Dr. Takanori Takebe	Tokyo Medical and Dental University	Japan	Hepato-biliary-pancreatic organoids to study development and disease
Dec. 1	Dr. Ichiro Manabe	Chiba University Graduate School of Medicine	Japan	Macrophages in the organ crosstalk and multimorbidity
Dec. 2	Dr. Ming Li	Sloan Kettering Institute	USA	Immunological mechanisms of cancer defense
Dec. 9	Dr. Kazuyoshi Ishigaki	Brigham and Women's Hospital, Harvard Medical School	USA	Immune system variations induced by genetic risk of autoimmunity
Dec. 16	Prof. Emma Teeling	University College Dublin	Ireland	Growing old yet staying young: do bats hold the secret of extended longevity?
Dec. 23	Dr. Hiroshi Nakagawa	RIKEN Center for Advanced Intelligence Project	Japan	Concept of guidelines for personal information management in medical research involving human subjects

Part 5

Data and Statistics

Publications 2020

Table: IMS Publications from January to December, 2020

Journal	Impact Factor (2019)	Number of Papers
Nature	42.8	11
Science	41.8	1
Nat Rev Immunol	40.4	1
Cell	38.6	2
Nat Biotechnol	36.6	2
Nat Genet	27.6	8
Immunity	22.6	2
Nat Immunol	20.5	1
Gastroenterology	17.4	1
Ann Rheum Dis	16.1	1
Nat Microbiol	15.5	1
Immunol Rev	13.9	1
Sci Immunol	13.4	1
Trends Immunol	13.4	5
Sci Adv	13.1	1
Eur Respir J	12.3	1
Nat Hum Behav	12.3	1
Nat Commun	12.1	23
Nat Struct Mol Biol	12.0	1
J Exp Med	11.7	2
Nucleic Acids Res	11.5	4
Genome Res	11.1	4
Mol Biol Evol	11.1	1
Genome Biol	10.8	1
Cell Death Differ	10.7	1
Methods Mol Biol	10.7	1
J Allergy Clin Immunol	10.2	1
Others		158
Total		**238**

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9 Ando Y, Kwon A, Shin JW. An Era of Single Cell Consortia. ***Exp Mol Med*** 52, 1409-1418 (2020)

10 Andrews L, Somasundaram A, Moskovitz J, Szymczak-Workman A, Liu C, Cillo A, Kurland B, Lin H, Normolle D, Moynihan K, Taniuchi I, Kirkwood J, Irvine D, Lipson E, Ferris R, Bruno T, Workman C, Vignali D. Resistance to PD1 blockade in the absence of metalloprotease-mediated LAG3 shedding. ***Sci Immunol*** 5, eabc2728 (2020)

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Budget, Personnel and Patents

IMS Budget FY2020

IMS Budget FY2020	JPY Million
Government funding for operations	3,806
External competitive funding	2,510
Total	**6,316**

Patents

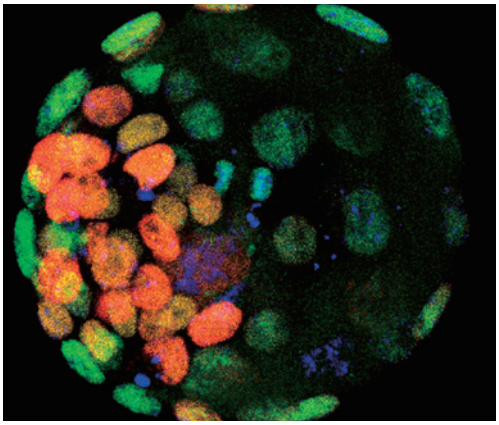
There were 58 patents registered from January to December, 2020.

Patents	Total	International patents	Domestic patents (Japan)
2020	58	52	6

Personnel FY2020

Category	Number
Director	1
Deputy Director	2
Senior Advisor	2
Team Leader	34
Unit Leader	1
Coordinator	5
Deputy Team Leader	7
Senior Scientist	22
Senior Research Scientist	4
Research Scientist	50
Postdoctoral Researcher	27
Special Fixed Term Contract Researcher	1
Special Postdoctoral Researcher	9
Research Fellow	14
Research Associate	10
Senior Technical Scientist	4
Technical Scientist	17
Expert Technician	16
Technical Staff I	69
Technical Staff II	48
International Program Associate	4
Junior Research Associate	21
Student Trainee	117
Intern	1
Research Administrator	6
Research Administrative Support Staff	5
Assistant	25
Part-time Staff	43
Senior Visiting Scientist	24
Visiting Scientist	224
Visiting Technical Scientist	17
Visiting Researcher	10
Temporary Staffing	18
Research Consultant	4
Consultant	2
Special Temporary Employee	1
Total	**866**

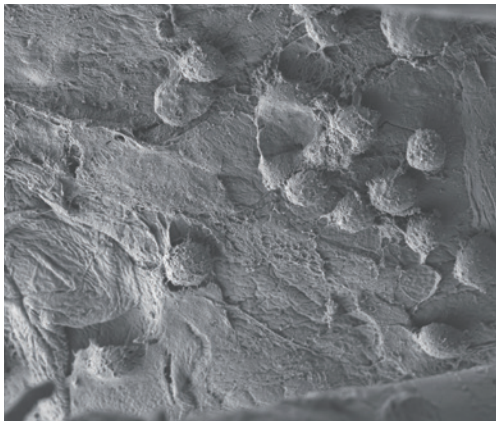
Original Photos of the Cover and Front Pages



Cover

A mouse blastocyst embryo immunostained for Cdx2 (green), Oct4 (yellow), Nanog (red), and DAPI (blue).

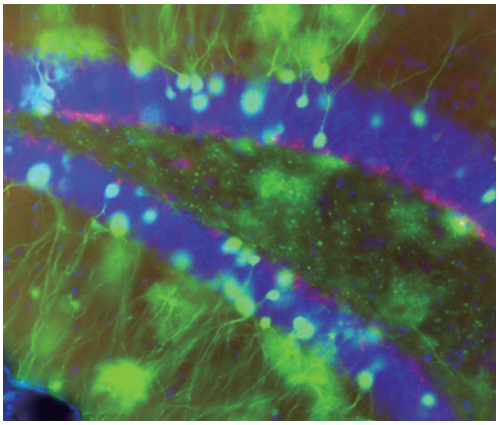
Credit to Dr. Chisayo Kozuka
YCI Laboratory for Metabolic Epigenetics



Front page of Part 1

SEM image of skin microbes on the stratum corneum of Tmem79-deficient mouse a spontaneous dermatitis model.

Credit to Dr. Yoshihiro Ito and Mr. Hachiro Iseki
Laboratory for Skin Homeostasis



Front page of Part 2

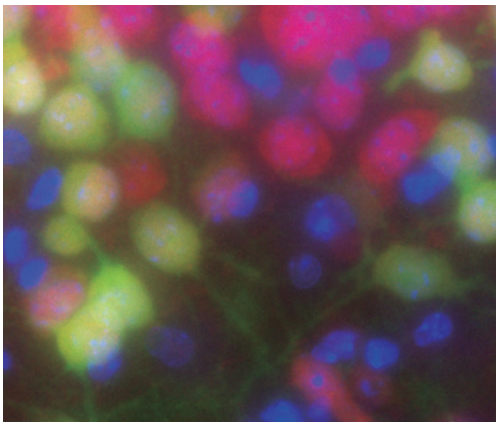
Borna disease virus expressing a GFP reporter (green) infects mouse dentate gyrus granule cells but not neuronal progenitor cells (red, Doublecortin).

Credit to Dr. Rie Koide
Genome Immunobiology RIKEN Hakubi Research Team

Front page of Part 3

A tadpole of the white-lipped tree frog (*Polypedates leucomystax*), which has a unique mitochondrial DNA with pseudogenized *atp8*.

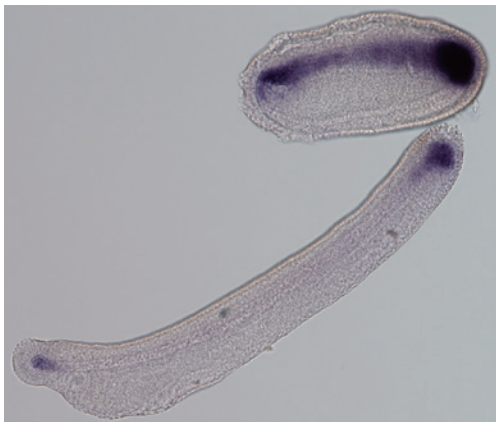
Credit to Dr. Yuuri Yasuoka
Laboratory for Comprehensive Genomic Analysis



Front page of Part 4

Borna disease virus expressing a GFP reporter (green) infects neurons (red, NeuN) of a mouse hippocampus.

Credit to Credit to Dr. Rie Koide
Genome Immunobiology RIKEN Hakubi Research Team

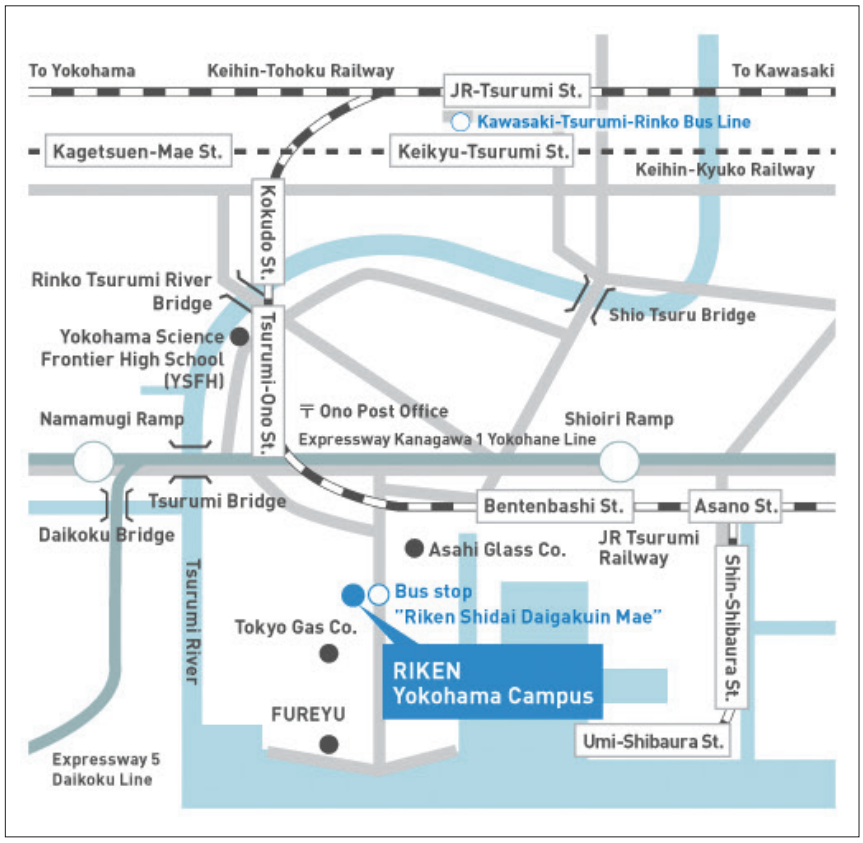


Front page of Part 5

Notochord expression of a *brachyury* gene in an amphioxus (*Branchiostoma floridae*, phylum Cephalochordata) neurula (top-right) and larva (bottom).

Credit to Dr. Yuuri Yasuoka
Laboratory for Comprehensive Genomic Analysis

Access to RIKEN Yokohama Campus



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.

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.

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