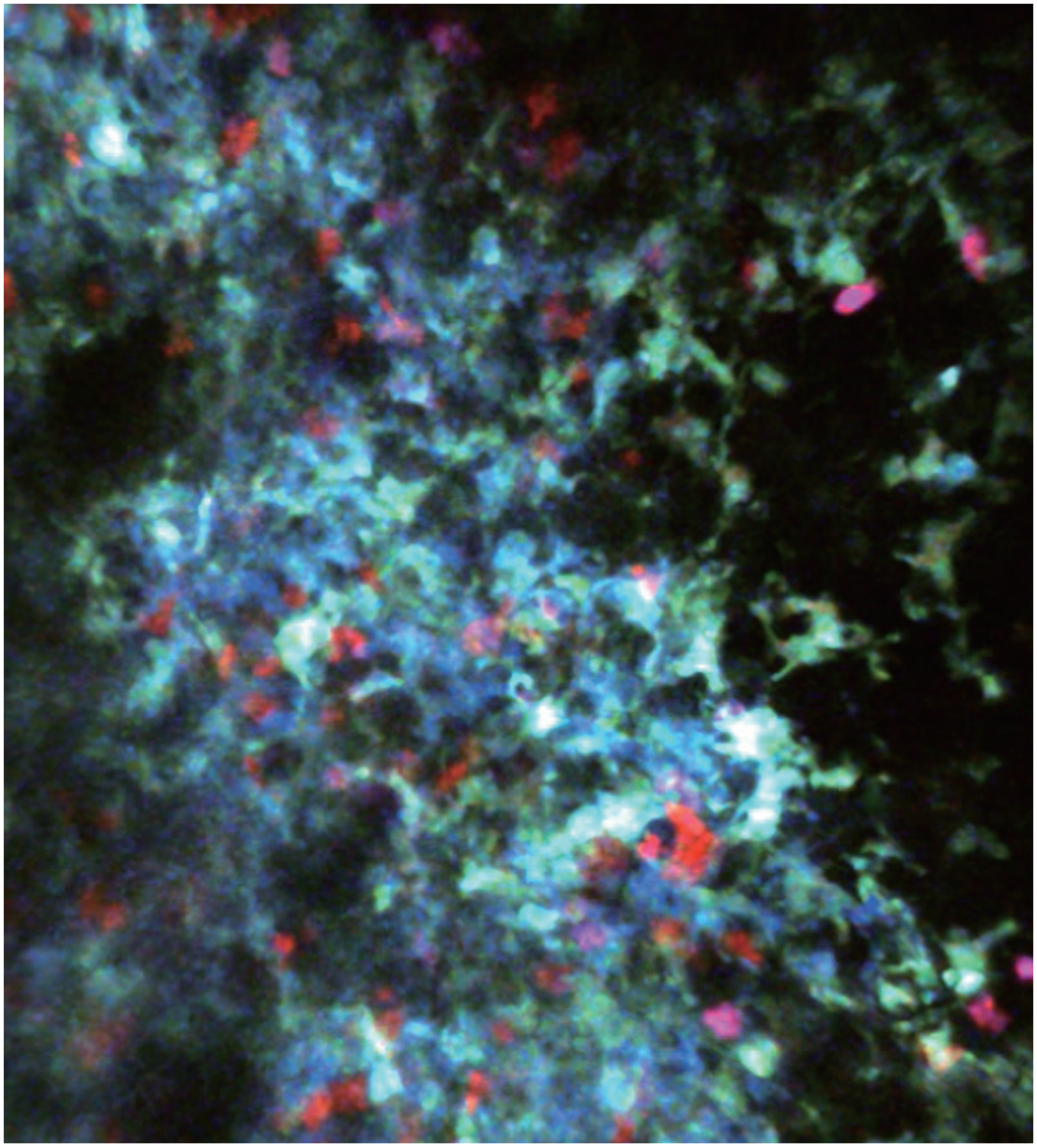

RIKEN IMS
Annual Report 2013

RIKEN Center for Integrative Medical Sciences

RIKEN Center for Integrative Medical Sciences Organization Chart

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Ryoji Noyori

Director (Acting)

Shigeo Koyasu

Deputy Director

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Takashi Saito
Haruhiko Koseki
Toshihiro Tanaka

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- Lab. for Cell Signaling: **Takashi Saito**
- Lab. for Lymphocyte Differentiation: **Tomohiro Kurosaki**
- Lab. for Transcriptional Regulation: **Ichiro Taniuchi**
- Lab. for Immune Cell System: **Shigeo Koyasu**
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- Lab. for Gut Homeostasis: **Kenya Honda**
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- Lab. for Inflammatory Regulation: **Takashi Tanaka**
- Lab. for Cytokine Regulation: **Masato Kubo**
- Lab. for Homeostatic Network: **Shigeo Koyasu**

Core for Precise Measuring and Modeling

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- Lab. for Integrative Genomics: **Osamu Ohara**
- Lab. for Disease Systems Modeling: **Hiroaki Kitano**
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- Lab. for Genotyping Development: **Michiaki Kubo**
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- Lab. for Bone and Joint Diseases: **Shiro Ikegawa**
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Program for Medical Innovations

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- Lab. for Immunotherapy: **Shin-ichiro Fujii**
- Lab. for Vaccine Design: **Yasuyuki Ishii**
- Lab. for Allergic Disease: **Toshiaki Kawakami**
- RIKEN-TORII Joint Research Team: **Masaru Taniguchi**
- Drug Discovery Antibody Platform Unit: **Toshitada Takemori**

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Director's Report

In 2012, RIKEN drafted a complete reorganization plan for the RIKEN's third 5-year term (2013-2018). The aim of the reorganization was to promote science and technology that leads to innovation, and leads to the discovery of solutions for critical scientific, technical and social issues, while still pursuing basic research.

The RIKEN Center for Integrative Medical Sciences (IMS) was newly established in April, 2013 by combining two existing research centers, RIKEN Research Center for Allergy and Immunology (RCAI 2001-2013) and RIKEN Center for Genomic Medicine (CGM 2008-2013). RCAI was established in 2001 as the first large research institute dedicated to allergy and immunology in Japan that is fully supported by the Japanese Government. CGM was established in 2008 by reorganization of RIKEN SNPs Research Center (SRC 2000-2008). SRC was a part of the Millennium Genome Projects initiated by the former Prime Minister, the late Keizo Obuchi. SRC successfully developed an accurate and high-throughput system for SNP analysis (genome-wide association study; GWAS). To accomplish the mission of discovering genetic elements that mediate human diseases and therapeutic effects and adverse reactions of various drugs, CGM conducted GWAS studies using more than 200,000 patients' samples for 47 diseases that were stocked in the BioBank Japan (BBJ).

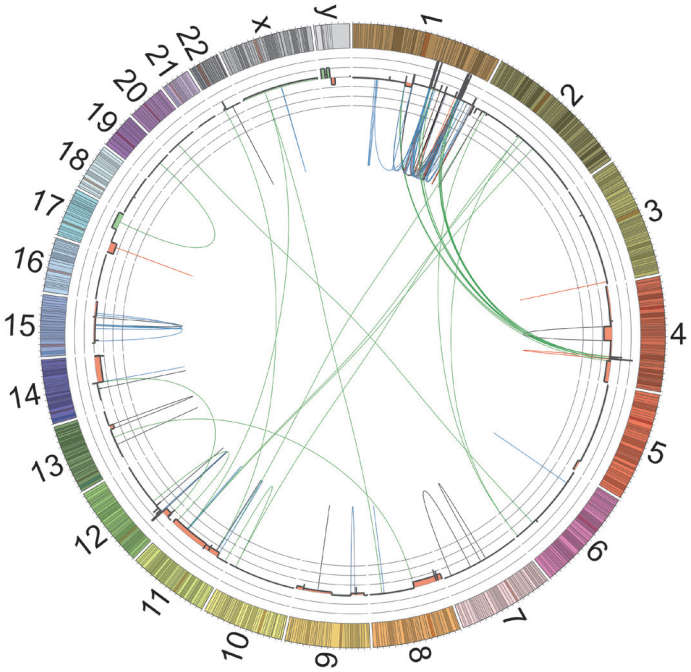
Both CGM and RCAI have been recognized worldwide as distinguished institutes with a large number of influential results in each field. CGM's approaches have uncovered the corelations between many human genetic polymorphisms and diseases. RCAI's approaches were extremely effective in elucidating molecular mechanisms by which certain genes regulate immune reactions using various gene-manipulated animal models. To understand the molecular mechanisms of human disease onset that are caused by gene alterations, we believed that amalgamation of these two approaches would be quite effective. Based on the history and achievements of two research centers, IMS integrates both mechanistic research developed in immunology and human genome research and transcends the existing scientific boundaries. IMS will also employ systems approaches and mathematical modeling expertise to create a new research area "integrative medical sciences."

The goal of IMS is to understand the robust system that maintains the homeostatic regulation of our body and to elucidate the mechanisms of disease onset as a consequence of malfunction of homeostasis. Homeostasis is regulated by robust gene networks that orchestrate the functions of cells, tissues and organs and the interplay between organs. The regulation of gene networks is modified by environmental factors affecting epigenetic modifications. Alteration of gene networks and interplay between cells and organs is induced by a combination of genetic factors and environmental factors and causes diseases if the degree of alteration proceeds beyond a certain threshold. We thus focus on the interplay of disease-related genes and environment factors, such as commensal microbiota affecting epigenetic alterations.

Elucidation of molecular mechanisms of disease onset by focusing on disease-related genes will uncover the target genes or target gene networks important for the prevention of diseases. Personal genomic information is also pivotal for the prediction of disease onset and avoidance of the adverse effect of certain drugs. Thus, IMS aims to elucidate how homeostasis is maintained and how its disruption initiates disease based on our knowledge of human genome research, which defines human genetic and epigenetic diversity and consequent disease susceptibility. IMS will contribute to the development of personalized preventive medicine and therapeutics.

IMS builds three cores and one program, Core for Homeostatic Regulation, Core for Precise Measuring and Modeling, Core for Genomic Medicine and Program for Medical Innovations. These divisions collaborate with each other and create platforms for 1) human genome analysis, 2) big data analysis and mathematical modeling, 3) disease models (e.g. humanized mice, human iPS cells), and 4) functional analysis using immunology and molecular and cellular biology approaches. These platforms will enable IMS to elucidate how the genetic and environmental alterations affect homeostasis. By understanding the disease onset mechanisms, IMS will apply our discoveries to predictive/preventive medicine and personalized medicine, and thus contribute to innovative medical science for the next generation.

Shigeo Koyasu
Director (Acting)
RIKEN Center for Integrative Medical Sciences (IMS)



Part 1

Lab Activities

Core for Homeostatic Regulation

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

The Core for Homeostatic Regulation is composed of 15 laboratories divided into four areas;

[1] Immune homeostasis: Cell signaling, Lymphocyte differentiation, Immune homeostasis and Metabolic homeostasis

[2] Lymphocyte development, Transcriptional regulation and Human disease model

[3] Mucosal immunity: Intestinal ecosystem, Mucosal immunity, Immune cell systems, Gut homeostasis and Immune crosstalk

[4] Allergy and inflammation: Skin homeostasis, Inflammatory regulation and Cytokine regulation

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

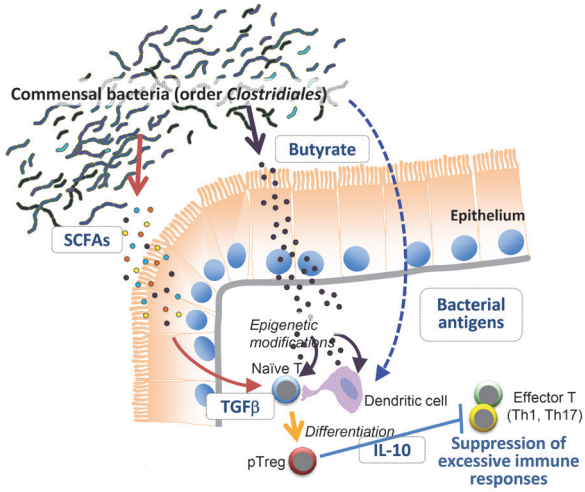


Figure: Induction of colonic regulatory T cells by fatty acids derived from gut microbiota.
Intestinal homeostasis is regulated by the interaction between gut microbiota and intestinal cells. Systemic analysis of the interaction elucidates the intestinal homeostasis and onset of mucosal diseases.

Core for Precise Measuring and Modeling

The ultimate goal of the Core for Precise Measuring and Modeling is to elucidate the mechanisms that govern human disease onset. This will be accomplished through collection of a wide variety of quantitative data to build a computational and predictive network of the process. Toward this end, this core must intensively collaborate with the Cores of Homeostatic Regulation and Genome Medicine as well as the program for Medical Innovations at IMS. Precise quantitative measurements are done for profiling of mRNA, protein (Laboratory for Integrative Genomics), and metabolites (Laboratory for Metabolomics) in a genome-wide manner and also for more dynamic properties of the biological systems by *in vivo*/*in vitro* bio-imaging (Laboratories for Tissue Dynamics, Molecular Live-Cell Quantification, and Cell Functional Imaging). The production of genetically engineered mice is another indispensable research activity in this core (Laboratories for Developmental Genetics and Immunogenetics). After being processed by bioinformatics (Laboratory for Integrated Bioinformatics), the datasets are used for modeling (Laboratories for Disease Systems Modeling and Integrated Cellular Systems). In particular, the Laboratory for Disease Systems Modeling plays a main role in systems biological research and software platform development at IMS. Because we have to derive a "Data-Model-Simulation-Validation" cycle to reach the ultimate goal, intra-core collaboration is essential. As the first leading project, the core is fully involved in the atopic dermatitis project at IMS. Needless to say, each laboratory in the core is also doing their own cutting-edge research to be prepared to address intriguing but unexplored questions in medical sciences.

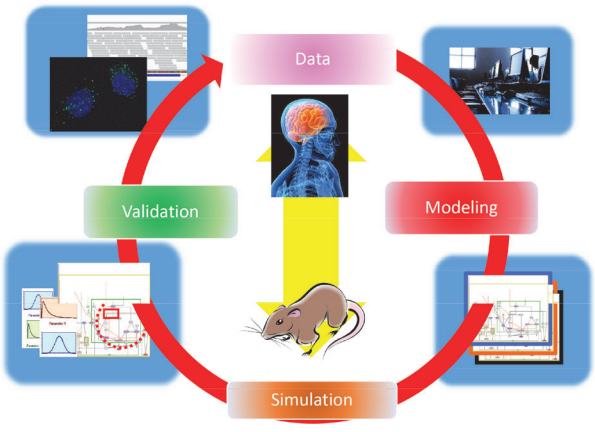


Figure: "Data-Model-Simulation-Validation (DMSV)" Cycle
A practical goal of the Core for Precise Measuring and Modeling is to implement the "DMSV" cycle in the IMS project in close collaboration with other cores at IMS.

Core for Genomic Medicine

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

To identify genetic variations related to disease susceptibility and drug responses, the Core for Genomic Medicine first showed the proof of concept of the genome-wide association study (GWAS) in 2002. To advance this strategy, the Core for Genomic Medicine has organized laboratories to facilitate comprehensive genomic research on common diseases. To produce comprehensive genomic information, the Laboratory for Genotyping Development is mainly working on large-scale SNP genotyping for GWAS and the Laboratory for Genome Sequencing Analysis is mainly working on whole genome sequencing of cancer genomes. To analyze large genomic variation data from many samples, the Laboratory for Statistical Analysis is mainly working on the analysis of GWAS data and the Laboratory for Medical Science Mathematics is mainly working on the analysis of cancer genome sequencing data. These laboratories are in close communication with each other to provide high quality genomic information analysis results to the research group of pharmacogenomics (Laboratory for Pharmacogenomics and Laboratory for International Alliance on Genomic Research), laboratories for disease-causing mechanisms (Laboratory for Cardiovascular Diseases, Autoimmune Diseases, Digestive Diseases, Bone and Joint Diseases, Endocrinology, Metabolism and Kidney Diseases, and Respiratory and Allergic Diseases) and many other collaborators worldwide for further analysis.

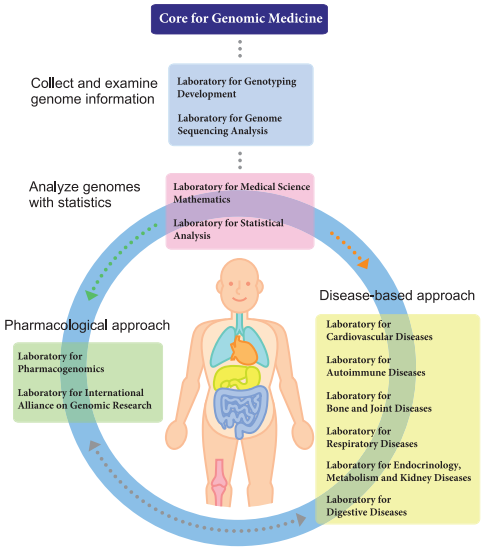


Figure: Twelve laboratories tackle the most advanced research
The Core for Genomic Medicine consists of four research groups and eight research teams. Each team is linked systematically with the others and works toward the implementation of personalized medicine.

Program for Medical Innovations

Seven original projects for clinical applications have been conducted in 2013: 1) A biochemical drug using a PEGylated Cryj-1/2 fusion recombinant protein for Cedar Pollinosis has been under development by the Torii pharmaceutical company and IMS. 2) A chemical compound recently developed by using the α-galactosylceramide (α-GalCer) analog, RCAI-X, selectively induces apoptosis of IgE but not IgG B cells and preferentially suppresses IgE production. It therefore potentially could be applied to any type of allergic disorder, such as pollenosis, food allergy, as well as allergic asthma. 3) NKT cell-targeted therapy for head and neck tumors has been conducted in collaboration with Professor Okamoto in Chiba University, and was authorized by the Japanese government as Advanced Medical Treatment B in 2013. 4) NKT cell-targeted therapy for stage IIA/IIB lung cancers after surgical resection has been conducted in collaboration with National Hospital Organizations. 5) The artificial adjuvant vector cell as an anti-tumor vaccine has been developed. This vaccine can be dosed with tumor antigen mRNA together with α-GalCer, activates both innate and acquired protective immunity, and induces long-term memory of more than one year in mice and dogs following a single administration. 6) The human iPS project for clinical use of *in vitro* generated NKT cells was accepted as the Center for Clinical Application Research (Type B) in the Research Center Network for Realization of Regenerative Medicine, Japan in 2013. 7) By using humanized mice, leukemia stem cells have been identified, and a drug candidate for effective treatment of leukemia has been developed.

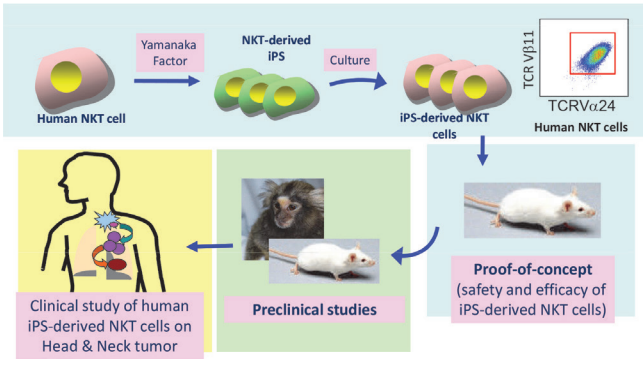
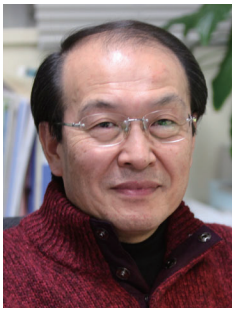


Figure: Human iPS project on cancer
The project is accepted as the Center for Clinical Application Research (Type B) in the Research Center Network for Realization of Regenerative Medicine, Japan.



Laboratory for Cell Signaling

Group Director: Takashi Saito

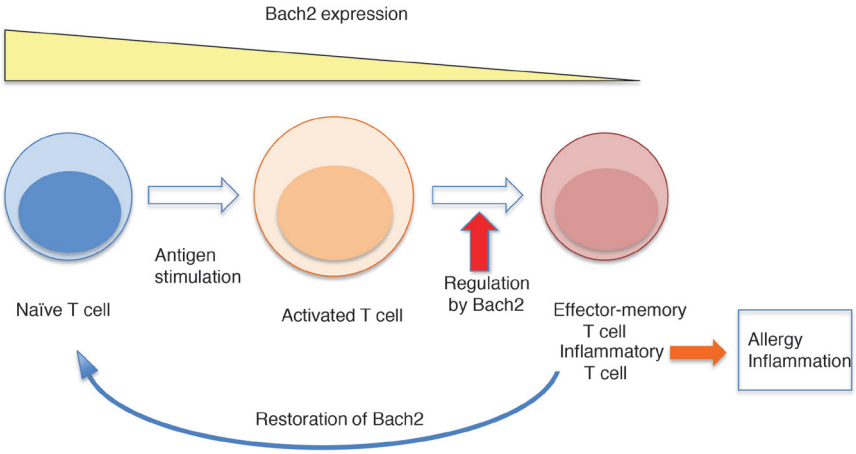


Figure: Regulation of generation of inflammatory T cells by Bach2.
Bach2 suppresses the expression of genes required to differentiate into Th2 and memory T cells, and maintains the naïve status of T cells. Bach2-deficient T cells express genes related to inflammatory/memory T cells, and re-expression of Bach2 resulted in a return to the phenotype of naïve T cells.

We have analyzed the mechanism and regulation of T cell activation and differentiation by analyzing the function of genes and signaling molecules whose expression is up-regulated during, or which regulate T cell activation and differentiation. Analyzing the genes whose expression is altered during T cell development, we identified Bach2. Bach-2 is transcriptional repressor and has been thought to be specific for B cells, but we found it is expressed equally well in T cells and to increase its expression during T cell maturation. Bach-2 deletion in T cells reduces naïve T cell numbers and increases memory-type T cells. The Bach2-/- naïve T cells highly express genes related to effector memory T cells, and rapidly produce Th2 cytokines upon T cell stimulation. These findings indicate that Bach2 suppresses effector memory-related genes to maintain the naive T-cell state and regulates generation of effector-memory T cells (Fig.).

We have analyzed the functional contribution of innate-related signals for T cell activation and function, particularly the function of TLRs expressed on T cells. We found that effector Th1 but not Th2 cells were directly activated through TLR2 for IFNγ production. Nucleic acids also induce T cell co-stimulation for cytokine production and proliferation, a process that is independent of TLR/RLRs. Unlike innate cells, not only CpG but also other forms of DNA could induce T cell co-stimulation, particularly when complexed with LL37 or histones. Nucleic acid-mediated T cell co-stimulation is induced by an as yet-unknown unique sensor and, physiologically, DNA released from dead cells at inflammation/infection sites induces Th2 differentiation by inducing IL-4 in naïve T cells, triggering allergic responses.

Recent Major Publications

Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death-1 forms negative costimulatory microclusters that directly inhibit T cell receptor signals by recruitment of phosphatase SHP2. ***J Exp Med*** 209, 1201-17 (2012)

Tsukumo S, Unno M, Muto A, Takeuchi A, Kometani K, Kurosaki T, Igarashi K, Saito T. Bach2 maintains T cells in naïve state by suppressing effector memory-related genes. ***Proc Natl Acad Sci*** 110, 10735-40 (2013)

Liang Y, Cucchetti M, Roncagalli R, Yokosuka T, Malzac A, Bertosio E, Imbert J, Nijman IJ, Suchanek M, Saito T, Wulfing C, Malissen B, Malissen M. Rltpr, a lymphoid lineage-specific actin-uncapping protein is essential for CD28 co-stimulation and regulatory T cell development. ***Nat Immunol*** 14, 858-66 (2013)

Invited Presentations

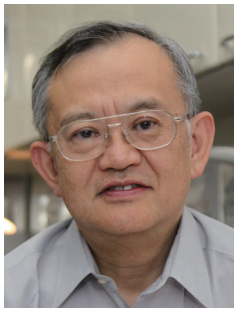
Saito T. Dynamic regulation and modulation of T cell activation. Immunology 2013: AAI Annual Meeting, Honolulu, USA. May 4th, 2013.

Saito T. Molecular dynamics for T cell activation and co-stimulation. Frontiers in Immunology Conference, Tokyo, Japan. August 2nd, 2013.

Saito T. Dynamic regulation of T cell activation and co-stimulation. 4th International Symposium: Regulators of Adaptive Immunity, Erlangen, Germany. September 28th, 2013.

Saito T. Dynamic regulation of T cell activation and co-stimulation. 43rd Annual Scientific Meeting of the Australiasian Society for Immunology (ASI ASM 2013), Wellington, New Zealand. December 2nd, 2013.

Saito T. Nucleic acids sensing by T cells initiates Th2 cell differentiation. Germany-Japan Immunology Seminar 2013, Shizuoka, Japan. December 7th, 2013.



Laboratory for Lymphocyte Differentiation

Group Director: Tomohiro Kurosaki

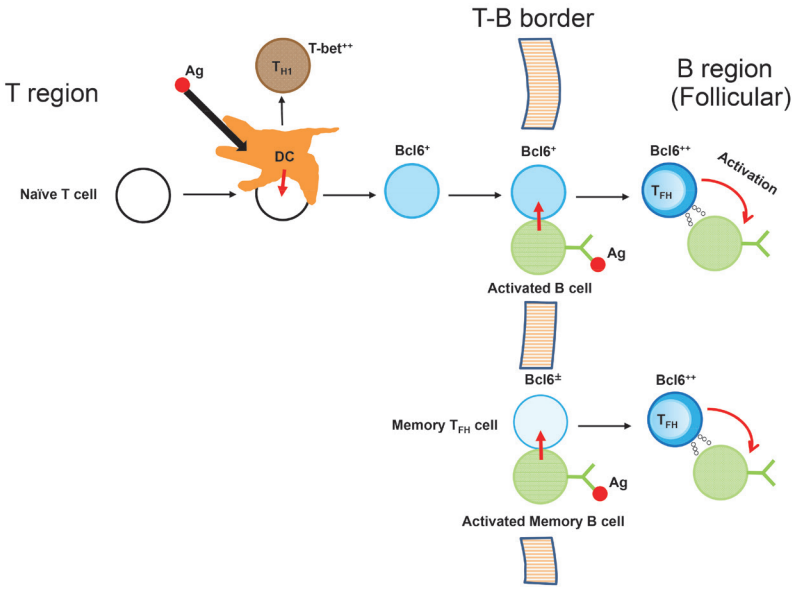


Figure: Bcl6 activation mechanisms in naive versus memory T cells
During primary immune responses, naïve T cells are primed by antigen-presenting dendritic cells and differentiate into effector cells. Some of the activated T cells up-regulate Bcl6 expression. The Bcl6$^+$ cells express the CXCR5 chemokine receptor, which leads them to the T-B border where cognate interactions between Bcl6$^+$ T cells and antigen-specific B cells occur. In contrast to primary responses, memory T_{FH} cells are activated directly by antigen-presenting memory B cells and quickly gain high levels of Bcl6 expression and re-differentiate into effector T_{FH} cells, which contribute to activation of memory B cells.

Memory humoral responses are typically more rapid, have a greater magnitude, and consist of antibodies of higher affinity than in the primary response. Our laboratory has now focused on clarifying the functional characterization of memory B cells and memory T cells and on revealing the mechanisms underlying the robustness of memory antibody responses.

Characterization of memory T_{FH} cells

CD4 T cells are critical for memory B cell generation and their subsequent activation. Previously, we demonstrated that CXCR5$^+$ CD4 T cells reside nearby the IgG1 memory B cells in the follicles (Aiba et al, PNAS, 2010) at the memory phase. Based on these results, we hypothesized that a fraction of antigen-specific effector T cells, especially T_{FH} cells, survives over the contraction phase and becomes memory CXCR5$^+$ T_{FH} cells, which participate in recall antibody responses. Thus, by using the transgenic TCR model, we first verified our idea. In addition, our data suggest that memory B cells function as APCs to rapidly induce Bcl6 in T_{FH} memory T cells, thereby contributing to robust humoral memory responses.

Regulation of Bach2 during immune responses

We previously proposed that reorganization of transcription factors (for instance, repression of Bach2) takes place during generation of IgG1 type memory B cells after primary antigen exposure, and is critical for rapid responsiveness of IgG1 memory B cells (BCR-extrinsic model) (Kometani et al, Immunity, 2013). To directly test this model, we have established mice in which Bach2 could be overexpressed or knocked out in an inducible manner. When Bach2 was overexpressed just before secondary responses, IgG1 type memory B cells became skewed toward generation of GC B cells rather than differentiating into plasma cells. Conversely, deletion of Bach2 promoted preferential differentiation into plasma cells. These results clearly suggest that the expression level of Bach2 is involved in the functions of memory B cells.

Recent Major Publications

Kometani K, Nakagawa R, Shinnakasu R, Kaji T, Rybouchkin A, Moriyama S, Furukawa K, Koseki H, Takemori T, Kurosaki T. Repression of the Transcription Factor Bach2 Contributes to Predisposition of IgG1 Memory B Cells toward Plasma Cell Differentiation. ***Immunity*** 39, 136-47 (2013)

Sasaki Y, Sano S, Nakahara M, Murata S, Kometani K, Aiba Y, Sakamoto S, Watanabe Y, Tanaka K, Kurosaki T, Iwai K. Defective immune responses in mice lacking LUBAC-mediated linear ubiquitination in B cells. ***EMBO J*** 32, 2463-76 (2013)

Capietto AH, Kim S, Sanford DE, Liehan DC, Hikida M, Kurosaki T, Novack DV, Faccio R. Downregulation of PLCγ2/β-catenin pathway promotes activation and expansion of myeloid-derived suppressor cells in cancer. ***J Exp Med*** (in press)

Invited Presentations

Kurosaki T. Calcium Signaling in B Lymphocytes. 5th International Conference on B cell and Autoimmunity, Como, Italy. August 20th, 2013.

Kurosaki T. Calcium Signaling in B Lymphocytes. Immune-related Pathologies: Understanding Leukocyte Signaling and Emerging therapies (IMPULSE 2013), Matrahaza, Hungary. September 2nd, 2013.

Kurosaki T. B cell intrinsic and extrinsic mechanisms for rapid responsiveness of IgG1 type memory B cells. Oversea Scholar Seminar, Tsinghua University, Beijing, China. November 14th, 2013.

Kurosaki T, Kometani K, Ise W, Shinnakasu R. B cell intrinsic and extrinsic mechanisms for rapid responsiveness of IgG1 type memory B cells. The 36th Annual Meeting of the Molecular Biology Society of Japan, Kobe, Japan. December 5th, 2013.

Kurosaki T. Calcium Signaling in B Lymphocytes. Keystone Symposia: Biology of B Cell Responses, Keystone, USA. February 11th, 2014.

Laboratory for Transcriptional Regulation

Group Director: Ichiro Taniuchi

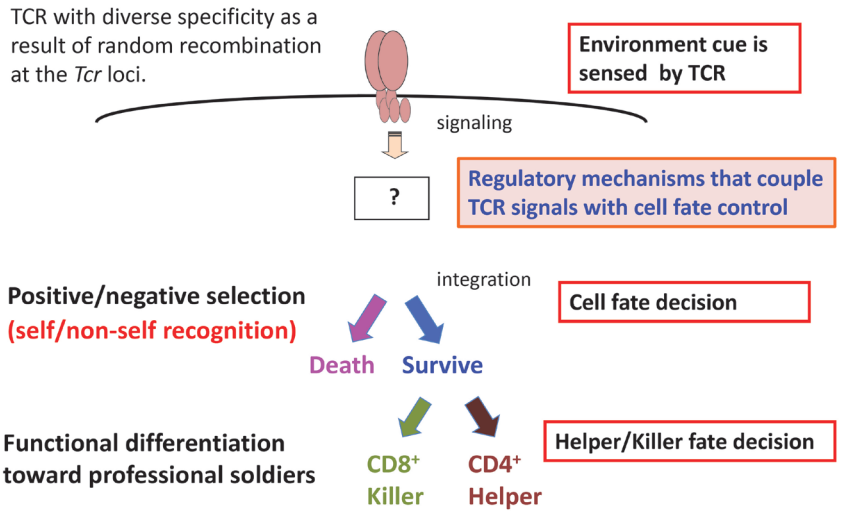


Figure: Toward understanding of mechanisms that couple TCR signals with cell fate decision
T lymphocytes sense environmental cues via the TCR. This information needs to be appropriately integrated into developmental programs that control cell fate. Studies in my laboratory focus on regulatory mechanisms that link environmental cues with cell fate determination.

One of major questions in developmental biology is how extracellular information is sensed by interface receptors and is integrated into a developmental program encoded by the genome. Besides such a profound question, current advances in our understanding of functional modulation of effector T cell subsets in different environments raised the challenging question of how retention and loss of developmental plasticity is counterbalanced during the initial commitment process. Research in my laboratory has been addressing these important issues by studying mechanisms that control helper versus cytotoxic T cell differentiation in the thymus as well as in the periphery. In particularly, we have focused on antagonistic interplay between two transcription factors, ThPOK and Runx. Our research goal is to advance our understanding of how external information, recognized by TCR in this case, is integrated into transcriptional and epigenetic control of these factors' expression in the initial lineage selection process in the thymus, and how peripheral environmental cues modulate their expression.

Our second objective is to unravel functions of Runx transcription factor complexes that act as heterodimers of Runx proteins and the non-DNA binding Cbfb protein. Runx complexes are evolutionally conserved and play multiple and central roles to control differentiation and function of many types of hematopoietic cells. Our goal is to reveal regulatory mechanisms that modulate function of Runx complexes as well as to provide insights into how Runx complexes regulate immune system development and immune responses. We are addressing these questions mainly by analyzing a series of mutant mouse strains harboring specific mutations in the Runx family genes and by identification and characterization of Runx interacting molecules including functional RNAs.

Recent Major Publications

Seo W, Ikawa T, Kawamoto H, Taniuchi I. Runx1/Cbfβ is essential for early B lymphocyte development through regulation of Ebf1 expression. ***J Exp Med*** 209, 1255-62 (2012)

Tanaka H, Naito T, Muroi S, Seo W, Chihara R, Miyamoto C, Kominami R and Taniuchi I. Epigenetic Thpok silencing limits the time window to choose CD4+ helper-lineage fate in the thymus. ***EMBO J*** 32, 1183-94 (2013)

Mucida D, Husain M.M, Muroi S, van Wijk F, Shinnakasu R, Naoe Y, Reis B, Huang Y, Lambolez F, Docherty M, Attinger A, Shui J.W, Kim G, Lena C, Sakaguchi S, Miyamoto C, Wang P, Atarashi K, Park Y, Nakayama T, Honda K, Ellmeier W, Kronenberg M, Taniuchi I* and Cheroutre H*. Transcriptional Reprogramming of Mature CD4 T helper Cells generates distinct MHC class II restricted Cytotoxic T Lymphocytes. ***Nat Immunol*** 14, 281-9 (2013)
*corresponding authors

Invited Presentations

Taniuchi I. Local Chromatin Loop in CD4/CD8 lineage Choice. 6th International Workshop of Kyoto T Cell Conference (KTCC2013), Kyoto, Japan. June 5th, 2013.

Taniuchi I. Reconstitution approach to understand regulatory mechanisms of the Zbtb7b and Cd8 gene expression. 4th Synthetic Immunology Workshop, Kyoto, Japan. November 16th, 2013.

Taniuchi I. Runx transcription factors and inflammation. The 63rd Annual Meeting of Japanese Society of Allergology, Tokyo, Japan. November 28th, 2013.

Laboratory for
Immune Cell System

Group Director: Shigeo Koyasu

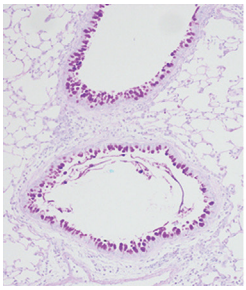
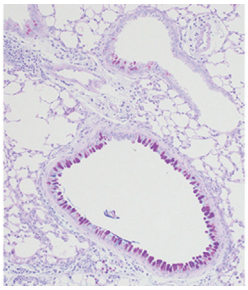
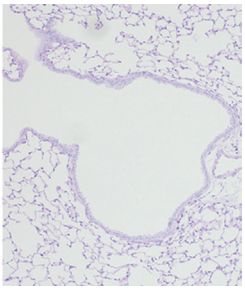




Figure: Pimozide attenuates corticosteroid resistance in a severe asthma model.
Mice were treated with IL-33 and TSLP for 3 days to induce asthmatic airway inflammation (left). Dexamethasone, a typical steroid used for treating asthma (middle), or a combination of dexamethasone and pimozide (right) were administered at 24 and 1 hr before IL-33+TSLP treatments. Shown are sections of the airways stained periodic acid–Schiff (PAS)-alcian blue to examine mucin secretion. It can be seen that dexamethasone alone is insufficient to suppress the airway inflammation characterized by thickening of airway epithelia and mucin secretion shown in violet (left and middle) but a combination of dexamethasone and pimozide effectively suppressed such asthmatic airway inflammation (right).

Recent Major Publications

Koyasu S, Moro K. Th2-type innate immune responses mediated by natural helper cells. ***Ann N Y Acad Sci*** 1283, 43-9 (2013)

Furusawa J, Moro K, Motomura Y, Okamoto K, Zhu J, Takayanagi H, Kubo M, Koyasu S. Critical role of p38 and GATA3 in natural helper cell function. ***J Immunol*** 191, 1818-26 (2013)

Kabata H, Moro K, Fukunaga K, Suzuki Y, Miyata J, Masaki K, Betsuyaku T, Koyasu S, Asano K. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. ***Nat Commun*** 4, 2675 (2013)

Invited Presentations

Koyasu S. Role of natural helper cell, a member for the group 2 ILC (ILC2), in steroid resistance of allergic inflammation. Keystone Symposia: Advances in the knowledge and treatment of autoimmunity, British Columbia, Canada. April 8th, 2013.

Koyasu S. Natural helper cells and type-2 innate immune responses. 5th Symposium & Master Classes on Mucosal Immunology: "Cytokines and border patrol", Rotterdam, Kingdom of the Netherlands. May 13th, 2013.

Koyasu S. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells in the inflamed airways. Aegean Conferences, 6th International Conference on Autoimmunity: Mechanism and Novel Treatments, Corfu, Greece. October 4th, 2013.

Moro K. Role of natural helper cells, a member of group 2 innate lymphoid cells. 12th FIMSA Advanced Training Course, Chiang Mai, Thailand. October 22nd, 2013.

Moro K. Natural Helper Cell "a new player in innate immune system". Annual Meeting of the Japanese Society for Immunology 2013, Chiba, Japan. December 12th, 2013.

We focus on a new lymphoid tissue in mouse, rat and human mesentery, fat-associated lymphoid cluster (FALC), and an innate lymphocyte population, the Natural Helper (NH) cell, a type of group 2 innate lymphoid cell (ILC2), both of which we discovered. NH cells constitutively produce low levels of IL-5, IL-6 and IL-13 and support the proliferation of B1 cells in the peritoneal cavity and IgA production by B cells expressing surface IgA. NH cells respond to IL-33 and a combination of IL-2 and IL-25 during helminth infection and produce large amounts of IL-5 and IL-13. IL-5 induces eosinophilia and IL-13 induces goblet cell hyperplasia during the innate phase of helminth infection (Moro et al., (2010) Nature 463: 540-544). In 2013, we demonstrated a critical role of Gata3 in the differentiation of NH cells and their expression of IL-5 and IL-13. IL-33 and a combination of IL-2 and IL-25 induced IL-5 and IL-13 production through the p38-Gata3 pathway. RORα is also expressed at high levels in NH cells. We showed that RORα is involved in the differentiation of NH cells but it is dispensable for cytokine production by mature NH cells. We also discovered in 2013 that NH cells are involved in steroid resistance of asthma. NH cells are as sensitive to corticosteroids as T cells and eosinophils but thymic stromal lymphopoietin (TSLP) synthesized during airway inflammation induced corticosteroid resistance of NH cells *in vitro* and *in vivo* by controlling phosphorylation of STAT5 and expression of Bcl-xL. Blockade of TSLP with a neutralizing antibody or blocking the TSLP/STAT5 signaling pathway with low molecular weight STAT5 inhibitors such as pimozide restored corticosteroid sensitivity of NH cells. Based on these results, the TSLP-STAT5 pathway could be a new therapeutic target in severe, corticosteroid-resistant asthma.

Laboratory for Human Disease Models

Group Director: Fumihiko Ishikawa

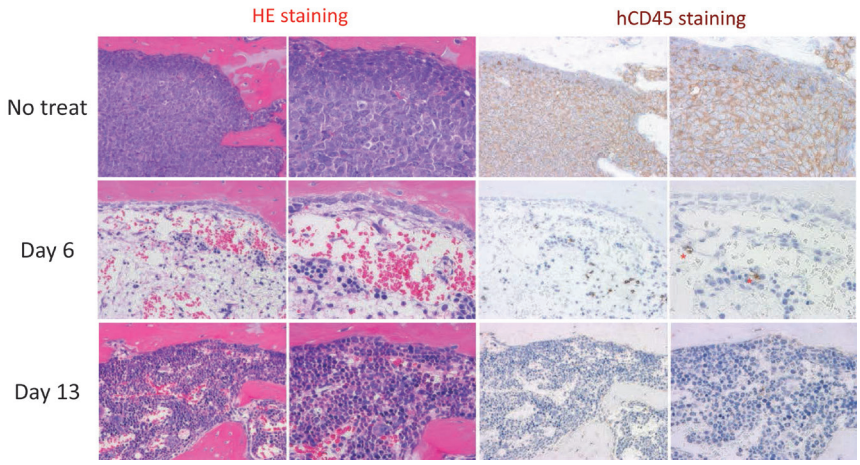


Figure: A pyrrolo-pyrimidine compound eliminates human AML stem cells.
Histopathological examination with HE staining and immunohistochemical staining demonstrated that *in vivo* administration of RK-20449 resulted in elimination of human AML cells in NSG bone marrow over time. Normal mouse erythrocytes and myeloid cells recovered at day 6 and at day 13 while the number of human AML cells in the bone marrow was decreased. Human AML cells were stained with an anti-hCD45 antibody (brown).

Our laboratory has been creating humanized mice, by injecting human cord blood HSCs into immune-deficient NOD/SCID/Il2rgKO (NSG) newborns. Though the NSG humanized mouse model has enabled us to achieve high levels of human hematopoietic chimerism in multiple organs, limitations remain due to the species barrier between human immune cells and the mouse microenvironment. To overcome such limitations, in the past few years we have been developing mice with a more humanized microenvironment. We previously reported that human $CD8^+$ T cells developed in HLA class I expressing NSG mice exhibited HLA-restricted functions such as cytokine production and cytotoxicity through recognition of EBV-infected B cells (Shultz, Saito, et al., PNAS 2010). Currently, we have been evaluating whether the HLA-expressing humanized mice could be a model to evaluate immune-therapy for malignancies.

In human leukemia research, to date, we reported that CD34+CD38- AML cells are largely cell cycle quiescent and are resistant to chemotherapy (Nature Biotechnology, 2007 & 2010). Furthermore, we found that HCK, a Src family kinase, was over-represented in human AML $CD34^+CD38^-$ cells compared with normal $CD34^+CD38^-$ HSCs (Science Translational Medicine 2010). Through large-scale library screening and *in silico* simulation for binding between HCK and small molecules, we identified a pyrrolo-pyrimidine compound, RK-20449, that can bind the ATP binding site of HCK and efficiently inhibit kinase activity of HCK. From *in vitro* experiments using 25 AML patient samples, we found that RK-20449 was effective especially against FLT3-ITD mutated AML cells. Finally, by using FLT3-ITD mutated AML engrafted humanized mice, we confirmed that *in vivo* administration of RK-20449 could almost completely eliminate human AML cells in the recipient circulation and in the recipient bone marrow (Fig.). We concluded that RK-20449 is a promising agent for targeting FLT3-ITD mutated AML stem cells (Saito et al. Science Translational Medicine 2013).

Recent Major Publications

Takagi S, Saito Y, Hijikata A, Tanaka S, Watanabe T, Hasegawa T, Mochizuki S, Kunisawa J, Kiyono H, Koseki H, Ohara O, Saito T, Taniguchi S, Shultz LD, Ishikawa F. Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation. ***Blood*** 119, 2768-2777 (2012)

Valent P, Bonnet D, De Maria R, Lapidot T, Copland M, Melo JV, Chomienne C, Ishikawa F, Schuringa JJ, Stassi G, Huntly B, Herrmann H, Soulier J, Roesch A, Schuurhuis GJ, Wohrer S, Arock M, Zuber J, Cerny-Reiterer S, Johnsen HE, Andreeff M, Eaves C. Cancer stem cell definitions and terminology: the devil is in the details. ***Nat Rev Cancer*** 12, 767-775 (2012)

Saito Y, Yuki H, Kuratani M, Hashizume Y, Takagi S, Honma T, Tanaka A, Shirouzu M, Mikuni J, Handa N, Ogahara I, Sone A, Najima Y, Tomabechi Y, Wakiyama M, Uchida N, Tomizawa-Murasawa M, Kaneko A, Tanaka S, Suzuki N, Kajita H, Aoki Y, Ohara O, Shultz LD, Fukami T, Gogo T, Taniguchi S, Yokoyama S, Ishikawa F. A pyrrolo-pyrimidine derivative targets human primary AML stem cells in vivo. ***Sci Transl Med*** 5, 181ra52 (2013)

Invited Presentations

Ishikawa F. Chemotherapy-Resistance of Human AML Stem Cells. 2013 USA-Japan Science Conference, Hawaii, USA. March 26th, 2013.

Ishikawa F. Targeting Chemotherapy-resistant AML Stem Cells. 1st Shanghai International Workshop on Stem Cells in Cancer, Shanghai, China. April 18th, 2013.

Ishikawa F. Niche for human leukemia stem cells. 54th meeting for Japan Society of Histochemistry & Cytochemistry, Tokyo, Japan. September 27th, 2013.

Ishikawa F. Developing Therapeutic Strategies Targeting AML Stem Cells. 4th International Workshop on Humanized Mice, Seoul, Korea. October 2nd, 2013.

Ishikawa F. Review talk on humanized mouse research. Annual Meeting of the Japanese Society for Immunology 2013, Chiba, Japan. December 11th, 2013.

Laboratory for
Intestinal Ecosystem

Group Director: Hiroshi Ohno

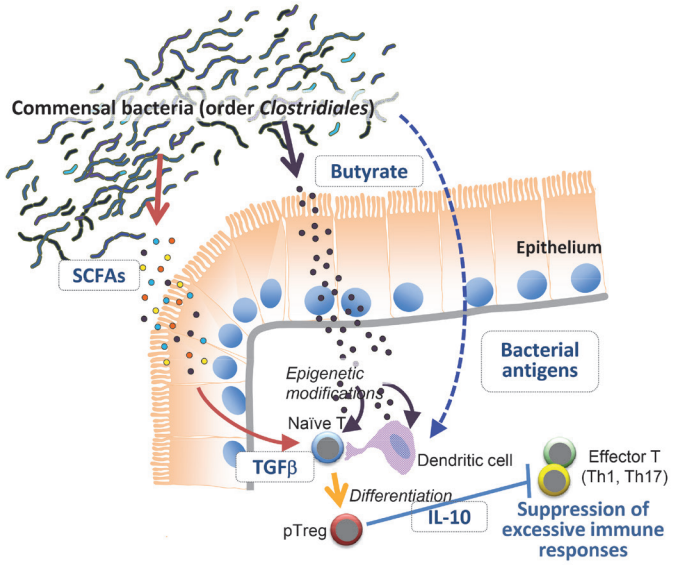


Figure: Schematic representation of the induction of colonic regulatory T-cell differentiation by gut microbial-derived butyrate
Butyrate, a short chain fatty acid produced as a result of fermentation by gut commensal microbiota of food-derived indigestible dietary carbohydrate fiber, induces differentiation of extrathymic regulatory T cells from naïve T cells in the colonic lamina propria. This is suggested to result mainly from epigenetic changes via the histone deacetylase-inhibiting capacity of butyrate in both naïve T cells and antigen-presenting dendritic cells. The combination of microbial short-chain fatty acids is also suggested to promote production of TGFβ, which is required for regulatory T-cell differentiation, by intestinal epithelial cells.

Gut microbiota play important roles in the normal physiology as well as pathology of the host. However, the gut does not unconditionally accept commensal microbiota. Our intestinal immune system somehow senses the kind and amount of bacteria existing in the gut lumen and tries to contain the total number and composition of the gut microbiota. The aim of this laboratory is to understand the mechanisms by which the host and its gut commensal microbiota interact, especially focusing on how gut microbes are delivered across the intestinal epithelial barrier to be recognized by the intestinal immune system, how gut microbiota shape host defense and immune systems, and how host-gut microbiota interactions affect host health and disease status.

The delivery of particulate antigens such as bacteria is thought to be mainly achieved by a unique epithelial cell subset, M cells, residing in a limited region of epithelial layer covering the lymphoid follicles of gut-associated lymphoid tissue such as Peyer's patches. We are studying the function and differentiation of M cells at the molecular mechanistic level. We have identified bacterial uptake receptors on M cells, such as glycoprotein 2 and cellular prion protein. We also have recently discovered that Spi-B, an Ets family transcription factor expressed in immature epithelial progenitor cells in the crypt, is essential for these cells to differentiate into M cells.

Regarding host-gut microbiota interactions, we employ a comprehensive multiple omics approach, combining exhaustive metagenomic, (meta)transcriptomic, and metabolomic analyses. By applying this approach, we have shown that genes encoding ATP-binding-cassette (ABC)-type carbohydrate transporters present in certain bifidobacteria contribute to protecting mice against death induced by enterohaemorrhagic *Escherichia coli* O157:H7. We have also found that a short chain fatty acid, butyrate, produced by gut microbiota is important for the induction of regulatory T cells from naïve T cells in the colonic lamina propria.

Recent Major Publications

Kanaya T, Hase K, Takahashi D, Fukuda S, Hoshino K, Sasaki I, Hemmi H, Knoop KA, Kumar N, Sato M, Katsuno T, Yokosuka O, Toyooka K, Nakai K, Sakamoto A, Kitahara Y, Jinnohara T, McSorley SJ, Kaisho T, Williams IR, Ohno H. The Ets transcription factor Spi-B is essential for the differentiation of intestinal M cells. ***Nat Immunol*** 13, 729-36 (2012)

Hase K, Nakatsu F, Ohmae M, Sugihara K, Shioda N, Takahashi D, Obata Y, Furusawa Y, Fujimura Y, Yamashita T, Fukuda S, Okamoto H, Asano M, Yonemura S, Ohno H. AP-1B-mediated protein sorting regulates polarity and proliferation of intestinal epithelial cells in mice. ***Gastroenterology*** 145, 625-35 (2013)

Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces colonic regulatory T cells. ***Nature*** 504, 446-50 (2013)

Invited Presentations

Ohno H. Differentiation and function of M cells, a unique subset of intestinal epithelial cells specialized for mucosal antigen-uptake. Immunology 2013: AAI Annual Meeting, Honolulu, USA. May 6th, 2013.Ohno H. Differentiation and function of M cells, a unique subset of intestinal epithelial cells specialized for mucosal antigen-uptake. The 16th International Congress for Mucosal Immunology: Plenary session, Vancouver, Canada. July 19th, 2013.Ohno H. Commensal microbe-derived butyrate epigenetically induces colonic regulatory T cell differentiation. 7th International Leukocyte Signal Transduction Conference, Kos, Greece. September 12th, 2013.

Laboratory for Mucosal Immunity

Team Leader: Sidonia Fagarasan

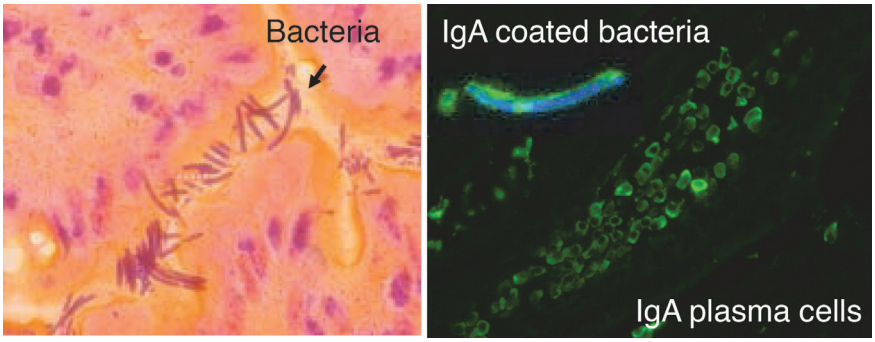


Figure: The coating of commensal bacteria with IgA facilitates maintenance of diversified bacterial species in the gut
A section of Peyer's patch Gram stained (left panel) showing mostly Gram positive segmented bacteria trapped in the mucus layer (yellow). Under normal conditions, a large fraction of the commensal bacteria are coated with IgAs produced by the plasma cells in the lamina propria (shown in the right panel).

Our previous studies demonstrated that the absence of immunoglobulin A (IgA), the major effector molecule of the adaptive immunity in the gut, or the impaired IgA selection in germinal centers (GC) due to deregulated T cell control, severely affects the balance of gut bacterial communities, resulting in massive activation of the immune system in the entire body. The absence of a subset of Foxp3$^+$ T cells induced by bacterial antigens also modifies the composition of the gut microbiota by evoking mucosal T_H2 inflammation. Interestingly, the Foxp3$^+$ T cells induce GC and IgA responses by generating GC T cells (T_{FH} and T_{FR} cells), and their depletion causes a rapid loss of specific IgA responses in the intestine. Together, all these observations pointed to the existence of a Foxp3-IgA axis in maintaining the gut microbiota balance. It remained unclear however, how these specific arms of the adaptive immune system mediate host-microbial interactions in the gut. We found that Foxp3$^+$ T cells contribute to diversification of the gut microbiota, particularly of species belonging to Firmicutes. The control of indigenous bacteria by Foxp3$^+$ T cells involved their regulatory functions outside and inside of the GCs, suppression of inflammation and regulation of IgA selection in Peyer's patches, respectively. The diversified and selected IgA repertoires generated in the presence of Foxp3$^+$ T cells in the GCs (T_{FR} cells) associate with specific coating of a large diversity of bacterial species and their maintenance rather than exclusion from the gut. By contrast, the IgAs generated in the absence of T_{FR} cells associate with abundant coating of bacteria with largely non-specific IgAs, reduced diversity and skewed gut microbiota. Thus, the adaptive immune system, through its cellular and molecular components required for immune tolerance, and diversification as well as selection of the antibody repertoire, mediates host-microbial symbiosis by controlling the richness and balance of bacterial communities required for homeostasis.

Recent Major Publications

Kawamoto S, Tran TH, Maruya M, Suzuki K, Doi Y, Tsutsui Y, Kato LM, Fagarasan S. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. Science 336, 485-9 (2012)

Kato LM, Kawamoto S, Maruya M, Fagarasan S. Gut TFH and IgA: key players for regulation of bacterial communities and immune homeostasis. Immunol Cell Biol 92, 49-56 (2014)

Magri G, Miyajima M, Bascones S, Mortha A, Puga I, Cassis L, Barra CM, Comerma L, Chudnovskiy A, Gentile M, Llige D, Cols M, Serrano S, Arostegui JI, Juan M, Yague J, Merad M, Fagarasan S, Cerutti A. Innate immune cells integrate stromal and immune signals to enhance antibody production by splenic marginal zone B cells. Nat Immunol (in press)

Invited Presentations

Fagarasan S. IgA synthesis: a form of immune adaptation extending beyond gut. Keystone Symposia: B Cell Development and Function (X1), Keystone, Colorado, USA. February 12th, 2013.

Fagarasan S. IgA synthesis: a form of immune adaptation extending beyond gut. 6th International Singapore Symposium of Immunology: From basic Immunology to effective immunotherapies, Singapore. June 6th, 2013.

Fagarasan S. TFR/TFH cells in mucosal immunity. Gordon Research Conferences: T follicular Helper Cells, Hong-Kong, China. July 23rd, 2013.

Fagarasan S. Symbiotic regulatory loop between Foxp3$^+$ T cells, IgA and bacteria in the gut. 15th International Congress of Immunology, Milan, Italy. August 27th, 2013.

Fagarasan S. Symbiotic regulatory loop between acquired immune system and gut microbiota. The 3rd lymphoid tissue meeting: Plenary Lecture, Rotterdam, The Netherlands. September 15th, 2013.

Laboratory for Gut Homeostasis

Team Leader: Kenya Honda

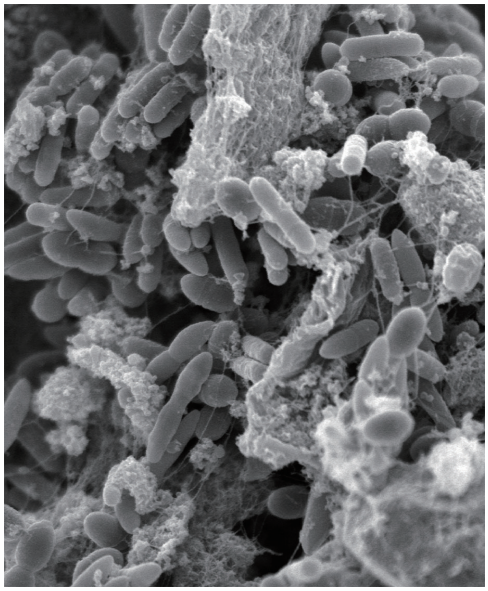


Figure: Treg-inducing 17 strains of Clostridia
The image is a scanning electron micrograph showing the proximal colon of germ-free mice colonized with the regulatory T cell-inducing human Clostridia strains that we have isolated (magnification ~5,000x). The community of Clostridia strains induces regulatory T cells and contributes to suppression of unfavorable inflammation and allergic responses.

The gut microbiota intimately interacts with the host immune system and influences pathogenesis of several immune disorders. $CD4^{+}Foxp3^{+}$ Treg cells are the most prominent regulatory cells. They are indispensable for maintaining immune tolerance and are also an emerging therapeutic target for inflammatory bowel diseases (IBD). Treg cells are most abundant in the intestinal mucosa at steady state. In germ-free (GF) or antibiotic-treated mice, the frequency of colonic Treg cells is significantly reduced and interleukin (IL)-10, an immune suppressive cytokine, expression by Treg cells is markedly decreased. We aimed to identify Treg-inducing bacterial strains derived from the human microbiota and test their potential to attenuate diseases such as colitis and allergy, thus enabling clinical translation of our previous findings. Starting from a complete human fecal sample from a healthy Japanese volunteer, which can typically include several hundred strains, a sequence of selection steps was applied to isolate Treg inducing strains. These steps included chloroform treatment, 20,000-fold dilution, and serial transplantation to GF mice using gnotobiotic techniques. These steps allowed us to narrow the number of bacterial strains down to 17 without sacrificing Treg-inducing potency. All 17 strains were successfully isolated, cultured, and stored in frozen stocks. All strains are members of Clostridia clusters IV, XIVa, and XVIII. When these 17 strains were mixed and inoculated into GF mice, they robustly induced Tregs in the colonic LP and displayed full Treg-inducing capacity, comparable to that of a complete fecal microbiome. The 17 strains act as a community to both provide bacterial antigens to T cells and to help polarize the T cells to become Tregs by inducing the generation of a TGF-β-rich environment. Furthermore, oral administration of the cocktail of 17 strains to adult mice attenuated disease in models of colitis and allergic diarrhea.We published these results in Nature.

Recent Major Publications

Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. ***Nature*** 499, 97-101 (2013)

Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K*. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. ***Nature*** 500, 232-6 (2013)*corresponding author

Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces colonic regulatory T cells. ***Nature*** 504, 446-50 (2013)

Invited Presentations

Honda K. Clostridia strains from human microbiota for Treg induction. Cell Symposia Microbiome and Host Health, Lisbon, Portugal. May 14th, 2013.

Honda K. Immunomodulation by the gut microbiota. Fondation Mérieux Conference: Targeting Commensal Flora to Better Shape Protective Immune Responses for Better Disease Prevention and Therapy, Veyrier-du-Lac France. June 12th, 2013.

Honda K. Regulation of T cells by the gut microbiota. FASEB Autoimmunity, Saxtons River, Vermont, USA. July 10th, 2013.

Honda K. Microbiota Regulation of T Cells in the Intestine. The 16th International Congress for Mucosal Immunology, Vancouver, Canada. July 19th, 2013.

Honda K. Regulation of Th17 and Treg cells by the gut microbiota. 15th International Congress of Immunology, Milan, Italy. August 27th, 2013.

Laboratory for Immune Homeostasis

Team Leader: Shohei Hori

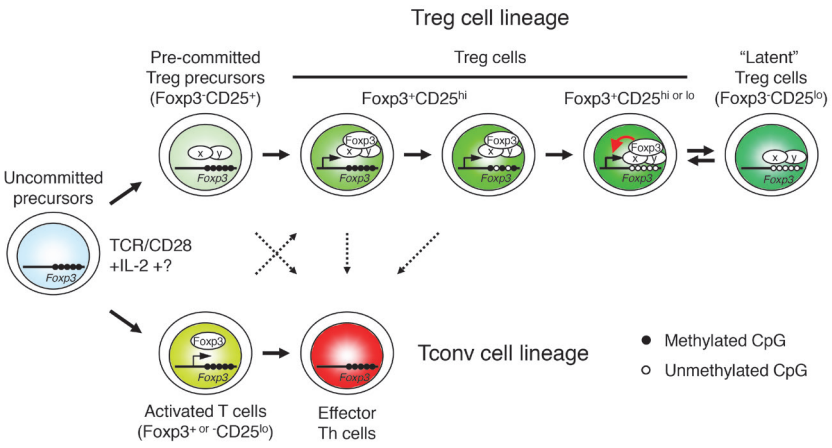


Figure: A model of regulatory T cell fate determination and maintenance
During Treg cell differentiation, uncommitted precursor cells adopt either Treg cell or conventional T (Tconv) cell fates upon activation, independently of Foxp3 expression. The commitment to the Treg cell fate is made before Foxp3 induction and executed by a transcription factor network elicited by extrinsic signals from the extracellular environment. The same signals also induce epigenetic modifications, including DNA demethylation of the *Foxp3* locus. Foxp3 is incorporated into the pre-existing transcription factor network and the resulting "Foxp3 interactome" establishes the characteristic Treg cell phenotype in cooperation with the epigenetic modifications. Although Treg cells may down-regulate Foxp3 expression under certain circumstances, these "latent" Treg cells retain the epigenetic memory of, and thus remain committed to, the Treg cell fate. On the other hand, when activated T cells express Foxp3 without engagement of the transcription factor network, Foxp3 by itself cannot establish the characteristic Treg cell phenotype. As a result, the activated Foxp3⁺ T cells readily lose Foxp3 expression, adopt the alternative Tconv cell fate and differentiate into effector Th cells.

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

We have performed cell fate mapping studies of Foxp3⁺ T cells to address the lineage stability of Treg cells and found that Foxp3 expression *per se* does not specify the Treg cell lineage. Thus, activated conventional T cells can promiscuously and transiently express Foxp3 and committed Treg cells can transiently and reversibly down-regulate Foxp3. Despite this phenotypic plasticity, Treg cells retain epigenetic memory of, and thus remain committed to, Foxp3 expression and suppressive functions. Our findings have established that Treg cells constitute a stable cell lineage committed to Foxp3-dependent suppressive functions.

We have also analyzed how Foxp3 gene mutations found in human IPEX impinge on Treg cells *in vivo* using knock-in mutagenesis in mice, and found that one mutation preferentially impairs Treg cell homeostasis in non-lymphoid tissues without affecting their differentiation and *in vitro* suppressive functions. Our analyses revealed that this defect is due to altered DNA binding specificity of the mutant Foxp3. By taking advantage of this unique animal model, we are currently investigating how Treg cells adapt to diverse and fluctuating tissue environments.

Recent Major Publications

Miyao T, Floess S, Setoguchi R, Luche H, Fehling HJ, Waldmann H, Huehn J, Hori S. Plasticity of Foxp3⁺ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. ***Immunity*** 36, 262-75 (2012)

Hori S. The Foxp3 interactome: a network perspective of Treg cells. ***Nat Immunol*** 13, 943-5 (2012)

Toker A, Engelbert D, Garg G, Polansky JK, Floess S, Miyao T, Baron U, Düber S, Geffers R, Giehr P, Schallenberg S, Kretschmer K, Olek S, Walter J, Weiss S, Hori S, Hamann A, Huehn J. Active demethylation of the Foxp3 locus leads to the generation of stable regulatory T cells within the thymus. ***J Immunol*** 190, 3180-8 (2013)

Invited Presentations

Hori S. Genetic control of regulatory T cell fitness in tissues. The 7th International Leukocyte Signal Transduction Conference, Kos, Greece. September 10th, 2013.

Hori S. Genetic control of regulatory T cell homeostasis in non-lymphoid tissues. The 3rd Bizan Immunology Symposium at the University of Tokushima "Immune System Development, Deviation, and Regulation", Tokushima, Japan. February 13th, 2014.

Laboratory for Skin Homeostasis

Team Leader: Masayuki Amagai

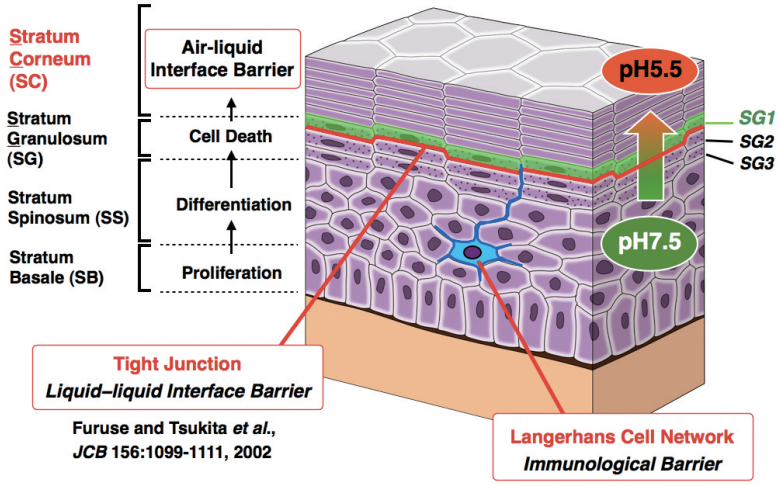


Figure: Three epidermal barrier components in the skin

Reviewed by Kubo, Nagao and Amagai, *JCI* 122:440-447, 2012

When the immune system meets external antigens in the skin, it tends to react to them. By contrast, when the immune system meets antigens in the gut, it tends to tolerate them. However, the exact mechanisms for these opposite immune reactions are still largely unknown. Our laboratory attempts to dissect and understand the skin as an immune organ. Especially we are studying skin barrier formation, function, and its dysfunction in atopic diseases.

Skin is composed of three components: epidermis, dermis and subcutaneous fat tissue. The epidermis is a keratinized stratified squamous epithelium and forms an effective barrier essential for preventing the invasion of microorganisms, chemical compounds and allergens. The epidermis is composed of four distinct layers; stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum (SC) from bottom to top (Fig.).

Among many elements of the skin barrier, we are focusing on the SC as an air-liquid barrier, tight junctions as a liquid-liquid barrier, and the Langerhans cell network as an immunological barrier (Fig.). Tight junctions are formed in the second layer of SG or SG2 cells and Langerhans cells extend their dendrites above these tight junctions to capture external antigens. Filaggrin deficiency, which is a predisposing factor for atopic disease, enhances SC penetration of external antigens.

The SC is 12 to 15 accumulated layers of corneocytes, which are terminally differentiated dead keratinocytes. Therefor, all the essential components for SC are produced in SG1 cells. We have established a method to isolate SG1 and SG2 cells and are characterizing SG1, 2-specific genes and proteins. It will be interesting to determine which proteases and/or protease inhibitors are key players in SC homeostasis and how their balance is regulated.

Recent Major Publications

Nagao K, Kobayashi T, Moro K, Ohyama M, Adachi T, Kitashima DY, Ueha S, Horiuchi K, Tanizaki H, Kabashima K, Kubo A, Cho YH, Clausen BE, Matsushima K, Suematsu M, Furtado GC, Lira SA, Farber JM, Udey MC, Amagai M. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. ***Nat Immunol*** 13, 744-52 (2012)

Kubo A, Shiohama A, Sasaki T, Nakabayashi K, Kawasaki H, Atsugi T, Sato S, Shimizu A, Mikami S, Tanizaki H, Uchiyama M, Maeda T, Ito T, Sakabe J, Heike T, Okuyama T, Kosaki R, Kosaki K, Kudoh J, Hata K, Umezawa A, Tokura Y, Ishiko A, Niizeki H, Kabashima K, Mitsuhashi Y, Amagai M. Mutations in SERPINB7, encoding a member of the serine protease inhibitor superfamily, cause Nagashima-type palmoplantar keratosis. ***Am J Hum Genet*** 93, 945-56 (2013)

Sasaki T, Shiohama A, Kubo A, Kawasaki H, Ishida-Yamamoto A, Yamada T, Hachiya T, Shimizu A, Okano H, Kudoh J, Amagai M. A homozygous nonsense mutation in the gene for Tmem79, a component for the lamellar granule secretory system, produces spontaneous eczema in an experimental model of atopic dermatitis. ***J Allergy Clin Immunol*** 132, 1111-1120. e4 (2013)

Invited Presentations

Matsui T. Skin-specific retroviral-like aspartic protease SASPase and evolution of mammalian skin. The Genetics Society of Japan, Tokyo, Japan. September 19th, 2013.

Amagai M. Skin barrier dysfunction and cutaneous sensitization in atopic disease. The 50th Annual Meeting of the Japanese Society of Pediatric Allergy and Clinical Immunology, Yokohama, Japan. October 19th, 2013.

Amagai M. Stratum corneum barrier dysfunction and atopic diseases. Annual Meeting of the Japanese Society for Immunology 2013, Chiba, Japan. December 11th, 2013.

Laboratory for Metabolic Homeostasis

Team Leader: Naoto Kubota

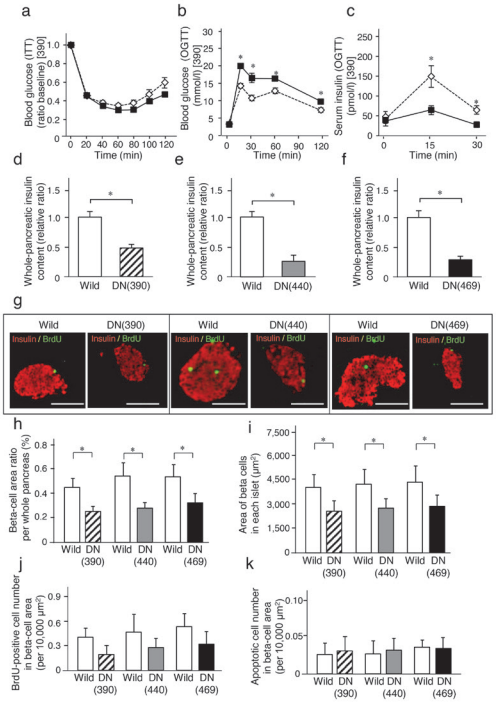


Figure: TCF7L2 expressed in pancreatic beta cells plays a crucial role in glucose metabolism through regulation of the beta cell mass.
Blood glucose levels (ratio baseline) during an insulin tolerance test (ITT).
(**a**) in 10-week-old wild-type (white diamonds) and B cell-specific dominant negative TCF7L2 transgenic (DN) mice (black squares) from each independent DN mouse line (n=12). Blood glucose (**b**) and serum insulin levels (**c**) during the oral glucose tolerance test (OGTT) in 11-week-old wild-type (white diamonds) and DN mice (black squares) from each line (n=8–12).Whole-pancreas insulin content (**d**–**f**) of 12-week-old wild-type (Wild) and DN mice from each line (n=12). Results for line 390 (**d**), 440 (**e**) and 469 (**f**). Immunohistochemical analysis of the pancreas for insulin (red) and BrdU (green) incorporation in 12-week-old mice (**g**). Scale bar, 100 μm. Beta cell area ratio per whole pancreas (**h**), area of the beta cells in each islet (**i**), number of BrdU-positive cells (**j**) and number of apoptotic cells (**k**) per beta cell area of 10,000 μm² in 12-week-old mice (n=8–10). Data are shown as mean ± SEM. *p<0.05, DN mice vs wild-type mice

Recent Major Publications

Kadowaki T, Ueki K, Yamauchi T, Kubota N. SnapShot: Insulin signaling pathways. ***Cell*** 148, 624- 624.e1 (2012)

Kadowaki T, Kubota N, Ueki K, Yamauchi T. SnapShot: physiology of insulin signaling. ***Cell*** 148, 834-834.e1 (2012)

Takamoto I, Kubota N, Nakaya K, Kumagai K, Hashimoto S, Kubota T, Inoue M, Kajiwara E, Katsuyama H, Obata A, Sakurai Y, Iwamoto M, Kitamura T, Ueki K, Kadowaki T. TCF7L2 in mouse pancreatic beta cells plays a crucial role in glucose homeostasis by regulating beta cell mass. ***Diabetologia*** (in press)

Invited Presentations

Kubota N. Molecular mechanisms of type 2 diabetes and insulin resistance. The 36th Naito Conference on Molecular Aspects of Energy Balance and Feeding Behavior, Sapporo, Japan. September 12th, 2013.

In recent years there has been a rapid increase in the incidence of type 2 diabetes in both Western and Asian countries. This high prevalence is most likely the result of a complex interplay between genetic, such as reduced insulin secretion, and environmental factors, such as high-fat diet and decreased physical activity. However, the precise molecular mechanisms underlying the development and progression of type 2 diabetes remained unclear. The goal of our team is to identify the molecular mechanism of insulin secretion and insulin resistance.

A) Molecular mechanism of insulin secretion

Most of the common variant single-nucleotide polymorphisms (SNPs) identified by genome-wide association study (GWAS) are associated with defective pancreatic islet function. However, the functional role of genes identified by GWAS remained unclear. We discovered that transcription factor 7-like 2 (TCF7L2), which confers the largest effect on the risk of type 2 diabetes in Western populations, plays a crucial role in glucose metabolism through regulation of the beta cell mass (Diabetologia in press).

B) Molecular mechanism of insulin resistance

Insulin resistance is defined as a condition in which physiological insulin signals are impaired or dysfunctional for some reason, such as ER stress, adipokine abnormality or chronic inflammation. Once insulin binds to insulin receptors, insulin receptor substrate (IRS)-1 and IRS-2 are activated and begin transmitting intracellular insulin signals. These result in various insulin-mediated activities, including glucose and lipid metabolism, protein synthesis and cell proliferation. Until now, we have been studying the role of IRS-1 and IRS-2, which show ubiquitous expression patterns (Diabetes 2000, Circulation 2003, J Clin Invest 2004, Cell Metab 2008, Cell Metab 2011, Cell 2012). We are currently working to elucidate the molecular mechanism of insulin resistance via IRS-1 and IRS-2 in cells and tissues such as liver, skeletal muscle, macrophages, and the central nervous system.

Laboratory for Immune Crosstalk

Team Leader: Hilde Cheroutre

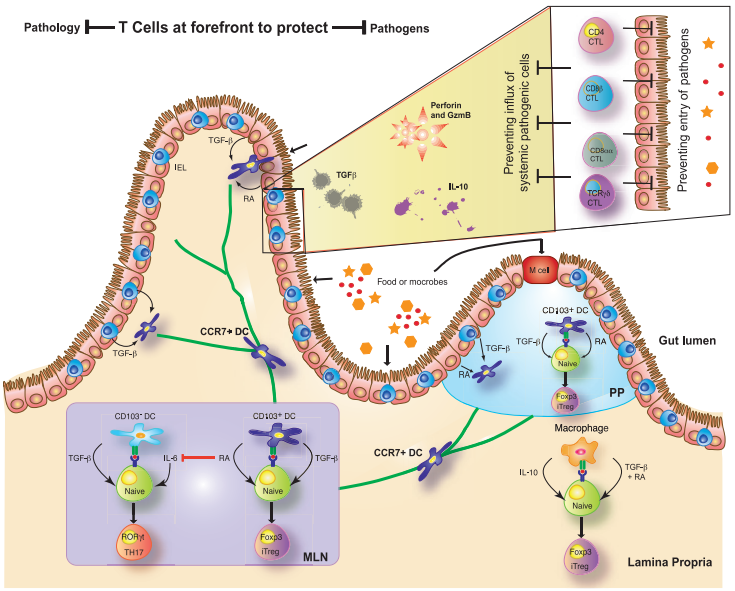


Figure: Intraepithelial T cells protect the mucosal barrier of the intestine from pathogen- and inflammation-induced pathology.
T cells that reside within the epithelium of the intestine are phenotypically heterogeneous but they are all specialized to protect the mucosal barrier against pathogen- and immune cell-induced pathology. In contrast to the T cells in the periphery, epithelial T lymphocytes are all antigen-experienced T cells that encountered their antigen initially during selection in the thymus (agonist selected CD8αα TCRαβ and TCRγδ precursor cells) or as mature cells in the periphery (CD8αβ CTL and CD4 CTL). Although the various epithelial T cell subsets display different antigen specificity and MHC restriction and although they follow different paths of effector differentiation, they all acquire cytolytic and regulatory capacity. The functional specialization of intraepithelial T lymphocytes adapts them to provide optimal protection in the face of preserving the integrity of the delicate mucosal barrier.

Recent Major Publications

Fu G, Casas J, Rigaud S, Rybakin V, Lambolez F, Brzostek J, Hoerter JA, Paster W, Acuto O, Cheroutre H, Sauer K, Gascoigne NR. Themis sets the signal threshold for positive and negative selection in T-cell development. ***Nature*** 504, 441-5 (2013)

Mucida D, Husain MM, Muroi S, van Wijk F, Shinnakasu R, Naoe Y, Reis BS, Huang Y, Lambolez F, Docherty M, Attinger A, Shui JW, Kim G, Lena CJ, Sakaguchi S, Miyamoto C, Wang P, Atarashi K, Park Y, Nakayama T, Honda K, Ellmeier W, Kronenberg M, Taniuchi I, Cheroutre H. Transcriptional reprogramming of mature CD4⁺ helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes. ***Nat Immunol*** 14, 281-9 (2013)

Invited Presentations

Cheroutre H. CD4 CTL: a major new player in health and disease. Immunology 2013: AAI Annual Meeting, Major Symposia A: Tissue-Resident Lymphocytes, Honolulu, USA. May 4th, 2013.

Cheroutre H. Mucosal T Cells in Inflammation and Homeostasis. The 16th International Congress for Mucosal Immunology: Pioneering Frontiers in Mucosal Regulation, Vancouver, Canada. July 20th, 2013.

Cheroutre H. Unique Adaptive Immune Defense at the Mucosal Frontline of the Intestine. 15th International Congress of Immunology, Symposium: Innate lymphocytes and mucosal Immunity, Milan, Italy. August 22nd-27th, 2013.

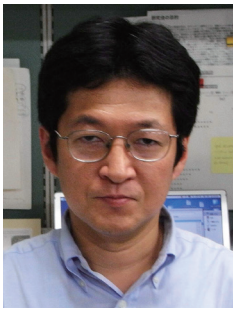
Cheroutre H. 2013 Grand Challenges Meeting: Vaccine Discovery and Translational scientific track session, Rio de Janeiro, Brazil. October 28th-30th, 2013.

Cheroutre H. 85th Anniversary Research Foundation Flanders (FWO): Session on Inflammation and Immunity, Ghent, Belgium. December 17th, 2013.

Our research continues to elucidate mechanisms of mucosal immune protection and regulation. In a recent study, we uncovered an unexpected degree of plasticity for CD4 T helper (Th) cells, which upon antigenic stimulation, are able to terminate the expression of the Th transcription factor, ThPOK, and differentiate into cytotoxic T lymphocytes (CTL). At steady state, CD4 CTL remain quiescent and express a self-regulated phenotype. However under challenging conditions, these cells have the potential to transform into potent inflammatory killer effector cells (Mucida *et al.*, *Nature Immunology* 2013).

Overall, based on the insights we are gaining from our research, a clear picture has begun to emerge showing that the immune defense of the intestine adapts to the local environment and specializes to provide the most efficient and immediate protection in the face of preserving the integrity of the most critical mucosal barrier of the body.

In another study, we are aiming to understand the various mechanisms and processes that lead to central tolerance. Our previous research showed that in addition to conventional selection, a process of so called "agonist" selection operates in the thymus, which preserves self-specific thymocytes and functionally differentiates these precursor cells to become beneficial pre-programmed protective or regulatory T cells. In an effort to understand what factors control the decisive checkpoint during thymic selection, we identified "*Themis*" as a critical switch (Guo *et al.*, *Nature* 2013). We are now trying to elucidate the molecular and cellular factors and events that connect the Pre-TCR and TCR signal strength with thymic selection and the fate decision of the developing T cells.



Laboratory for Inflammatory Regulation

Team Leader: Takashi Tanaka

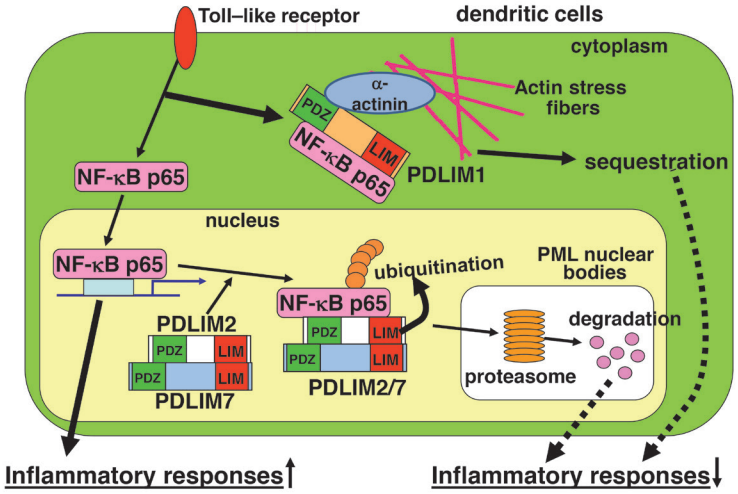


Figure: LIM is a new family of negative regulators of NF-κB signaling.
PDLIM2 and PDLIM7 are ubiquitin E3 ligases for the p65 subunit of NF-κB, form heterodimers and cooperatively promote p65 degradation. By contrast, PDLIM1 sequestrates p65 in the cytoplasm and inhibits its nuclear translocation, thereby suppressing NF-κB signaling.

The inflammatory response is an important host defense mechanism to sense and eliminate invading microbial pathogens. Dendritic cells first detect pathogens and activate the transcription factor NF-κB, which enters the nucleus and induces the expression of a series of inflammation-related genes. These initially helpful inflammatory responses must be terminated at the appropriate time point, otherwise excessive responses can damage normal tissue and may cause autoimmune or allergic diseases. Our research goal is to identify key regulators of inflammation-related signal transduction pathways and to clarify the complete picture of the molecular mechanisms for regulating inflammatory responses. We previously identified PDLIM2 (PDZ and LIM domain protein-2), a nuclear protein that belongs to a large family of LIM proteins, as one of the key factors negatively regulating inflammatory responses. We found that PDLIM2 is an E3 ubiquitin ligase for the STAT4 and STAT3 transcription factors, thereby suppressing Th1 and Th17 cell differentiation (Tanaka T, Immunity, 2005, Tanaka T, Sci. Signal., 2011). We have also demonstrated that PDLIM2 negatively regulates NF-κB activity and subsequent inflammatory responses, acting as a nuclear ubiquitin E3 ligase targeting the p65 subunit of NF-κB. (Tanaka T, Nat. Immunol., 2007). We now focus on PDLIM2 and other members of the LIM family. Recently we found that PDLIM7 and PDLIM1 are also negative regulators of NF-κB-mediated signaling. PDLIM7 was found to be an E3 ubiquitin ligase for p65. PDLIM7 and PDLIM2 form heterodimers and synergistically promote p65 degradation by the proteasome. On the other hand, PDLIM1 sequestrated p65 in the cytoplasm, possibly by interaction with actin stress fibers, through binding to α-actinin, an actin binding protein, and suppressed nuclear translocation of p65 protein. These studies should contribute to our understanding of the pathogenesis of human autoimmune and inflammatory diseases and the development of new therapeutic tools.

Recent Major Publications

Yan P, Fu J, Qu Z, Li S, Tanaka T, Grusby MJ, Xiao G. PDLIM2 suppresses HTLV-I Tax-mediated tumorigenesis by targeting Tax into the nuclear matrix for proteasomal degradation. ***Blood*** 113, 4370-80 (2009)

Tanaka T, Yamamoto Y, Muromoto R, Ikeda O, Sekine Y, Grusby MJ, Kaisho T, Matsuda T. PDLIM2 inhibits T Helper 17 cell development and granulomatous inflammation through degradation of STAT3. ***Sci Signal*** 4, ra85 (2011)

Sasaki I, Hoshino K, Sugiyama T, Yamazaki C, Yano T, Iizuka A, Hemmi H, Tanaka T, Saito M, Sugiyama M, Fukuda Y, Ohta T, Sato K, Ainai A, Suzuki T, Hasegawa H, Toyama-Sorimachi N, Kohara H, Nagasawa T, Kaisho T. Spi-B is critical for plasmacytoid dendritic cell function and development. ***Blood*** 120, 4733-43 (2012)

Invited Presentations

Tanaka T. Negative regulation of T-helper cell differentiation by LIM proteins. The 132th Annual Meeting of the Pharmaceutical Society of Japan, Hokkaido, Japan. March 30th, 2012.

Tanaka T. Clarifying the molecular mechanisms that regulate inflammatory responses. RIKEN - Novo Nordisk A/S Scientific Forum, Yokohama, Japan. April 19th, 2012.

Tanaka T. Negative regulation of inflammatory responses by LIM proteins. The 12th Biennial International Endotoxin & Innate Immunity Society Meeting, Tokyo, Japan. October 24th, 2012.

Tanaka T. Clarifying the molecular mechanisms that regulate inflammatory responses. The Osaka Minami Medical Center: Talk with the expert seminar, Osaka, Japan. October 11th, 2013.

Tanaka T. Regulation of inflammatory responses by LIM proteins. The 5th LJI & IMS-RCAI Workshop, Yokohama, Japan. October 31st, 2013.

Laboratory for Cytokine Regulation

Team Leader: Masato Kubo

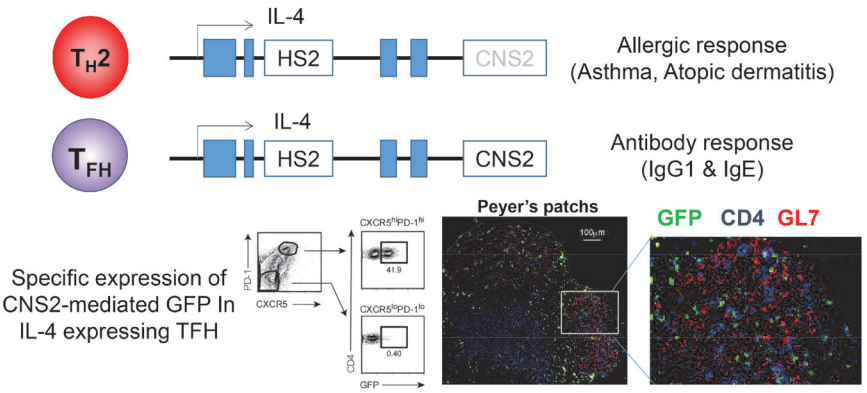


Figure: CNS2 is an essential enhancer element in T_{FH} cells but not in T_H2 cells.
The CNS active T cells in CNS2-regulated transgenic mice expressed several markers of T_{FH} cells, CXCR5, PD-1, and ICOS, which favored their localization in B cell follicles and germinal centers of Peyer's patches. The upper part of the figure depicts the genome structure of the *Il4* locus. The lower part shows the cell surface phenotype and localization of GFP$^+$ T_{FH} cells.

Recent Major Publications

Harada Y, Tanaka S, Motomura Y, Harada Y, Ohno S, Ohno S, Yanagi Y, Inoue H, Kubo M. The 3' enhancer CNS2 is a critical regulator of interleukin-4-mediated humoral immunity in follicular helper T cells. ***Immunity*** 36, 188-200 (2012)

Otsuka A, Nakajima S, Kubo M, Egawa G, Honda T, Kitoh A, Nomura T, Hanakawa S, Sagita Moniaga C, Kim B, Matsuoka S, Watanabe T, Miyachi Y, Kabashima K. Basophils are required for the induction of Th2 immunity to haptens and peptide antigens. ***Nat Commun*** 4, 1739 (2013)

Noti M, Wojno ED, Kim BS, Siracusa MC, Giacomin PR, Nair MG, Benitez AJ, Ruymann KR, Muir AB, Hill DA, Chikwava KR, Moghaddam AE, Sattentau QJ, Alex A, Zhou C, Yearley JH, Menard-Katcher P, Kubo M, Obata-Ninomiya K, Karasuyama H, Comeau MR, Brown-Whitehorn T, de Waal Malefyt R, Sleiman PM, Hakonarson H, Cianferoni A, Falk GW, Wang ML, Spergel JM, Artis D. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. ***Nat Med*** 19, 1005-13 (2013)

Invited Presentations

Kubo M. Role of Notch signal in the generation of follicular helper T cells (TFH) and memory T cell. The 1st International Immunological Memory and Vaccine Forum, Tokyo, Japan. January 29th, 2013.

Kubo M. Roles of follicular helper T (TFH) cells in antibody based protective immunity against influenza virus. Joint Symposium of the 21st International Symposium of Macrophage Molecular and Cellular Biology & the 78th Japanese Society for Interferon and Cytokine Research, Tokyo, Japan. May 21st, 2013.

Kubo M. Cytokine regulation in T follicular helper (TFH) cells. Gordon Research Conferences: T Follicular Helper Cells, Hong Kong, China. July 22nd, 2013.

Kubo M. Molecular Mechanism of Immune Responses. The 86th Annual Meeting of the Japanese Biochemical Society: International session, Yokohama, Japan. September 13th, 2013.

The expression profile of a group of genes related to T cell development and differentiation is ingeniously controlled by spatiotemporal processes. Epigenetic changes in *cis*-acting regions of the *Il13/Il4* locus are strongly associated with cytokine expression profiles during T_H2 differentiation. We generated a series of genetically targeted mice disrupting the *cis*-acting activity of conserved non-coding sequences (CNS) in the *Il4* locus to understand lineage-specific regulation of IL-4. The main role for T_H2-derived IL-4 has been thought to be in humoral immunity. However, we provided evidence that a recently-discovered subset of follicular T (T_{FH}) cells generates the IL-4 that promotes B cell differentiation into antibody producing cells. CNS2 is an essential enhancer element for IL-4 expression in T_{FH} cells but not in Th2 cells. Mice with a CNS2 deletion had a reduction in IgG1 and IgE production and in IL-4 expression by T_{FH} cells. We concluded that T_H2 cells are dispensable for antibody responses and that CNS2 is a specific enhancer element required for IL-4 expression by T_{FH} cells controlling humoral immunity.

We established a diphtheria toxin-based conditional deletion system using an *Il4* enhancer that we previously found was specific for IL-4 production by mast cells or basophils (Mas-TRECK and Bas-TRECK mice). Using the Bas-TRECK system, we examined the role of thymic stromal lymphopoietin (TSLP) in basophil development. We further focused on Eosinophilic esophagitis (EoE), a food allergy–associated inflammatory disease characterized by esophageal eosinophilia. We established a new mouse model of EoE-like disease that developed independently of IgE, but was dependent on TSLP and basophils, as targeting TSLP or basophils during the sensitization phase limited disease. Our data suggest that the TSLP-basophil axis contributes to the pathogenesis of EoE and could be therapeutically targeted to treat this disease.

Laboratory for Homeostatic Network

Team Leader: Shigeo Koyasu

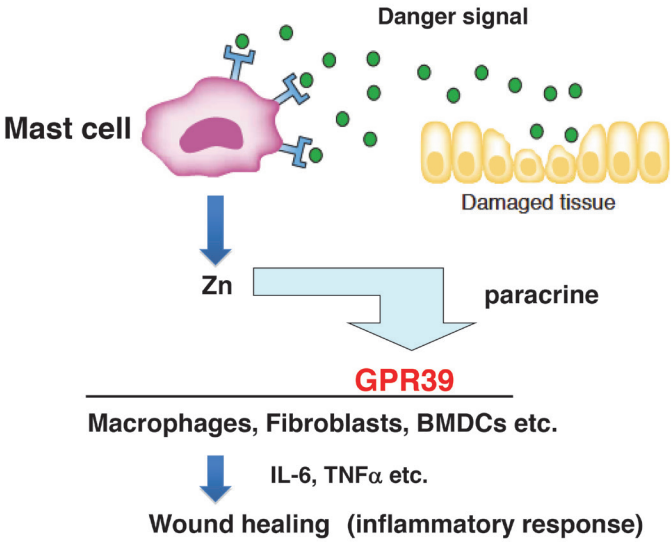


Figure: Zn is a newly identified inflammatory mediator.
Zinc is a mediator of the inflammatory response. After injury, endogenous inflammatory mediators or "danger signals" are released and activate mast cells, which then release Zn into the extracellular space. The released Zn directly binds to the GPR39 Zn receptor on immune-related cells and induces the expression of cytokines such as IL-6 and TNFα. These cytokines then contribute to inflammatory responses such as wound healing.

Zinc signaling is essential for B cell maintenance.

The immune system is influenced by vital zinc (Zn) status and Zn deficiency triggers lymphopenia and immunodeficiency, however the mechanisms of Zn-mediated lymphocyte maintenance remain elusive. We investigated the role of ZIP10, a Zn transporter expressed in B cells, on their development. Genetic ablation of *Zip10* in early B cell stages resulted in reduction of B cells, which showed increased caspase activity in parallel with decreases in intracellular Zn levels, indicating that ZIP10-mediated Zn signaling is essential for early B cell survival. Moreover, ZIP10 expression was regulated by JAK-STAT pathways, which was also correlated with STAT activation in human B cell lymphoma, implicating ZIP10 as a mediator of malignancy. Our results establish a role for ZIP10 in B cell survival during early development and underscore the importance of Zn signaling in immune system maintenance.

Zinc controls skin wound healing through the GPR39 / IL-6 axis.

Bedsores are painful skin lesions that affect many patients, including the increasing populations of elderly individuals and those afflicted with metabolic syndromes. The essential trace element Zn has an important role in accelerating skin-wound healing, but how it does so is unclear. We reported that Zn released from mast cells is required for wound healing. The released Zn binds to its receptor (called GPR39) on inflammatory cells, which release the inflammation-promoting molecule, IL-6. IL-6 in turn controls wound healing (Fig.). These findings explain why Zn is beneficial for treating skin disorders including wounds and burns.

Recent Major Publications

Yamasaki S, Hasegawa A, Hojyo S, Ohashi W, Fukada T, Nishida K, Hirano T. A Novel Role of the L-Type Calcium Channel α(1D) Subunit as a Gatekeeper for Intracellular Zinc Signaling: Zinc Wave. ***PLoS One*** 7, e39654 (2012)

Fukada T, Hojyo S, Furuichi T. Zinc Signal: Zinc signal: a new player in osteobiology. ***J Bone Miner Metab*** 31, 129-35 (2013)

Tamaki M, Fujitani Y, Hara A, Uchida T, Tamura Y, Takeno K, Kawaguchi M, Watanabe T, Ogihara T, Fukunaka A, Shimizu T, Mita T, Kanazawa A, Imaizumi MO, Abe T, Kiyonari H, Hojyo S, Fukada T, Kawauchi T, Nagamatsu S, Hirano T, Kawamori R, Watada H. The diabetes susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance. ***J Clin Invest*** 123, 4513-24 (2013)

Invited Presentations

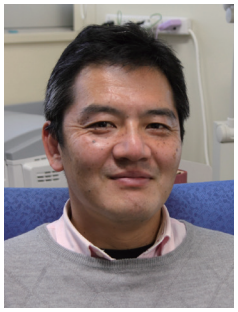
Fukada T. Physio-pathological roles of zinc signaling. The 86th Annual Meeting of the Japanese Pharmacological Society, Fukuoka, Japan. March 22nd, 2013.

Fukada T. New Horizon of Zinc Biology in Signaling, Homeostasis and Diseases. The 86th Annual Meeting of the Japanese Biochemical Society: International session, Yokohama, Japan. September 11th, 2013.

Nishida K. Role of zinc transporter Slc30a2/ZnT2 in normal wound healing. The 86th Annual Meeting of the Japanese Biochemical Society: International session, Yokohama, Japan. September 11th, 2013.

Nishida K. Metallothioneins control FcεRI-mediated IL-4 production in basophils. The meeting of the Japanese Research Group for Studies of Metalbioscience 2013, Shizuoka, Japan. September 27th, 2013.

Fukada T. Basic and Clinical aspects in Zinc and Degenerative diseases of Aging. 10th International Society for Trace Element Research in Humans (ISTERH) 2013, Tokyo, Japan. November 19th, 2013.



Laboratory for Developmental Genetics

Group Director: Haruhiko Koseki

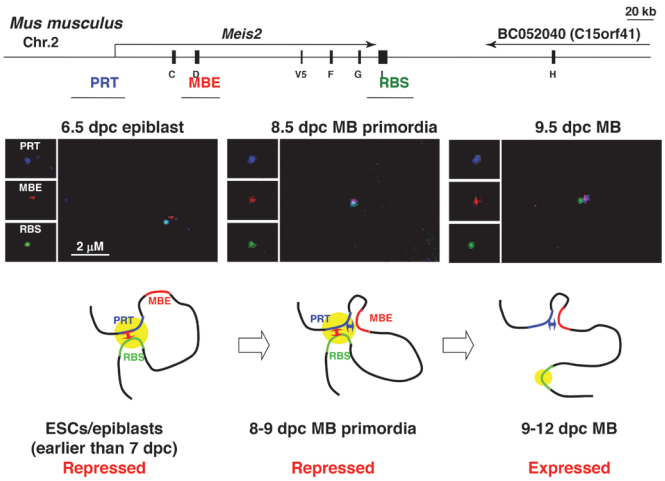


Figure: The *Meis2* promoter/enhancer association is preceded by promoter/enhancer/RBS tripartite interaction in midbrain.
(Top) The *Meis2* gene is schematically depicted and positions of FISH probes are shown. (Middle) Tri-color FISH images showing topological transition of promoter (PRT) (blue), MBE (red) and RBS (green) in 6.5 dpc epiblasts and midbrain of 16-20 and 22-26 somite stage embryos. Fluorescent signals from each channel in the regions indicated by dotted boxes are shown in insets. (Bottom) Graphic summary of promoter/enhancer/RBS DNA topology. Co-localization of RING1B foci with promoter (PRT), enhancer (MBE) or RBS at each stage is shown. Note that RBS constitutively associates with the RING1B foci.

The Developmental Genetics Research Group is pursuing a research program to elucidate the epigenetic regulation of organ development and stem cell functions by Polycomb group (PcG) proteins and DNA methylation mechanisms.

Regulation of large scale chromatin structures by Polycomb

The PcG Repressive Complex-1 (PRC1) forms microscopically visible clusters in nuclei; however, the impact of this cluster formation on transcriptional regulation and the underlying mechanisms that regulate this process remain obscure. We found that the sterile Alpha Motif (SAM) domain of a PRC1 core component, Phc2, plays an essential role for PRC1 clustering through head-to-tail macromolecular polymerization, which is essential for PRC1 clustering, recruitment of PRC1 to target genes and robust gene silencing activity. We propose a novel role for SAM domain polymerization in this repression by two distinct mechanisms, firstly, through capture and/or retaining of PRC1 at the PcG targets, and, secondly, by strengthening the interactions between PRC1 and PRC2 to stabilize transcriptional repression. Our findings reveal a previously unknown regulatory mechanism mediated by SAM domain polymerization for PcG-mediated repression of developmental loci that enables a robust yet reversible gene repression program during development.

Chromatin dynamics associated with activation of Polycomb-repressed genes

PcG proteins mediate repression of developmental regulators in a reversible manner. However, it is poorly understood how PcG-repressed genes are activated by developmental cues. We used the *Meis2* gene as a model to identify the role of a tissue-specific enhancer in removing PcG from its promoter. *Meis2* repression in early development depends on binding of RING1B, an essential E3 component of PcG, to its promoter, coupled with its association with another RING1B-binding site (RBS) at the 3' end of the *Meis2* gene. During early midbrain development, a midbrain-specific enhancer (MBE) transiently associates with the promoter/RBS, forming a promoter/MBE/RBS tripartite interaction in a RING1-dependent manner. Subsequently, RING1B-bound RBS dissociates from the tripartite, leaving promoter/MBE engagement to activate *Meis2* expression. This study therefore demonstrates the role of PcG and/or related factors in *Meis2* activation by regulating the topological transition of cis-regulatory elements.

Recent Major Publications

Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, Toyoda T, Ito T, Eskeland R, Bickmore WA, Vidal M, Bernstein BE, Koseki H. Histone H2A Mono-Ubiquitination Is a Crucial Step to Mediate PRC1-Dependent Repression of Developmental Genes to Maintain ES Cell Identity. ***PLoS Genet*** 8, e1002774 (2012)

Isono K, Endo TA, Ku M, Yamada D, Suzuki R, Sharif J, Ishikura T, Toyoda T, Bernstein BE, Koseki H. SAM Domain Polymerization Links Subnuclear Clustering of PRC1 to Gene Silencing. ***Dev Cell*** 26, 565-77 (2013)

Kondo T, Isono K, Kondo K, Endo TA, Itohara S, Vidal M, Koseki H. Polycomb Potentiates Meis2 Activation in Midbrain by Mediating Interaction of the Promoter with a Tissue-Specific Enhancer. ***Dev Cell*** 28, 94-101 (2014)

Invited Presentations

Koseki H. DNA methylation in T cells and gut immune homeostasis. 3rd McGill-RIKEN Workshop, Montreal, Canada. June 21st, 2013.

Koseki H. Epigetic regulation of spermiogenesis. The 10th Annual Meeting of Asian Reproductive Biotechnology Society, Phan Thiet, Vietnam. August 21st, 2013.

Koseki H. Activation of polycomb-repressed genes. Mouse Molecular Genetics Conference , Cambridge, UK. September 20th, 2013.

Koseki H. Activation of polycomb-repressed genes. Annual Meeting of the Japanese Society for Immunology 2013, Chiba, Japan. December 11th, 2013.

Laboratory for Integrative Genomics

Group Director: Osamu Ohara

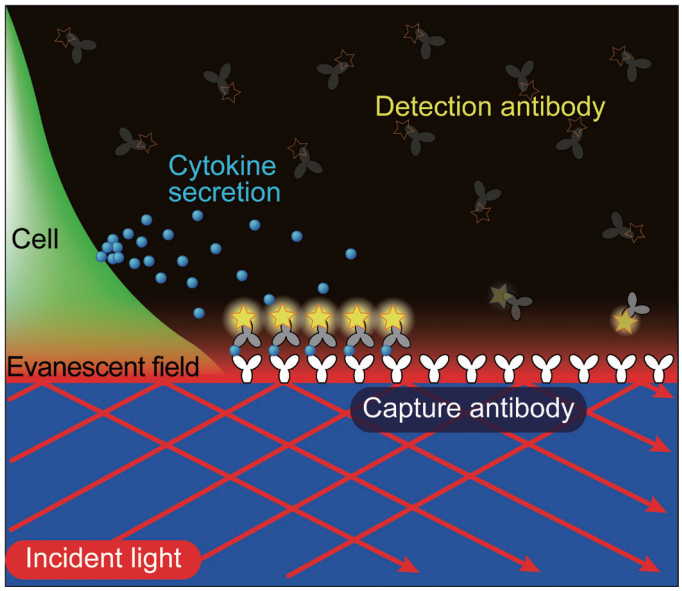


Figure: Real-time imaging of IL-1β secretion
A conceptual diagram of the platform for real-time imaging of protein secretion at single-cell resolution. Secreted protein was captured on site by antibody immobilized on the bottom of a microwell. Captured protein was immediately recognized by fluorescently-labelled detection antibody and visualized by total internal reflection illumination.

Since the preceding research center, Research Center for Allergy and Immunology, was launched more than 10 years ago, the very basic mission of the Laboratory for Integrative Genomics has consistently been to function as a "Gateway" to genomics for biologists coming from other fields. To achieve this mission, we have organized our research activities into three parts as follows: (1) central support activities; (2) strategic and collaborative research activities; and (3) exploratory research activities aimed at new technology development. Because this system has worked very well so far, we have continued to maintain it in the new IMS. As for the central support activities (1), we have made every effort to introduce new genomic technologies to the center according to the needs and the budget size of the center. The most prominent change in these three years is to incorporate massively parallel DNA sequencing (MPS) technology using a Roche 454 GS Junior, Illumina MiSeq and HiSeq1500. As for the strategic research activities (2), our group plays the role of "workhorse", which drives the measurement pipeline of center projects such as the Atopic Dermatitis project and the Primary Immunodeficiency (PID) project. In particular, we have developed a network of pediatricians in Japan and functioned as a hub of the network in the PID project. As for the collaborative research activities (3), we have carried out many intramural as well as extramural collaboration projects. As exploratory research activities (3), we have focused our efforts on development of "single-cell" measurement technologies. The most recent study enables us to monitor protein secretion processes from single cells in real-time (Fig.), which will be applied for analysis of autoinflammatory diseases.

Recent Major Publications

Shirasaki Y, Yamagishi M, Shimura N, Hijikata A, Ohara O. Toward an understanding of immune cell sociology: real-time monitoring of cytokine secretion at the single-cell level. ***IUBMB Life*** 65, 28-34 (2013)

Saito Y, Yuki H, Kuratani M, Hashizume Y, Takagi S, Honma T, Tanaka A, Shirouzu M, Mikuni J, Handa N, Ogahara I, Sone A, Najima Y, Tomabechi Y, Wakiyama M, Uchida N, Tomizawa-Murasawa M, Kaneko A, Tanaka S, Suzuki N, Kajita H, Aoki Y, Ohara O, Shultz LD, Fukami T, Goto T, Taniguchi S, Yokoyama S, Ishikawa F. A pyrrolo-pyrimidine derivative targets human primary AML stem cells in vivo. ***Sci Transl Med*** 5, 181ra52 (2013)

Kamae C, Nakagawa N, Sato H, Honma K, Mitsuiki N, Ohara O, Kanegane H, Pasic S, Pan-Hammarström Q, van Zelm MC, Morio T, Imai K, Nonoyama S. Common variable immunodeficiency classification by quantifying T-cell receptor and immunoglobulinκ-deleting recombination excision circles. ***J Allergy Clin Immunol*** 131, 1437-40.e5 (2013)

Invited Presentations

Ohara O. Systems immunology: Tackling the complexity of the immune system. 2012 Annual Meeting of the Japanese Society for Immunology, Kobe, Japan. December 7th, 2012.

Ohara O. Exploration of disease causative mutations on the basis of genomics. Annual Meeting of Japan Pediatric Society, Hiroshima, Japan. April 21st, 2013.

Ohara O. Perspectives of integrative genomics for understanding of dynamics of the biological system. The 40th Annual Meeting of the Japanese Society of Toxicology, Chiba, Japan. June 18th, 2013.

Ohara O. Post-GWAS animal models. 3rd Sardinian Summer School, Pula, Italy. September 9th, 2013.

Ohara O. Next-generation DNA sequencing technology and genomic analysis. 58th Annual Meeting of the Japan Society of Human Genetics, Sendai, Japan. November 23rd, 2013.

Laboratory for
Disease Systems Modeling

Group Director: Hiroaki Kitano

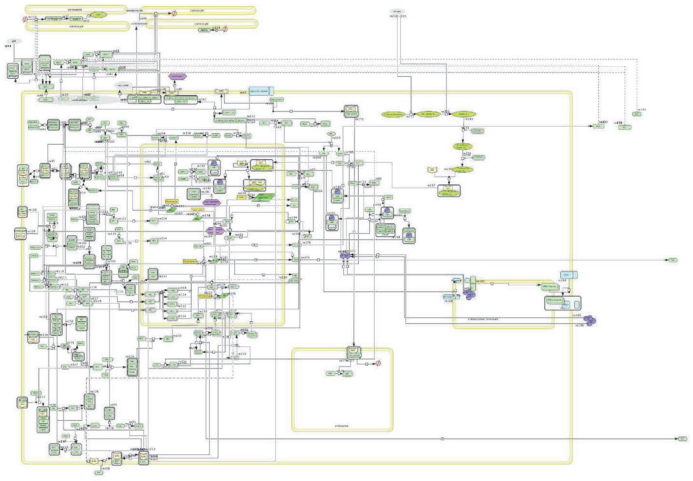


Figure: Partial map of the keratinocyte interaction network relevant to AD

LDSM is responsible for systems modeling, computational infrastructure development for IMS-focused projects, and exploratory research converging on the center's goals.

- Understanding atopic dermatitis (AD) using systems biology by combining bottom-up (detailed modeling) and top-down (high-throughput data analysis) strategies is one of our central activities.
  - We are developing a detailed molecular interaction map of intra- and intercellular interactions, focused on keratinocytes and immune cells. So far, a draft map for keratinocytes has been created; it continues to be updated based on current literature, databases and maps previously developed. The map will serve as the backbone for all our modeling and analysis tasks.
  - We are also developing a range of large-scale analysis tools to integrate various high-throughout data generated within IMS.
  - We are creating a hierarchical dynamical model of skin representing healthy and disease states thereby offering computational analysis of disease mechanisms and potential therapeutic intervention. Comprehensive model development is underway to construct a detailed model incorporating existing hypotheses within the current focus of experimental groups at IMS. Modeling will be extended to multi-scale settings.
- A large-scale molecular interaction map of the yeast stress response was created as a principal model of robustness inherent in eukaryotic organisms. Systemic network analysis of the map to unravel robustness and signal separation machinery of stress response pathways are underway.
- A new robustness analysis combining a gTOW assay as developed for a novel high-precision computational modeling for budding yeast, with possible extension to mammalian cells.
- In collaboration with RIKEN's FANTOM5 consortium, we are analyzing the Down Syndrome (DS) -iPS to neuron data using systems biology approaches to understand the time-dependent changes in the process of differentiation of a DS cell.
- Development of a series of computational infrastructures to support model development and data analysis at IMS is underway. "Project Rishi" aims to develop highly intelligent systems to scan existing literature to extract knowledge of molecular interactions. It is based on novel large-scale text mining combined with multi-strategy machine learning.

Recent Major Publications

Hase T, Ghosh S, Yamanaka R, Kitano H. Harnessing Diversity towards the Reconstructing of Large Scale Gene Regulatory Networks. ***PLoS Comput Biol*** 9, e1003361 (2013)

Ghosh S, Matsuoka Y, Asai Y, Hsin KY, Kitano H. Toward an integrated software platform for systems pharmacology. ***Biopharm Drug Dispos*** 34, 508-26 (2013)

Hsin KY, Ghosh S, Kitano H. Combining machine learning systems and multiple docking simulation packages to improve docking prediction reliability for network pharmacology. ***PLoS One*** 8, e83922 (2013)

Invited Presentations

Kitano H. Software platform for systems drug discovery. Innovative Medicines Initiative (IMI): Workshop on Translational Knowledge Management in Pharmaceutical R&D, Brussels, Belgium. July 12th, 2013.

Kitano H. Network-oriented drug discovery. Innovative Medicines Initiative (IMI): Workshop on Translational Knowledge Management in Pharmaceutical R&D, Brussels, Belgium. July 12th, 2013.

Kitano H. Systems Drug Discovery and Integrated Software Platform. Talk at ETH Zurich IMSB, Zurich, Switzerland. August 30th, 2013.

Kitano H. Mathematics for Planet Earth. Talks at RMIT Melbourne and University of New South Wales, Melbourne and Sydney, Australia. October 7th and 10th, 2013.

Kitano H. Grand Challenges of Systems Biology and Systems Biomedicine. World Health Summit 2013: Keynote Lecture, Berlin, Germany. October 22nd, 2013.

Laboratory for Immunogenetics

Team Leader: Hisahiro Yoshida

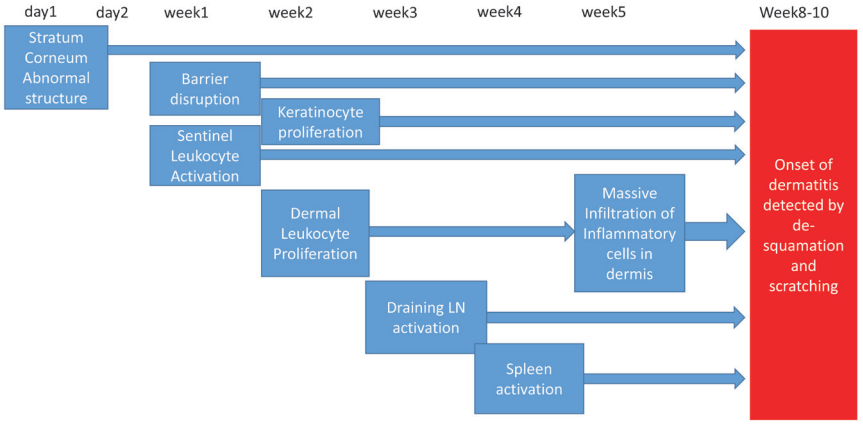


Figure: Pre-symptomatic disease development in the *Spade* mutant
Pre-symptomatic events occurring in the *Spade* mutant were detected by histological, cytological and molecular biological analysis and are summarized in the figure as longitudinal steps.

Recent Major Publications
Watarai H, Sekine-Kondo E, Shigeura T, Motomura Y, Yasuda T, Satoh R, Yoshida H, Kubo M, Kawamoto H, Koseki H, Taniguchi M. Development and function of invariant natural killer T cells producing T(h)2- and T(h)17-cytokines. ***PLoS Biol*** 10, e1001255 (2012)

Sugiyama M, Nakato G, Jinnohara T, Akiba H, Okumura K, Ohno H, Yoshida H. Expression pattern changes and function of RANKL during mouse lymph node microarchitecture development. ***Int Immunol*** 24, 369-78 (2012)

Invited Presentations
Yoshida H. Skin homeostasis and atopic dermatitis development. 8th RCAI-JSI International Symposium on Immunology, Yokohama, Japan. June 27th, 2013.

Yoshida H. Skin homeostasis and atopic dermatitis development. The 5th LJI & IMS-RCAI Workshop, Yokohama, Japan. October 30th, 2013.

Usually disease onset is detected by symptomatic observation, however, before the onset of the disease, there must be an accumulation of many imperceptible pathogenic events in the human body as part of pre-symptomatic disease development. If one could precisely monitor and understand the pre-symptomatic longitudinal multiple events proceeding in a healthy individual, it should be possible to predict the timing of disease onset and to take measures to prevent it. It will be beneficial for many of us in the coming era when everyone can know their own genome sequence information and genetic risk factors shortly after birth.

In our laboratory, we had been working to identify the genetic factors for allergic and immune disease development by phenotype screening of chemical mutagen, N-ethyl N-nitrosourea (ENU), induced mutant mice on a C57BL/6J background. We have established an animal model of atopic dermatitis in which the disease developed at approximately 8 weeks after birth, as detected by ear skin desquamation and scratching of the skin a few days after the first symptoms. This phase is followed by a Th2 immune bias detected by serum IgE, IgG1 and histamine level elevation 3 weeks later, and 8 weeks after that, a Th1 immune bias was also detected, with serum IgG2b and IgG2c elevation. At this final stage, the pathological findings identified a chronic skin inflammatory condition. Therefore we have named this mutant *Spade* (Stepwise progressive atopic dermatitis).

Using this disease model, we are now trying to precisely define the pre-symptomatic events occurring not only in skin lesions but also in systemic immune regulation, from birth to the onset of dermatitis. As shown in the figure, we have now found that distinct pre-symptomatic events occur independently in epidermis, dermis, draining lymph node, and spleen and that the accumulation of all these events leads to the onset of dermatitis in the late stage.

Laboratory for Integrated Bioinformatics

Team Leader: **Todd D. Taylor**

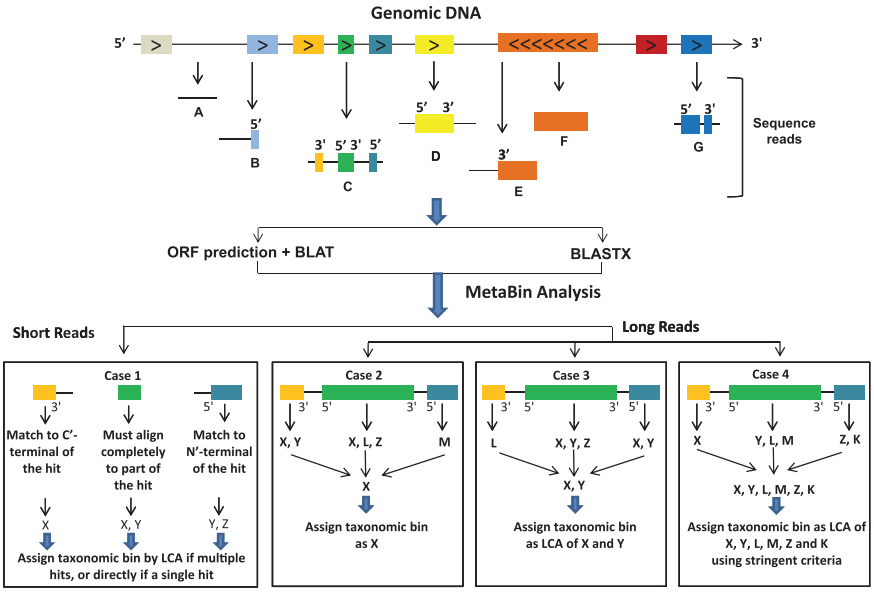


Figure: ORF-based approach for the taxonomic assignment of reads of different lengths derived from different regions of the genomic DNA
Read derived from an intergenic region (A), read containing a small 5′ region of an ORF (B), read containing two partial ORFs at the 5′ and 3′ terminals and a complete ORF in the middle (C), read containing only a single complete ORF (D), read containing a long partial ORF at one end (E), read obtained from within an ORF (F), read with sequencing error causing a single ORF to split into two smaller ORFs (G). X, Y, Z, K, L, and M are the genomes to which the ORFs showed matches. The taxonomic IDs of the species of these genomes are used for making the taxonomic assignments and for creating the taxonomic bins.

Recent Major Publications

Sharma VK, Kumar N, Prakash T, Taylor TD. Fast and accurate taxonomic assignments of metagenomic sequences using MetaBin. ***PLoS One*** 7, e34030 (2012)

Fukuda S, Toh H, Taylor TD, Ohno H, Hattori M. Acetate-producing bifidobacteria protect the host from enteropathogenic infection via carbohydrate transporters. ***Gut Microbes*** 3, 449-54 (2012)

Hariharan R, Simon R, Pillai MR, Taylor TD. Comparative analysis of DNA word abundances in four yeast genomes using a novel statistical background model. ***PLoS One*** 8, e58038 (2013)

Invited Presentations

Taylor TD. Metagenomics: an introduction and applications to health and the environment. School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. April 19th, 2013.

Taylor TD. Metagenomics and approaches to immunology. 8th RCAI-JSI International Symposium on Immunology, Yokohama, Japan. June 28th, 2013.

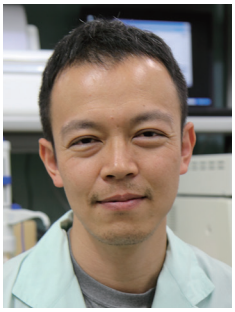
Taylor TD. Metagenomics and barcoding. Poznan Summer School of Bioinformatics: Molecular Evolution & Phylogenomics, Poznan, Poland. August 23rd, 2013.

Taylor TD. Online resources: databases, tools and pipelines; Introduction to the human genome project and metagenomics. 1st Workshop in Bioinformatics, Penang, Malaysia. December 16th, 2013.

The aims of the laboratory are to provide centralized bioinformatics support for the other research laboratories in this center, with a special emphasis on metagenomics and bacterial genomics related projects. We will develop an infrastructure of pipelines and tools for the efficient handling of both small-scale and large-scale datasets and for the analysis of various types of data produced from expression profile analysis, transcription network analysis, biomarker discovery analysis, and so on, with a special emphasis on metagenomic and metatranscriptomic analysis. Until recently, the main area of our research has been the analysis of various human health-and environmental- related metagenomics systems. We are developing powerful, high-throughput open-source tools and databases for the comprehensive analysis of metagenomic and bacterial genomic sequence data.

We have participated in various domestic and international metagenome, microbial genome, and other genome projects, some of which are still ongoing, including analysis of 1) the human gut microbiome in 13 healthy Japanese individuals, 2) termite gut bacteria and microbial fuel cell metagenomes, both which have the potential for waste degradation and clean energy production, and 3) several other bacteria from a wide variety of environments. My team has developed several tools for high-throughput metagenomic and bacterial genomic sequence analysis, including 1) iMetaSys, a comprehensive high-performance analysis pipeline and knowledgebase, 2) MetaBioME, a comprehensive platform to facilitate homology-based computational identification of novel homologous commercially useful enzymes (CUEs) from metagenomic datasets, and 3) MetaBin, a program for fast, accurate and highly sensitive taxonomic assignments of metagenomic sequences.

The ultimate goal of our research is to develop tools capable of efficiently processing and analyzing high-throughput datasets leading to the modeling and prediction of behaviors and interactions of whole environments of microbes, in association with their hosts (if applicable), metabolites, environmental conditions, and other relevant factors.



Laboratory for Tissue Dynamics

Team Leader: Takaharu Okada

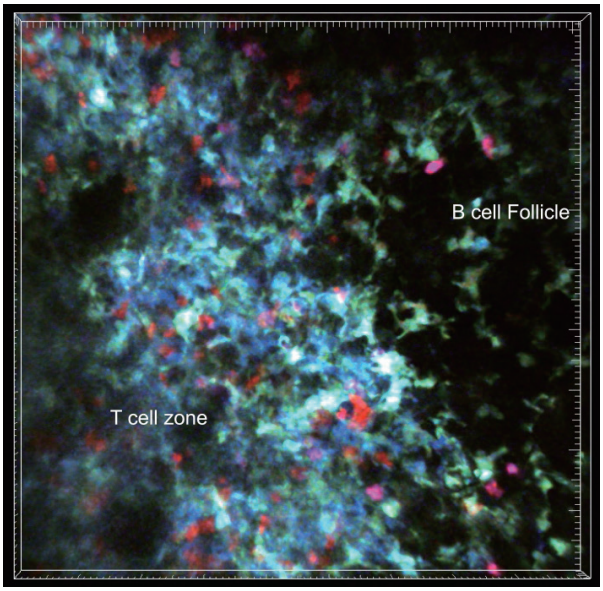


Figure: Intravital two-photon image of T cells and dendritic cells in the lymph node
Pink and red cells are antigen-specific CD4⁺ and CD8⁺ T cells, respectively. Blue and Green cells are different subsets of dendritic cells. Image volume: 400 µm x 400 µm x 50 µm.

The goal of the laboratory is to understand the mechanisms regulating cell migration and interactions in the tissues that shape adaptive immune responses. Currently, we have limited understanding of how generation of immunological memory and tolerance, two key features of adaptive immunity, are controlled by dynamic interactions among immune cells. For example, it is not understood how dynamics of B cells and helper T cells contribute to generation of humoral immune memory. As for cellular immunity mediated by cytotoxic T lymphocytes (CTLs), little is known about cell-cell interactions that regulate the CTL differentiation balance between effector and memory cells. Furthermore, cell-cell interactions that are required for peripheral tolerance of autoreactive B cells and CD8⁺ T cells are poorly understood.

As a strategy for tackling the above questions, we use real time imaging, in particular two-photon microscopy, to analyze cellular migration and interactions in the tissues. This microscopy method has been revealing striking dynamics of immune cells in various organs, underlining the importance of this approach to resolve the complexity of the immune system. By applying the imaging strategy to relevant mouse models, we aim to reveal immune cell dynamics that are critical for generation of immunological memory and tolerance. For this aim, we have been developing and studying mouse strains in which the dynamics of helper T cells specialized for B cell immune responses are perturbed, the differentiation balance of CTLs is disrupted, cross-presenting dendritic cells (DCs) are fluorescently labeled for *in vivo* imaging, or the dynamics and function of regulatory T (Treg) cell subsets are perturbed to break peripheral tolerance of autoreactive B cells and CD8⁺ T cells.

Recent Major Publications

Kitano M, Okada T. Four-dimensional tracking of lymphocyte migration and interactions in lymph nodes by two-photon microscopy. ***Methods Enzymol*** 506, 437-54 (2012)

Hirata E, Yukinaga H, Kamioka Y, Arakawa Y, Miyamoto S, Okada T, Sahai E, Matsuda M. In vivo fluorescence resonance energy transfer imaging reveals differential activation of Rho-family GTPases in glioblastoma cell invasion. ***J Cell Sci*** 125, 858-68 (2012)

Okada T, Moriyama S, Kitano M. Differentiation of germinal center B cells and follicular helper T cells as viewed by tracking Bcl6 expression dynamics. ***Immunol Rev*** 247, 120-32 (2012)

Invited Presentations

Okada T. Two-photon imaging of cellular dynamics during the adaptive immune response. RIKEN RCAI: Univ. Michigan Medical Center Joint Workshop, Ann Arbor, MI, USA. January 16th, 2013.

Okada T. 2-Photon imaging of adaptive immune responses. 2nd New Zealand-Japan Joint Immunology Workshop, Auckland, New Zealand. February 27th, 2013.

Okada T. S1PR2-driven retention of Tfh cells in the germinal center and its contribution to antibody responses. Gordon Research Conferences: T Follicular Helper Cells, Hong Kong, China. July 22nd, 2013.

Okada T. Imaging of dendritic cells important for cytotoxic T cells. The 3rd International Symposium by JSPS Core-to-Core Program: Cooperative International Framework in TGF-β Family Signaling, Matsuyama, Japan. October 29th, 2013.

Okada T. The role for follicular regulatory T cells in immune homeostasis. Annual Meeting of the Japanese Society for Immunology 2013: International Symposium 8, Chiba, Japan. December 12th, 2013.

Laboratory for Integrated Cellular Systems

Team Leader: Mariko Okada

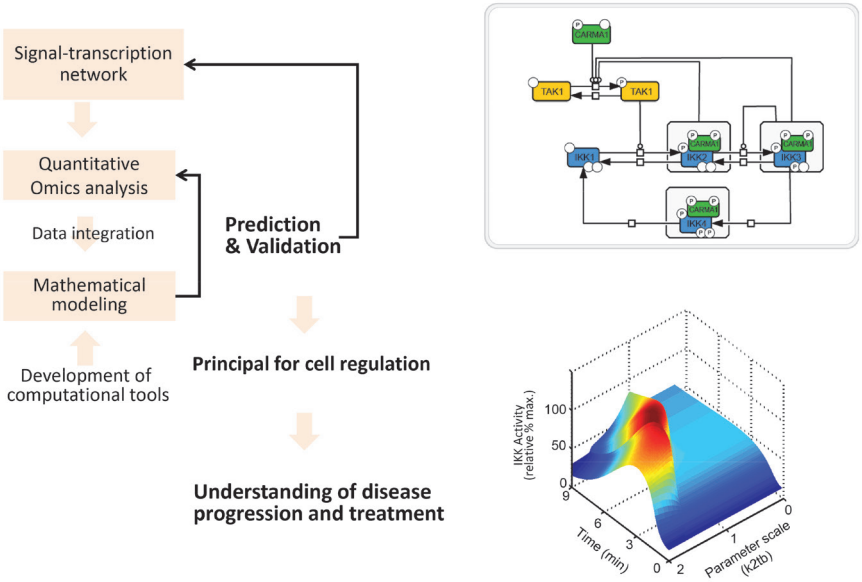


Figure: Modeling of a signal-transcription network
Measurement, data integration and modeling in a theoretical framework for understanding of signal-dependent cell fate control (right). Mathematical model and simulation of NF-κB network.

Recent Major Publications

Hiroshima M, Saeki Y, Okada-Hatakeyama M, Sako Y. Dynamically varying interactions between heregulin and ErbB proteins detected by single-molecule analysis in living cells. ***Proc Natl Acad Sci U S A*** 109, 13984-9 (2012)

Nomura M, Okada-Hatakeyama M. Phase responses of oscillating components in a signaling pathway. ***Front Physiol*** 4, 68 (2013)

Terada A, Okada-Hatakeyama M, Tsuda K, Sese J. Statistical significance of combinatorial regulations. ***Proc Natl Acad Sci U S A*** 110, 12996-3001 (2013)

Invited Presentations

Okada M. A switch in NF-κB immune signaling. The First Annual Winter q-bio Meeting, Honolulu, USA. February 21st, 2013.

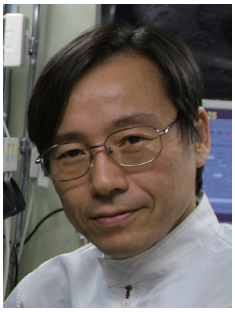
Okada M. Regulation of cancer signaling network. The 72nd Annual Meeting of the Japanese Cancer Association, Yokohama, Japan. October 3rd, 2013.

Shinohara H. Switch-like activity of NF-κB is generated by CARMA1-TAK1-IKKβ positive feedback loop in BCR-signaling. Gradients and Signalling: from chemotaxis to development, Okinawa, Japan. November 14th, 2013.

Okada M. Multi-layered regulation of intracellular signaling network. The 36th Annual Meeting of the Molecular Biology Society of Japan, Kyoto, Japan. December 3rd, 2013.

Okada M. Signaling dynamics and ON/OFF transcriptional regulation. The 3rd Open Symposium: Grant-in-Aid for Scientific Research on Innovative Areas- Protein Modifications in Pathogenic Dysregulation of Signaling, Tokyo, Japan. January 25th, 2014.

The aims of the laboratory are to define the general regulatory rules in signal transduction-transcriptional networks in cell determination processes and to apply this knowledge of regulatory principles to the understanding and treatment of human diseases. For this purpose, we perform quantitative measurements of the target biological system using various experimental methods and integrate these heterogeneous data by means of mathematical modeling. However, it is not always the case that preexisting computational methods and theory are satisfactory to analyze the dynamic behavior of a particular biological system. Therefore, we also develop computational algorithms that can be applied for the analysis of actual biological data. In 2013, we developed several mathematical models of signal-transcription networks in immune cell development and cancer. In B cell signaling, based on quantitative experiments and mathematical modeling, we identified a positive feedback loop and a key molecule that induces switch activation of the NF-κB transcription factor. We showed that a positive cooperativity in the signaling network plays an important role to determine individual cell fate (Science, accepted). We also developed several computational methods. A newly developed algorithm LAMP (limitless arity multiple-testing procedure) method enables us to identify combinatorial regulation of transcription factors from time-series transcriptome data. In comparison with existing methods that can find only two-motif combinations, our testing procedure contributed to finding larger fractions of regulatory pathways and TF complexes in human breast cancer. The method allows us to find hidden modes of transcriptional regulation critical for determination of specific cell stages.



Laboratory for Molecular Live-Cell Quantification

Team Leader: Makio Tokunaga

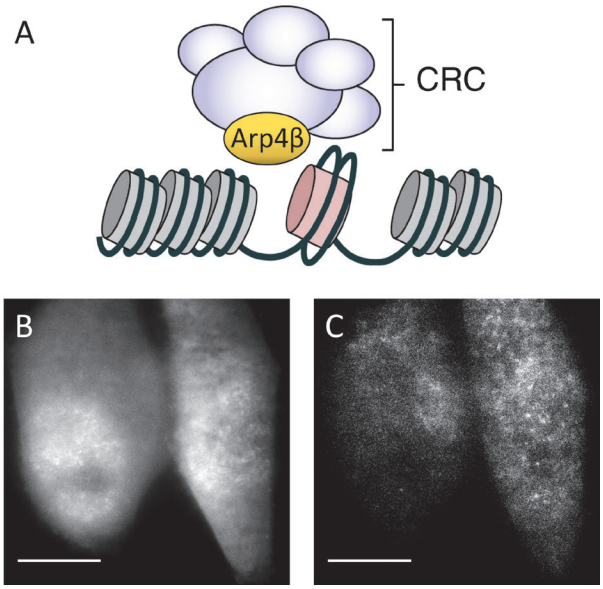


Figure: Actin-related protein (Arp4) is an essential component of the ATP-dependent chromatin-remodeling complexes (CRC).
(A) Arp4 is thought to be involved in transcription control and DNA damage repair. (B) (C) Fluorescence images of Arp4-EGFP expressing HeLa cells using HILO microscopy. Scale bar: 10 μm Arp4 is localized both in the nucleus and cytoplasm (C), while single molecules of Arp4 are detected in the nucleus (C).

Based on emerging techniques in molecular imaging, our long-term goal is the understanding of transcriptional regulation related to cell signaling. To achieve this goal we quantify the interactions of signaling molecules and transcriptional regulators.

TCR microcluster formation

T cell receptor (TCR) microclusters are important to initiate and sustain T cell activation. Although various signaling molecules were found enriched in microclusters, the mechanism of microcluster formation remains unclear. To visualize the dynamics of membrane proteins related to microcluster formation, we performed simultaneous single molecule imaging of TCR and the tyrosine phosphatase CD45 with GFP labeled microclusters. CD45 is known to function as an initiator of TCR activation and to be excluded from TCR microclusters. On the activated T cell surface, TCR molecules showed slower diffusion and longer residence time in microclusters than CD45, suggesting dynamic interaction of TCR molecules with the microcluster. We are planning to clarify the mechanism of TCR microcluster formation.

Transcriptional control by actin-related proteins

Actin-related proteins (Arps) are evolutionarily and structurally similar to actin. In the nucleus, Arp4 and actin are critical components of ATP-dependent chromatin-remodeling complexes, which regulate the dynamic modification of chromatin architecture. Arp4 is reported to play a key role as a regulator of chromatin remodeling, possibly through its direct binding to histones, however, the detailed mechanisms remain elusive. Aiming to clarify the dynamics of Arp4 in transcriptional control, we performed a quantitative imaging analysis of Arp4 in the nucleus. Both single molecule imaging by HILO microscopy and FRAP analysis revealed dynamic movements of Arp4. After stimulation with PMA, an activator of transcription, the residence time of Arp4 became shorter. These results suggest dynamic interactions of Arp4 with either chromatin-remodeling complexes or chromatin.

Recent Major Publications

Hotta K, Nashimoto A, Yasumura E, Suzuki M, Azuma M, Iizumi Y, Shima D, Nabeshima R, Hiramoto M, Okada A, Sakata-Sogawa K, Tokunaga M, Ito T, Ando H, Sakamoto S, Kabe Y, Aizawa S, Imai T, Yamaguchi Y, Watanabe H, Handa H. Vesnarinone Suppresses TNFα mRNA Expression by Inhibiting Valosin-containing Protein. ***Mol Pharmacol*** 83, 930-8 (2013)

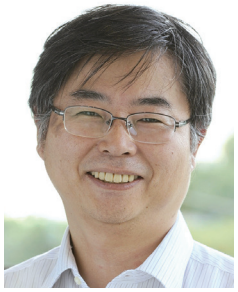
Asakawa H, Yang HJ, Yamamoto TG, Ohtsuki C, Chikashige Y, Sakata-Sogawa K, Tokunaga M, Iwamoto M, Hiraoka Y, Haraguchi T. Characterization of nuclear pore complex components in fission yeast Schizosaccharomyces pombe. ***Nucleus*** 5, 1-14 (2014)

Invited Presentations

Tokunaga M, Fukagawa A, Sakata-Sogawa K. Dynamic and entropic molecular interactions by single molecule studies. The 69th Annual Meeting of the Japanese Society of Microscopy: Symposium Session on technology of super-resolution microscopy and its application in biology, Suita, Japan. May 20th, 2013.

Tokunaga M. Single molecule imaging — TIRF and HILO microscopy —. The 21st Cell Biology Workshop: Training Course of Fluorescence Microscopy I, Kobe, Japan. August 10th, 2013.

Tokunaga M. Seeing the Dynamic Face of Life by viewing a Single Molecule with Light. Symposium open to the Public: The structure of Life Forms that manipulate their own DNA, Suita, Japan. August 25th, 2013.



Laboratory for Genotyping Development

Group Director: Michiaki Kubo

Figure: Illumina Human Genotyping Array system (left) and HiSeq 2500 System (right)

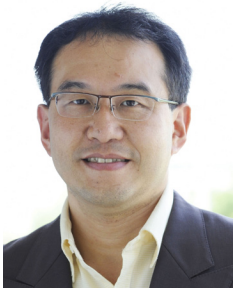
Our team established a high-throughput SNP genotyping system using a combined method of multiplex-PCR and the Invader assay during the era of the SNP Research Center (FY2000-2007). Using this system, our team contributed to the establishment of the JSNP database (http://snp.ims.u-tokyo.ac.jp/index_ja.html) and to the success of the International HapMap Project Phase 1 (http://hapmap.ncbi.nlm.nih.gov/). From FY2003, our team has been working as the main facility of genomic research for the BioBank Japan project and has generated a large amount of SNP genotyping data for the association studies of common diseases. In addition, since 2008 we have been performing genotyping of samples collected by NIH for pharmacogenetic study, Pharmacogenomics Research Network (PGRN), under the PGRN-RIKEN CGM Global Alliance (http://bts.ucsf.edu/pgrn-cgm/). Moreover, we developed a new genotyping method to detect copy number variation (RETINA) that was published in Human Mutation (29:182-9, 2008). Using RETINA, we developed a new genotyping method for the *CYP2D6* gene, which has many functional variations combined with copy number variations. Since CYP2D6 is associated with the metabolism of many drugs, CYP2D6 activity estimated by our method will help in establishing personalized drug treatment, the right drug and the right dose. We also developed a rapid SNP genotyping system for clinical research and are performing several clinical intervention studies based on the genotype information in the Genome-guided drug Treatment Optimization Program (G-TOP) funded by MEXT. Our hope is to implement Genomic Medicine that will optimize medical care and health by use of genomic information.

Recent Major Publications

Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, Qiu X, Sharma S, Takimoto A, Ogura Y, Jiang H, Yan H, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Hosono N, Tsuji T, Suzuki T, Sudo H, Kotani T, Yonezawa I, Londono D, Gordon D, Herring JA, Watanabe K, Chiba K, Kamatani N, Jiang Q, Hiraki Y, Kubo M, Toyama Y, Tsunoda T, Wise CA, Qiu Y, Shukunami C, Matsumoto M, Ikegawa S. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. ***Nat Genet*** 45, 676-9 (2013)

Ellinghaus D, Baurecht H, Esparza-Gordillo J, Rodríguez E, Matanovic A, Marenholz I, Hübner N, Schaarschmidt H, Novak N, Michel S, Maintz L, Werfel T, Meyer-Hoffert U, Hotze M, Prokisch H, Heim K, Herder C, Hirota T, Tamari M, Kubo M, Takahashi A, Nakamura Y, Tsoi LC, Stuart P, Elder JT, Sun L, Zuo X, Yang S, Zhang X, Hoffmann P, Nöthen MM, Fölster-Holst R, Winkelmann J, Illig T, Boehm BO, Duerr RH, Büning C, Brand S, Glas J, McAleer MA, Fahy CM, Kabesch M, Brown S, McLean WH, Irvine AD, Schreiber S, Lee YA, Franke A, Weidinger S. High-density genotyping study identifies four new susceptibility loci for atopic dermatitis. ***Nat Genet*** 45, 808-12 (2013)

Perera MA, Cavallari LH, Limdi NA, Gamazon ER, Konkashbaev A, Daneshjou R, Pluzhnikov A, Crawford DC, Wang J, Liu N, Tatonetti N, Bourgeois S, Takahashi H, Bradford Y, Burkley BM, Desnick RJ, Halperin JL, Khalifa SI, Langaee TY, Lubitz SA, Nutescu EA, Oetjens M, Shahin MH, Patel SR, Sagreiya H, Tector M, Weck KE, Rieder MJ, Scott SA, Wu AH, Burmester JK, Wadelius M, Deloukas P, Wagner MJ, Mushiroda T, Kubo M, Roden DM, Cox NJ, Altman RB, Klein TE, Nakamura Y, Johnson JA. Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. ***Lancet*** 382, 790-6 (2013)



Laboratory for Genome Sequencing Analysis

Team Leader: Hidewaki Nakagawa

Recent Major Publications

Akamatsu S, Takata R, Haiman CA, Takahashi A, Inoue T, Kubo M, Furihata M, Kamatani N, Inazawa J, Chen GK, Le Marchand L, Kolonel LN, Katoh T, Yamano Y, Yamakado M, Takahashi H, Yamada H, Egawa S, Fujioka T, Henderson BE, Habuchi T, Ogawa O, Nakamura Y, Nakagawa H. Common variants at 11q12, 10q26 and 3p11.2 are associated with prostate cancer susceptibility in Japanese. ***Nat Genet*** 44, 426-9 (2012)

Akamatsu S, Takahashi A, Takata R, Kubo M, Inoue T, Morizono T, Tsunoda T, Kamatani N, Haiman CA, Wan P, Chen GK, Le Marchand L, Kolonel LN, Henderson BE, Fujioka T, Habuchi T, Nakamura Y, Ogawa O, Nakagawa H. Reproducibility, performance, and clinical utility of a genetic risk prediction model for prostate cancer in Japanese. ***PLoS One*** 7, e46454 (2012)

Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, Arai Y, Takahashi H, Shirakihara T, Nagasaki M, Shibuya T, Nakano K, Watanabe-Makino K, Tanaka H, Nakamura H, Kusuda J, Ojima H, Shimada K, Okusaka T, Ueno M, Shigekawa Y, Kawakami Y, Arihiro K, Ohdan H, Gotoh K, Ishikawa O, Ariizumi S, Yamamoto M, Yamada T, Chayama K, Kosuge T, Yamaue H, Kamatani N, Miyano S, Nakagama H, Nakamura Y, Tsunoda T, Shibata T, Nakagawa H. Whole genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. ***Nat Genet*** 44, 760-4 (2012)

Invited Presentations

Nakagawa H. Comprehensive genomic analysis of liver cancers with biliary phenotype. International Symposium on Cholangiocarcinoma Tokyo 2013, Tokyo, Japan. February 9th, 2013.

Nakagawa H. Whole genome signatures of liver cancer and personalized medicine. Cancer genomics focused on lung cancer. Roche Korea: 12th Round Asia Oncology Forum 2013, Seoul, Korea. August 23rd and 24th, 2013.

Nakagawa H. Whole genome sequencing analysis of cancer and its interpretation. 72nd Annual Meeting of the Japanese Cancer Association, Yokohama, Japan. October 5th, 2013.

Nakagawa H. Whole genome sequencing analysis of liver cancer, forwarding to personalized medicine. BGI: 8th International Conference on Genomics, Shenzen, China. November 1st, 2013.

Nakagawa H. Whole genome sequencing analysis of liver cancer, forwarding to personalized medicine. Yonsei Institute 20th Annual International Symposium: A Roadmap to the Personalized Cancer Treatment, Seoul, Korea. November 8th, 2013.

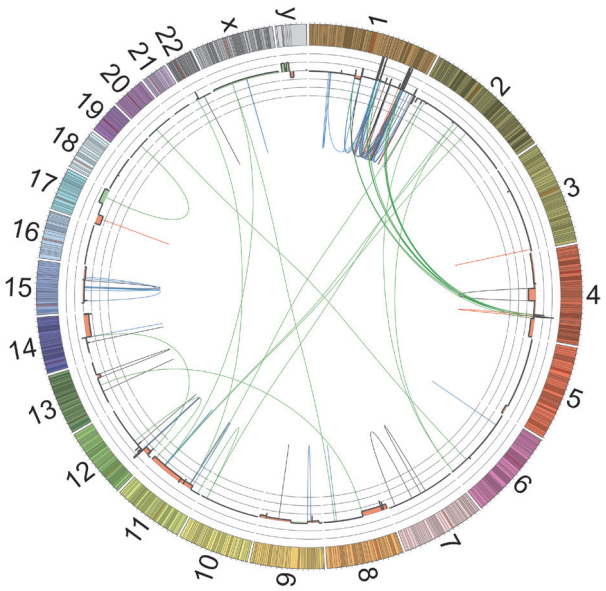


Figure: Whole genome structure of one virus-related liver cancer
Circos plot showing intra- or inter-chromosomal rearrangements and copy number alterations in each chromosome (1~22, X, and Y).

We have been organizing a cancer genome project for liver cancer as a Japanese ICGC project by collaborating with the National Cancer Research Center and the University of Tokyo. By operating three HiSeq sequencers at full capacity, as of 2013 we completed 270 whole genome sequencing (WGS) sets of liver cancers (x35 for cancer and x30 for blood). We and the Laboratory for Medical Science Mathematics performed mathematical analysis for the WGS dataset and demonstrated a comprehensive landscape of diverse phenotypes of liver cancers with multiple etiological backgrounds (virus-related and non-viral, hepatocellular carcinoma and biliary phenotype) for point mutations, short indels, copy-number alterations, structural variations, and virus integrations. On average 9,700 somatic substitutions and indels were called per each tumor, and mutational signatures of liver cancer, especially multi-centric (MC) tumors, indicated an influence of etiological background on somatic mutation patterns (Fujimoto *et al.* Nat Genet 2012) and a strong impact of chronic inflammation on the mutation process in cancer development. We systematically performed integrated and comparative analyses of whole genomes and transcriptomes (RNA-seq) of HBV-related liver cancers and their matched controls, which provided strong evidence that various types of genomic mutations could trigger diverse transcriptional changes. Some silent mutations in coding regions, deep intronic mutations and structural changes caused splicing aberrations, and HBV integrations generated diverse patterns of virus-human fusion transcripts. These comparative and complementary analyses provide a higher-resolution landscape of the cancer genome and can improve the interpretation of mutational consequences of genomic alterations. As of 2013, we also completed ~600 exome sequencing of several diseases including arrhythmia, autoimmune diseases, and rare tumors by collaborating with other teams within IMS. We were also involved with GWAS of prostate cancer and establishment of a risk estimation model for prostate cancer by using 16 common SNPs (Akamatsu *et al.* PLoS ONE 2012) and participated in global meta-analysis of prostate cancer GWAS.

Laboratory for Medical Science Mathematics

Group Director: Tatsuhiko Tsunoda

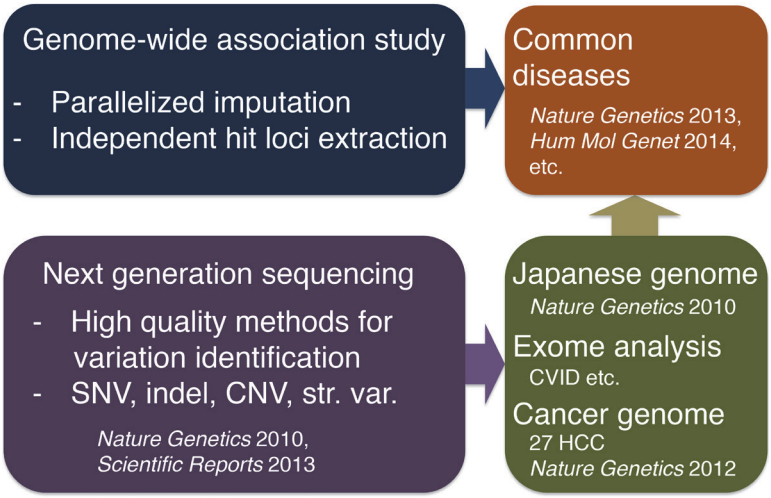


Figure: Development and application of our new analysis methods

Our research centers around the development of mathematical methods to enhance understanding of genetic variation and its impact on human diseases. To enhance ongoing GWAS analyses, we developed parallelized imputation pipelines to infer data for ungenotyped SNPs using 1000 Genomes haplotypes. Further, we developed methods to extract independent loci from the high-density data and applied these methods to a number of GWAS, e.g. [**3**]. We recently published a type 2 diabetes (T2D) study using these methods to search for novel variants and to better understand existing T2D association signals. That study discovered three novel loci associated with T2D (***Human Molecular Genetics***, 23: 239-246, 2014). Recently, massively parallel sequencing technology has allowed creation of comprehensive catalogs of genetic variation. However, to overcome relatively high sequencing error rates, sophisticated analysis methods are required. Expanding on our analysis of the first report of a Japanese individual's whole-genome sequence (***Nature Genetics*** 42: 931-936, 2010), we developed methods for detecting SNVs and short indels in whole genome and exome sequencing data for single sample calling [**2**]. The results showed high concordance with genotyping arrays, and Sanger sequencing revealed extremely low false positive and negative rates. As an International Cancer Genome Consortium (ICGC) member (***Nature*** 464: 993-998, 2010), we constructed an analytical pipeline based on this method to detect somatic alterations and viral-integration sites in cancer genomes. We reported our analysis of 27 hepatocellular carcinoma (HCC) genomes, 25 of which were associated with hepatitis B or C virus infection, including two sets of multicentric tumors, using this pipeline [**1**]. After adjusting for gene-length and background mutation frequency, we compiled a long-tailed list of recurrently mutated genes. Through gene set enrichment analysis of deleterious mutated genes, we found a significant overrepresentation of chromatin regulators. Furthermore, principal component analysis found that the substitution patterns of the 27 HCCs correlate with the etiological backgrounds from which the tumors developed.

Recent Major Publications

Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, Arai Y, Takahashi H, Shirakihara T, Nagasaki M, Shibuya T, Nakano K, Watanabe-Makino K, Tanaka H, Nakamura H, Kusuda J, Ojima H, Shimada K, Okusaka T, Ueno M, Shigekawa Y, Kawakami Y, Arihiro K, Ohdan H, Gotoh K, Ishikawa O, Ariizumi S, Yamamoto M, Yamada T, Chayama K, Kosuge T, Yamaue H, Kamatani N, Miyano S, Nakagama H, Nakamura Y, Tsunoda T, Shibata T, Nakagawa H. Whole Genome Sequencing of Liver Cancers Identifies Etiological Influences on Mutation Patterns and Recurrent Mutations in Chromatin Regulators. ***Nat Genet*** 44, 760-4 (2012)

Shigemizu D, Fujimoto A, Akiyama S, Abe T, Nakano K, Boroevich KA, Yamamoto Y, Furuta M, Kubo M, Nakagawa H, Tsunoda T. A practical method to detect SNVs and indels from whole genome and exome sequencing data. ***Sci Rep*** 3, 2161 (2013)

Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, Qiu X, Sharma S, Takimoto A, Ogura Y, Jiang H, Yan H, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Hosono N, Tsuji T, Suzuki T, Sudo H, Kotani T, Yonezawa I, Londono D, Gordon D, Herring JA, Watanabe K, Chiba K, Kamatani N, Jiang Q, Hiraki Y, Kubo M, Toyama Y, Tsunoda T, Wise CA, Qiu Y, Shukunami C, Matsumoto M, Ikegawa S. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. ***Nat Genet*** 45, 676-9 (2013)

Invited Presentations

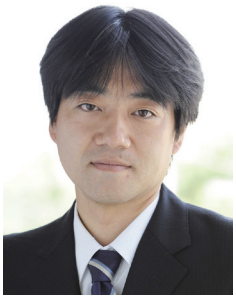
Tsunoda T. Genomic medicine's milestones and future. International Conference on Bioinformatics 2012 (InCoB 2012): Keynote lecture, Bangkok, Thailand. October 3rd, 2012.

Tsunoda T. Supercomputing accelerates genomic medicine. 4th Biosupercomputing Symposium: International Symposium for Next-Generation Integrated Simulation of Living Matter (ISLiM), Tokyo, Japan. December 5th, 2012.

Tsunoda T. Whole genome sequencing and comprehensive mutation analysis of liver cancer. 2013 SNUCRI & SNUCH Cancer Symposium, Jeju, Korea. May 3rd, 2013.

Tsunoda T. Whole Genome Approach Is Revolutionizing Medicine. The 10th International Workshop on Advanced Genomics, Tokyo, Japan. May 21st, 2013.

Tsunoda T. Whole genome and exome sequence analysis. The 58th Annual Meeting of the Japan Society of Human Genetics, Sendai, Japan. November 23rd, 2013.



Laboratory for Statistical Analysis

Team Leader: Atsushi Takahashi

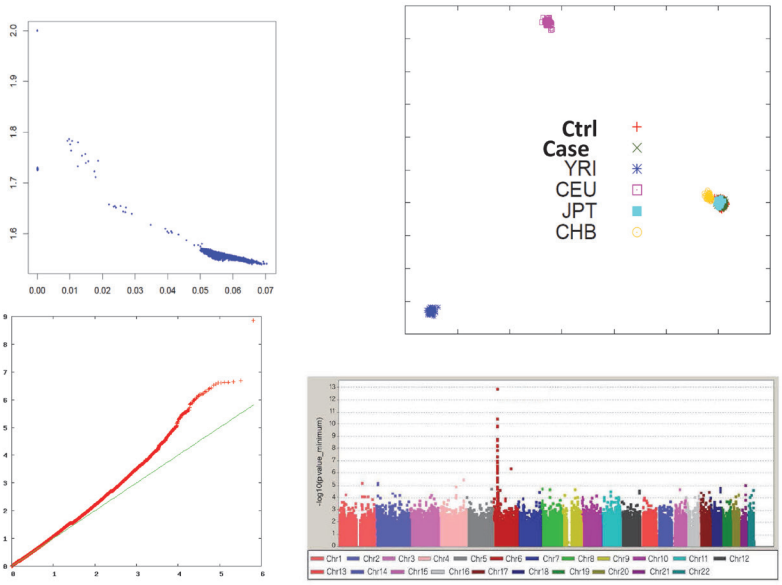


Figure: Representative results of GWAS Analysis

The mission of our laboratory is to clarify the mechanisms of human diseases and traits from the viewpoint of statistics and informatics. Recent technology developments have enabled us to investigate human variations over the entire genome. Massive amounts of genomic data are available, and we try to identify the genes associated with diseases/traits through performing analysis of these data.

Our center searches for genes associated with diseases and drug reactions. Our laboratory is in charge of performing GWAS and selecting candidate SNPs associated with diseases. We are performing many quality controls on the SNP data based on statistical genetics and statistics to obtain interpretable results. Then our laboratory is performing case-control association studies or quantitative analyses.

The Biobank Japan project has collected approximately 200,000 patients with 47 diseases, along with phenotype information. IMS has genotyped a huge number of the SNPs of individuals in the Biobank Japan. By using these data, we try to find new loci associated with diseases by GWAS. We also have constructed and developed GWAS systems in IMS to perform the GWAS in a short time. Individual genotype data have been accumulated, and there are very large amounts of genomic data. Therefore, we have constructed a data system to manage all these data. We are in charge of GWAS and statistical analysis and collaborate in many projects/consortiums to find novel loci associated with diseases, drug reactions and traits by GWAS

We are attempting to identify causal loci related to monogenic diseases by using next generation sequencing. We are in charge of development of new methods and systems to perform such analysis from the viewpoint of statistics and informatics.

Recent Major Publications

Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, Sakashita M, Yamada T, Fujieda S, Tanaka S, Doi S, Miyatake A, Enomoto T, Nishiyama C, Nakano N, Maeda K, Okumura K, Ogawa H, Ikeda S, Noguchi E, Sakamoto T, Hizawa N, Ebe K, Saeki H, Sasaki T, Ebihara T, Amagai M, Takeuchi S, Furue M, Nakamura Y, Tamari M. Genome-Wide Association Study Identifies Eight New Susceptibility Loci for Atopic Dermatitis in the Japanese Population. ***Nat Genet*** 44, 1222-6 (2012)

Low SK, Chung S, Takahashi A, Zembutsu H, Mushiroda T, Kubo M, Nakamura Y. Genome-Wide Association Study of Chemotherapeutic Agent-Induced Severe Neutropenia/Leucopenia for Patients in Biobank Japan. ***Cancer Sci*** 104, 1074-82 (2013)

Low SK, Takahashi A, Ashikawa K, Inazawa J, Miki Y, Kubo M, Nakamura Y, Katagiri T. Genome-Wide Association Study of Breast Cancer in the Japanese Population. ***PLoS One*** 8, e76463 (2013)

Invited Presentations

Takahashi A. Informatics in Genomic Epidemiology. The 58th Annual Meeting of the Japan Society of Human Genetics, Sendai, Japan. November 21st, 2013.

Laboratory for Pharmacogenomics

Group Director: Taisei Mushiroda

Recent Major Publications

Kiyotani K, Mushiroda T, Imamura CK, Tanigawara Y, Hosono N, Kubo M, Sasa M, Nakamura Y, Zembutsu H. Dose-adjustment study of tamoxifen based on CYP2D6 genotypes in Japanese breast cancer patients. ***Breast Cancer Res Treat*** 131, 137-45 (2012)

Kiyotani K, Mushiroda T, Tsunoda T, Morizono T, Hosono N, Kubo M, Tanigawara Y, Imamura CK, Flockhart DA, Aki F, Hirata K, Takatsuka Y, Okazaki M, Ohsumi S, Yamakawa T, Sasa M, Nakamura Y, Zembutsu H. A genome-wide association study identifies locus at 10q22 associated with clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients in Japanese. ***Hum Mol Genet*** 21, 1665-72 (2012)

Province MA, Goetz MP, Brauch H, Flockhart DA, Hebert JM, Whaley R, Suman VJ, Schroth W, Winter S, Zembutsu H, Mushiroda T, Newman WG, Lee MT, Ambrosone CB, Beckmann MW, Choi JY, Dieudonné AS, Fasching PA, Ferraldeschi R, Gong L, Haschke-Becher E, Howell A, Jordan LB, Hamann U, Kiyotani K, Krippl P, Lambrechts D, Latif A, Langsenlehner U, Lorizio W, Neven P, Nguyen AT, Park BW, Purdie CA, Quinlan P, Renner W, Schmidt M, Schwab M, Shin JG, Stingl JC, Wegman P, Wingren S, Wu AH, Ziv E, Zirpoli G, Thompson AM, Jordan VC, Nakamura Y, Altman RB, Ames MM, Weinshilboum RM, Eichelbaum M, Ingle JN, Klein TE. CYP2D6 Genotype and Adjuvant Tamoxifen: Meta-analysis of Heterogeneous Study Populations. ***Clin Pharmacol Ther*** 95, 216-27 (2014)

Invited Presentations

Mushiroda T. Genetics and Genome-Guided Personalized Medicine in Japan. The 2nd Meeting of South East Asian Pharmacogenomics Research Network (SEAPharm), Bangkok, Thailand. January 15th, 2013.

Mushiroda T. Future direction of pharmacogenomics: identification of genes associated with drug responses using GWAS. The 20th Annual Meeting of the Japanese Society for Gene Diagnosis and Therapy, Hamamatsu, Japan. July 20th, 2013.

Mushiroda T. Avoidance of severe drug-induced skin rash based on pharmacogenomics. The 23rd Annual Meeting of the Japanese Society of Pharmaceutical Health Care and Sciences, Sendai, Japan. September 22nd, 2013.

Mushiroda T. Individualization of anticancer therapeutics based on genome-wide association study. The 72nd Annual Meeting of the Japanese Cancer Association: Core Symposia - Pharmacogenomics in Oncology, Yokohama, Japan. October 4th, 2013.

Mushiroda T. Individualization of drug therapy based on genome-wide association study. Joint Congress of the 23rd Annual Meeting of the Japanese Society of Clinical Neuropsychopharmacology and the 43rd Annual Meeting of the Japanese Society of Neuropsychopharmacology, Okinawa, Japan. October 25th, 2013.

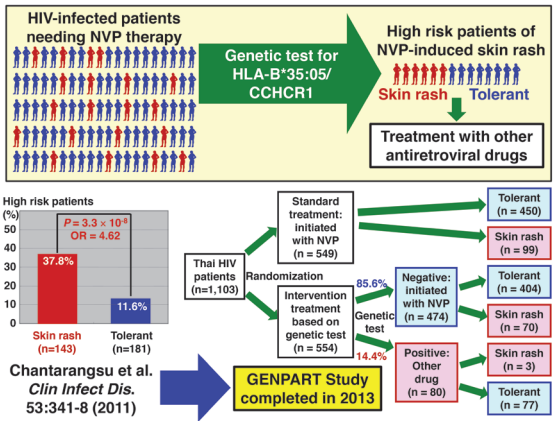


Figure: Strategy of a pharmacogenomics-guided approach to avoid adverse drug reactions: focusing on genomic biomarkers associated with risk of skin rash induced by nevirapine (NVP), an anti-HIV therapeutic reagent

A prospective clinical trial, GENPART Study was conducted in order to validate a prediction method for the NVP-induced skin rash using genetic testing of HLA-B*35:05 and an SNP of CCHCR1, rs1576. Advance genetic testing significantly reduced the prevalence of NVP-induced skin rash from 18% in the control group to 13% in the intervention group (P = 0.031), indicating the medical usefulness of genetic testing using the HLA-B*35:05 and CCHCR1 rs1576 markers.

Adverse drug reactions (ADRs) are often unpredictable, owing to the fact that responses to drugs vary among different individuals. However, it is believed that applying knowledge of pharmacogenomics (PGx) to clinical treatment can help to improve predictions of efficacy and/or toxicity of drugs, leading to appropriate therapeutic regimens for individual patients and to contribute to improvement of our medical care. In fact, the U.S. Food and Drug Administration (FDA) recommended genotyping of polymorphisms in drug-metabolizing enzymes and HLA prior to drug administration for avoidance of severe ADRs for several drugs, such as irinotecan, atomoxetine, carbamazepine and abacavir. In attempts to identify genomic biomarkers that predict efficacy or risk of ADRs for various drugs, such as neutropenia and leucopenia induced by cancer chemotherapeutic agents and skin rash induced by anti-epileptics, we conduct genome-wide association studies (GWAS) using single-nucleotide polymorphisms (SNPs), which are the most abundant polymorphisms in the human genome. To date, we have identified several "probable valid" genomic biomarkers (HLA-B*35:05/CCHCR1 for nevirapine -induced skin rash, VKORC1/CYP2C9 for warfarin maintenance dosage, HLA-A*31:01 for carbamazepine-induced skin rash, and CYP2D6 for tamoxifen efficacy) that will be useful for PGx-guided drug therapy. In order to establish PGx-based individualization of drug therapy, advantages of the genomic biomarkers should be demonstrated. Thus, we have conducted and are conducting prospective clinical trials that can evaluate the medical utility and cost-effectiveness of genetic testing. If physicians can predict in advance which patients are more susceptible to ADRs, they could use alternative drugs or take particular care during the course of treatment to prevent the skin rash at an early stage, leading to safe and patient-friendly personalized treatment.

Laboratory for International Alliance on Genomic Research

Team Leader: Ming Ta Michael Lee

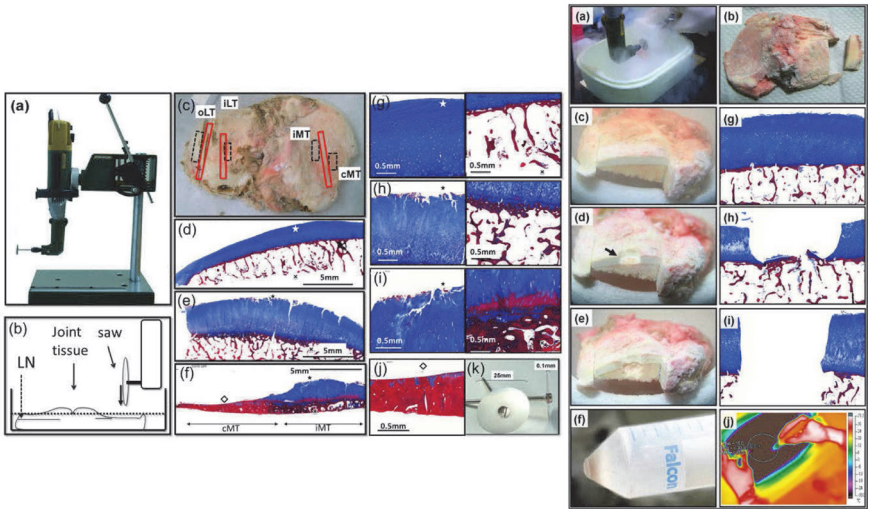


Figure: A New tool for joint tissue sectioning and separation of articular cartilage and subchondral bone

Left panel: Custom tools for joint tissue sectioning that can cut any region of interest and a four-region model system representing disease progression of OA. Right Panel: sectioning and grinding for separating the overlaying cartilage and underlying subchondral bone.

Recent Major Publications

Cornejo-García JA, Liou LB, Blanca-López N, Doña I, Chen CH, Chou YC, Chuang HP, Wu JY, Chen YT, Plaza-Serón Mdel C, Mayorga C, Guéant-Rodríguez RM, Lin SC, Torres MJ, Campo P, Rondón C, Laguna JJ, Fernández J, Guéant JL, Canto G, Blanca M, Lee MT. Genome-wide association study in NSAID-induced acute urticaria/angioedema in Spanish and Han Chinese populations. ***Pharmacogenomics*** 14, 1857-69 (2013)

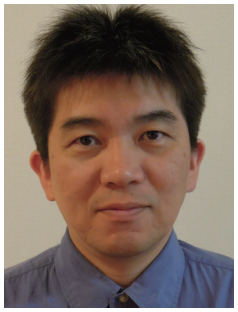
Chou CH, Wu CC, Song IW, Chuang HP, Lu LS, Chang JH, Kuo SY, Lee CH, Wu JY, Chen YT, Kraus VB, Lee MT. Genome-wide Expression Profiles of Subchondral Bone in Osteoarthritis. ***Arthritis Res Ther*** 15, R190 (2013)

Chen CH, Lee CS, Lee MT, Ouyang WC, Chen CC, Chong MY, Wu JY, Tan HK, Lee YC, Chuo LJ, Chiu NY, Tsang HY, Chang TJ, Lung FW, Chiu CH, Chang CH, Chen YS, Hou YM, Chen CC, Lai TJ, Tung CL, Chen CY, Lane HY, Su TP, Feng J, Lin JJ, Chang CJ, Teng PR, Liu CY, Chen CK, Liu IC, Chen JJ, Lu T, Fan CC, Wu CK, Li CF, Wang KH, Wu LS, Peng HL, Chang CP, Lu LS, Chen YT, Cheng AT. Variant GADL1 and Response to Lithium Therapy in Bipolar I Disorder. ***N Engl J Med*** 370, 119-28 (2014)

Invited Presentations

Lee MT. Applications of Pharmacogenetics. The 2nd Meeting of South East Asian Pharmacogenomics Research Network (SEAPharm), Bangkok, Thailand. January 15th, 2013.

The main aim of our laboratory is to identify genetic associations with diseases; drug induced adverse events or drug efficacy. It is hoped that the discoveries from our research will identify useful biomarkers that could be used to predict drug-induced adverse events, guide drug use and could also be used in disease prediction/diagnosis. Another main focus of this laboratory is to promote collaborations both within Japan and internationally. We have set up collaborations/consortium in Asia (Southeast Asian Pharmacogenetics Consortium, SEAPharm) and Europe (Genomic Medicine Alliance) to foster collaboration with RIKEN and among the participating research groups. We also actively recruit young scientists from overseas to work at RIKEN and carry out research in SNP-based approaches, statistical analysis and biological analysis. Our goal is to establish an international genetic research network that will make the Center for Integrative Medical Sciences a world leader in personalized medicine. Currently, our group's main focus is on Pharmacogenetics (PGx) and the genetic study of complex diseases. For PGx studies, we aim to establish a functional analysis platform that not only allows us to study the interactions between HLA and drugs, but can also be used to confirm the findings from genetic analysis for severe ADRs, many of which involve the HLA molecules. In addition, we will use genome-wide association study to identify genetic variants associated with 1. Anti-infectious drug-induced liver injuries. 2. Phenytoin and co-trimoxazole induced Stevens-Johnson Syndrome (SJS) and Toxic epidermal necrolysis. 3. Adverse reactions to non-steroidal anti-inflammatory drugs. For complex diseases, we aim to identify genetic variants associated with Hippocampal Sclerosis in Thais and are performing an epigenetic and biomarker study of osteoarthritis (OA).



Laboratory for Cardiovascular Diseases

Group Director: Toshihiro Tanaka

Recent Major Publications

Onouchi Y, Ozaki K, Burns JC, Shimizu C, Terai M, Hamada H, Honda T, Suzuki H, Suenaga T, Takeuchi T, Yoshikawa N, Suzuki Y, Yasukawa K, Ebata R, Higashi K, Saji T, Kemmotsu Y, Takatsuki S, Ouchi K, Kishi F, Yoshikawa T, Nagai T, Hamamoto K, Sato Y, Honda A, Kobayashi H, Sato J, Shibuta S, Miyawaki M, Oishi K, Yamaga H, Aoyagi N, Iwahashi S, Miyashita R, Murata Y, Sasago K, Takahashi A, Kamatani N, Kubo M, Tsunoda T, Hata A, Nakamura Y, Tanaka T. A genome-wide association study identifies three new risk loci for Kawasaki disease. ***Nat Genet*** 44, 517-21 (2012)

Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, Arking DE, Müller-Nurasyid M, Krijthe BP, Lubitz SA, Bis JC, Chung MK, Dörr M, Ozaki K, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagoner DR, Magnani JW, Wakili R, Clauss S, Rotter JI, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Völker U, Völzke H, Milan DJ, Hofman A, Boerwinkle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N, Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann HE, Witteman JC, Kao WH, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjögren M, Newman AB, Liu Y, Gollob MH, Melander O, Tanaka T, Stricker BH, Felix SB, Alonso A, Darbar D, Barnard J, Chasman DI, Heckbert SR, Benjamin EJ, Gudnason V, Kääb S. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. ***Nat Genet*** 44, 670-5 (2012)

Invited Presentations

Ozaki K, Tanaka T. Genetic background of cardiovascular diseases. Taiwan Human Genetics Society 2013 Spring Symposium: Taiwan-Japan Joint Symposium on BioBank and Genomic Medicine, Taipei, Taiwan. May 25th, 2013.

Tanaka T. Genetic Epidemiology ~understanding human diversity~. Seminar at Health Sciences University of Mongolia, Ulaanbaatar, Mongolia. September 2nd, 2013.

Ozaki K, Tanaka T. Genetic background of myocardial infarction. International Conference on Personalized Medicine and Global Health 2013, Astana, Kazakhstan. October 17th, 2013.

Onouchi Y. Genetic Factors of Kawasaki Disease. The 2nd Oriental Congress of Pediatrics, Shanghai, China. October 19th, 2013.

Onouchi Y. Genetic studies of multifactorial disease. 58th Annual Meeting of the Japan Society for Premature and Newborn Medicine, Kanazawa, Japan. December 1st, 2013.

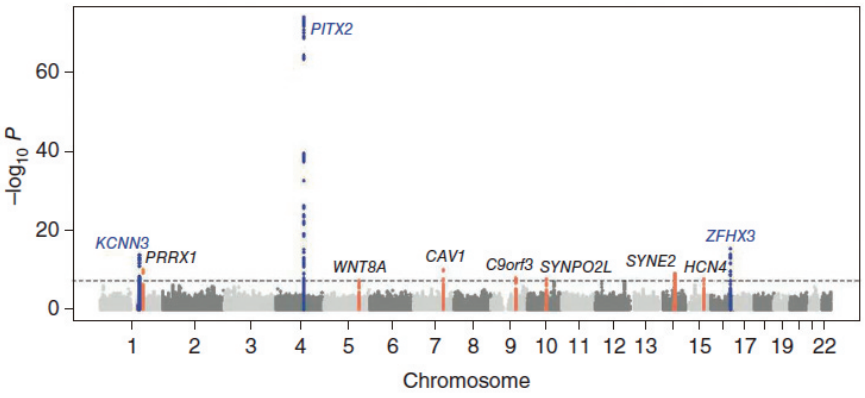


Figure: Manhattan plot of meta-analysis results for genome-wide association with atrial fibrillation

Since heart diseases represent more than 15% of the cause of death in the Japanese population and more than 20% of the total medical expenses in Japan, it is socially important to discover the mechanisms of these disorders. We have been the first to reveal genetic background effects in myocardial infarction (MI), atrial fibrillation (AF), and Kawasaki disease (KD) by comprehensive genetic analyses of the Japanese population, followed by functional *in vitro* analyses. We also began studies to identify genetic factors for arteriosclerosis obliterans, or peripheral artery disease (ASO). At present, our laboratory focuses on these four cardiovascular diseases. Our ultimate goal is to provide novel diagnostic/therapeutic approaches to such patients. To this end, we are extending our research area from genetics to molecular biology, including *in vivo* analyses of genetically engineered mice. For MI, we are searching for novel MI susceptibility genes, followed by functional analyses of these genes *in vitro* and *in vivo*, including knock-out or transgenic mouse techniques. We have just started to develop a screening system to search for novel drugs that might prevent coronary restenosis after percutaneous coronary intervention by inhibiting cellular BRAP- I kappa B interactions. For AF, we are performing functional analyses of AF susceptible genes *in vitro* and *in vivo*. For ASO, we have identified two novel loci associated with the disease and are currently performing fine mapping of these loci and functional assessment of the associated SNPs. For KD, we are trying to identify genetic risk factors of resistance to IVIG therapy and developing coronary artery lesions.

Laboratory for Autoimmune Diseases

Team Leader: Kazuhiko Yamamoto

Recent Major Publications

Okada Y, Shimane K, Kochi Y, Tahira T, Suzuki A, Higasa K, Takahashi A, Horita T, Atsumi T, Ishii T, Okamoto A, Fujio K, Hirakata M, Amano H, Kondo Y, Ito S, Takada K, Mimori A, Saito K, Kamachi M, Kawaguchi Y, Ikari K, Mohammed OW, Matsuda K, Terao C, Ohmura K, Myouzen K, Hosono N, Tsunoda T, Nishimoto N, Mimori T, Matsuda F, Tanaka Y, Sumida T, Yamanaka H, Takasaki Y, Koike T, Horiuchi T, Hayashi K, Kubo M, Kamatani N, Yamada R, Nakamura Y, Yamamoto K. A genome-wide association study identified AFF1 as a susceptibility locus for systemic lupus eyrthematosus in Japanese. ***PLoS Genet*** 8, e1002455 (2012)

Myouzen K, Kochi Y, Okada Y, Terao C, Suzuki A, Ikari K, Tsunoda T, Takahashi A, Kubo M, Taniguchi A, Matsuda F, Ohmura K, Momohara S, Mimori T, Yamanaka H, Kamatani N, Yamada R, Nakamura Y, Yamamoto K. Functional variants in NFKBIE and RTKN2 involved in activation of the NF-κB pathway are associated with rheumatoid arthritis in Japanese. ***PLoS Genet*** 8, e1002949 (2012)

Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Yoshida S, Graham RR, Manoharan A, Ortmann W, Bhangale T, Denny JC, Carroll RJ, Eyler AE, Greenberg JD, Kremer JM, Pappas DA, Jiang L, Yin J, Ye L, Su DF, Yang J, Xie G, Keystone E, Westra HJ, Esko T, Metspalu A, Zhou X, Gupta N, Mirel D, Stahl EA, Diogo D, Cui J, Liao K, Guo MH, Myouzen K, Kawaguchi T, Coenen MJ, van Riel PL, van de Laar MA, Guchelaar HJ, Huizinga TW, Dieudé P, Mariette X, Louis Bridges Jr S, Zhernakova A, Toes RE, Tak PP, Miceli-Richard C, Bang SY, Lee HS, Martin J, Gonzalez-Gay MA, Rodriguez-Rodriguez L, Rantapää-Dahlqvist S, Arlestig L, Choi HK, Kamatani Y, Galan P, Lathrop M, Eyre S, Bowes J, Barton A, de Vries N, Moreland LW, Criswell LA, Karlson EW, Taniguchi A, Yamada R, Kubo M, Liu JS, Bae SC, Worthington J, Padyukov L, Klareskog L, Gregersen PK, Raychaudhuri S, Stranger BE, De Jager PL, Franke L, Visscher PM, Brown MA, Yamanaka H, Mimori T, Takahashi A, Xu H, Behrens TW, Siminovitch KA, Momohara S, Matsuda F, Yamamoto K, Plenge RM. Genetics of rheumatoid arthritis contributes to biology and drug discovery. ***Nature*** 506, 376-81 (2014)

Invited Presentations

Yamamoto K. From Genetics to Functional Insights into the Pathogenesis of Rheumatoid Arthritis. The Advanced in Targeted Therapies Meeting, Nice, France. April 12th, 2013.

Yamamoto K. Genetic information and functional understanding in Rheumatoid Arthritis. Symposium of the Asia Pacific League of Associations for Rheumatology, Bali, Indonesia. August 31st, 2013.

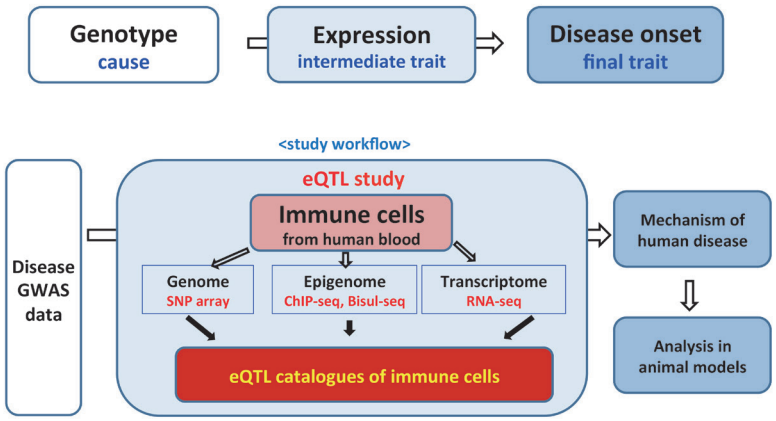


Figure: Understanding autoimmune diseases through eQTL studies
We focus on the expression of genes as an intermediate trait to understand the mechanism of autoimmune diseases. We perform eQTL studies for each immune cell type by using next generation sequencing technologies such as RNA-seq. By combining the data from GWAS and eQTL studies, we will unravel the mechanism of disease.

Most autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus, are multifactorial diseases involving both genetic and environmental factors. The aim of our laboratory is to elucidate the etiology of these autoimmune diseases by dissecting their genetic aspect. RA is one of the most common autoimmune diseases with inflammatory arthritis. While the *HLA-DRB1* gene polymorphism is the major determinant of RA susceptibility, several groups worldwide including our own have performed genome-wide association studies (GWAS) to find non-HLA risk loci. However, each individual GWAS lacked statistical power and a substantial proportion of risk loci remained undiscovered. In world-wide collaborations, we performed meta-analyses of GWAS and found more than 100 risk loci for RA so far.

As GWAS could only indicate the presence of disease-associated variants in the loci, we further investigated these candidate loci to seek disease-causal variants and elucidate their biological relevance to RA. Previous expression quantitative trait loci (eQTL) studies that examined association between genetic variants and gene expression levels have suggested that the majority of autoimmune loci are eQTLs, where disease causing variants affect expression of the responsible genes. Therefore, we think it is essential to focus on "gene expression" as an intermediate trait to dissect the etiology of "disease onset" (Fig.). We are currently undertaking genome and transcriptome analysis of each immune cell type from Japanese individuals to establish eQTL catalogues of human immune cells. Furthermore, as the precise function of the responsible genes *in vivo* has been not yet clarified for most of the candidate loci, we are investigating the function of these genes including *PADI4*, *CD244* and *FCRL3* by using disease-model mice. Our goal is to unravel the pathological mechanisms of disease as well as to facilitate the development of new therapies.

Laboratory for Digestive Diseases

Team Leader: Kazuaki Chayama

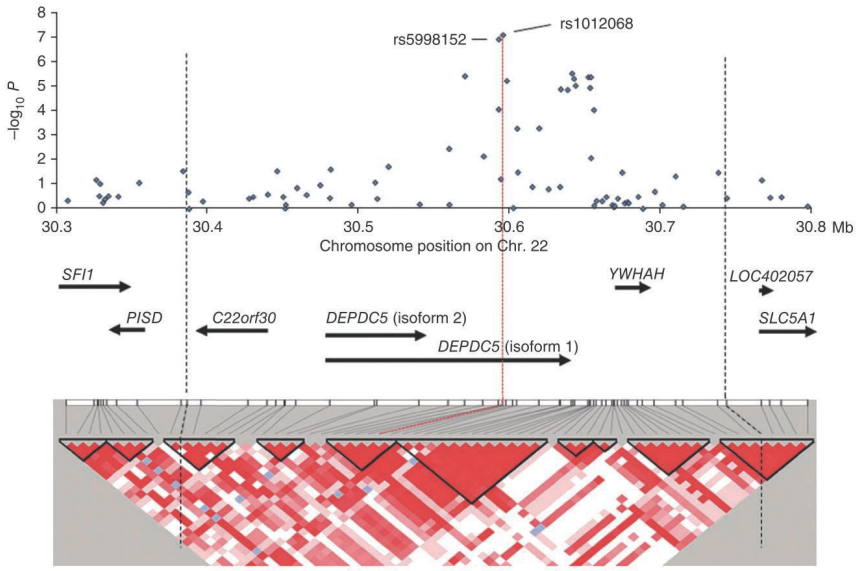


Figure: Case-control association plots and linkage disequilibrium (LD) map and genomic structure of the *DEPDC5* region on chromosome 22q12.2-3
Blue diamonds represent -log10 P obtained from the GWAS and fine mapping (upper panel). The LD map based on D' was drawn using the genotype of the cases and controls in the GWAS samples (lower panel). The candidate region is indicated by two black dashed lines. The landmark SNP (rs1012068) is indicated by the red dotted line.

The objectives of our laboratory are: 1. Identification of genes related to the effects and side effects of antiviral agents, 2. Identification of genes related to HCV-related hepatocellular carcinoma, and 3. Identification of genes related to chronic HCV infection.

Hepatitis C virus (HCV) infection is one of the major causes of chronic liver diseases, which may lead to liver cirrhosis and hepatocellular carcinoma (HCC). Type-I interferons have been used as antiviral agents for HCV infection. Recent GWAS, including ours, have shown that variants in the inosine triphosphatase (*ITPA*) gene are associated with ribavirin induced anemia during pegylated interferon plus ribavirin combined therapy for HCV infection, which may lead to dose reduction or therapy discontinuation. On the other hand *IL28B* variants have been found to be associated with treatment outcome of the therapy.

We assessed efficacy and predictive factors for sustained virological response (SVR) for teraplevir-based triple therapy and published a prediction model for the outcome of pegylated interferon plus ribavirin combination therapy (Chayama, J Infect Dis 2011; Ochi, J Infec Dis 2012).

To identify risk factors for HCV related HCC, we conducted a genome-wide study and identified one intronic SNP in the *DEPDC5* locus associated with HCC risk (rs1012068, combined P=1.27 $\times 10^{-13}$, odds ratio: 1.75) (Miki, Nat Genet 2011).

To identify genetic risk factors for chronic HCV infection, we performed GWAS in Japanese chronic HCV patients and controls. We found one intronic SNP in the *HLA-DQ* locus associated with chronic HCV infection (combined P = 3.59 $\times$ 10^{-16}, odds ratio: 0.79). Subsequent analysis revealed another SNP rs1130380 with a stronger association (odds ratio: 0.72). This SNP causes an amino acid substitution in the HLA-DQB1 protein specific to the DQB1*03 allele, suggesting that a common amino acid substitution in HLA-DQB1 affects susceptibility to chronic HCV infection in the Japanese population (Miki, PLOS ONE 2013 in press).

Recent Major Publications

Okazaki A, Hiraga N, Imamura M, Hayes CN, Tsuge M, Takahashi S, Aikata H, Abe H, Miki D, Ochi H, Tateno C, Yoshizato K, Ohdan H, Chayama K. Severe necroinflammatory reaction caused by natural killer cell-mediated Fas/Fas ligand interaction and dendritic cells in human hepatocyte chimeric mouse. ***Hepatology*** 56, 555-66 (2012)

Shi N, Hiraga N, Imamura M, Hayes CN, Zhang Y, Kosaka K, Okazaki A, Murakami E, Tsuge M, Abe H, Aikata H, Takahashi S, Ochi H, Tateno-Mukaidani C, Yoshizato K, Matsui H, Kanai A, Inaba T, McPhee F, Gao M, Chayama K. Combination therapies with NS5A, NS3 and NS5B inhibitors on different genotypes of hepatitis C virus in human hepatocyte chimeric mice. ***Gut*** 62, 1055-61 (2013)

Miki D, Ochi H, Takahashi A, Hayes CN, Urabe Y, Abe H, Kawaoka T, Tsuge M, Hiraga N, Imamura M, Kawakami Y, Aikata H, Takahashi S, Akuta N, Suzuki F, Ikeda K, Kumada H, Karino Y, Toyota J, Tsunoda T, Kubo M, Kamatani N, Nakamura Y, Chayama K. HLA-DQB1*03 Confers Susceptibility to Chronic Hepatitis C in Japanese: A Genome-Wide Association Study. ***PLoS One*** 8, e84226 (2013)

Invited Presentations

Chayama K. Treatment of Chronic Hepatitis C with First Generation Protease Inhibitors in Asian Population: Telaprevir-Based Triple Therapy in Japanese Population. Taiwan Digestive Disease Week, Taipei, Taiwan. October 5th, 2013.

Chayama K. HCV Genotype, Variations Associated with IFN and DAA Resistance. Taiwan Association for the Study of the Liver (TASL) Conference, Taipei, Taiwan. December 6th, 2013.

Laboratory for
Bone and Joint Diseases

Team Leader: Shiro Ikegawa

Recent Major Publications

Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, Qiu X, Sharma S, Takimoto A, Ogura Y, Jiang H, Yan H, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Hosono N, Tsuji T, Suzuki T, Sudo H, Kotani T, Yonezawa I, Londono D, Gordon D, Herring JA, Watanabe K, Chiba K, Kamatani N, Jiang Q, Hiraki Y, Kubo M, Toyama Y, Tsunoda T, Wise CA, Qiu Y, Shukunami C, Matsumoto M, Ikegawa S. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. ***Nat Genet*** 45, 676-9 (2013)

Nakajima M, Mizumoto S, Miyake N, Kogawa R, Iida A, Ito H, Kitoh H, Hirayama A, Mitsubuchi H, Miyazaki O, Kosaki R, Horikawa R, Lai A, Mendoza-Londono R, Dupuis L, Chitayat D, Howard A, Leal GF, Cavalcanti D, Tsurusaki Y, Saitsu H, Watanabe S, Lausch E, Unger S, Bonafé L, Ohashi H, Superti-Furga A, Matsumoto N, Sugahara K, Nishimura G, Ikegawa S. Mutations in B3GALT6, which encodes a glycosaminoglycan linker region enzyme, cause a spectrum of skeletal and connective tissue disorders. ***Am J Hum Genet*** 92, 927-34 (2013)

Song YQ, Karasugi T, Cheung KM, Chiba K, Ho DW, Miyake A, Kao PY, Sze KL, Yee A, Takahashi A, Kawaguchi Y, Mikami Y, Matsumoto M, Togawa D, Kanayama M, Shi D, Dai J, Jiang Q, Wu C, Tian W, Wang N, Leong JC, Luk KK, Yip SP, Cherny SS, Wang J, Mundlos S, Kelempisioti A, Eskola PJ, Männikkö M, Mäkelä P, Karppinen J, Järvelin MR, O'Reilly PF, Kubo M, Kimura T, Kubo T, Toyama Y, Mizuta H, Cheah KS, Tsunoda T, Sham PC, Ikegawa S, Chan D. Lumbar disc degeneration is linked to a carbohydrate sulfotransferase 3 variant. ***J Clin Invest*** 123: 4909-17 (2013)

Invited Presentations

Ikegawa S. Genetic study of lumbar disc herniation A Japanese study. 1st Meeting of the International Spine and Pain Consortium (ISPC), Hong Kong, China. June 28th, 2013.

Ikegawa S. Genetics of bone and joint disease: From genome to personalized medicine. ASBMR 2013 Annual Meeting, Baltimore, USA. October 4th, 2013.

Ikegawa S. Genetic risk factors for common skeletal disorders. Croucher foundation: advanced study institutes symposium, Hong Kong, China. December 18th, 2013.

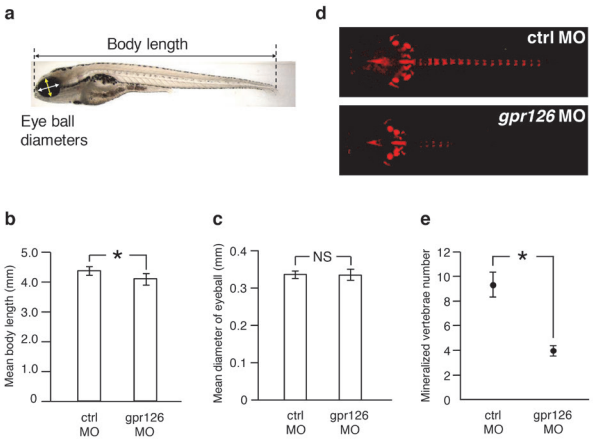


Figure: Phenotypes of a zebrafish morphant (MO) of *gpr126*

(a) A lateral view of zebrafish with measurements of body length and eyeball diameter. The eyeball diameter is an average of the maximum (white arrow) and minimum (yellow arrow) diameters. The body length (b) and eyeball diameter (c) of *gpr126* and control MOs at 14 dpf. Alizarin red staining for the skeleton (d) and the number of mineralized vertebrae (e) at 14 dpf. Vertebral development is significantly delayed in *gpr126* MO. *P< 0.01; NS: not significant.

We are now mainly working on the identification of new susceptibility genes for adolescent idiopathic scoliosis (AIS). AIS is a complex three-dimensional spinal deformity that occurs during the pubertal growth spurt. AIS is the most common pediatric skeletal disease, affecting > 2% of school-age children. The etiology of AIS remains largely unknown; however, many clinical and genetic studies suggest a contribution of genetic factors in the development of AIS.

We conducted a GWAS of AIS in Japanese and identified a single locus on chromosome 10q24.31 that surpassed a genome-wide significance level of $P < 5 \times 10^{-8}$ (Takahashi *et al.* Nat Genet 2011). The association of the locus was subsequently replicated in two independent Chinese populations and our own international meta-analysis (Sharma *et al.* manuscript under review). The locus has provided the most compelling evidence of association with AIS to date.

To identify additional AIS susceptibility loci, we extended our GWAS. Through a step-wise association study which included a total of ~1,800 cases and ~26,000 controls, we identified a new AIS locus on chromosome 6q24.1 in Japanese ($P = 2.25 \times 10^{-10}$) (Kou *et al.* Nat Genet 2013). The most significantly associated SNP was in *GPR126* (G protein-coupled receptor 126) and its association was replicated in Han Chinese and Caucasian populations (combined $P = 1.23 \times 10^{-14}$). *GPR126* is highly expressed in cartilage and its knockdown in zebrafish caused delayed ossification of the developing spine (Fig.). Our results should provide further insights into AIS etiology and pathogenesis.

Laboratory for Endocrinology, Metabolism and Kidney Diseases

Team Leader: Shiro Maeda

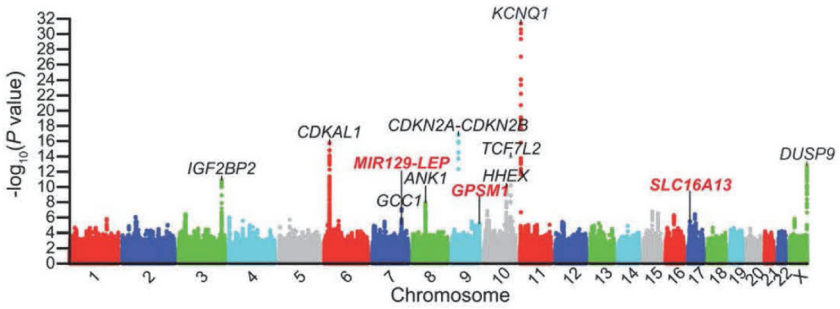


Figure: Manhattan plot for the discovery analysis of directly genotyped and imputed SNPs in 5,976 T2D cases and 20,829 controls
Known loci that reached genome-wide significance ($P < 5 \times 10\text{-}8$) in the discovery analysis (5,976 T2D cases and 20,829 controls) are indicated in black font and the three loci that reached genome-wide significance in the combined analysis of discovery and follow-up analyses (19,094 cases and 31,417 controls) are indicated in red.

Research activities for type 2 diabetes (T2D):

We have performed extended analyses of our previous GWAS (Nat Genet, 2010) by increasing the number of examined SNPs to 2,229,890 SNPs with genotype imputation, and identified the *ANK1* locus as a new locus for T2D (Imamura *et al.* Hum Mol Genet, 2012). In an effort of the Asian Genetic Epidemiology Network (AGEN), 8 new loci were identified by a meta-analysis of East Asian GWAS (Cho *et al.* Nat Genet, 2012). We further performed a larger scale Japanese GWAS for T2D using 6,209,637 SNPs in 26,805 Japanese individuals (5,976 T2D and 20,829 controls). Combined discovery and follow-up analyses (30,392 cases and 34,814 controls) identified three new loci, *MIR129-LEP, GPSM1* and *SLC16A13* (Fig.).

To assess the clinical utility of GWAS-derived T2D susceptibility variants in the Japanese population, we constructed a genetic risk scores (GRS) by summing the susceptibility alleles of 49 SNP loci for T2D and examined the association of the GRS with the disease by receiver operating characteristic (ROC) analyses using a logistic regression model. The area under the curve (AUC) for GRS alone (model-1) and for age, sex, and BMI (model-2) was 0.624 and 0.743, respectively. Addition of the GRS to model-2 resulted in a small but significant increase in the AUC (ΔAUC = 0.03, $p = 7.99 \times 10^{-15}$). These results indicate that currently available genetic information slightly improves disease prediction ability, but is not sufficiently robust for translation into clinical practice (Imamura *et al.* J Clin Endocrinol Metab, 2013).

Other research activities:

We have also participated in the identification of susceptibility loci for diabetic nephropathy, a biomarker for visceral obesity, and causal mutations for autosomal dominant polycystic kidney disease in Japanese populations.

Recent Major Publications

Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, Takeuchi F, Wu Y, Go MJ, Yamauchi T, Chang YC, Kwak SH, Ma RC, Yamamoto K, Adair LS, Aung T, Cai Q, Chang LC, Chen YT, Gao Y, Hu FB, Kim HL, Kim S, Kim YJ, Lee JJ, Lee NR, Li Y, Liu JJ, Lu W, Nakamura J, Nakashima E, Ng DP, Tay WT, Tsai FJ, Wong TY, Yokota M, Zheng W, Zhang R, Wang C, So WY, Ohnaka K, Ikegami H, Hara K, Cho YM, Cho NH, Chang TJ, Bao Y, Hedman ÅK, Morris AP, McCarthy MI, DIAGRAM Consortium, MuTHER Consortium, Takayanagi R, Park KS, Jia W, Chuang LM, Chan JC, Maeda S, Kadowaki T, Lee JY, Wu JY, Teo YY, Tai ES, Shu XO, Mohlke KL, Kato N, Han BG, Seielstad M. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in East Asians. ***Nat Genet*** 44, 67-72 (2011)

Imamura M, Maeda S, Yamauchi T, Hara K, Yasuda K, Morizono T, Takahashi A, Horikoshi M, Nakamura M, Fujita H, Tsunoda T, Kubo M, Watada H, Maegawa H, Okada-Iwabu M, Iwabu M, Shojima N, Ohshige T, Omori S, Iwata M, Hirose H, Kaku K, Ito C, Tanaka Y, Tobe K, Kashiwagi A, Kawamori R, Kasuga M, Kamatani N, Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium, Nakamura Y, Kadowaki T. A single-nucleotide polymorphism in *ANK1* is associated with susceptibility to type 2 diabetes in Japanese populations. ***Hum Mol Genet*** 21, 3042-9 (2012)

Imamura M, Shigemizu D, Tsunoda T, Iwata M, Maegawa H, Watada H, Hirose H, Tanaka Y, Tobe K, Kaku K, Kashiwagi A, Kawamori R, Maeda S. Assessing the clinical utility of a genetic risk score constructed using 49 susceptibility alleles for type 2 diabetes in a Japanese population. ***J Clin Endocrinol Metab*** 98, E1667-73 (2013)

Invited Presentations

Maeda S, Imamura M. Genetic characteristics of type2 diabetes in the Japanese. 47th Annual Post Graduate Course: Advance in Diabetology, Yokkaichi, Japan. February 16th, 2013.

Maeda S. Genetic Study of type 2 diabetes in Japan. Taiwan Human Genetics Society 2013 Spring Symposium: Taiwan-Japan Joint Symposium on BioBank and Genomic Medicine, Taipei, Taiwan. May 25th, 2013.

Laboratory for Respiratory and Allergic Diseases

Team Leader: Mayumi Tamari

Recent Major Publications

Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, Sakashita M, Yamada T, Fujieda S, Tanaka S, Doi S, Miyatake A, Enomoto T, Nishiyama C, Nakano N, Maeda K, Okumura K, Ogawa H, Ikeda S, Noguchi E, Sakamoto T, Hizawa N, Ebe K, Saeki H, Sasaki T, Ebihara T, Amagai M, Takeuchi S, Furue M, Nakamura Y, Tamari M. Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. ***Nat Genet*** 44, 1222-6 (2012)

Tomita K, Sakashita M, Hirota T, Tanaka S, Masuyama K, Yamada T, Fujieda S, Miyatake A, Hizawa N, Kubo M, Nakamura Y, Tamari M. Variants in the 17q21 asthma susceptibility locus are associated with allergic rhinitis in the Japanese population. ***Allergy*** 68, 92-100 (2013)

Ellinghaus D, Baurecht H, Esparza-Gordillo J, Rodríguez E, Matanovic A, Marenholz I, Hübner N, Schaarschmidt H, Novak N, Michel S, Maintz L, Werfel T, Meyer-Hoffert U, Hotze M, Prokisch H, Heim K, Herder C, Hirota T, Tamari M, Kubo M, Takahashi A, Nakamura Y, Tsoi LC, Stuart P, Elder JT, Sun L, Zuo X, Yang S, Zhang X, Hoffmann P, Nöthen MM, Fölster-Holst R, Winkelmann J, Illig T, Boehm BO, Duerr RH, Büning C, Brand S, Glas J, McAleer MA, Fahy CM, Kabesch M, Brown S, McLean WH, Irvine AD, Schreiber S, Lee YA, Franke A, Weidinger S. High-density genotyping study identifies four new susceptibility loci for atopic dermatitis. ***Nat Genet*** 45, 808-12 (2013)

Invited Presentations

Tamari M. Genome-wide association study of adult asthma. The 53rd Annual Meeting of the Japanese Respiratory Society, Tokyo, Japan. April 20th, 2013.

Tamari M. Genetic Study of Allergic Diseases. Taiwan Human Genetics Society 2013 Spring Symposium: Taiwan-Japan Joint Symposium on BioBank and Genomic Medicine, Taipei, Taiwan. May 25th, 2013.

Tamari M. Genome-wide association study of atopic dermatitis. The 112th Annual Meeting of the Japanese Dermatological Association: Educational Lectures - Atopic dermatitis, Kanagawa, Japan. June 15th, 2013.

Tamari M. Genomics in Allergic Disease. European Academy of Allergy and Clinical Immunology & World Allergy Organization World Allergy and Asthma Congress: Symposium 24 World Allergy Forum - Omics in Allergic Disease, Milan, Italy. June 23th, 2013.

Tamari M. Genome-Wide Association Study of Allergic Diseases. 8th RCAI-JSI International Symposium on Immunology 2013, Yokohama, Japan. June 28th, 2013.

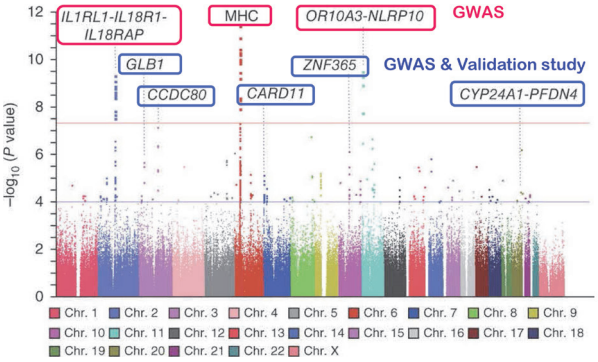
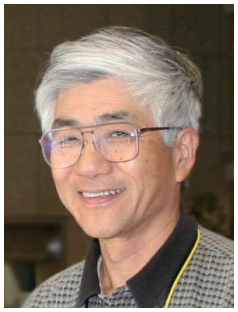


Figure: Manhattan plot of the GWAS for Japanese atopic dermatitis
We conducted a GWAS and a validation study. A total of eight new atopic dermatitis susceptibility loci were identified with genome-wide significance: *IL1RL1-IL18R1-IL18RAP*, the MHC region, *OR10A3-NLRP10, GLB1-CCR4, CCDC80, CARD11, ZNF365-EGR2* and CYP24A1-PFDN4.

The aim of our project is to improve our understanding of the pathophysiology of human respiratory and allergic diseases, which are caused by a combination of genetic and environmental factors. To elucidate the genetic components, we have conducted genome-wide association studies (GWASs) and identified several genetic loci for bronchial asthma and atopic dermatitis (Nat. Genet. 2011 & 2012). A recent immunochip analysis for atopic dermatitis using European populations revealed four new susceptibility loci, 4q27 (*IL2-IL21*), 11p13 (*PRR5L*), 16p13.13 (*CLEC16A-DEXI*) and 17q21.32 (*ZNF652*). We conducted a replication study using a Japanese population and confirmed the associations at the 11p13, 16p13.13 and 17q21.32 loci (Nat. Genet. 2013). Candidate genes identified by the GWASs and immunochip analysis suggest roles for barrier functions, innate-adaptive immunity, IL-1 family signaling, regulatory T cells, and the vitamin D pathway in the pathogenesis of allergic diseases. To investigate whether polymorphisms identified by the genetic studies could affect the susceptibility to and clinical phenotypes of diseases, we will conduct functional analyses and serological studies.

The first GWAS of asthma in the European population identified a locus on chromosome 17q21.1. Subsequent replication studies have identified genetic variants on the 17q21.1 locus associated with asthma in different ethnic populations. We reported that genetic variants in the 17q21.1 locus were associated with allergic rhinitis and were strongly correlated in *cis* with transcript levels of *ORMDL3* in Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines in a Japanese population (Allergy 2013).We participate in the Global Alliance for Pharmacogenomics. We have conducted GWASs and identified several loci that are involved in individual asthma treatment response.

We will conduct further cross-disciplinary studies combining genetics, immunology and clinical epidemiology for translation of research into clinical practice.



Laboratory for Immune Regulation

Group Director: Masaru Taniguchi

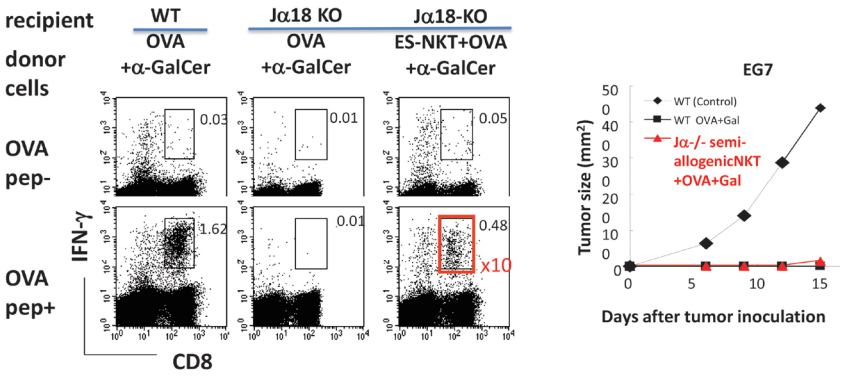


Figure: Anti-tumor adjuvant effects mediated by semi-allogeneic (129xB6) F1 ES-NKT cells
Semi-allogeneic (129xB6) F1 ES-derived NKT cells were transferred into NKT-KO mice followed by stimulation with OVA and α-GalCer/DCs. A significant increase in the number of OVA-CD8T cells and suppression of OVA-bearing tumor growth was observed. Thus, semi-allogeneic NKT cells can function *in vivo* even if they are eliminated in a few days by the host immune response.

Recent Major Publications

Watarai H, Sekine-Kondo E, Shigeura T, Motomura Y, Yasuda T, Satoh R, Yoshida H, Kubo M, Kawamoto H, Koseki H, Taniguchi M. Development and Function of Invariant Natural Killer T cells Producing TH2- and TH17-cytokines. ***PLoS Biol*** 10, e1001255 (2012)

Oh-Hora M, Komatsu N, Pishyareh M, Feske S, Hori S, Taniguchi M, Rao A, Takayanagi H. Agonist-Selected T Cell Development Requires Strong T Cell Receptor Signaling and Store-Operated Calcium Entry. ***Immunity*** 38, 881-95 (2013)

Fujii S, Shimizu K, Okamoto Y, Kunii N, Nakayama T, Motohashi S, Taniguchi M. NKT cells as an ideal anti-tumor immunotherapeutic. ***Front Immunol*** 4, 409 (2013)

Invited Presentations

Taniguchi M. NKT Cell-mediated Adjuvant Activity on Antitumor Responses. Federation of Clinical Immunology Societies (FOCIS2012), Vancouver, Canada. June 22nd, 2012.

Taniguchi M. NKT cells as an ideal target for anti-tumor immunotherapy. 15th International Congress of Immunology, Milan, Italy. August 25th, 2013.

Taniguchi M. Characterization of the cell with NKT cell potential in DN1 thymic fraction. 7th International Symposium on CD1 and NKT cells, Tours, France. September 14th, 2013.

Taniguchi M. Discovery of NKT cells and their clinical application. 12th International Symposium on Sjögren's Syndrome, Kyoto, Japan. October 9th, 2013.

Taniguchi M. RIKEN IMS-RCAI Medical Innovation Programs based on the NKT cell biology. Germany-Japan Immunology Seminar 2013, Shizuoka, Japan. December 7th, 2013.

Identification of NKT cell precursors: Different from the present notion of NKT cell development after CD1d-selection at the DP stage, we identified an NKT precursor in the DN1e stage (DN1eP) in the adult and fetal thymus of $CD1d^{-/-}$ and wild-type mice. The DN1eP have unique gene expression profiles that differ from NKT cells. The DN1eP already express key Th1-like signature genes prior to CD1d selection and preferentially develop into Th1-type NKT cells in the presence of CD1d. The development of DN1eP is dependent on IL-7R, PLZF and SAP, while that of DP precursors is not. Our findings reveal a novel NKT cell developmental pathway that is different from the DP pathway.

Clinical trials of NKT-targeted therapy on advanced lung cancer (stage IIIB, IV and recurrent tumor) after surgery/chemotherapy/radiation therapy, and in head and neck tumors: The NKT-targeted cell therapy was approved by the Japanese government for advanced lung cancer in 2011 and for head and neck tumors in 2013, because the therapy improved their prolonged median survival time (29.6Mo vs 4.6Mo for lung cancer and SD or PD in 100% patients treated). From 2013, we are collaborating with Chiba University Hospital on head and neck tumors and the National Hospital Organization on Stage IIA/IIB lung cancer after surgical tumor resection.

iPS-derived NKT cells: For the future clinical application of iPS-derived NKT cells, we made the following important observations: a) NKT cells did not induce GvHD. b) Semi-allogeneic NKT cells induced a significant increase in the number of CD8 T cells and suppression of tumor growth. Thus, semi-allogeneic NKT cells can function *in vivo* even if they are eliminated in a few days by the host immune system.

Laboratory for Immunotherapy

Team Leader: Shin-ichiro Fujii

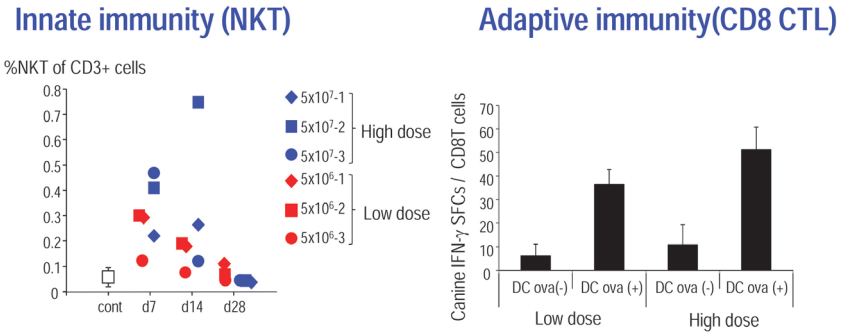


Figure: Immune response in dogs immunized with artificial adjuvant vector cells
Two doses of ova-expressing artificial adjuvant vector cells (low dose; $5x10^6$, high dose; $5x10^7$) were administered to 3 dogs. The number of iNKT cells was evaluated by staining with murine CD1d-dimer/Gal and canine CD3 Ab (left). The function of $CD8^+$ T cells was assessed by IFN-γ ELISPOT after stimulation with autologous Ag-loaded DCs (right).

The aims of the laboratory are to extend our basic studies for advancing immunotherapy and translational research, from basic studies back and forth to the bedside in the field of cancer. For this, we have been conducting three NKT cell-related projects. In this system, the synthetic glycolipid, α-galactosylceramide (α-GalCer), is presented by CD1d molecules to invariant NKT lymphocytes. When NKT cells are activated in this manner, they have unique immunostimulatory features that include the rapid production of IFN-γ and NK cell activation, followed by innate immunity-mediated antitumor effects.

First, we have made attempts to establish a strategy linking innate and adaptive immunity. For this purpose, we have been studying processes of activation of adaptive immunity through full maturation of DCs soon after the activation of NKT cells *in vivo*. In addition, we are investigating adequate delivery systems that have the potential to enhance antitumor immunity. We have developed artificial adjuvant vector cells as new type of drug delivery system composed of NKT ligand and tumor associated antigen, linking innate and adaptive immunity. Since this project was accepted by the RIKEN translational program, we are making efforts to work toward preclinical studies that will ultimately lead to clinical trials (Fig.). As a second project, we have been working in a collaborative study with the RIKEN iPS-group to establish iPS-NKT cells. In this project, we focus on the preparation of primary NKT cells as the starting material for generating NKT cell-derived iPS and on the analysis of the function of iPS-derived NKT cells. Third, in our previous collaboration with Chiba University, we obtained successful immunological and clinical results using α-GalCer-loaded dendritic cell (DC/Gal) therapy in advanced lung cancer patients. To continue and extend this project, after a framework agreement, we have been launching a joint clinical phase I /IIa study of NKT cell therapy with the National Hospital Organization (NHO) for early stage lung cancer patients after surgical tumor resection. In this study, we play an important role in the analyses of immune responses.

Recent Major Publications

Shimizu K, Mizuno T, Shinga J, Asakura M, Kakimi K, Ishii Y, Masuda K, Maeda T, Sugahara H, Sato Y, Matsushita H, Nishida K, Hanada K, Dorrie J, Schaft N, Bickham K, Koike H, Ando T, Nagai R, Fujii S. Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust in situ immune responses by dendritic cells. ***Cancer Res*** 73, 62-73 (2013)

Shimizu K, Asakura M, Shinga J, Sato Y, Kitahara S, Hoshino K, Kaisho T, Schoenberger SP, Ezaki T, Fujii S. Invariant NKT cells induce plasmacytoid DC cross-talk with conventional DCs for efficient memory $CD8^+$ T cell induction. ***J Immunol*** 190, 5609-19 (2013)

Fujii S, Shimizu K, Okamoto Y, Kunii N, Nakayama T, Motohashi S, Taniguchi M. NKT cells as an ideal anti-tumor immunotherapeutic. ***Front Immunol*** 4, 409, 1-7 (2013)

Invited Presentations

Fujii S. iNKT cell-triggered in vivo DC targeting immunotherapy. 2nd New Zealand-Japan Joint Immunology Workshop, Auckland, New Zealand. February 26th, 2013.

Fujii S. Development of artificial adjuvant vector cells targeting dendritic cells in situ. Dokkyo Medical School, Tochigi, Japan. April 8th, 2013.

Fujii S. Development of Artificial Adjuvant Vector Cells linking Innate and Adaptive Immunity. The 2nd SCIENCE in Shinagawa, Tokyo, Japan. April 20th, 2013.

Laboratory for Vaccine Design

Team Leader: Yasuyuki Ishii

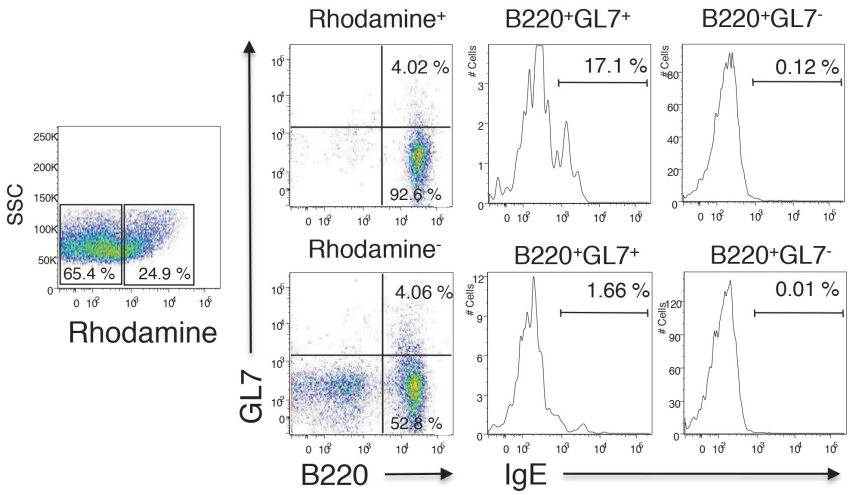


Figure: Analysis of IgE+ B cells in the germinal center
OVA-sensitized C57BL/6 mice were injected intravenously with rhodamine-liposomal α-GalCer. Splenocytes were prepared 1 h after the injection, stained with anti-B220, anti-GL7 and anti-IgE mAbs and analyzed by flow cytometry.

Invariant natural killer T (iNKT) cells perform immunoregulatory roles such as the suppression of IgE responses as well as having adjuvant roles in host defense. Although the mechanism of immune activation by iNKT cells is well understood, that of immune suppression remains unclear. Here, we show that the delivery of α-galactosylceramide (α-GalCer), a representative iNKT cell ligand, to splenic B220-positive cell subsets including IgE-expressing B cells leads to IL-21 expression by iNKT cells and results in the IgE isotype-specific suppression. Fluorescent-labeled liposomal α-GalCer was preferentially incorporated into $B220^{+}CD21^{high}CD23^{low}$ cell subsets expressing a high level of CD1d rather than into CD11b and/or CD11c-positive cells. Splenic iNKT cells migrated and co-localized with the α-GalCer-presenting B cells in a CXCL16-dependent manner. The expression of IL-21 by iNKT cells after the co-localization was remarkably diminished by blockade of the CXCR6/CXCL16 interaction with an anti-CXCL16 neutralizing antibody. Furthermore, the suppression of the IgE responses seen in mice administered the liposomal α-GalCer was restored by treatment with the anti-CXCL16 antibody. Unlike off-target cells of the liposomal α-GalCer, the major target B cells, expressed IgE, IL-21 receptor and Bmf mRNAs, and were highly sensitive to IL-21-induced apoptosis *in vitro*. IgE-expressing cell subsets among the liposomal α-GalCer-targeted B cells included $B220^{+}GL7^{+}$ germinal center B cell subsets (Fig.). These results collectively suggest that *in vivo* IgE isotype-specific suppression by liposomal α-GalCer administration could be caused by IgE B-specific apoptosis induced by IL-21 produced by activated iNKT cells.

Recent Major Publications

Shimizu K, Mizuno T, Shinga J, Asakura M, Kakimi K, Ishii Y, Masuda K, Maeda T, Sugahara H, Sato Y, Matsushita H, Nishida K, Hanada K, Dorrie J, Schaft N, Bickham K, Koike H, Ando T, Nagai R, Fujii S. Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust in situ immune responses by dendritic cells. ***Cancer Res*** *73,* 62-73 (2013)

Hirai T, Ishii Y, Ikemiyagi M, Fukuda E, Omoto K, Namiki M, Taniguchi M, Tanabe K. A Novel Approach Inducing Transplant Tolerance by Activated Invariant Natural Killer T Cells with Co-stimulatory Blockade. ***Am J Transplant*** 14, 554-567 (2014)

Invited Presentations

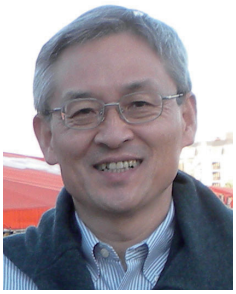
Ishii Y. Research for oral allergy vaccines inducing immune tolerance. 2nd New Zealand-Japan Joint Immunology Workshop, Auckland, New Zealand. February 26th, 2013.

Ishii Y. Development effective vaccine strategies to launch promising new generation vaccine candidates. 7th Annual World Vaccine Congress Asia 2013, Singapore. June 19th, 2013.

Ishii Y. A novel allergy therapy using IgE-suppressive drugs generated in RIKEN. Symposium: Development of the biomedicine using a plant - the present condition and future, Tokyo, Japan. November 8th, 2013.

Ishii Y. New approaches of causal therapies for allergic diseases. 4th Kanagawa Mirai Forum, Yokohama, Japan. November 9th, 2013.

Ishii Y. Clinical applications of transplantation tolerance induced by invariant natural killer T cells. 99th Ochanomizu Cancer Academia Forum, Tokyo, Japan. November 27th, 2013.



Laboratory for Allergic Disease

Team Leader: Toshiaki Kawakami

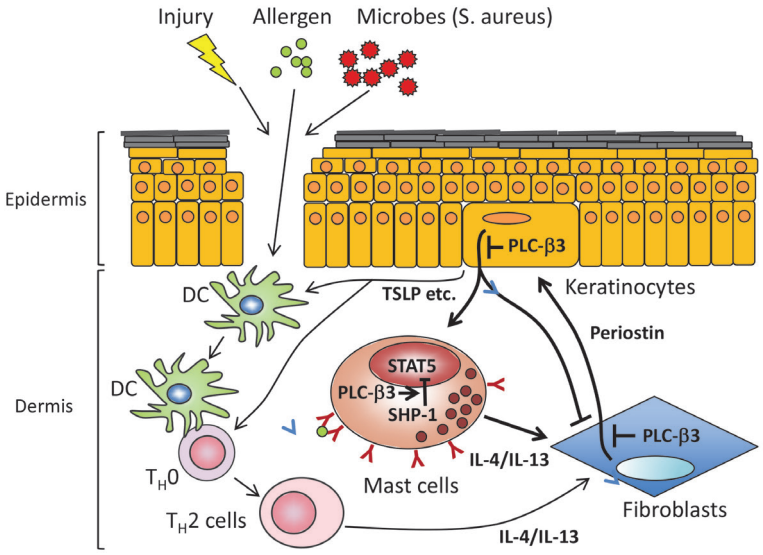


Figure: Hypothetical vicious cycle of allergic skin inflammation
Allergen-specific T_H2 cells stimulate production/secretion of periostin by fibroblasts. Periostin in turn then stimulates keratinocytes to produce and secrete TSLP and other inflammatory cytokines. In this scheme, T_H2 cells seem to be required for initial epidermal overexpression of TSLP. Once sustained overexpression of TSLP is established, mast cells may play a more important role in persistent dermatitis than T_H2 cells. PLC-β3 can regulate activities of the cellular elements of this network, such as proliferation of mast cells, periostin production/secretion by fibroblasts and TSLP production/secretion by keratinocytes. Our data also suggest the presence of a feedback loop for inhibition of fibroblast periostin production by TSLP, and that PLC-β3 in fibroblasts is required for this feedback inhibition. DC, dendritic cell; T_H0, naïve $CD4^+$ T cells.

Recent Major Publications

Ando T, Matsumoto K, Namiranian S, Yamashita H, Glatthorn H, Kimura M, Dolan BR, Lee JJ, Galli SJ, Kawakami Y, Jamora C, Kawakami T. Mast cells are required for full expression of allergen/SEB-induced skin inflammation. ***J Invest Dermatol*** 133, 2695-705 (2013)

Kawakami T, Ando T. Salmonella's masterful skill in mast cell suppression. ***Immunity*** 39, 996-8 (2013)

Ando T, Xiao W, Gao P, Namiranian S, Matsumoto K, Tomimori Y, Hong H, Yamashita H, Kimura M, Kashiwakura JI, Hata TR, Izuhara K, Gurish MF, Roers A, Rafaels NM, Barnes KC, Jamora C, Kawakami Y, Kawakami T. Critical role for mast-cell Stat5 activity in skin inflammation. ***Cell Rep*** 6, 366-76 (2014)

Invited Presentations

Kawakami T. Phospholipase C-beta3 in allergic diseases. 2nd Allergy & Respiratory Drug Discovery Conference, San Diego, California, USA. February 1st, 2013.

Kawakami T. Phospholipase C-beta3 in immune cells. 2013 International Symposium: PI-PLC Activity and Signaling, Ulsan, Korea. July 19th, 2013.

Kawakami T. Hospholipase C-beta3 in atopic dermatitis. 7th International Leukocyte Signal Transduction Conference, Kos, Greece. September 11th, 2013.

Kawakami T. Phospholipase C-β3 as a tumor suppressor and a guardian of the immune system. The 5th LJI & IMS-RCAI Workshop, Yokohama, Japan. October 30th, 2013.

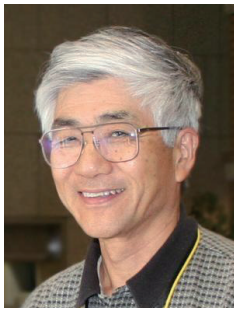
Kawakami T. Phospholipase C in immune cells. NHRI & IBMS Joint Conference: 2013 International Conference of Inflammation, Cancer and Metabolic Disorders, Zhunan, Miaoli, Taiwan. November 4th, 2013.

Role of histamine-releasing factor (HRF) in allergic diseases

HRF is a cytokine-like protein that can stimulate histamine release and cytokine (IL-4 and IL-13) production/secretion from IgE-sensitized basophils and mast cells. HRF-like activities are found in bodily fluids during the late phase of allergic reactions. We recently demonstrated that some, but not all, IgE and IgG molecules interact with HRF with low affinity. By mapping the binding sites on both HRF and IgE/IgG molecules, we developed competitive inhibitors of HRF-IgE (or IgG) interactions. Using these inhibitors, we showed that HRF promotes allergic inflammation in mouse models of anaphylaxis and asthma (Kashiwakura et al., 2012). We are now investigating the role of HRF in food allergy. Using a standard food allergy model, which is mast cell-, IgE-, and FcεRI-dependent, our initial experiments suggest that HRF is involved in the promotion of the allergic reactions (unpublished). We have been measuring HRF and HRF-reactive IgGs in sera of food allergy patients before and after the rapid oral induction of tolerance (ROIT). Another area of research is on HRF-related human mRNA sequences (unpublished). We are now studying whether the HRF-related proteins encode by these transcripts affect HRF functions.

Pathogenic mechanisms of atopic dermatitis (AD)

AD is a chronic pruritic inflammatory skin disease. In the AD project, we have been studying the cellular and molecular mechanisms using our previously established *in vivo* AD induction model and the spontaneously occurring AD-like skin lesions in phospholipase C (PLC)-β3-deficient mice. As we recently showed the importance of PLC-β3-mediated Stat5 regulation in mast cells in causing AD (Ando et al., 2014; Fig.), we are now studying whether Jak family members as upstream regulators are involved in the regulation of Stat5 in mouse and human mast cells.



RIKEN-TORII Joint Research Team

Team Leader: Masaru Taniguchi

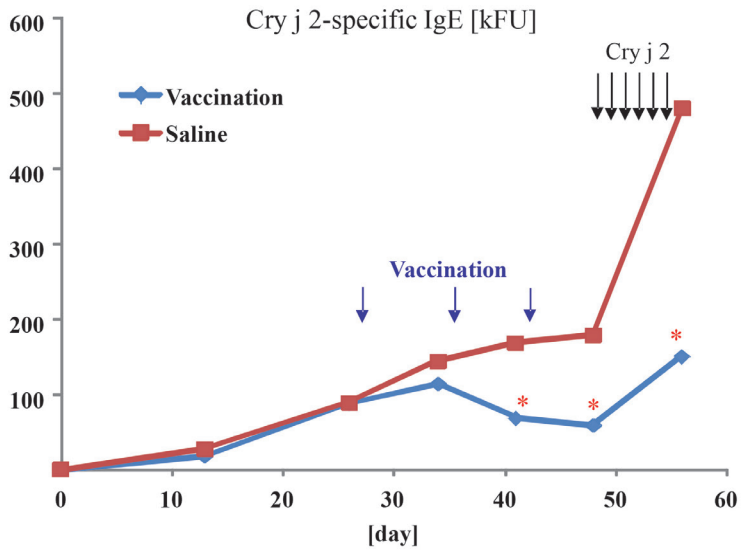


Figure: Subcutaneous vaccination significantly inhibits the increase of Cry j 2-specific IgE after Cry j 2 intranasal sensitization.
The recombinant vaccine was administered subcutaneously into Cry j 2-sensitized mice three times and the mice were again sensitized with Cry j 2 intranasally after the vaccination. The increase of Cry j 2-specific IgE after the last challenge with Cry j 2 was significantly attenuated in the vaccinated mice compared with the control mice with no-vaccination (saline). This figure shows the mean Cry j 2-specific IgE titer in each group. *: p<0.05 by Mann-Whitney U-test.

Recent Major Publications

Fujimura T, Okamoto Y, Taniguchi M. Therapeutic effects and biomarkers in sublingual immunotherapy: a review. ***J Allergy (Cairo)*** 2012, 381737 (2012)

Japanese cedar pollinosis is a common allergy in Japan. Antigen-specific immunotherapy (SIT) is considered to be the only curative treatment for allergy, and only a crude extract from Japanese cedar pollen has been approved for clinical use by the Ministry of Health, Labour and Welfare in Japan. Vaccines using allergoids and modified Cry j 1, a major allergen of Japanese cedar pollen, have been developed and used in pre-clinical trials; however, none of them has been commercially provided for medical use. This situation is due to poor clinical outcomes at later stage clinical trials or the inability to find a cooperative pharmaceutical company that will introduce them onto the market. To fill the critical gap between basic research and the later stage of drug development, IMS-RCAI established a program named 'exchange zone', where RIKEN, universities, hospitals, and pharmaceutical companies work together for drug development, including allergy vaccines especially for Japanese cedar pollinosis. Based on this program, RIKEN and TORII pharmaceutical Co., Ltd. set up a joint research laboratory in RCAI in May 2010 and began to develop the SIT vaccine.

For the vaccine, recombinant technology is used to conjugate two major allergens from Japanese cedar pollen, namely Cry j 1 and Cry j 2, and then the protein is further modified with polyethylene glycol to prevent binding with immunoglobulin E (IgE) and to improve its solubility.

Systemic injections of the vaccine into Cry j 1 or Cry j 2-sensitized mice prevented the increase of serum antigen-specific IgE after systematic or local sensitization with native Cry j 1 or Cry j 2 in a dose dependent manner. To improve the therapeutic effects of the vaccine, elucidation of the therapeutic mechanisms and identification of biomarkers are important issues. Therefore, we are looking for biomarkers to monitor therapeutic responses during or after the vaccination.

Drug Discovery Antibody Platform Unit

Unit Leader: Toshitada Takemori

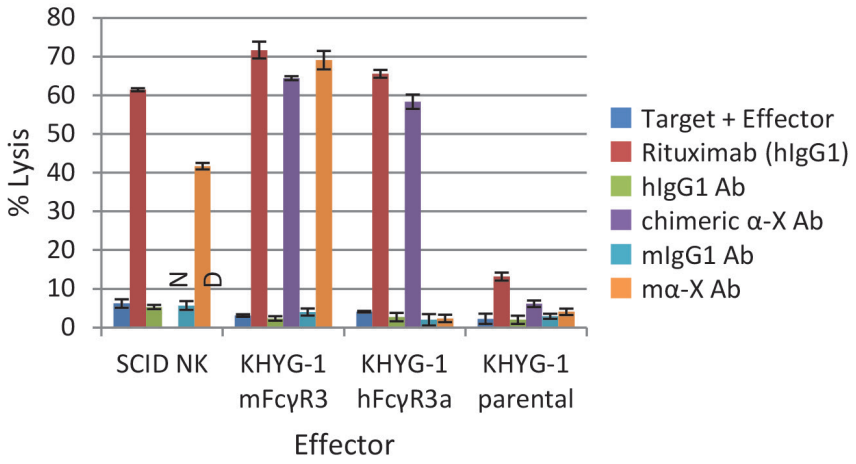


Figure: ADCC activity estimated using the KHYG NK cell line
ADCC activity by Rituximab (as a positive control) and mouse and chimeric anti-X mAbs against cancer cells measured by utilizing NK cells from spleens of SCID mice and NK cell lines as effectors.

The use of monoclonal antibodies (mAbs) for cancer therapy has achieved significant success in recent years. Our laboratory, linked to the RIKEN Program for Drug Discovery and Medical Technology Platforms (DMP), is preparing new mAbs for cancer and other diseases by collaborations within and outside of RIKEN. Along with mAb production, we clarify the activity of mAbs obtained in our laboratory in terms of whether they could be applicable in medical therapies by using several model systems, prior to further analysis on the clinical side.

Tumor cell killing is mediated through direct action of the antibody (through receptor blockade or agonist activity), complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC). The antibody Fc is essential for mediating tumor cell killing through CDC and ADCC, but the antibody V-region is also important for the killing activity with respect to the recognition sites and affinity for the target molecules.

In 2013, in order to isolate a large number of candidate mAb with ADCC activity from a hybridoma screening, we prepared a human NK cell line that expresses a mouse or human FCγIII receptor as the effector cells for the assay (Fig.). This strategy is very efficient as it enables us to measure ADCC activity by a minute amount of Abs in the culture supernatant at any time point without a complex process. Accordingly we are able to preferentially select ADCC-positive clones during the initial hybridoma culture, prior to the antibody purification. Furthermore, we established a mouse model to evaluate the anti-cancer activity of mAbs selected by *in vitro* analysis.

The successful development of candidate antibodies for the clinic involves a complex process of scientific and preclinical evaluations, with a convergence of cancer biology and immunology. We hope to develop antibodies for the clinic, particularly leukemic cancers, in collaboration with clinical researchers.

Recent Major Publications

Ato M, Takahashi Y, Fujii H, Hashimoto S, Kaji T, Itamura S, Horiuchi Y, Arakawa Y, Tashiro M, Takemori T. Influenza A whole virion vaccine induces a rapid reduction of peripheral blood leukocytes via interferon-α-dependent apoptosis. ***Vaccine*** 31, 2184-90 (2013)

Kometani K, Nakagawa R, Shinnakasu R, Kaji T, Rybouchkin A, Moriyama S, Furukawa K, Koseki H, Takemori T, Kurosaki T. Repression of the transcription factor Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation. ***Immunity*** 39, 136-47 (2013)

Kaji T, Furukawa K, Ishige A, Toyokura I, Nomura M, Okada M, Takahashi Y, Shimoda M, Takemori T. Both mutated and unmutated memory B cells accumulate mutations in the course of the secondary response and develop a new antibody repertoire optimally adapted to the secondary stimulus. ***Int Immunol*** 25, 683-95 (2013)

Invited Presentations

Takemori T. CD4 memory T cell development is licensed by Bcl6 and accomplished by expression of a group of genes mediated by cognate B cell interactions. The 36th Annual Meeting of the Molecular Biology Society of Japan, Kobe, Japan. December 5th, 2013.

Central Facilities

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

FACS Laboratory

The FACS Lab provides a range of support for flow cytometry and cell sorting, procedures that are essential for not only immunological experiments but also a wide variety of biological experiments. The FACS Lab has added new FACS Aria and reduced the number of FACS Vantages. In addition to FACS, the lab installed ImageStreamX, a device that combines flow cytometry with the visual detail of microscopy in a single platform and CyTOF, a mass-spectometry-based cytometer that has potential for analyzing more than 30 markers simultaneously with metal-labelled antibodies.

For the users of the FACS machines (cell analyzers and cell sorters), Tomomi Aoyama and Noriko Yoza cover various services: (1) *Technical support and training*: In 2013, the facility offered 16 technical courses (8 for cell sorting and 8 for cell analysis). Courses were held at 3 different levels, Calibur basic, Canto II and Aria basic. A total of 66 researchers took the courses in 2013. (2) *Cell sorting operation service*: The FACS Lab provides a cell sorting operation service, in which researchers can ask an experienced operator to conduct the sorting experiment. In 2013, 293 such operation services were provided by the lab. (3) *Management/ maintenance of FACS machines*: FACS machines are available for registered users 24 hours a day and reservations are accepted up to one month in advance through an internal website. In addition to the in-house FACS Lab staff, engineers from Becton Dickinson visit once a week to provide maintenance and technical support.

Table: Instruments in the FACS Lab.

Machine types	Machines	# of machines
FACS cell analyzer	Calibur Cantoll	4 2
FACS cell sorter	Aria I/II Aria III Vantage (Diva)	3 2 1
Mass-cytometer	CyTOF	1
Imaging flow cytometer	ImageStreamX	1

Confocal Laboratory

The Confocal Laboratory provides equipment for cell and tissue imaging, and coordinates technical support by Leica Microsystems. There are seven laser-scanning fluorescence microscopes available to IMS researchers.

1. Inverted SP2 system with visible lasers for single-photon excitation and a femtosecond Ti:Sa laser for two-photon excitation.
2. Inverted SP2 system with visible lasers for single-photon excitation including a 405 violet laser. This microscope is equipped with a chamber system that controls CO2 concentration, temperature and humidity for live cell imaging.
3. Inverted SP5 system with visible lasers for single-photon excitation including a 405 violet laser.
4. Upright SP2 system with visible and UV lasers for single-photon excitation.
5. Inverted SP8 system with visible lasers for single-photon excitation. SP8 is Leica's newest system with improved optics.
6. Upright SP5 system with twin femtosecond Ti:Sa lasers for two-photon excitation. This system utilizes resonant scanners that enable high-speed acquisition of large z-stacks for live tissue imaging.
7. Inverted SP5 II system with a femtosecond Ti:Sa laser for two-photon excitation. This system utilizes resonant scanners. The laser system is equipped with an optical parametric oscillator (OPO) that enables two-photon imaging by long wavelength excitation (1100-1300 nm).

Figure: Left: SP5 confocal microscope (#3). Right: SP5 II two-photon microscope with a femtosecond Ti:Sa laser plus an OPO (#7).

Genomics Laboratory

The Laboratory for Integrative Genomics also serves as a technical support service lab which provides genome- and proteome-wide analyses for the scientific research community in the Center for Integrative Medical Sciences (IMS). We offer a variety of services to suit the needs of laboratories in various biological fields. These services include DNA sequencing, proteomics analysis, multiplex suspension array (Luminex technology), DNA microarray (Affymetrix /Agilent), cDNA/genomic clone distribution, and Primer/labeled probe distribution for qRT-PCR analysis of immune cells (see Table). As part of our advanced technologies on demand, we provide comprehensive interrogation of the nucleic-acid based information in a cell at single-base resolution with Illumina HiSeq1500 and proteomic approaches using AB SCIEX TripleTOF 5600. With the unbiased approach using this sequencer, we have been identifying transcription units, alternative splice sites and transcription factor binding sites, as well as performing mapping/genome annotation. Our mass spectrometry system will make it possible to take quantitative proteomic approaches in various immunological studies. These technologies will help to illuminate additional hidden features of the dynamic genomic and proteomic landscape that are regulated by both genetic and epigenetic pathways in all organisms. Together with the Laboratory for Integrated Bioinformatics, we also carry out primary processing of large amounts of data from DNA sequencers and mass spectrometers for IMS users.

Table: Services provided by the Genomics Lab in 2013

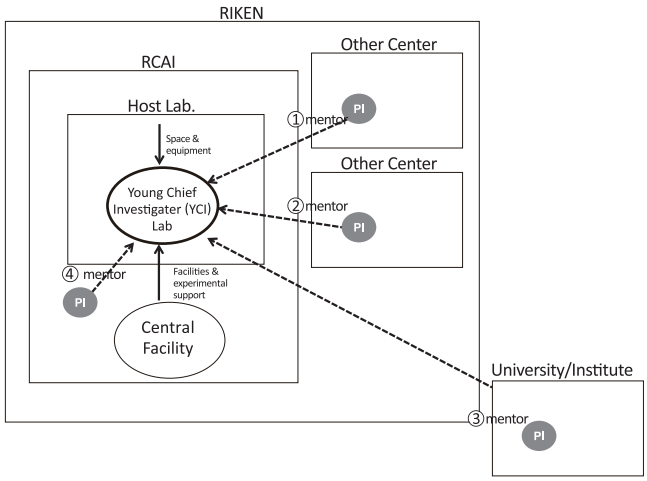
	# of samples	# of teams
Next-generation DNA sequencing		
Illumina HiSeq1500	731	20
Proteimics		
Two-dimentional electrophoresis	1	1
Mass Spectrometry Analysis	2	2
Multiplex suspension array	2,802	11
Affymetrix Genechip (Exon array, Gene array, miRNA array)		
Human	90	2
Mouse	153	11
Total	243	11
Sanger DNA sequencing		
36cm capillary	9,068	18
50cm capillary	5,610	15
Total	14,678	33
cDNA clone delivery	11	4
Primer/labeled probe delivery	58	2

Animal Facility

We have been maintaining over 45,000 mice in the SPF area, 1,750 mice in an isolated area and several germfree mice. We have newly introduced mouse lines into the SPF area by a combination of *in vitro* fertilization (IVF) and embryo transfer and generated cryostocks of genetic resources for 642 lines. We also maintain relatively large colonies of several commonly used strains such as NOD/SCID/γCKO mice, Rag1KO and cre deleters and provided them to users on demand. We have also provided technical assistance to generate knockouts (126 lines) and transgenic mice (6 lines). We have been undergoing renovation of an internally available database for genetic resources.In addition, we have launched a new activity to improve the efficacy of transplantation of human hematopoietic stem cells into NOD/SCID/γCKO mice by "humanizing" the host strain. For this purpose, we have introduced large genomic fragments containing human genes encoding MHC, cytokines, adhesion molecules, virus receptors and others into NOD/SCID/γCKO mice. Up to now, we have established 15 BAC transgenics and confirmed the expression of human genes on a C57BL/6 background and begun back-crossing these mice onto the NOD/SCID/γCKO mice using the speed-congenic method. To adapt to increasing demands for the use of humanized animals, we have started a new facility primarily for humanized mice, which is fully equipped with autoclaves and cage washing systems on the 1st floor of the north building. In this new facility, we can maintain up to 900 cages (up to 4,500 mice) in either isolators or bio-bubbles. More recently, we have added a new activity to create and maintain germ-free and gnotobiote mice in collaboration with Dr. Hiroshi Ohno (Laboratory for Intestinal Ecosystem), Dr. Sidonia Fagarasan (Laboratory for Mucosal Immunity) and Dr. Kenya Honda (Laboratory for Gut Homeostasis). We performed sanitization of standard strains and knockout mice at a pace of 1-2 strain(s) per month.

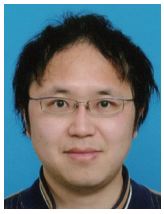
Photo: New animal facility for humanized mice

Young Chief Investigator Program



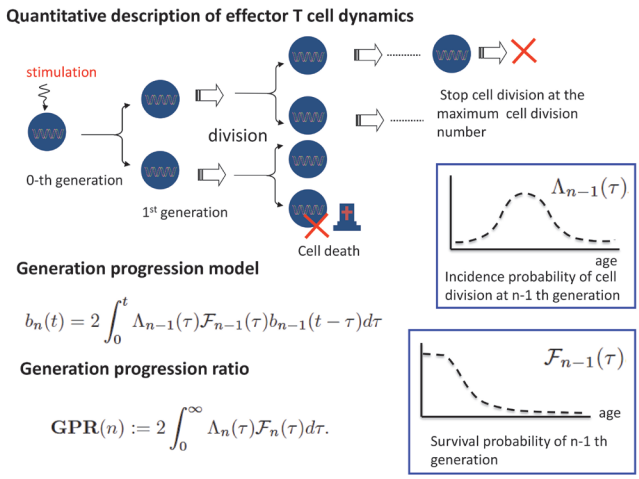
The Young Chief Investigator Program aims to provide a career path for young investigators who conduct multidisciplinary research that will bridge immunology with other research fields. In this program, the selected Young Chief Investigator (age below 40) will head an independent research laboratory 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 (Fig.) The YCI Program Committee considers necessary changes in the Center's support for each YCI and discusses the relevance and value of each YCI research project as part of the core research projects at IMS.

There will be an initial 5-year appointment with the possibility of extending for an additional 2 years after evaluation by the Director and an internal committee. At that point, a Young Chief Investigator can leave IMS to take a position at another institution or be promoted to another type of position within IMS.



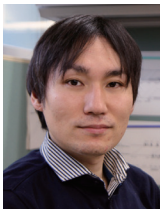
YCI Laboratory for Mathematical Modeling of Immune System

Young Chief Investigator: Shinji Nakaoka



In our laboratory, we undertake several mathematical works that are indispensable for construction of multi-scale mathematical models. In this year, we especially focused on a quantitative study of T cell population dynamics (Fig.). On the basis of label-based measurement data, such as BrdU, to track cell division and death, we proposed a general framework that allows incorporating general distributions for the timing of cell division and death. Specifically, we proposed a mechanistic mathematical model, called a generation progression model (GPM), that is described by a system of Volterra integral equations. Formulation by ODEs corresponds to a special case of GPM with an exponentially distributed kernel. A formulation developed in mathematical demography is employed to track the history of cell divisions. We newly defined a quantity called generation progression ratio (GPR) which provides a quantitative measure for the expected number of daughter cells generated from their survived mother cell. As an application of GPM and GPR, a time-course measured data for the transient growth of lymphocytes at the single cell level was used to quantitatively characterize the expansion and contraction phase.

Figure: A scheme of a generation progression model and a generation progression ratio



YCI Laboratory for
Stem Cell Competency
Young Chief Investigator: Hayato Kaneda

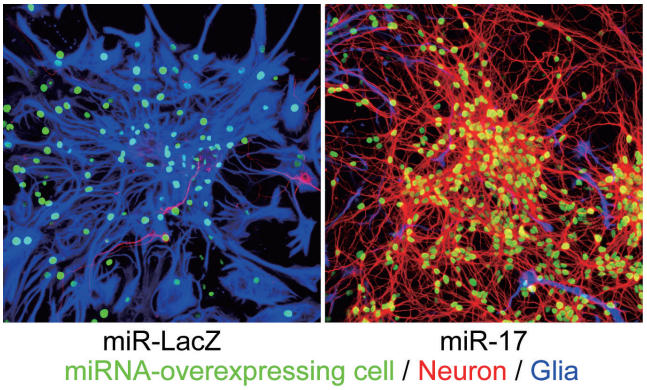


Figure: Restoration of neurogenic potential in development-progressed gliogenic NSPCs by overexpression of miR-17.
Embryonic stem cell-derived NSPCs can sequentially generate specific types of neurons and glial cells *in vitro* in their proper *in vivo* order. Overexpression of miR-17 inhibited the acquisition of gliogenic competence and forced stage-progressed NSPCs to regain neurogenic competence.

Adult tissue stem cells (TSCs) are impaired in their functions with age. TSC dysfunction and decreased regenerative capacity are involved, at least in part, in disturbances of tissue homeostasis, e.g., inefficient muscle repair, reduced bone mass, neurodegenerative diseases, and dysregulation of hematopoiesis. Therefore, the restoration of TSC functions is expected to contribute to recovery of tissue homeostasis and improvements in our health.

Previously, we identified the "competence change", which is responsible for the responsiveness of neural stem/progenitor cells (NSPCs) to extrinsic signals (Naka H et. al. *Nat Neurosci* 2008). Moreover, further investigation revealed that the competence regulation enabled us to control neurogenic-to-gliogenic transition and restore neurogenic potential in developmentally-progressed gliogenic NSPCs (Fig.) (Naka-Kaneda H et. al. *Proc Natl Acad Sci USA* 2014). Based on these findings, we have been investigating stem cell aging. Competence regulation is also involved in the aging of other TSCs, such as the decline in lymphopoiesis by hematopoietic stem cells and in the osteogenesis ability of mesenchymal stem/stromal cells. We aim to elucidate the central molecular machinery of stem cell aging and its influence on tissue homeostasis and in turn to develop a method for functional recovery of aged stem cells and restoration/maintenance of tissue homeostasis.

YCI Laboratory for
Immune Regeneration
Young Chief Investigator: Tomokatsu Ikawa

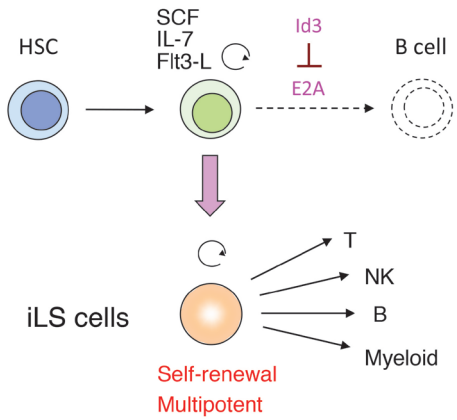


Figure: Induced Leukocyte Stem (iLS) cells
We have established multipotent progenitors that have self-renewal activity and multipotency. This was done by overexpressing Id3 in hematopoietic stem/progenitor cells and culturing the cells under B cell differentiating conditions. Id3 suppresses E2A activity, and the multipotent progenitors acquire self-renewal activity. The cells extensively proliferate for at least several months, still maintaining their multipotency.

T, B and NK lymphocytes are generated from pluripotent hematopoietic stem cells (HSCs) through a successive series of lineage restriction processes. Many regulatory components, such as transcription factors, cytokines/cytokine receptors, and signal transduction molecules orchestrate the cell fate specification and determination. Above all, transcription factors play a key role in regulating lineage-associated gene programs. Although many essential transcription factors, such as PU.1, Ikaros, GATA3, TCF-1, Bcl11b, E2A, EBF1 and Pax5 have been implicated in regulating the cell fate choice of lymphoid lineages, molecular mechanisms underlying the generation of these patterns during cell fate determination remain unexplored because of an absence of suitable experimental systems. We have recently established an ideal system in which we can examine gene regulatory networks during lymphoid lineage specification from HSCs (Fig.). This novel system enabled the analysis of a large set of regulatory molecules that control the generation of T and B lymphocytes. It can also be applied for *ex vivo* expansion of human hematopoietic stem/progenitors, which will be required for immune cell therapy or transplantation of HSCs. Thus, the aims of our study are 1) from a basic science perspective, to elucidate the mechanisms that orchestrate cell fate specification, commitment and differentiation during lymphocyte development and 2) from a clinical medicine perspective, to establish a novel method to expand human hematopoietic stem/progenitors for the development of HSC transplantation as a clinical strategy.

YCI Laboratory for
Integrative Genomics
Young Chief Investigator: Katsuyuki Shiroguchi

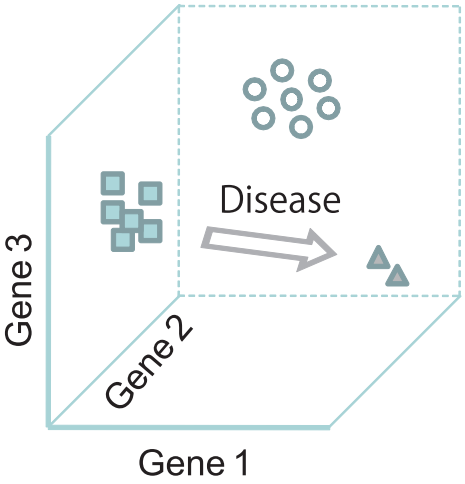


Figure: Detection of homeostasis destruction by genomics study
The destruction of homeostasis can be detected by accurate counting of RNA molecules in single cells. Each symbol corresponds to a single cell.

Quantitative Genomics at the Single Cell & Single Molecule Level

In order to investigate at the molecular level how homeostasis is destroyed at the beginning of a disease and how compensation occurs while responding to an external perturbation before the break, we wish to visualize the distribution of cell states by accurate system-wide measurements with single molecule and/or single cell resolution. To visualize the distribution and its shift, we are working on multiple projects with development of highly accurate quantification methods. One of them is to digitally count the copy number of RNA molecules genome-wide using our technique under development and a next generation sequencer. This method provides highly reproducible quantification even for low-expressed RNAs and the absolute RNA copy number per cell, which should allow us to find small differences between samples whose phenotypes are only slightly different. In addition, I believe that the absolute counting of cellular molecules or rates of biological events will become more important, especially in a coming era, since it makes possible the integrative interpretation with other data obtained by different measurements. We will work on developing such a technique to "see" something unknown, which may contribute to understanding biological systems and diseases.

YCI Laboratory for
Cellular Bioenergetic Network
Young Chief Investigator: Toshimori Kitami

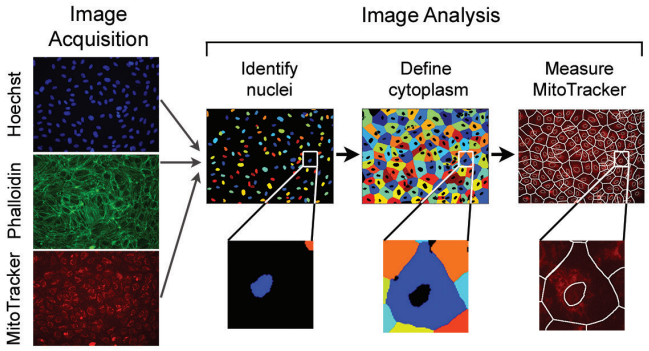


Figure: Schematic of image-based assay for mitochondria using automated microscopy
High-throughput automated microscopy is one of many cutting-edge technologies available at IMS-RCAI.

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

Our new laboratory, established in FY2013, hopes to take advantage of the immunology expertise of IMS-RCAI and our expertise in mitochondrial biology, chemical screening, and genomics to explore the interface between immunity, mitochondria, and complex diseases.

RIKEN International Program Associate (IPA)

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

The IPA students who studied at IMS in 2013 were

Yue Ren (Jilin University, China) studied in the Laboratory for Immune Regulation.
Mohamed El Sherif Gadelhaq Gadelhaq Badr (Tokyo Medical and Dental University) from Edypt studied in the Laboratory for Cell Signaling.
Mei Suen Kong (Universiti Sains Malaysia, Malaysia) studied in the Laboratory for Cell Signaling.
Cheng Chung Yong (Universiti Sains Malaysia, Malaysia) studied in the Laboratory for Intestinal Ecosystem.

RIKEN Foreign Postdoctoral Researcher (FPR) Program

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

In 2013, **Wooseok Seo** studied in the Laboratory for Transcriptional Regulation as a RIKEN FPR.

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, 20 JRA students studied in IMS.

Norihiko Inoue (Laboratory for Integrated Cellular Systems)
Chie Kano (Laboratory for Intestinal Ecosystem)
Misao Hanazato (Laboratory for Intestinal Ecosystem)
Masanaka Sugiyama (Laboratory for Inflammatory Regulation)
Hisashi Wada (Laboratory for Transcriptional Regulation)
Takashi Ikeno (Laboratory for Tissue Dynamics)
Satoshi Koga (Laboratory for Immune Cell System)
Ryuichi Murakami (Laboratory for Immune Homeostasis)
Yujiro Yamamoto (Laboratory for Genome Sequencing Analysis)
Yuji Nagano (Laboratory for Gut Homeostasis)
Akihiko Kimura (Laboratory for Immune Homeostasis)
Akemi Fujiwara (Laboratory for Intestinal Ecosystem)
Yuuhou Najima (Laboratory for Human Disease Models)
Rikiya Ishikawa (Laboratory for Intestinal Ecosystem)
Yusuke Sato (Laboratory for Immunotherapy)
Tomohiro Miyai (Laboratory for Immune Cell System)
Rumiko Ono (Laboratory for Inflammatory Regulation)
Takaharu Sasaki (Laboratory for Immune Cell System)
Yuma Sakamoto (Laboratory for Bone and Joint Diseases)
Atsushi Ono (Laboratory for Digestive Diseases)

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

Shimpei Kawamoto (Laboratory for Mucosal Immunity)
Saya Moriyama (Laboratory for Tissue Dynamics)
Shinsuke Ito (Laboratory for Developmental Genetics)
Hirokazu Tanaka (Laboratory for Transcriptional Regulation)

Award winners 2013


Photo 1


Photo 2


Photo 3


Photo 4


Photo 5


Photo 6


Photo 7


Photo 8

Ichiro Taniuchi (photo 1), Group Director of the Laboratory for Transcriptional Regulation, received the 16th Japanese Society for Immunology (JSI) Award for his study, "Transcriptional control of T cell development".

Hidewaki Nakagawa (photo 2), Team Leader of the Laboratory for Genome Sequencing Analysis, received the Princess Takamatsu Cancer Research Fund Award for his study, "Whole genome sequencing analysis of intrahepatic cholangiocarcinoma".

Fumihiko Ishikawa (photo 3), Group Director of the Laboratory for Human Disease Models, received the JSPS (Japan Society for the Promotion of Science) Prize for his study, "Development of Humanized Mouse System Enabling *in vivo* Investigation of Human Leukemia and Therapeutic Approach".

Atsushi Takahashi (photo 4), Team Leader of the Laboratory for Statistical Analysis, and **Yuta Kochi** (photo 5), Senior Research Scientist of the Laboratory for Autoimmune Diseases, received the Japan Society of Human Genetics (JSHG) Encouragement Award in November 2013.

Sidonia Fagarasan (photo 6), Team Leader of the Laboratory for Mucosal Immunity, and **Kenya Honda** (photo 7), Team Leader of the Laboratory for Gut Homeostasis, received the NISTEP Award from the National Institute of Science and Technology Policy (NISTEP), under the direct jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology (MEXT). They were selected for their outstanding contributions to elucidating the interactions between the immune system and microbes at the mucosal barrier.

Koji Ueda (photo 8), Senior Research Scientist in the Laboratory for Genome Sequencing Analysis, received the Japan Human Proteome Organization Young Researcher's Award for his study, "Glycol-proteomics technology and tumor marker research by glycol-proteomics".

Hidewaki Nakagawa, Team Leader of the Laboratory for Genome Sequencing Analysis, received the Takeda Science Foundation Award for his study, "Identification of prostate cancer-susceptibility genes /variants and their association with diet".

Kohei Kometani, Research Scientist in the Laboratory for Lymphocyte Differentiation, received the Japanese Society for Immunology Young Investigator Award for his study, "Unveiling molecular mechanism of B cell activation and subsequent plasma cell differentiation".

Takashi Kanaya, Research Scientist in the Laboratory for Intestinal Ecosystem, received the Research Encouragement Award from the Japan Bifidus Foundation on June 14th, 2013.

Naoka Ito-Nagato, Research Scientist in the Laboratory for Immune Regulation, and Tomomitsu Hirota, Research Scientist in the Laboratory for Respiratory and Allergic Diseases, received the Abstract Award at the European Academy of Allergy and Clinical Immunology –World Allergy Organization (EAACI-WAO) Congress 2013 held in Milan, Italy, June-July 2013.

Mari Tenno, Research Scientist in the Laboratory for Transcriptional Regulation, received the poster award for her research on "Unique role of Cbfb2 variant in Langerhans cell development" at the 13th International Workshop on

Langerhans Cells, held on October 10th-13th, 2013, in Amsterdam, the Netherlands.

Shintaro Hojyo, Research Scientist in the Laboratory for Laboratory for Homeostatic Network, received the Suzuki Koichi Memorial Award at the 86th Annual Meeting of the Japanese Biochemical Society in October 2013.

Tomohiro Miyai, Junior Research Associate in the Laboratory for Homeostatic Network, received the excellent presentation award at the meeting of the Japanese Research Group for Studies of Metalbioscience 2013 on September 27th.

Siew-Kee Low, Postdoctoral Researcher in the Laboratory for Statistical Analysis, was selected as a European Society of Human Genetics (ESHG) Poster Award Finalist in June 2013.

Shimpei Kawamoto, Special Postdoctoral Researcher in the Laboratory for Mucosal Immunity, **Takashi Kanaya,** Research Scientist in the Laboratory for Intestinal Ecosystem and **Raoul Eduardo Vizcardo Sakoda**, Research Scientist in the Laboratory for Developmental Genetics received the Tadamitsu Kishimoto International Travel Award for their presentations at the 15th International Congress of Immunology held in Milan, Italy.

Koji Atarashi, Senior Research Scientist in the Laboratory for Gut Homeostasis, and **Tomomitsu Hirota**, Research Scientist in the Laboratory for Respiratory and Allergic Diseases, received RIKEN Research Incentive Awards. **Tomoyuki Ishikura**, Technical Staff member in the Laboratory for Developmental Genetics received a RIKEN Technology Incentive Award in March, 2014.

Two graduate students studying at IMS won Best Poster Awards at the RIKEN Noyori Summer School held in Kobe on September 6th-7th, 2013. **Yuho Najima**, Junior Research Associate in the Laboratory for Human Disease Models, received the Medical Science Prize for his presentation "Induction of human WT1-specific $CD8^+$ T cells in an HLA class I TG NSG mice". **Takaharu Sasaki**, Junior Research Associate in the Laboratory for Immune Cell System, received the Biology Prize for his presentation "Role of Natural Helper Cells in Obesity."

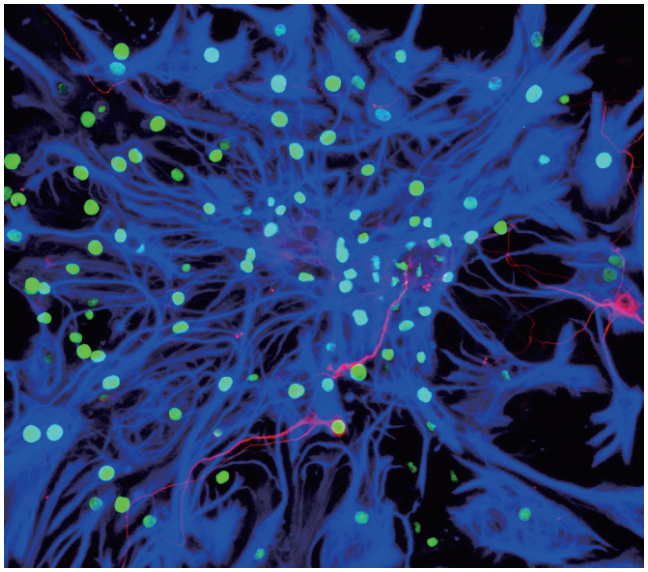
The IMS Excellent Paper of the Year 2013 award was given to the following four papers:

Dr. Yukihiro Furusawa, Mr. Yuuki Obata, Dr. Shinji Fukuda, Dr. Koji Hase and Dr. Hiroshi Ohno Laboratory for Intestinal Ecosystem "Commensal microbe-derived butyrate induces colonic regulatory T cells." ***Nature*** Vol. 504, pp.446-450, 2013.

Dr. Yoriko Saito and Dr. Fumihiko Ishikawa Laboratory for Human Disease Models "A pyrrolo-pyrimidine derivative targets human primary AML stem cells in vivo." ***Science Translational Medicine*** Vol. 5, 181ra52, 2013.

Dr. Koji Atarashi, Dr. Takeshi Tanoue and Dr. Kenya Honda Laboratory for Gut Homeostasis "Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota." ***Nature*** Vol. 500, pp.232-236, 2013.

Dr. Ikuyo Kou, Dr. Yohei Takahashi and Dr. Shiro Ikegawa Laboratory for Bone and Joint Diseases "Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis." ***Nature Genetics*** Vol. 45, pp.676-679, 2013.



Part 2

Research Projects

Intestinal Bacteria Show 'Community Spirit'

The collaborative effects of multiple bacterial strains in the gut may help prevent the onset of certain inflammatory diseases

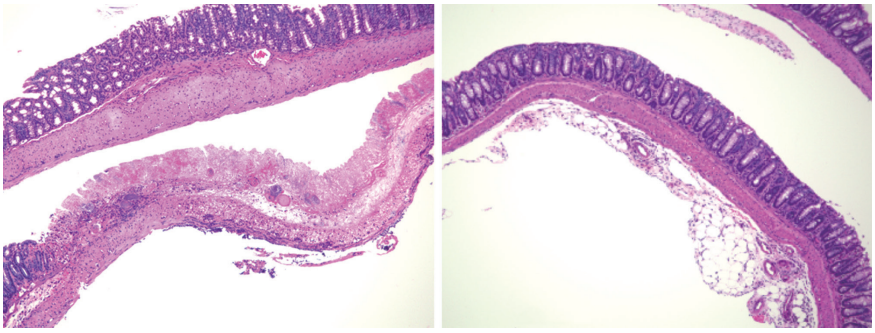


Figure: Inflammatory damage to the intestinal epithelium in a mouse model of colitis (*left*) is greatly reduced after oral administration of a mixture of 17 Clostridia strains (*right*).
Reproduced from Ref. © 2013 K. Atarashi et al.

At first, it may sound alarming to learn that a population of bacteria in your gut is conspiring to suppress your immune system—however, this is actually good news. By identifying the strains responsible, a research team led by Kenya Honda (Laboratory for Gut Homeostasis) may have uncovered a promising avenue of treatment for certain inflammatory disorders.

Immune cells known as regulatory T (Treg) cells are in part responsible for preventing the immune system from overreacting to foreign molecules or attacking healthy tissue. It is well established that immune function is affected by the diverse microbial community within the digestive tract, and Honda's team previously discovered that bacteria belonging to the genus Clostridium act on this particular immune pathway in mice to exert a strong anti-inflammatory effect.

"We showed that they were responsible for triggering production of Treg cells in the colon of mice," says Honda, "and that oral administration of these strains protected mice against colitis and systemic allergic responses."

Honda and his colleagues have now verified the existence of an equivalent bacterial population in humans. They obtained a stool sample from a healthy volunteer and subjected it to the same purification regimen that yielded the Clostridia subpopulation identified in mice. When these bacteria were transplanted into the colons of 'germ-free' mice, in which the normal population of gut bacteria is entirely absent, they exerted a potent immunomodulatory effect. Through systematic analysis of this microbial cohort, the researchers zoomed in on a specific subset of 17 distinct Clostridia strains.

These strains collectively secrete a host of signaling molecules that promote Treg cell activation. "None of the organisms alone were nearly as potent as when they were in consortium," says Honda. "This suggests that cooperation between the strains is essential to their therapeutic effects." The collective benefit also appears to pertain to humans; analysis of the gut 'microbiome' in healthy patients versus individuals with ulcerative colitis revealed that all 17 strains were present at significantly lower levels in the latter group.

Accordingly, oral administration of this 17-microbe 'cocktail' greatly mitigated intestinal inflammation in mouse models of allergic diarrhea and ulcerative colitis (Fig. 1), suggesting the potential for a more 'natural' treatment of such conditions in humans. "A substantial number of patients don't benefit from existing drugs, which also have considerable adverse effects," says Honda. "We want to clinically test our hypothesis that reconstituting these bacteria to normal levels in patients may help restore immune tolerance and resolve chronic inflammatory processes."

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Original paper
Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., Kim, S., Fritz, JV., Wilmes, P., Ueha, S., Matsushima, K., Ohno, H., Olle, B., Sakaguchi, S., Taniguchi, T., Morita, H., Hattori, M., Honda, K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. ***Nature*** 500, 232–236 (2013).

Friends in Low Places Preserve Gut Health

Bacteria living in the mammalian intestine help to digest dietary fiber, generating metabolites that also control gut inflammation

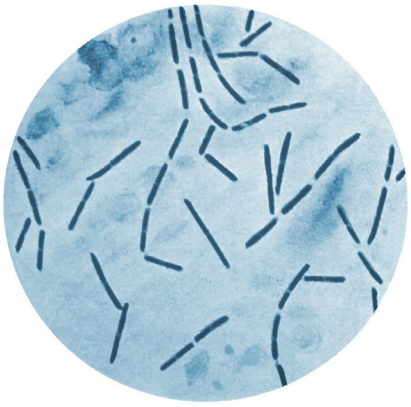

Figure: Commensal bacteria belonging to the Clostridiales order help to control inflammation in the mammalian gut.
Image courtesy of the Centers for Disease Control and Prevention

The bacterial communities that live in our intestine should not be considered freeloaders—they contribute substantially to our well-being in a number of ways, including assisting in the breakdown of otherwise indigestible dietary fiber. Hiroshi Ohno (Laboratory for Intestinal Ecosystem) and colleagues have discovered a mechanism by which this digestive assistance also helps to prevent gut inflammation.

Bacteria belonging to the order *Clostridiales* are known to metabolize indigestible dietary fiber to produce metabolites, such as short-chain fatty acids (SCFAs). Ohno's team found that mice lacking gut commensal bacteria including Clostridiales exhibit bloating within the cecum—the start of the large intestine—after consuming a high-fiber diet. However, this problem could be repaired by transplanting a Clostridiales-enriched gut bacterial community (Fig.).

In other recent work, Honda and his colleagues demonstrated that these same bacteria promote the differentiation of regulatory T (T_{reg}) cells, which specifically prevent the immune system from overreacting (detailed in the previous page). Ohno suspected that this and his most-recent observation might be connected. "This led us to hypothesize that bacterial metabolism of dietary fiber may be the cause of T_{reg} induction," he says.

After searching for metabolic products that were elevated following consumption of a high-fiber diet, the researchers focused on one SCFA, butyrate, as a likely candidate. Butyrate proved capable of converting immature 'naive' T cells into T_{reg} cells in culture, and a maize starch diet that had been chemically enriched with butyrate invoked a similarly potent T_{reg} differentiation within the mouse colon. The same butyrate-enriched maize starch diet failed to elicit T_{reg} differentiation in mice lacking commensal microbes, suggesting that in addition to butyrate, bacterial components are required as an antigen to be recognized by naive T cells.

Butyrate modulates the activity of histone deacetylase to modify epigenetic status of chromatin via histone acetylation, which can dramatically affect gene expression, and Ohno and his colleagues found the upregulation of gene encoding the Foxp3 transcription factor essential for T_{reg} differentiation. "Bacterial butyrate affected the epigenetic status of naive T cells to propel their differentiation into T_{reg} cells within the colonic tissue," he says. This resulted in a strong protective effect, and the increased numbers of T_{reg} cells that developed following consumption of a butyrate-enriched diet ameliorated inflammation in a mouse model of colitis.

These findings may thus reveal a critical component of the pathology of human inflammatory bowel diseases as well as a potential means for treatment. Ohno and his colleagues now hope to explore whether the same mechanism is also relevant in the inflammatory response to food allergies.

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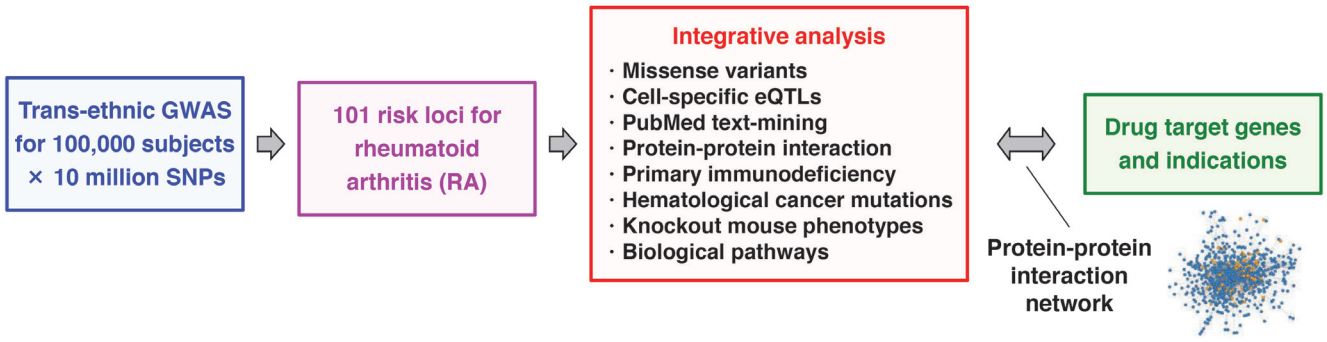
Original paper
Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T. Takahashi, M., Fukuda, N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., Clarke, JM., Topping, DL., Tomita, M., Hori, S., Ohara, O., Morita, T., Koseki, H., Kikuchi, J., Honda, K., Hase K., Ohno, H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. ***Nature*** 504, 446–450 (2013)

Rheumatoid Arthritis Research Shows the Potential of Large-scale Genetic Studies for Drug Discovery



(Okada et al. *Nature* 2013.)

The study, conducted by Dr. Robert M. Plenge from the Harvard Medical School and the Broad Institute in the USA and Dr. Yukinori Okada from the Laboratory for Autoimmune Diseases, collaborating with colleagues from 70 institutions worldwide, is published in the journal Nature.

Genome-wide association studies are a method employed by scientists to identify the genes contributing to human disease. The current Nature study is the first to demonstrate that integrating the information provided by genome-wide association studies with existing datasets of genomic and biological information, such as drug targets, can assist in the discovery of drugs to cure human disease.

Rheumatoid arthritis is an autoimmune disease leading to inflammation of the joints and affecting 0.5-1% of adults in the developed world. The disease is thought to be caused by a complex combination of genetic and environmental factors and several genes have been shown to be associated with the disease. However, most of the findings were based on single population studies, and no large-scale trans-ethnic study had been carried out to date.

The international team performed a genome-wide association study meta-analysis on a total of over 100,000 subjects of European and Asian descent - 29,880 rheumatoid arthritis patients and 73,758 controls – by analysing around 10 million genetic variants called single nucleotide polymorphism (SNPs). They identified 42 new regions in the genome (loci) that are associated with rheumatoid arthritis, bringing the total number of known rheumatoid arthritis loci to 101.

By conducting bioinformatics studies integrating existing datasets with this new information, the researchers were able to pinpoint 98 genes in these 101 loci that could potentially contribute to the onset of rheumatoid arthritis. By integrating their findings with existing drug databases they demonstrated that these genes indeed possess many overlapping regions with the genes targeted by approved rheumatoid arthritis drugs – although this wasn't known when the drugs were developed. The team identified existing drugs used to treat cancer that also target rheumatoid arthritis genes and could potentially be used as therapy for the disease, such as CDK4/6 inhibitors.

The bioinformatics study also reveals that there is significant overlap between the genes involved in rheumatoid arthritis, human primary immunodeficiency disorders and blood cancers.

"This study sheds light on the fundamental genes, pathways and cell types that contribute to the onset of rheumatoid arthritis and provides evidence that the genetics of rheumatoid arthritis can provide important information for drug discovery," conclude the authors."While there are previous anecdotal examples, our study provides a systematic approach by which human genetic data can be efficiently integrated with other biological information to derive biological insights and drug discovery," they add.

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http://www.riken.jp/en/pr/press/2013/20131226_1/

Original paper
Okada, Y. Wu, D., Trynka, G., Raj, T., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Yoshida, S., Graham, RR., Manoharan, A., Ortmann, W., Bhangale, T., Denny, JC., Carroll, RJ., Eyler, AE., Greenberg, JD., Kremer, JM., Pappas, DA., Jiang, L., Yin, J., Ye, L., Su, DF., Yang, J., Xie, G., Keystone, E., Westra, HJ., Esko, T., Metspalu, A., Zhou, X., Gupta, N., Mirel, D., Stahl, EA., Diogo, D., Cui, J., Liao, K., Guo, MH., Myouzen, K., Kawaguchi, T., Coenen, MJ., van Riel, PL., van de Laar, MA., Guchelaar, HJ., Huizinga, TW., Dieudé, P., Mariette, X., Bridges SL Jr., Zhernakova, A., *et al.* Genetics of rheumatoid arthritis contributes to biology and drug discovery. ***Nature*** 506, 376-381, 2013

Connecting the Genetic Dots in Connective Tissue Disorders

Scientists identify a mutated gene responsible for a spectrum of skeletal and connective tissue disorders

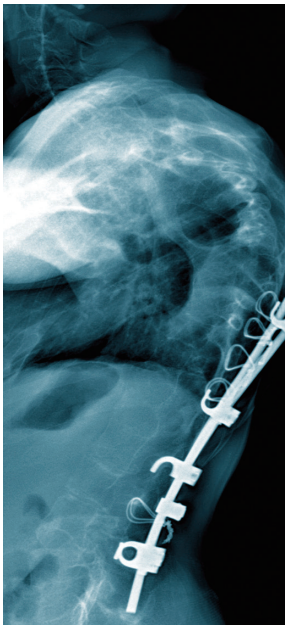

Figure: Spinal x-ray of an SEMD-JL1 patient.
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The disorder known as spondyloepimetaphyseal dysplasia with joint laxity type 1, or SEMD-JL1, is characterized by skeletal abnormalities and loose ligaments that result in spinal misalignment and respiratory problems. The genetic basis of SEMD-JL type 2, a related disorder, was recently determined, but the genetic underpinnings of the type 1 form of the disease remain unknown.

By studying the genomes of seven people with SEMD-JL1, a large international research team led by Shiro Ikegawa from the Laboratory for Bone and Joint Diseases has now identified a gene that, when mutated, is responsible not only for SEMD-JL1 but also a range of other bone and connective tissue defects.

Noriko Miyake, who joined Ikegawa's team from Yokohama City University, discovered the gene by sequencing the entire protein-coding region of the genomes of seven Japanese people with SEMD-JL1 from six unrelated families. Using targeted sequencing to confirm initial 'hits', Masahiro Nakajima, a member of Ikegawa's lab, showed that all of the subjects, in addition to an eighth Vietnamese individual with SEMD-JL1, had mutations in a gene called *B3GALT6* on the short arm of chromosome 1. This gene codes for a type of enzyme known as a galactosyltransferase II, which is involved in the synthesis of the proteoglycan linker region that helps form the structural cement of connective tissue. The researchers found that mutations in *B3GALT6* caused disease in a recessive fashion, meaning that all of the affected individuals had inherited two faulty copies of the gene.

The researchers then noticed that some of the individuals with SEMD-JL1 in the study exhibited many of the same clinical characteristics as those found in people with another connective tissue disorder—the progeroid form of Ehlers-Danlos syndrome (EDS), which is characterized by a defect in the synthesis of collagen. To investigate this further, they sequenced the *B3GALT6* gene in four people with progeroid-form EDS of unknown genetic cause. All four subjects carried mutations in the *B3GALT6* gene.

"B3GALT6 enzyme deficiency results in a wide variety of disorders," says Ikegawa. "These diseases have been considered to belong to totally different categories of disease, but actually are a spectrum of disorders affecting bone, cartilage, muscle, tendon, ligament, and skin. Our findings will enable genetic diagnosis of these diseases." The researchers anticipate that the findings could open the door to future therapies for disorders related to *B3GALT6*.

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Original paper
Nakajima, M., Mizumoto, S., Miyake, N., Kogawa, R., Iida, A., Ito, H., Kitoh, H., Hirayama, A., Mitsubuchi, H., Miyazaki, O. Kosaki, R., Horikawa, R., Lai, A., Mendoza-Londono, R., Dupuis, L., Chitayat, D., Howard, A., Leal, GF., Cavalcanti, D., Tsurusaki, Y., Saitsu, H., Watanabe, S., Lausch, E., Unger, S., Bonafé, L., Ohashi, H., Superti-Furga, A., Matsumoto, N., Sugahara, K., Nishimura, G., Ikegawa, S. Mutations in *B3GALT6*, which encodes a glycosaminoglycan linker region enzyme, cause a spectrum of skeletal and connective tissue disorders. ***Am J Hum Genet.*** 92, 927–934 (2013)

Getting Ahead of the Curve

A gene involved in spinal development may contribute to a common childhood disease responsible for spinal curvature

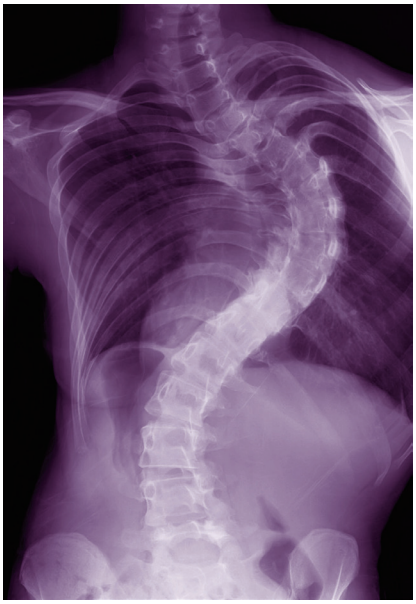

Figure: Spinal x-ray of a child with AIS.
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Adolescent idiopathic scoliosis (AIS) is the most common pediatric skeletal disease, causing complex rotational deformity of the spine in approximately 2% of school-age children worldwide. Recent studies have implied that AIS may, at least in part, be caused by genetics, but the pathogenesis of AIS still remains poorly understood. An international research team led by Shiro Ikegawa from the Laboratory for Bone and Joint Diseases has now identified another gene that may contribute to AIS by altering spinal development.

Ikegawa's group previously identified a gene associated with AIS in Japanese populations. To further reveal the genetics underlying the disease, Ikuyo Kou from Ikegawa's lab conducted a genome-wide association study including over 27,000 Japanese individuals. In this study, Kou identified another single nucleotide polymorphism (SNP) that is significantly associated with AIS in Japanese subjects. Additional testing showed that the association was replicated in Han Chinese and European populations, marking the first time a SNP associated with AIS has been identified in distinct populations.

Kou also determined that the SNP is located in the gene *GPR126*, which encodes a receptor protein. *GPR126* is known to be associated with the development of the sheaths that insulate nerve fibers, but its other functions are largely unknown. By examining the tissue-specific expression of *GPR126*, the researchers found that it is expressed in spinal cartilage, implying a role in the development of the spine.

Using zebrafish, Long Guo and Chisa Shukunami who joined Ikegawa's team from Kyoto University knocked down *GPR126* expression to investigate the role of the gene in vertebrate development. They found that zebrafish with lower levels of the GPR126 protein had shorter bodies and their vertebrae showed delayed bone formation, or osteogenesis. "Our zebrafish knockdown studies indicate the importance of GPR126 in bone tissue growth and formation, and raise the possibility that abnormal spinal development and growth induce AIS," explains Kou.

GPR126 is also known to be associated with shorter trunk length and reduced height in European populations, which coincides with the new findings. "Their associations are in the same direction; the susceptibility allele is the same for both AIS and shortened trunk," says Kou.

Although the identification of *GPR126* sheds new light on the pathogenesis of AIS, the two genes implicated so far explain only ~1% of the variation in AIS traits. Further studies therefore remain necessary to determine precisely how mutations of *GPR126* lead to AIS in humans.

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Original paper
Kou, I., Takahashi, Y., Johnson, T. A., Takahashi, A., Guo, L., Dai, J., Qiu, X., Sharma, S., Takimoto, A., Ogura, Y. Jiang, H., Yan, H., Kono, K., Kawakami, N., Uno, K., Ito, M., Minami, S., Yanagida, H., Taneichi, H., Hosono, N., Tsuji, T., Suzuki, T., Sudo, H., Kotani, T., Yonezawa, I., Londono, D., Gordon, D., Herring, JA., Watanabe, K., Chiba, K., Kamatani, N., Jiang, Q., Hiraki, Y., Kubo, M., Toyama, Y., Tsunoda, T., Wise, CA., Qiu, Y., Shukunami, C., Matsumoto, M., Ikegawa, S. Genetic variants in *GPR126* are associated with adolescent idiopathic scoliosis. ***Nat Genet.*** 45, 676–679 (2013)

Compound that Could Prevent Acute Blood Cancer Relapse Identified

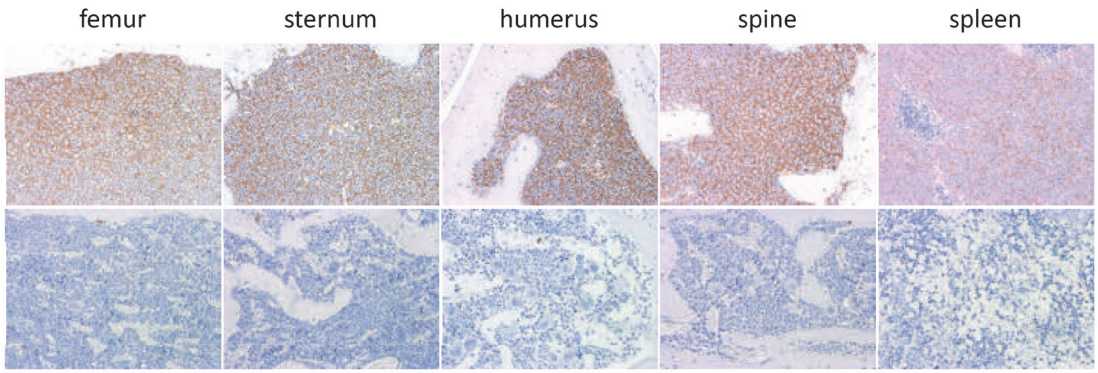

Figure: Eradication of AML cells from bone marrow (*in brown*) following treatment with small molecule RK-20449 (*lower panels*).

The research group led by Fumihiko Ishikawa (Laboratory for Human Disease Models) identified a compound that could be used as a new treatment to prevent relapse in acute myeloid leukemia patients.

In a study published in *Science Translational Medicine*, they showed that this compound reduces the risk of relapse in a mouse model of the human disease. They report that this compound could be most active in patients that carry a mutation lowering their chances of recovery.

Acute myeloid leukemia (AML) is an acute type of blood cancer that starts in the blood-forming cells in the bone marrow. AML is the most common type of acute leukemia in adults.

While many patients are able to fight off the disease at first with conventional chemotherapy, long-term outcomes in the majority of patients are poor due to disease relapse.

"To improve patient outcomes, it is crucial to understand the mechanisms of AML relapse and to develop effective treatment strategies to reduce AML relapse," explains Ishikawa.

Over the last decade, bone marrow cells called leukemia stem cells (LSC) have been recognized as key players in human AML pathogenesis as well as in chemotherapy resistance and relapse. Previous studies have suggested that LSCs might cause relapse if they are not properly eliminated by conventional chemotherapy.

By transplanting LSCs obtained from AML patient samples into immune-deficient newborn mice, Ishikawa and his team developed a mouse model for AML, which they used to study AML and LSCs.

Using this model, they were able to identify a protein (HCK) present in higher quantities in human AML LSCs than in normal blood-forming stem cells, and that could be used as a target for therapeutic agents against human AML LSCs.

In the present study, the researchers screened a library of tens of thousands of small molecules that could act as therapeutic agents by specifically inhibiting HCK. They isolated one small molecule that was highly active against patient-derived AML LSCs grown in culture. To assess the potential of this molecule for therapeutic development, they administered it to their mouse model of AML. They found that administration of this molecule resulted in a significant reduction of human AML cells in the blood of the mice, as well as a reduction of human AML LSCs in the bone marrow of the mice (Fig.).

In particular, in mice engrafted with human AML derived from patients with the FLT3-ITD mutation, one of the mutations associated with worse clinical outcomes, the administration of the small molecule led to nearly complete elimination of both AML LSCs and non-stem AML cells in the bone marrow of multiple bones (femur, tibia, sternum and spine) as well as the spleen and peripheral blood.

"These findings suggest that treatment with this small molecule may help reduce relapse in AML patients," conclude the authors.

"However, more work is needed before this small molecule can be delivered to patients as a therapeutic agent. We now plan to proceed with a more in-depth biochemical and pharmacologic characterization of this compound in the lab, to find out whether it is safe and to determine which subset of AML patients could benefit from it. Ultimately, we hope to develop a drug that can be used in the clinic," adds Ishikawa.

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http://www.riken.jp/en/pr/press/2013/20130418_1

Original paper
Saito, Y., Yuki, H., Kuratani, M., Hashizume, Y., Takagi, S., Honma, T., Tanaka, A., Shirouzu, M., Mikuni, J., Handa, N. et al. A pyrrolo-pyrimidine derivative targets human primary AML stem cells *in vivo*. ***Sci Transl Med.*** 5, 181ra52 (2013).

Genetic Variations May Help Identify Best Candidates for Preventive Breast Cancer Drugs

Newly discovered genetic variations may help predict breast cancer risk in women who receive preventive breast cancer therapy with the selective estrogen receptor modulator drugs tamoxifen and raloxifene, a Mayo Clinic-led study has found. The study is published in the journal Cancer Discovery.

"Our findings are important because we identified genetic factors that could eventually be used to select women who should be offered the drugs for prevention," said James Ingle, M.D., an oncologist at Mayo Clinic.

Dr. Ingle and collaborators at the National Surgical Adjuvant Breast and Bowel Project (NSABP) and the RIKEN Center for Integrative Medical Sciences (IMS) conducted a genome-wide association study involving 592 patients who developed breast cancer while receiving preventive therapy and 1,171 matched controls. Participants were selected from 33,000 women enrolled in the NSABP breast cancer prevention trials. This research was supported by a Pharmacogenomics Research Network grant from the National Institute of General Medical Sciences and the National Cancer Institute.

The researchers analyzed participants' DNA to identify variations in their genetic makeup and identified two genetic variations, or single nucleotide polymorphisms (SNPs), that were associated with breast cancer risk in or near the genes ZNF423 and CTSO.

They discovered that women with favorable variations in these genes were more likely to respond to preventive therapy with the drugs while women with unfavorable variations may not. In addition, women with unfavorable variations had a five-fold increased risk of developing breast cancer.

Dr. Ingle says the recent guidelines by the U.S. Preventive Services Task Force emphasize that selective estrogen receptor modulators (SERM) therapy with tamoxifen and raloxifene can lower a woman's risk for developing breast cancer. However, there currently is no way to know which women will benefit from the therapy.

"This is a major step toward truly individualized prevention of breast cancer," says Dr. Ingle. "Our findings provide clear direction as to which women are likely and which are unlikely to benefit from tamoxifen or raloxifene." Dr. Ingle says the findings provide the basis for a reinvigoration of research efforts in breast cancer prevention.

The researchers also studied breast cancer cell lines with the most common variation and the less common variation of the SNPs. They found that in cells with the most common variation of the SNPs, estrogen increased expression of both ZNF423 and CTSO and the expression of BRCA1, a gene associated with breast cancer risk. Estrogen did not increase expression of these genes in cells that had the less common form of the SNPs. Importantly, however, when tamoxifen or raloxifene were added to estrogen, there was a striking reversal in the patterns of expression of ZNF423 and BRCA1. In cells with the less common ZNF423 SNP, expression of ZNF423 and BRCA1 rose dramatically. This reversal in expression patterns provides a potential explanation for the decreased occurrence of breast cancer in women undergoing SERM therapy who carry this SNP.

NIH's National Institute of General Medical Sciences and National Cancer Institute funded this research through grants U19GM61388, P50CA116201, U10CA37377, U10CA69974, U24CA114732 and U01GM63173.

This article is reproduced from Mayo Clinic News network
http://newsnetwork.mayoclinic.org/discussion/genetic-variations-may-help-identify-best-candidates-for-preventive-breast-cancer-drugs-2a78c6

Original paper
Ingle JN, Liu M, Wickerham DL, Schaid DJ, Wang L, Mushiroda T, Kubo M, Costantino JP, Vogel VG, Paik S, Goetz MP, Ames MM, Jenkins GD, Batzler A, Carlson EE, Flockhart DA, Wolmark N, Nakamura Y, Weinshilboum RM. Selective Estrogen Receptor Modulators and Pharmacogenomic Variation in ZNF423 Regulation of BRCA1 Expression: Individualized Breast Cancer Prevention. ***Cancer Discov.*** 3, 812-825 (2013)

Drug Treatment Improves Survival of Insulin-producing Cells

Pretreatment with a calcium-blocking drug improves the effectiveness of islet transplantation for diabetes in mice

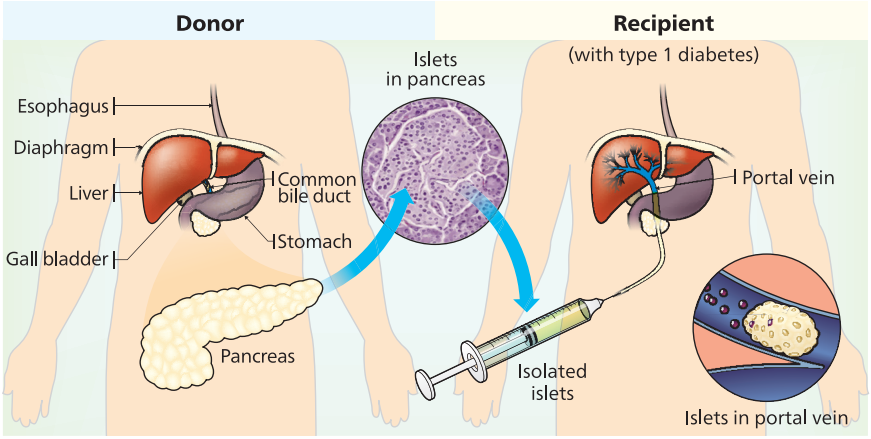

Figure: The process of clinical islet transplantation.
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The transplantation of insulin-producing islet cells from a donor pancreas could help diabetes sufferers avoid the need for daily insulin injections. However, the use of this experimental procedure is hampered by an immune response in the recipient that often rejects the transplanted islet cells. Masaru Taniguchi from Laboratory for Immune Regulation and Yohichi Yasunami from Fukuoka University have now led research that has improved the efficiency of the procedure by pretreating the islet cells with a drug that blocks the sodium–calcium exchanger (NCX) protein.

Islet transplantation often involves the use of islet cells from two or three different donors in order to achieve sufficient cell engraftment. "This low efficiency has been a major obstacle facing clinical islet transplantation," says Taniguchi.

Taniguchi, Yasunami and their respective lab groups set out to improve the islet transplantation procedure by pretreating islet cells prior to transplantation with a drug that blocks the NCX protein. This pretreatment protected the cells from innate immune responses in the liver, the site of islet transplantation, which led to longer-term survival of the cells in mouse models of diabetes.

"Pretreatment of donor islets with an NCX inhibitor prior to transplantation prevents early loss of transplanted islets and affords a new strategy to improve the efficiency of islet transplantation," says Taniguchi. Notably, the method allows for improved engraftment efficiencies without requiring transplant recipients to take any additional anti-rejection drugs. "Our new strategy to target donor islets does not add further risks to recipients," notes Taniguchi.

The drug pretreatment is thought to work by stopping NCX from boosting the intracellular levels of calcium ions. Normally, this calcium influx leads to low oxygen conditions, which trigger the release of a protein called high-mobility group box 1 (HMGB1) from islets soon after their transplantation. HMGB1 in turn activates immune cells, causing the early loss of transplanted islets. The NCX-blocking drug, called SEA0400, can prevent this cascade of events.

After transplanting pretreated and untreated islets into diabetic mice, the research team found that mice receiving SEA0400-treated islets displayed normal blood sugar control. In contrast, the control animals, which received the same number of untreated islets, experienced elevated blood sugar levels due to a lack of functional insulin-producing cells, which presumably had been attacked by the immune system. This same effect was seen whether the transplanted islets were human or murine in origin.

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Original paper
Mera, T., Itoh, T., Kita, S., Kodama, S., Kojima, D., Nishinakamura, H., Okamoto, K., Ohkura, M., Nakai, J., Iyoda, T., Iwamoto, T., Matsuda, T., Baba, A., Omori, K., Ono, J., Watarai, H., Taniguchi, M., Yasunami, Y. Pretreatment of donor islets with the $Na^+/Ca2^+$ exchanger inhibitor improves the efficiency of islet transplantation. ***Am J Transplant.*** 13, 2154-2160 (2013)

Fighting Asthma Drug Resistance

The poor efficacy of corticosteroid treatments in some patients with severe asthma could be overcome by blocking the action of an inflammatory protein

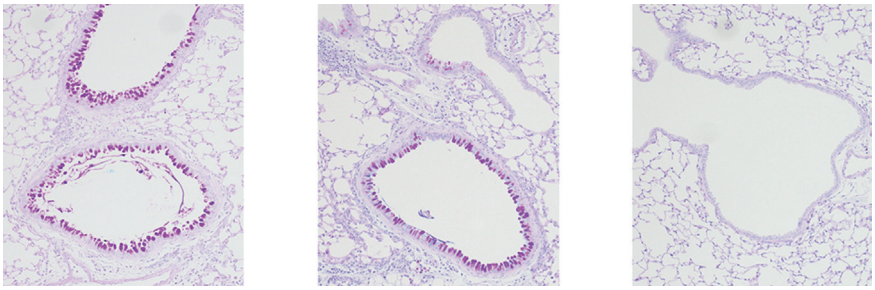

Figure: Asthmatic airway inflammation in mice induced by IL-33 and TSLP (*bright pink, left*) cannot be reduced by steroid treatment (*center*) but can be reduced when steroid treatment is augmented with the STAT5 inhibitor pimozide (*right*).
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Current asthma treatments include drugs that open up the tubes of the lungs and corticosteroids that fight lung inflammation. Some patients, however, are stubbornly resistant to corticosteroids, limiting the therapies available to them. Shigeo Koyasu and Kazuyo Moro from the Laboratory for Immune Cell System, in collaboration with Hiroki Kabata of the Keio University School of Medicine and Koichiro Asano of Tokai University and co-workers, have now found that the inflammatory protein called thymic stromal lymphopoietin (TSLP) and its downstream signaling molecules play key roles in the resistance of natural helper (NH) cells, a member of group 2 innate lymphoid cells (ILC2s), to the anti-inflammatory effects of corticosteroid treatment.

Asthma is a chronic disease of the airways characterized by persistent lung inflammation. The condition is known to be driven by the production of type 2 cytokines, such as interleukin (IL)-5 and IL-13, by NH cells.

The researchers developed a mouse model for this condition by administering an allergen and the pro-inflammatory cytokine IL-33 to mice. The treatment caused an increase in NH cells and mucus production in the lung, similar to the response observed in the lungs of asthmatic patients. Mice treated with corticosteroids did not show any improvement, indicating that the condition was resistant to the drug.

Koyasu and his colleagues found that the lungs of the mice that received the allergen and IL-33 also had higher levels of TSLP. Separately, they found that the addition of TSLP to NH cells cultured in the presence of IL-33 induced the proliferation of NH cells, even when corticosteroids were present. These findings suggest that TSLP causes NH cells to become resistant to the anti-inflammatory effects of corticosteroid treatment.

In other cell culture experiments the researchers found that in the absence of TSLP, NH cells died more readily under corticosteroid treatment, indicating that an antibody that blocks TSLP could eliminate corticosteroid resistance.

The team then discovered that in the presence of TSLP, NH cells activate the intracellular signaling protein STAT5. Pimozide, widely used as an anti-psychotic drug, is known to inhibit STAT5. Experiments confirmed that pimozide did indeed restore the susceptibility of NH cells to corticosteroids in cell culture (Fig.)

The research provides strong evidence that pimozide could be used to eliminate resistance to corticosteroids for the treatment of asthma. "Because pimozide has already been in clinical use for a while, this drug could be applied to patients with corticosteroid-resistant severe asthma after appropriate clinical studies are conducted," explains Koyasu.

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Original paper
Kabata, H., Moro, K., Fukunaga, K., Suzuki, Y., Miyata, J., Masaki, K., Betsuyaku, T., Koyasu, S., & Asano, K. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. ***Nature Commun.*** 4, 2675 (2013).

Rapid Recall to Fight Familiar Foes

By tinkering with immune cell development, researchers learn how the body mounts an accelerated response to recurring threats

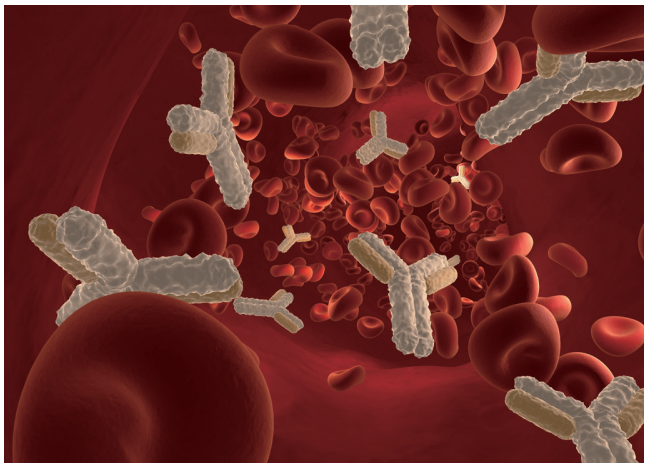

Figure: Memory B cells quickly recognize threats previously encountered by the immune system to mount a rapid and robust antibody response.
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The immune system's first encounter with a potential threat is a valuable learning experience. Through a process of genetic recombination, our immune B cells can produce a wide array of B cell receptor (BCR) molecules, each recognizing a distinct molecular target. When a naive B cell bumps into its specific target, it initiates an immune response that yields antibodies with the same specificity as its BCR, but also gives rise to 'memory cells' that can quickly recognize the target if it appears in the future. Working with genetically modified mice, Tomohiro Kurosaki's team, Laboratory for Lymphocyte Differentiation, has now gained insight into how memory cells mobilize.

"Memory B cells achieve rapid and robust antibody production during a secondary immune response, but its molecular mechanism was unknown," explains Kohei Kometani, a researcher in Kurosaki's lab and lead author of the study.

Naive B cells typically produce BCRs and antibodies that belong to the immunoglobulin M (IgM) class of proteins. In contrast, memory B cells produce an alternative immunoglobulin G (IgG) subtype of BCRs and antibodies through an additional gene recombination process called 'class switching'. Some researchers believe that the IgG form of BCR contains structural elements that stimulate the memory B cell response, while others have favored cellular mechanisms besides IgG-induced signaling.

In initial experiments, the researchers worked with IgM naive and IgG memory cells that specifically recognize a known antigen, 4-hydroxy-3-nitrophenylacetyl (NP). As expected, the former cells proliferated in response to NP, while the latter promptly developed into antibody-secreting plasma cells, as occurs in a typical secondary immune response. To determine whether IgG is specifically responsible, Kurosaki's group generated cloned mice derived from an IgG memory cell that recognizes NP.

The resulting animals produced NP-specific IgG naive B cells, which do not normally occur in nature. Remarkably, these cells responded to NP by proliferating in essentially the same fashion as IgM naive cells, suggesting that IgG alone does not drive the memory response. A comparative analysis of gene expression revealed an alternative mechanism, controlled by a protein called Bach2. "We found that Bach2 is reduced in memory B cells, and is important for their enhanced antibody production," says Kometani.

By revealing this IgG-independent mechanism, these results should help resolve the long-standing debate over memory cell function. However, this finding is just a starting point, and Kurosaki's group is now engaged in exploring the upstream factors that switch off Bach2 production in memory cells.

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Original paper
Kometani, K., Nakagawa, R., Shinnakasu, R., Kaji, T., Rybouchkin, A., Moriyama, S., Furukawa, K., Koseki, H., Takemori, T. & Kurosaki, T. Repression of the transcription factor Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation. ***Immunity*** 39, 136–147 (2013).

Helping Cells Make a Commitment

Multiple levels of gene inhibition help determine and maintain the developmental destiny of immune cells

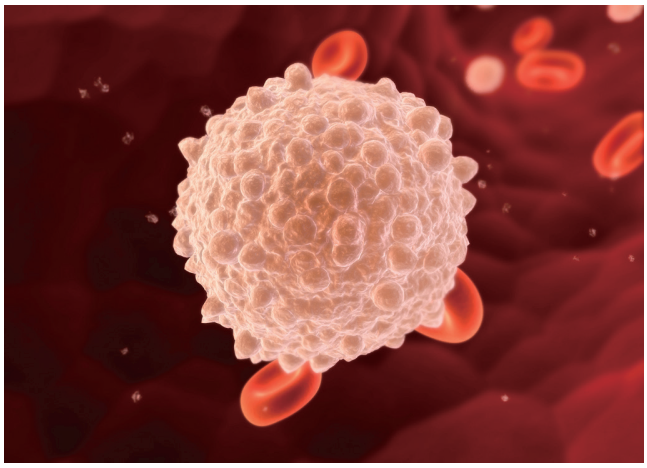

Figure: Researchers at RIKEN have investigated the role of the *Thpok* gene in the development of the two classes of immune T cells.
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Immune T cells can be broadly categorized into two classes: cytotoxic T lymphocytes (CTLs), which directly kill infected or cancerous cells, and helper T cells, which coordinate the overall immune response. Immature T cells commit to one or the other lineage via a process called positive selection, and generally remain locked into their developmental fate from that point onward. The Laboratory for Transcriptional Regulation led by Ichiro Taniuchi has now gained insight into how these cells remain dedicated to their career path.

It is known that the *Thpok* gene is a critical activator of helper T cell development, and gets switched off in developing CTLs by a regulatory region called the silencer. However, Taniuchi's team determined that the silencer only provides the initial deactivation signal, and requires further support to make its orders 'official'.

Many genes undergo what is known as epigenetic regulation through the addition of various chemical modifications to the chromosome. For instance, different patterns of 'histone methylation' can either activate or repress nearby genes. The researchers determined that while the silencer facilitates the accumulation of repressive methylation patterns at the *Thpok* gene in CTL precursors, helper T cell precursors exhibit activating methylation patterns. When the researchers selectively deleted the silencer in cells that had already committed to CTL development, *Thpok* remained repressed, indicating that these marks are critical to long-term inhibition of this gene.

Taniuchi and his colleagues found that by engineering additional copies of the silencer region into the *Thpok* gene, they could induce repression in helper T cells where the gene would otherwise be active. If introduced into T cell precursors, these additional silencer copies caused the accumulation of repressive histone methylation marks normally observed in CTLs, and disrupted normal helper T cell development. This demonstrates the critical role of the silencer in establishing T cell fate."Our finding that the epigenetic processes that stably silence *Thpok* can occur independently of commitment to the cytotoxic-lineage is striking," says Hirokazu Tanaka, lead author of the study.

Importantly, their work also suggests how these instructions might be overridden. Previous studies have suggested that prolonged signaling via the T cell receptor (TCR) specifically promotes helper T cell development, and these prolonged signals may work by preventing inhibitory modifications that would otherwise accumulate through the influence of the *Thpok* silencer. "Our findings provide for the first time an epigenetic view as to why persistent TCR signals are necessary," says Tanaka.

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Original paper
Tanaka, H., Naito, T., Muroi, S., Seo, W., Chihara, R., Miyamoto, C., Kominami, R., Taniuchi, I. Epigenetic *Thpok* silencing limits the time window to choose CD4$^+$ helper-lineage fate in the thymus. ***EMBO J.*** 32, 1183–1194 (2013)

Building Blocks Help Silence Genes

Head-to-tail connections in gene-repressing complexes maintain proper DNA regulation during embryo development and tumor formation

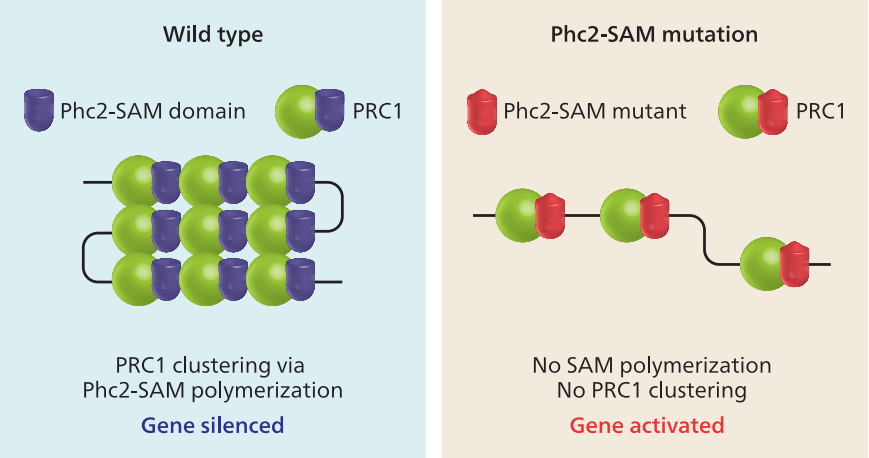

Figure: A functional SAM domain is needed to facilitate PRC1 clustering and PcG-mediated gene silencing.
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Polycomb-group (PcG) proteins play an important role in controlling gene expression. Complexes containing PcG proteins are thought to inhibit or 'silence' gene activity by localizing to specific targets in the genome and remodeling how DNA is wound up into chromosomes, but the exact mechanism by which these complexes repress gene activity remains poorly understood. Kyoichi Isono, Haruhiko Koseki and colleagues from the Laboratory for Developmental Genetics have now pinpointed the part of a critical PcG protein complex that is essential for maintaining a robust yet reversible gene repression program during both mammalian development and cancer progression.

Isono, Koseki and their colleagues set out to identify the formation mechanism of a cluster of PcG proteins known as Polycomb-group repressive complex-1 (PRC1). They focused their attention on a particular domain within one of the molecules in the PRC1 complex: the sterile alpha motif (SAM) of polyhomeotic-like protein 2 (Phc2). The SAM domain helps to keep Phc2 proteins in the same orientation, facilitating head-to-tail, building-block-like linking of repeated copies of the PRC1 complex (Fig.)

The team created human cells designed to express Phc2 carrying a mutation in the SAM domain. This mutation prevented PRC1 binding but did not affect the basic assembly of each complex. Nonetheless, the researchers observed a substantial reduction in PRC1 cluster or 'body' formation in the cell nucleus, indicating that the construction of linked repeats of PRC1, driven by SAM domain polymerization, may be necessary for proper functioning of the complex. In embryonic mice genetically engineered to possess a mutant SAM domain, Isono, Koseki and their team observed defects in the developing skeleton, further supporting the notion that the aberrant PRC1 bodies were not maintaining proper gene regulation in the absence of the SAM domain.

The findings illuminate a key aspect of mammalian embryo formation. According to Isono, however, the results could also have implications that go well beyond the understanding of basic developmental processes. "The repressive function of PRC1 has a strong impact on not only pluripotency and differentiation of stem cells but also tumorigenesis," he says. "The mechanism that underlies SAM polymerization and depolymerization could be useful for regenerative medicine and cancer therapy."

A particularly exciting potential therapeutic target might be compounds that destroy the interactions between SAM domains, which could halt tumor growth in cancer patients. "I believe we can develop such drugs by conducting microscopic screenings for PcG body morphology," Isono says.

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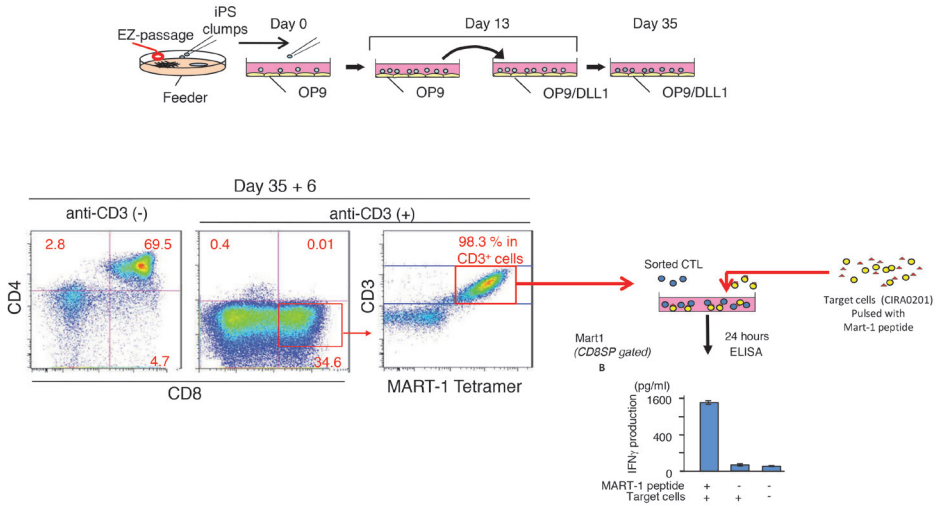
Original paper
Isono, K., Endo, T. A., Ku, M., Yamada, D., Suzuki, R., Sharif, J., Ishikura, T., Toyoda, T., Bernstein, B. E. & Koseki, H. SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. ***Dev Cell.*** 26, 565–577 (2013).

iPS Project

Induced pluripotent stem (iPS) cells possess tremendous therapeutic potential in the fields of both regenerative medicine and immune therapy. We have started an activity to apply iPS technology to mouse and human immunology research and therapeutic development. On a collaborative basis with individual research activities in RCAI, the core facility for iPS research is engaged in developing efficient protocols to reprogram various lymphocytes and induce differentiation of iPS cells into lymphoid lineage cells. This activity is partly supported by JST.

This year, the facility established iPS cells from mature cytotoxic T cells specific for the melanoma epitope MART-1. JKF6 cells are long-term cultured tumor infiltrating lymphocytes that were originally derived from a melanoma patient and have been maintained at the Surgery Branch of the National Cancer Institute. JKF6 cells are specific for the complex of MART-1-peptide and HLA-A*02:01, and can be visualized as MART-1-tetramer$^+$ cells by flow cytometry. These MART-1-specific T cells were transduced with Yamanaka factors, after which they established clones that form colonies with human ESC-like morphology (Vizcardo R., et al., 2013). When co-cultured with OP9/DLL1 cells, the MART-1-iPS cells efficiently generated CD8$^+$ T cells, and more than 90% of these cells were specific for the original MART-1 epitope. Stimulation of these CD8$^+$ T cells with HLA-A*02:01-expressing cells pulsed with MART-1-peptide resulted in the secretion of IFNγ. The present study thus provides a novel method for cloning and expanding functional CD8$^+$ T cells specific for a given antigen, which can potentially be applied for immune cell therapy against cancer.

Figure: iPS based approach for regeneration of functional antigen-specific CTLs.
MART-1 derived iPSCs were separated into small clumps and plated on OP9 feeder cells. On day 13, cells were transferred to co-culture with OP9-DLL1 feeder cells. On day 35, cells were stimulated by anti-CD3 mAb for inducing CD8$^+$ cells. After stimulation, CD8$^+$ CD3$^+$ MART-1 tetramer$^+$ cells were sorted for an INFγ production assay. They were co-cultured with a human EBV-transformed lymphoblastoid cell line expressing HLA-A*02:01 with or without MART-1 peptide for 24hr. These cells produced a substantial amount of INFγ in the presence of the specific peptide.



Identification of Novel Causative Genes for Common Variable Immunodeficiency

To understand the mechanisms responsible for homeostasis in the human immune system, we sought to identify novel causative genes for common variable immunodeficiency (CVID), through a collaboration with Tokyo Medical and Dental University (TDMU). CVID is the most frequent symptomatic primary immunodeficiency encountered in adults.

To this end, we have carried out whole exome analysis (WEA) of DNA samples obtained from five patients with CVID and their family members. After conventional WEA with HiSeq 2000, variant calling, gene annotation, and elimination of single nucleotide variants at IMS, we identified one to eight potential somatic changes for each patient. One of the genes identified as disease-causing was a gene that has been reported as responsible for a severe type of CVID.

We also identified a few candidate heterologous mutations that were each only found in a single patient or in a patient and the mother. These mutations have not been reported in the literature, and their function in the human immune system is yet to be explored. TMDU will sequence the identified candidate genes in the >200 CVID samples in their cohort, and IMS investigators are in the process of determining their functions in the immune system by using different models.

Modeling Skin Diseases

Laboratory for Immunogenetics
Laboratory for Integrative Genomics
Laboratory for Disease Systems Modeling
Laboratory for Integrated Bioinformatics
Laboratory for Developmental Genetics
Laboratory for Metabolomics
Laboratory for Immune Homeostasis
Laboratory for Cytokine Regulation
Laboratory for Respiratory and Allergic Diseases

The pathogenesis of human diseases is a highly complex process because of the complexity of not only pathogenetic insults and homeostatic responses but also of the structures of organs and tissues that include many cells of different origins. To tackle such complexity, we have initiated center-wide projects to understand the pathogenesis of atopic dermatitis (AD), autoimmune diseases, primary immunodeficiency and others, in which multiple research groups work interactively and synergistically to achieve their common objectives. In each project, we first aim to understand molecular and cellular networks underlying homeostasis of each organ/tissue. We have initiated this project with skin because GWAS data for AD and related diseases and several unique dermatitis model animals are already available. Skin is a complex and highly dynamic organ where there are continuous processes of proliferation, differentiation, migration, and death of cells in addition to constant surveillance by immune and neural systems. This implies that highly integrative efforts, from basic research to clinical applications, are required to model skin homeostasis.

In order to unify the expertise of the various IMS laboratories in a coordinated manner, it is important to thoroughly considered project planning with specific milestones. Our primary goal in the AD project, and hence the initial milestone, is to use a combined computational and experimental approach to investigate the molecular interaction network leading to the onset of AD, its underlying dynamics and how they play a role in the progression of skin dysfunction in AD.

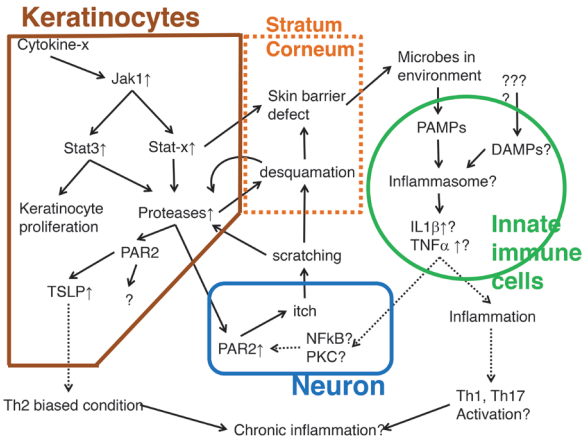


Figure: Molecular and cellular events preceding atopic dermatitis onset in mouse skin tissue
A genetic factor, a point mutation in the Jak1 signal transduction molecule, induces protease over-expression in the epidermis and induces sequential events in various cells in skin tissue under the influence of environmental factors and ultimately results in atopic dermatitis.

Collaboration with the SardiNIA Project

The SardiNIA project was originally launched by the National Institute on Aging (NIA) in NIH (US) and Italian investigators (https://sardinia.irp.nia.nih.gov/) to unravel the genetic processes involved in age-related traits and diseases in people living on the Mediterranean island of Sardinia. As an extension of the SardiNIA project, they have focused on immune diseases and recently elucidated human genetic variations regulating immune cell levels in health and disease (*Cell*, 2013, 155(1):242-256). However, how variations in immune cell levels eventually result in immune diseases, e.g. autoimmunity, still remains unclear. Thus, to bridge the gap between immune cell levels and disease etiology, we have begun to collaborate with the SardiNIA group through generation of mice with genetic variations corresponding to those found in humans: Based on the human genetic variations identified by the SardiNIA group, genetically engineered mice have been designed and are under development at IMS. In addition, we have performed protein-protein interaction assays to evaluate the impact of the genetic variation(s) in the protein-coding region identified by the SardiNIA genome cohort at the molecular level. In this regard, the collaboration with the SardiNIA group serves as an extramural version of the on-going IMS projects, which aim to fill the gap between research in humans and mice. Thus, we have very good reasons to collaborate with the SardiNIA group toward our shared goal. We expect this tri-lateral collaboration will considerably enhance the international visibility of our "integrative medical science (IMS)" approach using animal models.

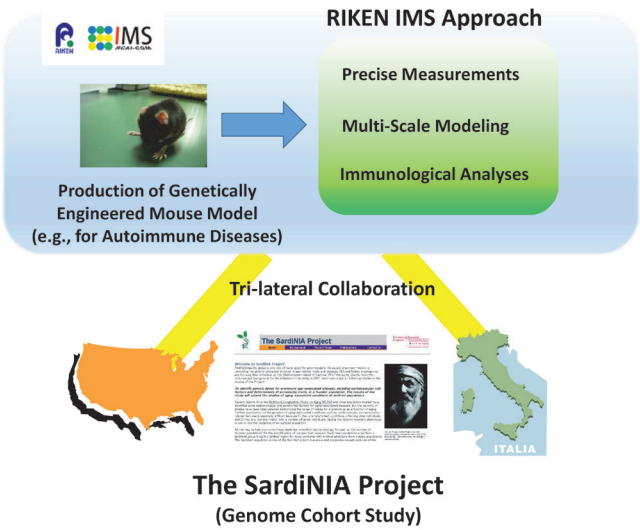


Figure: IMS Approach in a Tri-Lateral Collaboration with the SardiNIA Project

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

IMS aims to contribute to the identification of new treatments for cancer and other diseases by promoting collaboration with DMP. The DMP was founded in RIKEN in 2010 in order to support all phases of development of new therapeutics, from the discovery of promising targets to the identification of potential lead compounds such as small molecules and antibodies, and the acquisition of intellectual property rights to drugs and technologies that can then be brought to the development phase. The implementation of drug discovery requires different technology, thus DMP established several medical technology platforms that promote research and development. IMS contributes to this effort in several ways, including by setting up a facility for development of antibody drugs (Drug Discovery Antibody Platform Unit lead by Toshitada Takemori).

IMS now has five programs in association with DMP, including cancer treatment with NKT cells (Masaru Taniguchi), allergy prophylaxis (Masaru Taniguchi), Artificial adjuvant vector cells (Shin-ichiro Fujii), Leukemia treatment drugs targeting leukemic stem cells (Fumihiko Ishikawa), and a Mucosal Vaccine delivery System (Hiroshi Ohno). Some of programs will provide the transfer of potential drug candidates to pre-clinical and clinical phases of drug development.

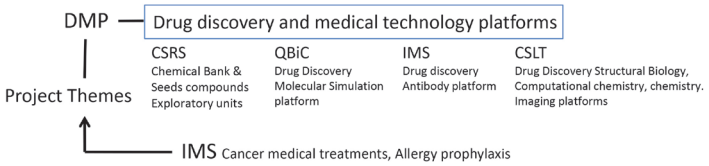


Figure: IMS links to the DMP project

Humanized Mouse Research

We have created humanized mice by injecting human cord blood hematopoietic stem cells (HSCs) into immune-deficient NOD/SCID/Il2rgKO (NSG) newborns. Though the NSG humanized mouse model has enabled us to achieve high levels of human leukocyte engraftment in multiple organs, limitations remain due to species barriers between human immune cells and the mouse microenvironment. In the last few years, in collaboration with Dr. Leonard Shultz at the Jackson Laboratory, the IMS Laboratories for Human Disease Models, Developmental Genetics, and Integrative Genomics have been trying to develop mice with a humanized microenvironment. By infecting humanized mice with EBV, we have shown the development of HLA-restricted human $CD8^+$ T cells in HLA class Iexpressing NSG mice (PNAS 2010). We are currently evaluating whether a peptide vaccine can elicit an Ag-specific CTL response in the HLA class I TG NSG mice.

For humanized BM environment, we have developed NSG mice expressing two distinct splice variants of human SCF, the membrane-bound (SCF220) and soluble (SCF248) forms. In both strains, we found significantly higher engraftment of human $CD45^+$ leukocytes in the bone marrow compared with conventional NSG mice (Blood 2012). In addition, expression of human SCF in the mouse environment has overcome human B cell-dominant engraftment in the recipient mouse BM that has been considered as one of the major differences between humanized mouse BM and native human BM. We aim to clarify the potentially distinct roles of membrane-bound and soluble forms of SCF in supporting human HSC/hematopoietic progenitor cell development and in human granulocyte development.

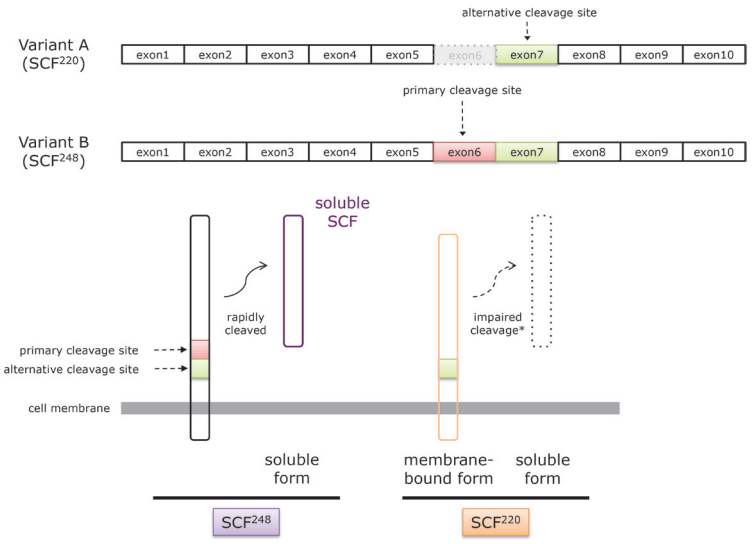


Figure: Development of NSG mice expressing human SCF

We have created new strains of NSG mice expressing two distinct spice variants of stem cell factor (SCF), membrane-bound form and soluble form. One of the variants (Variant A: SCF220) lacks the primary cleavage site, therefore human SCF is not secreted into the BM but remains as a membrane bound form.

NKT Project

NKT cells are known to enhance immune responses. The medical innovation groups in IMS have launched projects aimed at application of NKT cells to cancer and allergic disease as follows.

Based on previous success using NKT ligand, α-galactosylceramide (α-GalCer)-loaded dendritic cells (DCs) against advanced lung cancer, we started a new collaboration with the National Hospital Organization of a randomized phase IIa trial in early stage lung cancer and also a phase IIa trial for head and neck cancer, in collaboration with Chiba University. Second, based on two previous studies in which we demonstrated the efficacy of human iPS-derived T cells (Cell Stem Cell 2013) and mouse iPS-NKT cells (JCI 2010), we will establish human iPS-NKT cells. This IMS iPS project on NKT cell-targeted therapy has been accepted as a Center for Clinical Application Research in the Research Center Network for Realization of Regenerative Medicine, Japan in 2013. Third, we previously established artificial adjuvant vector cells against cancer that are composed of tumor mRNA and α-GalCer leading to activation of both innate and adaptive immunity. We have been working on preclinical studies with the human artificial adjuvant vector cells and have begun discussions about preclinical studies with the Pharmaceuticals and Medical Devices Agency (PMDA). Forth, we have developed a new chemical compound that selectively induces apoptosis of IgE B cells, resulting in the preferential suppression of IgE production. A contract between a pharmaceutical company and us for a drug applicable for asthma, pollinosis or food allergy has been made.

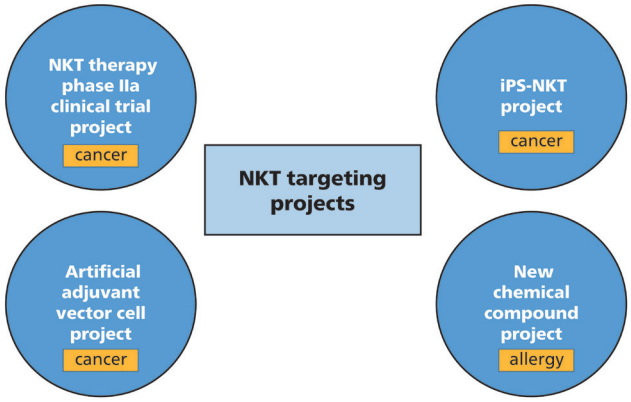


Figure: NKT projects in IMS as translational research
Four different NKT translational research projects have been launched in IMS. Three projects are for cancer treatment and one project is for treatment of allergic diseases.

Allergy Project

A chemical compound that selectively induces suppression of total IgE production has been developed. This compound carries an α-GalCer analog, RCAI-X, and selectively targets marginal B cells (MZB), but not DCs or macrophages. These B cells, after taking up the compound, express IgE mRNA, IL-21 receptor, and CXCL16 to recruit NKT cells. The NKT cells activated by RCAI-X presented by the MZB cells produce IL-21. The NKT cell production of IL-21 in turn acts on these MZB cells to induce phosphorylation of Bmf, causing it to detach from the dynein light chain and bind with Bcl-2, inhibiting its function and inducing apoptosis of the IgE B cells. Bmf expression in IgG B cells is very low, therefore, the compound only affects IgE B cells and not IgG B cells. The RCAI-X compound, even at doses in the 1-100 ng/kg ranges, resulted in significant suppression of not only the antigen-specific secondary IgE but also of total IgE production (Fig.). A contract with a pharmaceutical company (Kaken Pharmaceutical Co. Ltd.) was completed in 2013 to develop a drug applicable for asthma, pollinosis or food allergy. The project is supported by the RIKEN Drug Discovery and Medical Technology Platforms and the Scientific Research Fund from Health and Welfare.

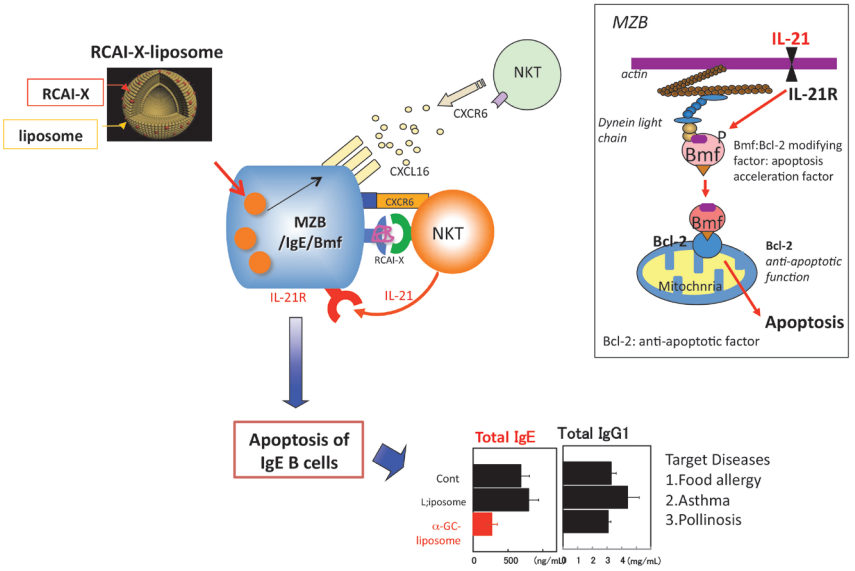


Figure: Development of a compound mediating IgE-specific suppression by RCAI-X-liposomes

Influenza Project

Influenza is a major life-threatening infectious disease in our society. Although the current vaccination method effectively reduces the risk of death, the following issues still remain.

1. What are the host factors that contribute to pathogenesis during the acute phase of infection?

Pathogenesis developed during infection varies among individuals. Several reports have suggested that dysregulation of immune responses might contribute to the pathogenesis. To understand the contribution of immune responses to pathogenesis, we have taken a systems biological approach. Gene expression profiles of subsets of tissue-invaded immune cells over time after infection are generated and analyzed.With this large dataset, we expect to identify new biomarkers, especially to predict the risk of death. The new biomarkers should enable us to prevent deaths from influenza by combining them with existing drugs.

2. Generating a next-generation vaccine against a broad range of influenza viruses.

Several groups have succeeded in isolating human and rodent antibodies that are broadly reactive with various influenza virus strains such as H1N1 and H5N1, findings that point to the feasibility of developing a universal vaccine. These antibodies mostly recognize the stem region of the Hemagglutinin (HA) protein, a region that is well-conserved at the amino acid sequence level. We are designing synthetic peptides to induce B cell antibody responses that can broadly neutralize influenza virus infections. Three teams from the research fields of immunology/biochemistry, computer science and structural biology are running this project, which aims to generate a next-generation vaccine for any upcoming pandemics resulting from antigenic shift and drift (Figure).

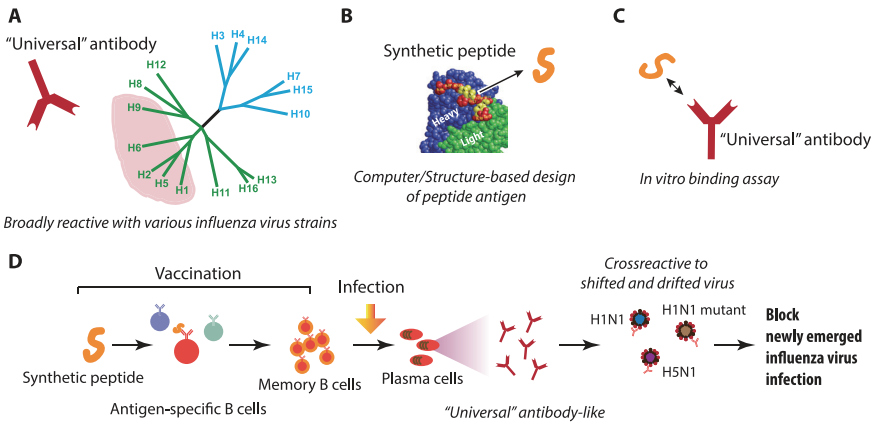


Figure: Crafting a universal vaccine for pandemic Influenza
A) Cross-reactivity of a "Universal" antibody. B) Numerous influenza peptides are designed and their affinities for "universal" antibody are predicted *in silico*. C) Selected peptides are subjected to biochemical binding analyses using the universal antibody. D) Potential peptides for the vaccine will be evaluated for their ability to generate "universal" antibody *in vivo* and to protect animals, and ultimately humans, from infection with various influenza virus strains.

PGRN-CGM International Collaborative Studies

The U.S. NIH Pharmacogenomics Research Network (PGRN) is a consortium of research groups funded as individual cooperative agreements by the NIH. PGRN investigators are top researchers from U.S. academic institutions and conduct studies of variation in human genes relevant to drug metabolism, pharmacokinetics and pharmacodynamics, and the relationship of the genetic variation to drug responses. Principal investigators of the PGRN and RIKEN Center for Genomic Medicine (now RIKEN IMS Core for Genomic Medicine: CGM) held a series of discussions on the need to accelerate discoveries in pharmacogenomics (PGx) and launched the Global Alliance of Pharmacogenetics (GAP) in 2008.

In this international collaboration, the PGRN has been successfully assembling an abundance of DNA samples from well-phenotyped patients receiving specific drugs and drug combinations in clinical trials conducted in the U.S. The CGM focuses on high-throughput genome-wide SNP scan with technological and methodological expertise to identify genetic factors associated with drug responses, risk of severe adverse drug reactions and non-response to medications. Together, the PGRN-CGM capitalizes on these strengths to advance discoveries in PGx research. More than 30 collaborative studies for various drug responses are ongoing to identify genomic biomarkers, which will develop better and safer medications and realize the dream of global personalized medicine.

Figure: Pharmacogenomics Research Network (PGRN) - RIKEN IMS Core for Genomic Medicine (CGM) strategic alliance.
Please visit http://bts.ucsf.edu/pgrn-cgm/

Collaboration with Asian Institutes and SEAPharm

One of the aims of the Laboratory of International Alliance on Genomic Research is to promote research collaborations around the globe. We have established connections with the Institute of Biomedical Sciences at Academia Sinica in Taiwan where we will explore several diseases and pharmacogenetic studies (PGx) together. In addition, we will also work closely with the Taiwan Biobank to study complex diseases and PGx across the two populations. It has been noticed that rare adverse skin reactions called SJS/TENS occur at a much higher frequency in East Asian and Southeast Asian populations and that these drug induced adverse reactions have strong genetic associations. To tackle this problem, we established the South East Asian Pharmacogenomics Research Network (SEAPharm) together with five Asian countries (Korea, Indonesia, Malaysia, Taiwan, and Thailand). The aim of the collaborative effort is to identify significant PGx events important to the region, so that we can identify genetic markers associated with these adverse drug reactions, which could then lead to a reduction in these events. Our first project will be to study the genetic associations of phenytoin-induced SJS/TEN. In addition, we also aim to understand how the identified genetic markers lead to the adverse events. It is hoped that through the discoveries from our collaborative efforts, we will identify useful biomarkers that can be used to predict drug-induced adverse events, guide drug use and be useful in disease prediction/diagnosis.

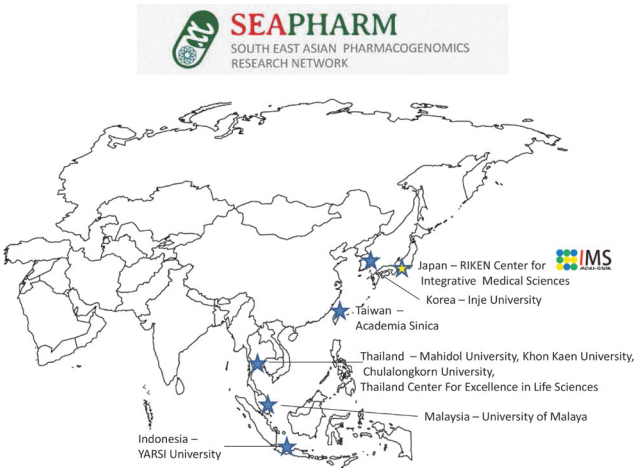


Figure: South East Asian Pharmacogenomics Research Network (SEAPharm)

International Cancer Genome Consortium (ICGC)

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

ICGC has been organized to launch and coordinate a large number of research projects that have the common aim of comprehensively elucidating the genomic changes present in many types of cancers. Its primary goals are to generate comprehensive catalogues of genomic abnormalities in different cancer types and to make the data available to the entire research community with minimal restrictions. At the end of 2013, 71 cancer genome projects across 16 countries and the EU were ongoing, and the ICGC released the genomic data from 8,532 cancer samples as Release 14 (September 26th, 2013). Our RIKEN group has been involved with the genome project of liver cancer, which is one of the most common and deadly cancers worldwide, especially in Asia. We performed whole genome sequencing (WGS) of 270 liver cancers and called somatic mutations by using our in-house pipeline. We deposited WGS data of 153 liver cancers so far, and released them as a Japanese ICGC project. As an internal working group, we are involved with benchmark comparison studies, where eleven genome centers analyze the same raw sequence data or the same DNA by each of their pipelines and sequencing platforms to compare their results of somatic mutation identification. ICGC has launched a "pan-cancer" genome project, where 2000~ cancer WGS data will be analyzed in the same pipeline by a world-wide collaboration. We are contributing to this ambitious project as a member of technical working group and data center, as well as by providing 300 WGS data.

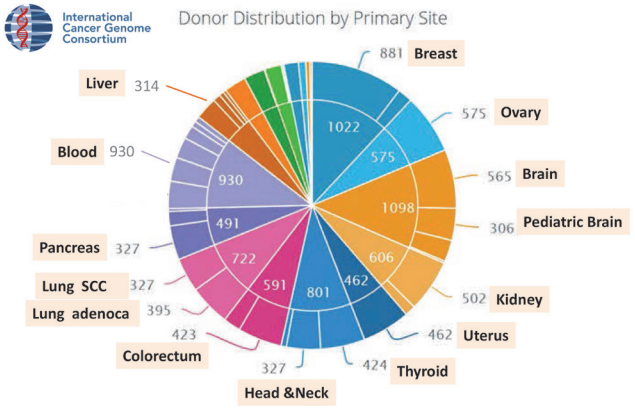


Figure: ICGC Data Release 14 (September 26th, 2013)

The BioBank Japan Project

The BioBank Japan project was started as a leading project of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in 2003 [Project leader: Yusuke Nakamura (FY2003-2011), Michiaki Kubo (FY2011-present)]. In the first 5-year period (FY2003-2007), this project constructed the BioBank Japan, which collected DNA, serum and clinical information from 200,000 patients who suffered from at least one of the 47 target diseases. In the second 5-year period (FY2008-2012), this project performed GWAS for various diseases using the samples stored in the BioBank Japan and identified many susceptibility genes for various diseases and drug responses. In the third period of 5 years from 2013, this project is expanding the BioBank infrastructure to collect DNA and clinical information from an independent cohort of 100,000 patients who suffer from at least one of 38 target diseases, including kidney cancer, dementia and depression as new disease targets. In collaboration with other national projects, BioBank Japan will further promote genomic research and move forward to apply its findings to clinical research.

Website: http://www.biobankjp.org/index.html

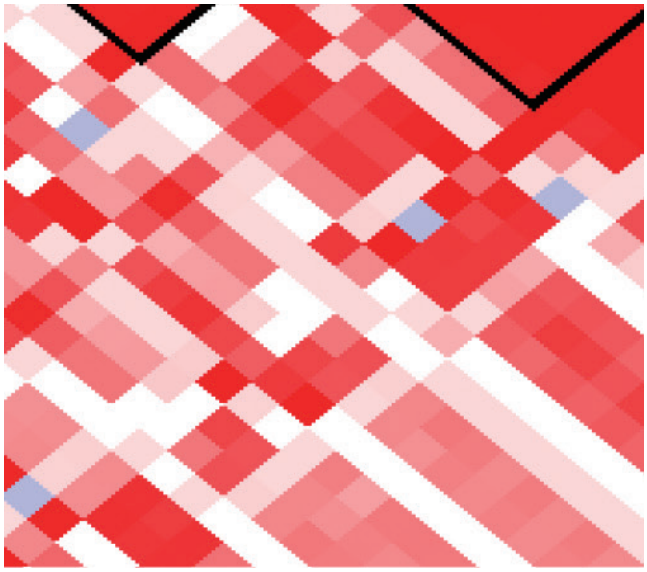
Photo: DNA Storage
Photo: Blood Serum Storage

Genome-guided drug Therapy Optimization Project (G-TOP)

The Genome-guided drug Therapy Optimization Project was started in December, 2011 as a top-down project of MEXT to validate the clinical utility, clinical efficacy and cost-effectiveness of pharmacogenomic research findings by clinical intervention study and to implement pharmacogenomic testing into clinical use [Project leader: Michiaki Kubo (FY2011-present)]. Currently, this project conducts three clinical interventional studies for the prevention of Carbamazepine-induced skin rush (GENCAT), genome-guided dose adjustment of Warfarin for safer anticoagulation (GENCAT), and genome-guided dose adjustment of tamoxifen for breast cancer therapy (TARGET-1). The Core for Genomic Medicine and the BioBank Japan organize all three projects in collaboration with many universities and hospitals in Japan. The Research Group for Pharmacogenomics (Group Director: Mushiroda) also manages this project.

Website: http://www.biobankjp.org/pgx/index.html

Figure: Website of the Genome-guided drug Therapy Optimization Project (G-TOP)



Part 3

Events

RIKEN IMS Summer Program (RISP) 2013

IMS was delighted to continue the RISP, now RIKEN IMS Summer Program, in 2013 after starting our new institute. RISP started in 2006 with the goal of offering training in current topics in immunology as well as providing networking opportunities on an international scale for young immunologists. It was also geared to encourage future collaboration and postdoctoral training experiences in Japan, so that RISP has included an internship program in which the participants perform research in the IMS laboratories. RISP is now co-organized by the Chiba University Leading Graduate School Program.

In RISP 2013, forty-seven graduate students/postdoctoral fellows from 18 countries and six students from Chiba University gathered at Yokohama from June 21st to 28th. The RISP 2013 program started with a tour of IMS research facilities, which include advanced two-photon microscopes, HILO microscope for single molecule imaging and conventional cell sorting instruments as well as a new CyTOF mass spec-based sorter. This was followed over the next four days by 12 lectures by Japanese and foreign distinguished scientists and oral and poster presentations by RISP students. The RISP program ended with participation in an annual international symposium, co-organized by IMS-RCAI and the Japanese Society for Immunology.

RISP 2013 was a success, as all of the students indicated in the RISP evaluation survey that they would recommend the program to colleagues and would encourage them to apply for a position at IMS-RCAI. IMS is planning to continue RISP, updating some of the lecture topics as necessary to keep pace with recent developments in this rapidly moving field of life science.

The 8th IMS-JSI International Symposium on Immunology 2013

The 8th IMS-JSI International Symposium on Immunology, hosted by the RIKEN Center for Integrative Medical Sciences (IMS), in conjunction with the Japanese Society for Immunology (JSI), was held on June 27-28 in Yokohama. This was the first international event since the launch of IMS in April 2013, which was in transition from RCAI, Research Center for Allergy and Immunology (2001-2013). Eighteen internationally-recognized speakers presented their cutting-edged research at the symposium entitled "Interface between Immune System and Environment", which attracted more than 350 participants. The symposium consisted of five sessions: Interface between gut and immune system; Interface between the lung and immune system; Interface between the skin and immune system; Molecular mediator between environment and immune system; Metagenomic analysis of the human immune system. Many different subpopulations of immune cells are active on the front line at the barriers of our body, such as skin, intestinal and bronchial mucosa. Microbiota and their metabolites affect these immunological actions relevant to both innate and acquired immunity, and might be linked to human diseases. Structural microenvironments established at the barriers, such as mucosa-associated lymphoid tissues, also appear to play a role in immunological memory functions of the acquired immune system. The research field of "Interface between Immune System and Environment" brings us a novel view to better understand the immune system and human diseases. Furthermore, expansion of large datasets from metagenomic analysis will be a powerful resource for the research community in this field.

The 8th PGRN-RIKEN Strategic Alliance Meeting

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

On June 19-20, 2013, CGM hosted the 8th PGRN-RIKEN Strategic Alliance Meeting at the Business Support Floor of the Landmark Tower Yokohama, in Yokohama, Japan. Some 40 participants attended this meeting, and it provided a valuable forum for exchanging information on ongoing collaborative activities: Aromatase Inhibitors and Bone Fractures, Adverse Cardiovascular Outcomes and New-onset Diabetes after Antihypertensive Therapy, Pharmacogenetics of Prostacyclin Dosing in Pulmonary Arterial Hypertension, Colorectal Cancer Pharmacogenetics, and SSTI Pharmacogenomics. Participants from PGRN and RIKEN also had in-depth discussions and shared ideas about their research projects. In addition, participants explored new collaborative research proposals as well as data analysis issues. Finally, it was agreed to hold this meeting on a regular basis to further enhance collaborations between PGRN and CGM, and the meeting successfully concluded.

LJI & IMS Workshop

The 5th joint workshop between LJI (La Jolla Institute of Allergy and Immunology, formerly abbreviated as LIAI) and IMS "New Horizon in Immune Regulation towards Disease Intervention" was held on October 30-31 in the RIKEN Yokohama campus. The workshop featured 11 speakers from both institutes. The acting director of IMS, Dr. Koyasu started by introducing the outline of IMS, and this was followed by the introduction of LJI by the director Dr. Kronenberg. Both speakers stressed the importance of continuation the mutual interactions and collaborations. The workshop had four scientific sessions on the 1st day: Development and function of T cells, Allergy and inflammation I, Regulation and intervention of diseases, Signal regulation of diseases, and three sessions on the 2nd day: Mucosal regulation and microbiota, Allergy and inflammation II, Systems and Immunology.

The topics covered broad aspects in lymphocyte development, signal transduction, mucosal regulation, allergy and inflammation, and integrative approaches to understand immune responses. One of the most cutting edge topics was the mucosal regulation of immune responses by the microbiota. In addition, the speakers from both institutes emphasized the study of diseases, particularly allergic skin diseases, inflammation, cancer, and diabetes.

Based of the facts that both institutes have similar goals, targets, and aspects of research and that two LJI investigators (Cheroutre and Kawakami) are now also PIs in IMS beginning this year, the two institutes promised to develop further strong relationships and collaborations.

Harvard Summer School 2013

IMS offers a summer internship program for undergraduate students from Harvard University. In this program students do a research internship in the IMS laboratories and have basic immunology lectures by IMS PIs and a Japanese language course. They also participate in the RIKEN IMS Summer Program (RISP) and the International Symposium on Immunology, organized by IMS and JSI. The participants receive course credit from Harvard. In 2013, we had three students from Harvard University (James Carey, Albert Li and Kathleen Wallace), and one from Brown University (Marjorie Palmeri) from June 3 to August 12.

Carey conducted his research project in the Laboratory for Immune Regulation (Dr. Taniguchi), Li in the Laboratory for Immunogenetics (Dr. Yoshida), Wallace in the Laboratory for Vaccine Design (Dr. Ishii), and Palmeri in the Laboratory for Cytokine Regulation (Dr. Kubo). During their internships, the students had numerous discussions with IMS researchers and, at the end of the program, they gave oral presentations describing their scientific results.

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

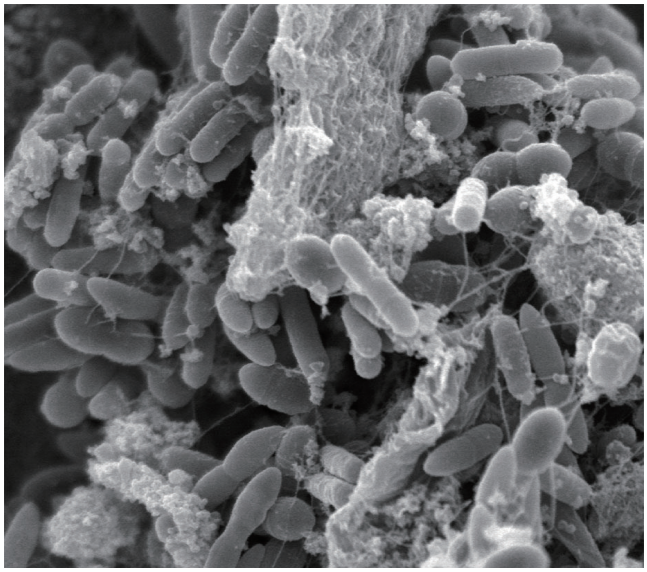
Photo: Cultural Exchange at Science Frontier High School

Adjunct Professorship Programs

IMS collaborates with and accepts graduate students from 11 domestic university graduate schools. There are now a total of 35 adjunct professors/associate professors in IMS (Table), and 118 students studied at IMS in 2013. On May 25, IMS held a briefing session on adjunct graduate school programs. Thirteen students participated from Kyushu, Kyoto, Kanagawa, Tokyo, Saitama, and even from Ireland. The session provided an opportunity for students to visit and talk directly with lab leaders and to consider their future directions.

Table: Adjunct professorship programs

Graduate Program	Affiliated RCAI Investigator
Graduate School of Frontier Bioscience, Osaka University	Tomohiro Kurosaki (Professor), Ichiro Taniuchi (Visiting Professor), Keigo Nishida (Visiting Associate Professor)
Graduate School of Medicine, Osaka University	Takashi Saito (Visiting Professor), Toshiyuki Fukada (Visiting Associate Professor)
Department of Immunology, Graduate School of Medicine, Chiba University	Takashi Saito (Visiting Professor), Haruhiko Koseki (Visiting Professor), Hiroshi Ohno (Visiting Professor), Shinichiro Fujii (Visiting Associate Professor), Yasuyuki Ishii (Visiting Associate Professor), Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Pharmaceutical Sciences, Chiba University	Osamu Ohara (Visiting Professor)
School of Biomedical Science, Tokyo Medical and Dental University	Takashi Saito (Visiting Professor)
Graduate School of Medicine, Yokohama City University	Michiaki Kubo (Visiting Professor), Shiro Ikegawa (Visiting Professor), Mayumi Tamari (Visiting Professor), Tatsuhiko Tsunoda (Visiting Professor), Hidewaki Nakagawa (Visiting Professor), Shiro Maeda (Visiting Professor), Taisei Mushiroda (Visiting Professor), Atsushi Takahashi (Visiting Professor)
Graduate School of Medical Life Science, Yokohama City University	Hiroshi Ohno (Visiting Professor), Haruhiko Koseki (Visiting Professor), Mariko Okada (Visiting Professor), Takaharu Okada (Visiting Associate Professor), Kazuyo Moro (Visiting Associate Professor)
Research Institute of Biological Sciences, Tokyo University of Science	Masato Kubo (Professor), Osamu Ohara (Visiting Professor), Shohei Hori (Visiting Associate Professor), Tadashi Yokosuka (Visiting Associate Professor)
Graduate School of Medicine, Kyoto University	Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Medicine, Keio University	Masayuki Amagai (Professor), Shigeo Koyasu (Visiting Professor), Haruhiko Koseki (Visiting Profefssor)



Part 4

Data and Statistics

Publlications 2013

Table: IMS Publlications Jan-Dec, 2013

Journals	Impact Factor (2012)	Number of Papers
Lancet	39.06	1
Nature	38.60	5
Nat Genet	35.21	5
Nat Rev Immunol	33.13	1
Science	31.03	1
Nat Immunol	26.99	4
Cell Stem Cell	25.32	2
Nat Med	24.30	1
Immunity	19.80	7
Mol Cell	15.28	1
J Exp Med	13.21	1
Dev Cell	12.86	2
Gastroenterology	12.82	1
J Clin Invest	12.81	3
Cell Host Microbe	12.61	1
J Allergy Clin Immunol	12.05	3
Am J Hum Genet	11.20	1
Sci Transl Med	10.76	1
Gut	10.73	1
Cancer Discov	10.14	1
Nat Commun	10.02	2
Proc Natl Acad Sci U S A	9.74	7
Annu Rev Genomics Hum Genet	9.50	1
EMBO J	9.49	2
Blood	9.06	3
Cancer Res	8.65	1
PLoS Genet	8.52	2
Diabetes	7.90	1
Hum Mol Genet	7.69	1
Am J Gastroenterol	7.55	1
Oncogene	7.36	1
Clin Pharmacol Ther	6.85	3
Diabetologia	6.49	2
J Clin Endocrinol Metab	6.43	1
Philos Trans R Soc Lond B Biol Sci	6.23	1
Development	6.21	1
Int J Cancer	6.20	1
J Invest Dermatol	6.19	1
Am J Transplant	6.19	1
Haematologica	5.94	1
Allergy	5.88	1
Breast Cancer Res	5.87	1
FASEB J	5.70	2
J Med Genet	5.70	1
J Immunol	5.52	7
Neoplasia	5.47	1
Hum Mutat	5.21	1
Other Journals		98
Total		**189**

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Guest Lectures 2013

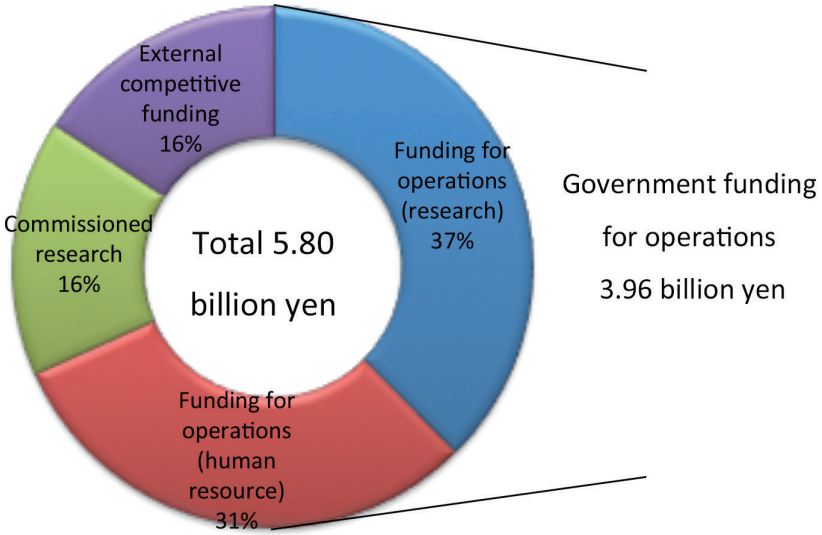
Table: Guest Lectures Jan-Dec, 2013

Date	Speaker	Affiliation	Country	Title
23-Jan-13	Dr. Shinichi Nishikawa	Center for Developmental Biology, RIKEN	Japan	Development of Hematopoietic Stem Cell: A final scenario
28-Jan-13	Dr. Becca Asquith	Imperial College London	UK	Efficiency of the CD8+ T cell response to persistent viral infection
15-Feb-13	Dr. Wilfred T.V. Germeraad	GROW School for Oncology and Developmental Biology Maastricht University	Neth.	Development of new Immunotherapies for cancer
07-Mar-13	Dr. Denis Duboule	Department of Genetics and Evolution, University of Geneva	Italy	Long range regulation of HoxD genes during mammalian development
07-Mar-13	Dr. Yusuke Miyanari	Institute of Genetics and Molecular and Cellular Biology (IGBMC)	France	Reprogramming and nuclear dynamics
13-Mar-13	Dr. Eugene Oltz	Washington University School of Medicine	USA	Defining the Malignant Epigenome in Follicular Lymphoma
02-Apr-13	Dr. Akira Imamoto	The Ben May Department for Cancer Research,The University of Chicago	USA	The CRK gene family: A tale of a small family business for mesenchymal property management
10-Apr-13	Dr. Koichi S Kobayashi	College of Medicine, Texas A&M Health Science Center	USA	Regulation of MHC class I and class II pathways by NLR family Proteins
15-Apr-13	Dr. Tsvee Lapidot	Dept. of Immunology, The Weizmann Institute of Science	Israel	Stem cell interactions with the BM microenvironment
27-May-13	Dr. Yumi Nakamura	Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School	USA	Understanding the link between Staphylococcus aureus colonization and atopic dermatitis
05-Jun-13	Dr. Fubito Nakatsu	Howard Hughes Medical Institute, Yale University School of Medicine	USA	Phosphoinositide metabolism at the plasma membrane: From signaling to membrane dynamics
07-Jun-13	Dr. Yoshiyuki Asai	Okinawa Institute of Science and Technology Graduate University	Japan	Tutorial of PhysioDesigner and Flint
13-Aug-13	Dr. Takeshi Egawa	Washington University School of Medicine	USA	Requirements for transcription factors in regulation of T cell activation
05-Sep-13	Dr. Patrick Varga-Weisz	Babraham Institute	UK	Chromatin Dynamics and Epigenetic Stability
27-Sep-13	Dr. Naoto Kubota	Graduate School of Medicine, The University of Tokyo	Japan	The roles of IRS-1 and IRS-2 in the regulation of glucose and lipid metabolism
07-Oct-13	Dr. David Scott	Department of Medicine, Uniformed Services University for the Health Sciences	USA	Gene therapy for tolerance, IgG fusion proteins and specific Tregs
11-Oct-13	Dr. Masahira Hattori	Graduate School of Frontier Sciences, The University of Tokyo	Japan	Ethnic variances of the human gut micro biome
16-Oct-13	Dr. Huh, Jun R	University of Massachusetts Medical School	USA	Small molecule inhibitors of RORγt: their development to study the function of inflammatory immune cells
17-Oct-13	Dr. Toshiaki Maruyama	Abwiz Bio Inc.	Japan	The use of phage display for the development of monoclonal antibodies from immune libraries as research reagents, diagnostics and therapeutics
21-Oct-13	Dr. Kaoru Saijo	School of Medicine, University of California, San Diego	USA	Nuclear Receptor-mediated Regulation of Neuroinflammation
14-Nov-13	Dr. Masatsugu Ohora	Medical Institute of Bioregulation, Kyushu University	Japan	Calcium signaling and T cell development
09-Dec-13	Mr. Minoru Yano	IDT-MBL KK.	Japan	Improved methods/reagents for target enrichment in NGS
18-Dec-13	Dr. Takashi Ebihara	Howard Hughes Medical Institute, Washington University Medical Center	USA	NK cell licensing and development: How do NK cells acquire functional competence?

Budget, Personnel and Patents

IMS Budget FY2013	JPY Million
Government funding for operations	3,963
Commissioned research	921
External competitive funding	917
Total	**5,801**

An additional supplementary budget (4,400 million yen) was provided in 2013 for commissioned research "Order-made Medicine Realization Program".



Personnel FY2013

Category	Number
Acting Director	1
Special Advisor	2
Deputy Director	4
Group Director	8
Team Leader	26
Deputy Team Leader	1
Coordinator	2
Senior Scientist	30
Postdoctoral Researcher	18
Research Scientist	44
Research Associate	13
Research Fellow	8
Special Postdoctoral Researcher	4
Foreign Postdoctoral Researcher	1
International Program Associate	4
Junior Research Associate	20
Student Trainee	94
Senior Technical Scientist	2
Technical Scientist	6
Technical Staff	137
Assistant	39
Consultant	10
Senior Visiting Scientist	9
Visiting Scientist	137
Visiting Technician	33
Temporary Employment	11
Total	**664**

Patents 2013

There were 19 patents filed from January to December, 2013.

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Cover image: Intravital two-photon
image of T cells and dendritic cells in the lymph node.
Image courtesy of Laboratory for
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