PROGRESS REPORT
Division of Cell Fate Regulation

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Division of Cell Fate Regulation

Chairman: Ryo Goitsuka, Ph.D., D.V.M.

After many rewarding years, my research group, the Goitsuka lab, has been officially closed on March 31, 2025. Since I launched this lab in 2006, we have been on a journey to understand the interaction of long-lived cells, such as hematopoietic stem/progenitor cells and embryo-derived B cells, with their surrounding specialized microenvironments, namely, niches. The success of our research projects would not have been possible without the support of our collaborators, students, and administrative staff. Although the lab is closing, the impact of the research and the spirit of innovation will endure through the future endeavors of its alumni. Thank you for being part of this incredible journey. Best wishes are extended to everyone in their next chapters.

The J chain acts as a critical regulator for intestinal IgA$^+$ plasma cell differentiation

The J chain is essential for IgA dimer formation and poly-Ig receptor binding. However, it shows no homology to any known protein structure, and there are no family molecules, the function of the J chain is incompletely understood, regardless of its immunological importance. We thus sought to examine the functions of the J chain in intestinal IgA-secreting plasma cells with the use of the reporter allele, in which a splice acceptor, EGFP-2A-CreERT2 cassette, and a poly-A sequence are knocked into the first intron of the J chain gene. In the pups harboring homozygous reporter alleles that lack the intact J chain protein (JKO), EGFP$^+$ IgA$^+$ cells were detected only at the base of the intestinal lamina propria, but absent in the villi, where EGFP$^+$ IgA$^+$ cells were abundant in heterozygous pups before weaning. RNA-seq analyses revealed that JKO EGFP$^+$ IgA$^+$ cells lack expression of CXCR5, a ligand of which CXCL13 is selectively expressed at the tip of the villi, suggesting the inability to migrate to the tip of the intestinal villi. To understand the life of cells expressing the J chain before weaning, newborn pups simultaneously having the Rosa26-tdTomato allele were treated with tamoxifen within 24 hours after the birth, and reporter expression in EGFP$^+$ IgA$^+$ plasma cells was analyzed at 10 weeks after the birth. In the intestinal lamina propria, approximately 20-30% of IgA$^+$ plasma cells were tdTomato$^+$ EGFP$^+$, whereas tdTomato$^+$ plasma cells expressing either IgA or IgM were almost absent in other tissues, including the bone marrow, spleen, Peyer's patches, and mesenteric lymph nodes. These tdTomato$^+$ EGFP$^+$ IgA$^+$ plasma cells disappeared by the loss of the J chain. Gene expression profiles differed between tdTomato$^+$ EGFP$^+$ and tdTomato$^-$ EGFP$^+$ cell populations, implying the distinct nature of intestinal IgA$^+$ plasma cells before and after weaning. Of note, IgA$^+$ plasma cells derived from before weaning express IL-18 and P2rx1, the former involving intestinal immune tolerance and the latter functioning as a sensor for extracellular ATP potentially provided by intestinal epithelial cells. Taken together, these findings indicate that intestinal IgA$^+$ plasma cells developed before weaning require the J chain to reach the niche of the villi, which leads to the persistent residence at the tip of the villi throughout life, and that these cells maintain the immune tolerance in the intestinal microenvironment.

Collaborators:

Fujisaki K., Okazaki S., Ogawa S., Kon, S.

Publications

Nozaki, Y., Kobayashi, M., Fukuoh, T., Ishimatsu, M., Narita, T., Taki, K., Hirao, Y., Ayabe, S., Yokoyama, M., Otani, Y., Mizunoe, Y., Matsumoto, M., Ohno, N., Kaifu, T., Okazaki, S., Goitsuka, R., Nakagawa, Y., Shimano, H., Iwakura, Y., and Higami, Y.: Mipep deficiency in adipocytes impairs mitochondrial protein maturation and leads to systemic inflammation and metabolic dysfunctions. ***Sci. Rep***., 15 (1):12839. (2025)

Fujisaki K., Okazaki S., Ogawa S., Takeda M., Sugihara E., Imai K., Mizuno S., Takahashi S., and Goitsuka R.: B cells of early-life origin defined by RAG2-based lymphoid cell tracking under native hematopoietic conditions. ***J. Immunol.***, 213(3):296-305. (2024)

Sawabe A., Okazaki S., Nakamura A., Goitsuka R., and Kaifu T.: The orphan G protein-coupled receptor 141 in myeloid cells functions as an inflammatory suppressor. ***J. Leukoc. Biol.***, 115(5):935-945. (2024)

Division of Cell Fate Regulation

Masayuki Sakurai, Ph.D.

Research Interest: *Deciphering Molecular Mechanisms of Nucleobase Modification:*

In the gene expression process—the "central dogma of life," in which the necessary genes are transcribed from DNA to RNA and translated into proteins as functional entities—proper gene expression is maintained by diverse regulatory mechanisms at each step. One such regulatory mechanism is the chemical modification of the four bases [A, G, C, T(U)] of DNA and RNA, which themselves encode genetic information. Our laboratory aims to elucidate biological phenomena that ultimately arise from changes in the chemical structures of these nucleobases at the molecular level.

Research Topics

We currently focus on the modification of adenosine (A) into inosine (I, Ino) through deamination. In canonical Watson–Crick base pairing, which is essential for the central dogma, A pairs with thymine (T) or uridine (U) as an [A:T(U)] pair, and guanosine (G) pairs with cytidine (C) as a [G:C] pair. After deamination, however, inosine forms base pairs with C as G does. Consequently, base pairing is altered from A:T(U) to Ino:C, which is equivalent to an A-to-G change in the genetic information and is called the A-to-I editing mechanism.

This mechanism was discovered in metazoans and is mediated by adenosine deaminases acting on double-stranded RNAs (ADARs), which use intracellular double-stranded RNA as a substrate. ADARs play important roles in transcript sequence diversification, alternative splicing, RNA–protein interactions, translation efficiency, and innate immunity against exogenous RNAs. Historically, ADARs were thought to act specifically on double-stranded RNA. However, recent work including our own has demonstrated that ADARs can also edit both RNA and DNA strands in RNA:DNA hybrids, thereby mediating A-to-I RNA editing and A-to-I DNA editing. This finding suggests that mammals harbor an intrinsic A-to-G base-editing mechanism in genomic DNA driven by A-to-I DNA editing.

We view RNA-driven editing of the chemical structures of nucleobases in DNA and RNA as a regulatory layer that rewires and optimizes the central dogma. We aim to establish this as a new research field, "nucleic acid editing biology," in which RNA—historically overlooked in the central dogma—is repositioned as the mediator that links DNA, RNA, and proteins. Our major research goals are (i) to establish technologies for labeling, purifying, and identifying inosine sites in RNA and DNA strands, which previously has been extremely difficult; (ii) to elucidate how DNA:RNA hybrid formation and inosinylation regulate gene expression and genome dynamics; and (iii) to develop artificial methods for site-specific introduction or inhibition of A-to-I editing in RNA and DNA for applications in medicine and genetic engineering.

Establishment of labeling and purification techniques for inosine-modified nucleic acids (ICLAMP)

Detecting inosine on DNA—where only two molecules per locus exist from homologous chromosomes—or on lowly expressed RNAs has long been a major technical challenge. To overcome this, we have developed detection and identification methods that exploit the unique chemical properties of inosine.

We previously developed the ICE method, which uses an inosine-specific cyanoethylation reaction to distinguish inosine from guanosine even after PCR amplification, achieving ~97% accuracy and compatibility with next-generation sequencing. However, the sensitivity of this method depends on transcript abundance, limiting its application to genomic DNA and low-abundance regulatory noncoding RNAs. We further advanced this work by establishing a fluorescent and functional tag labeling technique that selectively modifies inosine in both RNA and DNA. We optimized conditions for inosine-specific fluorescent labeling and other functional group tagging in nucleic acids and succeeded in affinity purification of inosine-containing nucleic acid molecules based on these tags. We named this technology ICLAMP (inosine-chemical labeling and affinity molecular purification), filed a patent application, and reported the main results in FEBS Letters. Using ICLAMP coupled with next-generation sequencing, we have begun identifying inosine sites in low-abundance RNAs and DNA from cultured human cells and mouse tissues and are currently analyzing and optimizing these datasets.

Collaborators:

Yuxi Yang (Sakurai Lab); Takeshi Wada, Kazuki Sato (Tokyo University of Science); Michiaki Hamada, Chao Zeng (Waseda University); Hiroki Ueda, Yuriko Sakaguchi, Ayaka Murayama, Tsutomu Suzuki (The University of Tokyo).

Inter-individual variation in A-to-I RNA editing and database construction

We aim to clarify inter-individual, sex-specific, and ethnic differences in A-to-I RNA (and DNA) editing. A-to-I editing represents a reconstruction layer of the central dogma that cannot be inferred from genome or transcriptome sequencing alone. We hypothesize that previously overlooked differences in editing patterns contribute to variations in cancer susceptibility, disease incidence, lifespan, and overall health, by finely tuning gene expression at the level of base modification.

As a foundation, we are building a database of A-to-I editing sites suitable for comparative analyses. Using commercially available RNA samples and our ICLAMP/ICE-seq platform (inosine chemical labeling coupled to next-generation sequencing), we have begun comprehensive identification of editing sites and constructed pilot datasets. In cultured cells (HeLa and HEK293T), we have modulated ADAR expression levels (knockdown or overexpression) and performed pilot ICE-seq analyses to validate the sensitivity and specificity of our pipeline for detecting differential editing.

We are performing the pilot experiments and refine a computational pipeline to detect, filter, and score A-to-I editing sites and to evaluate their reliability. Next, we will apply ICE-seq to RNA from paired cancer and adjacent non-cancer tissues obtained from research-use sample repositories, to characterize both cancer–normal differences and inter-individual variation in editing. In parallel, we will analyze RNA resources from healthy, cancer, and disease cohorts to define editing signatures associated with specific phenotypes or molecular mechanisms.

Collaborators:

Yuxi Yang, Mai Kubota (Sakurai Lab); Michiaki Hamada, Chao Zeng (Waseda University).

ADAR-mediated regulation of DNA:RNA hybrids and R-loop dynamics

This project focuses on how ADAR proteins recognize and bind double-stranded RNA and DNA:RNA hybrid strands in cells and how this regulates A-to-I editing and genome function. We pay particular attention to R-loops, structures formed when newly transcribed RNA remains hybridized to the template DNA strand, leaving the non-template DNA strand single-stranded. R-loops are implicated in genome instability and transcriptional regulation and represent a likely site for A-to-I DNA editing on DNA:RNA hybrids.

Phenotypic analyses under ADAR suppression in cultured cells revealed increased phosphorylation of γH2AX, RPA32, and DNA-PKcs, indicating enhanced DNA damage and activation of DNA repair pathways. Phosphorylation analyses of Cyclin B1, Histone H3, CDC2, and PLK1 further demonstrated mitotic arrest and apoptosis under ADAR knockdown conditions. In HeLa cell nuclei, R-loop localization overlaps with that of ADAR, and ADAR suppression leads to an accumulation of R-loops. We are now dissecting the molecular mechanism underlying mitosis-specific arrest observed upon ADAR suppression. We have found that genes involved in the spindle checkpoint, genes controlling mitotic entry, and cohesin components form complexes with ADAR in M phase, and that protein phosphorylation plays an important role in this regulatory process.

To clarify ADAR dynamics in M phase, we have performed DNA-IP and RNA-IP using ADAR followed by next-generation sequencing, revealing that ADARs accumulate at centromeric regions specifically in mitosis. We are currently testing whether RNA is required in this mechanism by comprehensively mapping R-loop regions. Transcriptome analysis under ADAR suppression identified the transcription factor EGR as the most strongly upregulated gene, and we are examining how ADAR and R-loops regulate EGR transcription.

Collaborators/

Yuxi Yang, Ryotaro Yanoshita, Yuki Minato, Kokone Hasegawa, Daiki Sakamoto, Mai Kubota and Eito Ichihashi (Sakurai Lab); Michiaki Hamada (Waseda University); Kazuko Nishikura (The Wistar Institute).

"RNA Reincarnation": dynamics of double-stranded RNA and DNA:RNA hybrids in A-to-I editing

Traditionally, RNA transcribed from DNA has been viewed as following a one-way path: splicing, translation, and eventual degradation. Recent findings suggest, however, that RNA degradation products can influence the transcription of other genes. We call this cyclic flow—from RNA "death" via degradation to "rebirth" as new transcription—"RNA reincarnation", conceptualized as a circular central dogma. We hypothesize that RNA reincarnation endows gene expression networks with robust yet flexible responses to environmental changes and may represent a fundamental principle underlying differentiation and disease.

To obtain direct evidence for RNA reincarnation, we are constructing experimental systems to trace how degradation-derived RNAs base-pair with genomic loci they regulate. Specifically, we fused the deaminase domain of ADAR to UPF1, a key factor in RNA decay, to introduce A-to-I editing marks into degradation-derived RNAs, thereby labeling them. Construction of expression vectors for this UPF1–ADAR deaminase fusion is in progress.

In parallel, we have created expression vectors for a fusion of the ADAR deaminase domain with NF90, a protein that binds double-stranded RNA and RNA:DNA hybrids, and confirmed its deaminase activity.

Collaborators:

Terutaka Kubota (Sakurai Lab); Michiaki Hamada (Waseda University); Nobuyoshi Akimitsu (The University of Tokyo).

"Artificial A-to-I editing of target nucleic acids and therapeutic applications

Recently, RNA editing oligonucleotides have been developed that can introduce A-to-I RNA editing at specific adenosines using nucleic-acid-based designs alone. Such molecules enable transient rewriting of genetic information, allowing (i) correction of pathogenic mutations and (ii) functional modulation of oncogenes without altering their expression levels.

Efficient in vivo delivery is another critical component of nucleic acid therapeutics. Our collaborator, Dr. Takeshi Wada (Tokyo University of Science), has developed Fol-Dab, an innovative delivery system that selectively delivers nucleic acid therapeutics to cancer cells. Because RNA editing nucleic acids form double-stranded structures, they are promising candidates for Fol-Dab–based delivery. By combining RNA editing and cancer cell-selective delivery, we aim to establish a novel technology that performs target-specific RNA editing only in cancer cells.

We constructed a genome-integrated reporter system in cultured human cells in which successful A-to-I editing leads to expression of green fluorescent protein (GFP), enabling real-time measurement of editing efficiency. By mimicking the flanking sequences of naturally occurring ADAR targets with very high editing efficiency, we designed guide sequences that, in vitro, are edited more efficiently by ADAR1 than by other ADAR family members. We are now applying this design to repair a G>A mutation in the endogenous mRNAs by A-to-I editing in cultured cells.

Collaborators:

Yuxi Yang, Koki Takemura, Terutaka Kubota (Sakurai Lab); Takeshi Wada (Tokyo University of Science); Masatora Fukuda (Fukuoka University); Kumiko Tei (The University of Tokyo).

Publications

- Yang, Y., Sakurai, M. "Detection technologies for A-to-I RNA editing: facilitating functional exploration of editing sites", *WIREs RNA* 16:e70014. [DOI: 10.1002/wrna.70014, PMID: 40223708] (2025)


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