# Annual Report (2024)

## No.36
## The NOVARTIS Foundation (Japan) for the Promotion of Science

2024年度

# 財団年報 第36号

公益財団法人 ノバルティス科学振興財団

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Chairman of the Board of Trustees

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# Introduction

Kuniaki Takata, Ph.D.
Chairman of the Board of Trustees

This annual report includes research reports from the 36th Novartis Research Grant recipients (research from April 2023 to March 2024: 39 research grants and 5 research meetings).

Looking back on the history of life science, the development of molecular biology that began with the elucidation of the DNA double-helix structure by Watson and Crick was epoch-making. The determination of amino acid sequences by cloning, experimental manipulation of protein expression, and other techniques have revealed the functions of biomolecules one after another. It is now possible to describe and analyze in detail the functions and interactions of cells, tissues, and organs in terms of not only biology, but also chemistry, physics, mathematics, and others. Now, with the rapid development of digital technology and AI, new knowledge is being generated using the vast amount of information obtained. We are truly living in an exciting era in which the frontiers of knowledge in the life world are moving rapidly.

The Novartis Foundation (Japan) for the Promotion of Science was established in 1987 with a donation of 1 billion yen from Ciba-Geigy AG (now Novartis AG) of Switzerland. The purpose of the Foundation is clearly stated in its Articles of Incorporation as "to promote science by encouraging creative research in the natural sciences, thereby contributing to the improvement of the health and welfare of the people. In addition, a document titled "Prospectus for Establishment" written in the year of the foundation's establishment states that the foundation "seeks to promote and support creative research in the natural sciences, which will be the axis of science in the coming 21st century, thereby contributing to the welfare of mankind" and that the foundation "provides financial support for research and offers opportunities for cross-border exchange. The policy of the Foundation is to "promote creative research in the natural sciences and thereby contribute to the welfare of humanity. Based on this policy, the Foundation has provided a total of 1,989 grants over the past 37 years, amounting to approximately 2.26 billion yen. In these times of rapid change, the Foundation is determined to return to its roots and support the excellent research that will open the way to the next era, whether it be for those who are developing their own original ideas or for those who are considering new projects.

This annual report summarizes the results of the excellent research supported by the Foundation. These are admirable accomplishments achieved in the limited time of one year. The list of past recipients of the Foundation's grants includes many leading researchers in their fields, such as Dr. Hideki Shirakawa, who received the Nobel Prize in Chemistry, and Dr. Tasuku Honjo, who received the Nobel Prize in Physiology or Medicine. We hope that the recipients of this grant will use the results of their research to make even greater strides in their fields. I would like to express my deepest gratitude again to the selection committee members who selected these outstanding research projects, and to Novartis Pharma K.K., the donor, and all those involved in supporting the activities of the Foundation.

# はじめに

代表理事　高田　邦昭

本年報には、第 36 回ノバルティス科学振興財団の研究助成金を受けられた方々の研究報告（2023 年４月～ 2024 年３月の研究：研究奨励金 39 件、研究集会 5 件）を収録しました。

生命科学の歴史を振り返って見ると、ワトソン、クリックによる DNA 二重らせん構造の解明から始まった分子生物学の発展は画期的でした。クローニングによるアミノ酸配列の決定、たんぱく質発現の実験的な操作等により、生体分子機能が次々と明らかになりました。そして、細胞、組織、器官のはたらきや相互作用を、生物学のみならず、化学、物理学、数学等のことばで記述し、詳細に解析することが可能となりました。今や、デジタル技術と AI の急速な発展により、得られた膨大な情報を使って新たな知が生み出されつつあります。まさに私たちは、生命世界における知のフロンティアが急速に動いている刺激的な時代を生きていると言えます。

本財団は 1987 年に、スイス、チバガイギー社（現ノバルティスファーマ社）からの 10 億円のご寄附をもとに設立されたものです。財団の目的は、定款に「自然科学における創造的な研究の奨励等を行うことにより、学術の振興を図り、もって国民の健康と福祉の向上に寄与する」と明記されています。また、財団設立の年に記された「財団設立の趣意」と題する文書には、財団が「来るべき 21 世紀の科学の軸となる自然科学の創造的研究の振興助成をはかり、以って人類の福祉に寄与できれば」とあり、「研究のための資金的な助成、並びに国境を越えた交流の場の提供」がうたわれています。このような方針のもと、37 年間で総計 1989 件、金額にして約 22.6 億円の助成を行ってきました。日々目まぐるしい変化の起こる現在においてこそ、財団は改めてこの原点に立ち返り、独自のアイデアを膨らませている方、新たなプロジェクトを考えている方などの、次の時代を拓く優れた研究を支援して行きたいと考えています。

この年報には本財団が支援した優れた研究の成果をまとめています。一年間という限られた時間の中で達成した立派な業績です。過去に当財団の助成を受けた方々のリストには、ノーベル化学賞を受賞した白川英樹博士やノーベル医学・生理学賞を受賞した本庶佑博士をはじめ各分野をリードする研究者の名前が多数見られます。今回助成を受けた方々が、この研究成果を契機としてさらに大きく飛躍されることを祈念いたします。これらの優れた研究を選考していただいた選考委員の皆様や、出捐者であるノバルティスファーマ社をはじめとして財団の活動を支えて下さっている関係者の皆様に改めて深く感謝いたします。

# Ⅱ.

# Reports from the Recipients of Novartis Research Grants

# Y Chromosomal Genes Protective against Heart Failure

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## Summary Abstract

Our previous work has shown that the presence of mosaic loss of Y chromosome (LOY) in leukocytes is causally associated with increased risk for heart failure. In the current study, we show that LOY macrophages from the failing hearts of humans with dilated cardiomyopathy exhibit widespread changes in gene expression that correlate with fibroblast activation in the myocardium, and we identify the ubiquitously transcribed tetratricopeptide Y linked (*Uty*) gene in leukocytes as a causal locus for accelerated progression of heart failure in male mice with LOY.

***Key Words*** : Heart failure, hematopoietic loss of Y chromosome, UTY epigenetic regulator, CRISPR/Cas9

## Introduction

Our recent research has substantiated a potential causal link between LOY in leukocytes and heart failure. In both humans and mice, loss of the entire Y chromosome in leukocytes has been correlated with adverse cardiovascular outcomes. A key question is whether the cardiovascular effects of LOY are simply a consequence of the aneuploid condition or whether the Y chromosome encodes genes that are required for cardiovascular homeostasis.

## Results

**Disruption of *Uty* in blood cells accelerates cardiac dysfunction in the TAC model**

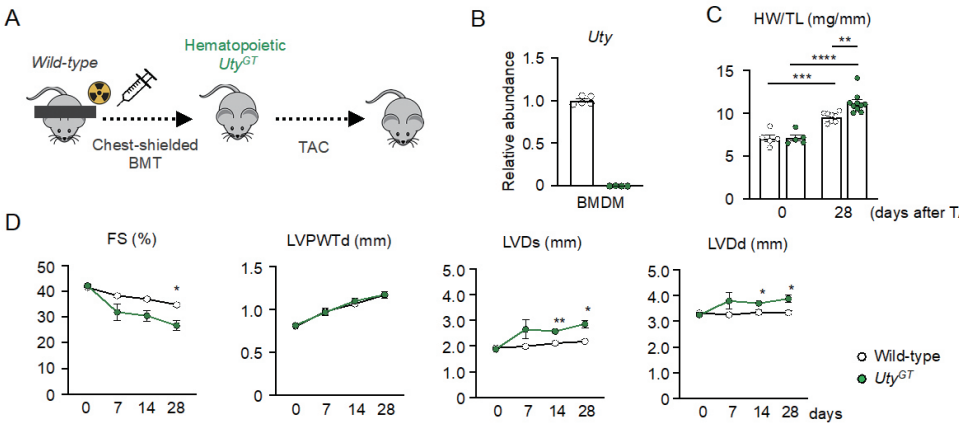
*Uty* gene trap ($Uty^{GT}$) mice were used to further test whether *Uty* gene disruption in hematopoietic cells worsens cardiac dysfunction post-TAC. Whereas the CRISPR/Cas9-disruption of *Uty* targeted exon 1, the $Uty^{GT}$ mice were engineered by placing an inactivating gene trap in the fourth intron of the *Uty* gene. The absence of *Uty* transcripts in $Uty^{GT}$ mice was verified in whole bone marrow cells, whole peripheral blood cells, and peritoneal macrophages. Expression of *Utx*, the *Uty* homolog on the X chromosome, as well as *Ddx3x*, *Eif2s3x* and *Kdm5c*, were unaffected by the disruption of *Uty* in these mice.

Mice underwent BMT with either $Uty^{GT}$ or control cells and were then assessed for the severity of cardiac dysfunction after TAC. In these experiments, thoraces of recipient mice were shielded during the irradiation preconditioning to minimize the confounding effects of radiation on the heart. By employing this strategy, blood donor chimerism levels ranging from 50 to 70% were achieved. As observed in the CRISPR/Cas9-based screen that targeted the *Uty* locus, mice transplanted with $Uty^{GT}$ hematopoietic cells lacked detectable *Uty* transcript expression in bone marrow derived macrophages and displayed greater reductions in cardiac function as determined by echocardiographic analysis after TAC, and elevated cardiac hypertrophy as indicated by an increase in the ratio of heart weight-to-tibia length. *Uty* deficiency did not affect circulating populations of leukocytes, nor did it affect

body weight or lung weight in the TAC model.

Mice transplanted with *Uty*$^{GT}$ hematopoietic stem cells exhibit trends of increased expression of transcripts encoding *Nppa* and a higher ratio of *Mhc b/a* in their hearts compared to control mice. The myocardial transcript levels of fibrosis marker genes, including collagen 1a1 (*Col1a1*), collagen 3a1 (*Col3a1*), and matrix metalloproteinase-2 (*Mmp-2*), were elevated in mice transplanted with *Uty*$^{GT}$ cells, suggesting that hematopoietic *Uty* disruption leads to an increase in myocardial fibrosis. Furthermore, flow cytometric analysis of heart cells revealed that the number of CD45$^-$CD31$^-$MEF-SK4$^+$ fibroblasts was higher in the hearts from TAC-treated mice transplanted with hematopoietic *Uty*$^{GT}$ cells compared with control. Consistent with these findings, histological analysis revealed greater fibrosis in the left ventricle of mice transplanted with *Uty*$^{GT}$ bone marrow cells after TAC, while there was no detectable difference in the number of cardiac endothelial cells (defined as CD45$^-$CD31$^+$). Taken together, these results provide additional support for the hypothesis that hematopoietic disruption of the *Uty* locus accelerates cardiac dysfunction in an experimental model of myocardial fibrosis.

Figures




**A.** Generation of CRISPR/Cas9-free hematopoietic *Uty*-disrupted mice with preserved cardiac microenvironment: male C57BL/6 CD45.1 (Pepboy) mice were lethally irradiated (with chest shielding) and reconstituted with bone marrow cells collected from *Uty*$^{GT}$ transgenic (CD45.2) or wild-type (CD45.2) mice. **B.** Transcript levels of *Uty* in bone marrow-derived macrophages (wild-type $n$ = 5, *Uty*$^{GT}$ $n$ = 4). **C.** Heart weight relative to tibia length at 0 and 28 days after the TAC procedure (day 0: wild-type $n$ = 5, *Uty*$^{GT}$ $n$ = 5, day 28: wild-type $n$ = 8, *Uty*$^{GT}$ $n$ = 9; two-way ANOVA post hoc Tukey). **D.** Sequential echocardiographic analysis of mice transplanted with wild- type or *Uty*$^{GT}$ cells. Repeated measurement was performed at the indicated time points after TAC (wild-type $n$ = 9, *Uty*$^{GT}$ $n$ = 7; two-way repeated-measures ANOVA post hoc Sidak). Data are presented as mean values ± s.e.m. *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001, ****$P$ < 0.0001.

To extend these analyses, a series of experiments employed *Ddx3y*-knockout mice in the bone marrow transplantation/TAC model. These mice lacked detectable Ddx3y transcript in bone marrow, but the expression of *Ddx3x* and other X chromosome-encoded genes were unaffected. In contrast to findings with *Uty*$^{GT}$ bone marrow, transplantation with *Ddx3y* knockout bone marrow had no effect on cardiac function, heart weight or other markers of cardiac failure in the TAC model.

## Discussion & Conclusion

The disruption of *Uty* exacerbated the cardiac dysfunction and hypertrophy observed in the TAC model. The importance of the *Uty* locus in the LOY effect was corroborated by experiments showing that mice transplanted with *UtyGT* bone mar- row cells exhibited exacerbated cardiac dysfunction in response to TAC compared to their wild-type counterparts. *Uty* is a member of the histone demethylase protein family of epigenetic regulators. While its demethylase activity is lower than that of its paralog, *Utx*, recent studies show that *Uty* can also modulate epigenetic processes through phase separation-mediated regulation of gene expression. Collectively, we identify the ubiquitously transcribed tetratricopeptide Y-linked (*Uty*) gene in leukocytes as a causal locus for an accelerated progression of heart failure in male mice with LOY.

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   Dirsuption of the *Uty* epigenetic regulator locus in hemtopoietic cells phenocopies the profibrotic attributes of Y chromosome loss in heart failure. ([#]first authors, [*]corresponding authors)
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   ***Science.*** 377(6603):292-297. 2022.

## 一般の皆様へ

男性は加齢と共に、血液細胞の中のＹ染色体を失うことがあります。このことを「Ｙ染色体の喪失（LOY）」と呼んでいます。私たちの以前の研究では、このＹ染色体の喪失が心不全を悪化させることがわかっていましたが、その具体的な理由は明らかではありませんでした。

今回の研究で、血液細胞のＹ染色体に含まれる「Uty」という遺伝子が失われることが、心不全を悪化させる原因の一つかもしれないことが示されました。この遺伝子の喪失がどのように心不全に影響を与えるのかを詳しく理解することで、心不全の治療法に新たな光が当たるかもしれません。これは男性だけでなく女性にとっても良いニュースです。

# Development of *in vivo* gene therapy using HSC-targeting lipid nanoparticles

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## Summary Abstract

*In vivo* gene therapy could be an ideal treatment for the diseases caused by defective stem cells with pathogenic mutations, such as the congenital bone marrow failure syndromes (BMFS). In this study, we developed the methods to edit genome in hematopoietic stem cells (HSCs) using lipid nanoparticle (LNP) or virus-like particle (VLP). We have also generated a knockin mouse with *Mecom* mutations as a model for BMFS. These results will be the basis for the future development of the *in vivo* gene therapy to treat BMFS patients.

***Key Words*** : Lipid nanoparticle, Genome editing, Hematopoietic Stem cells

## Introduction

Congenital bone marrow failure syndromes (BMFS) are a group of rare genetic disorders that affect the ability of bone marrow to produce blood cells. Radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT) syndrome is one of the BMFS characterized by the congenital fusion of the forearm bones, which is largely caused by missense mutations in the *MECOM* gene. Currently, hematopoietic stem cell transplantation is the only treatment option to cure BMFS. Therefore, there is a strong medical need to develop gene therapy to repair the pathogenic mutation of BMFS.

# Results

## (1) LNP-based genome editing in hematopoietic stem cells

Messenger RNA (mRNA) has emerged as a new category of therapeutic agents for the treatment of various diseases. To function in vivo, mRNA requires safe, effective, and stable delivery systems that protect the nucleic acid from degradation and that allow cellular uptake and release of mRNA. Lipid nanoparticles (LNPs) have attracted considerable attention for the delivery of mRNA to target cells (**Ref.1**). We have developed hematopoietic stem cell (HSC)-targeting LNPs that can efficiently deliver mRNAs into human HSCs. Using the HSC-targeting LNPs, we introduced Cas9 mRNA together with non-targeting (NT) or TP53-targeting sgRNAs (sgTP53-A and B) into human umbilical cord blood CD34+ cells, which contain many HSCs. The sgTP53-transduced HSCs, but not the NT-transduced HSCs, became resistant to the TP53-activating drug DS5272(**Ref.2**) (**Figure 1**). The successful knockout of the TP53 gene was also confirmed by sequencing analysis.

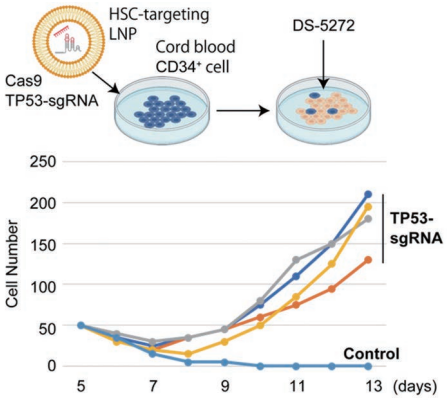
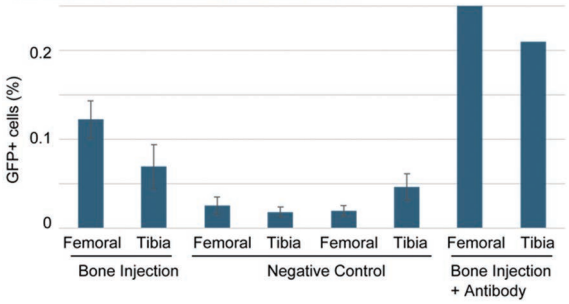



**Figure 1**. HSC-targeting LNP containing Cas9-mRNA and TP53-targeting sgRNA induced TP53 depletion in human cord blood CD34+ cells.

We then administered the HSC-targeting LNPs encapsulated with GFP mRNA to immunodeficient NSG mice that had previously been transplanted with human HSCs. Bone marrow cells were harvested 24 hours after LNP injection to evaluate GFP expression in human CD34+ HSCs. We confirmed the presence of GFP+ cells in human cells (**Figure 2**), indicating successful in vivo LNP-based RNA delivery to human HSCs.

**Figure 2**. HSC-targeting LNP-mediated in vivo transfer of GFP-mRNA into human cord blood cells in mice bone marrow.




In addition to the LNP-based genome editing, we also developed a virus-like particle (VLP)-based genome editing technology (**Ref.3**) in HSCs. Using the VLP, we successfully induced depletion of TP53 in mouse bone marrow cKit+ cells and leukemia cells in vitro. This VLP-based genome editing technology could also be used for the in vivo gene editing of HSCs in future studies.

## (2) Generation and analysis of MECOM-mutant knockin mice

MECOM is a nuclear transcription factor essntial for the proliferation of hematopoietic stem cells (HSCs) and myeloid leukemia cells (Ref. 4). MECOM comprises N- and C-terminal zinc finger domains (ZFDs) and includes binding motifs for the corepressor CtBP to regulate gene expression. Recent genetic studies have linked germline MECOM variants to thrombocytopenia, radioulnar synostosis, and bone marrow failure, collectively known as MECOM-associated syndromes (Ref. 5). Although the mutations are concentrated in the C-terminal ZFD, the impact of these mutations on MECOM function has been unclear. Our research indicates that the C-terminal ZFD is the primary DNA-binding domain of MECOM

and that disease-associated mutations eliminate its DNA-binding capability. Additionally, we discovered that MECOM functionally antagonizes GATA2 via C-terminal ZFD-mediated DNA binding and CtBP interaction, thus promoting myeloid leukemogenesis while inhibiting mast cell differentiation.

We also generated mutant MECOM knockin mice with a C-terminal ZFD mutation (mouse R751W), corresponding to the MECOM-R750W identified in patients with MECOM-associated syndromes. Homozygous mutant mice died before embryonic day 14.5 (E14.5). Heterozygous mutant mice ($MECOM^{WT/R750W}$) developed normally and showed no apparent hematopoietic defects in the peripheral blood over one and a half years of observation. However, we observed a significant decrease in the frequency of the $Lineage^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$ (LSK) fraction, which contains HSCs, in the fetal liver at E14.5. Additionally, there was a reduction in the frequency of the SLAM-LSK fraction, which contains long-term HSCs, in the adult bone marrow of $MECOM^{WT/R751W}$ mice (Figure 3). $B220^{+}$ B-cells were also slightly reduced in the bone marrow of $MECOM^{WT/R751W}$ mice, whereas the frequencies of Gr-1+ and Ter119+ cells did not significantly change. Thus, $MECOM^{WT/R751W}$ mice replicate the HSC defects and B-cell deficiency seen in some patients with MECOM-associated syndromes. However, the relatively mild phenotype of the $MECOM^{WT/R751W}$ mice suggests that additional factors are required for the full development of MECOM-associated syndromes.

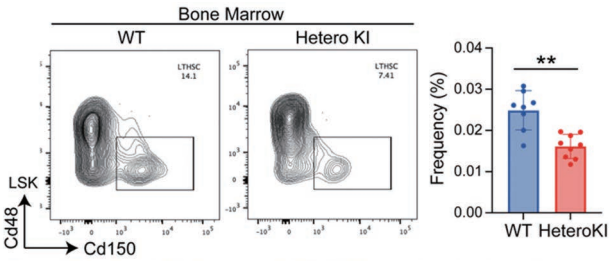



**Figure 3**. Reduced HSCs in mutant MECOM hetero knockin (KI) mice. $Lin^{-}Sca\text{-}1^{+}cKit^{+}CD150^{+}CD48^{-}$ HSCs were reduced in bone marrow of $MECOM^{WT/R715W}$ mice.

## Discussion & Conclusion

In vivo gene therapy could be an ideal treatment for the diseases caused by defective stem cells with pathogenic mutations, such as congenital bone marrow failure syndromes (BMFS). HSCs whose pathogenic mutation is repaired by the gene therapy are expected to outcompete defective HSCs in BMFS patients. Therefore, the repair of such pathogenic mutations in a small number of HSCs should show sufficient therapeutic effects for the BMFS patients. In this study, we demonstrated that our HSC-targeting LNPs containing Cas9 and sgRNAs can edit the genome in vitro and transfer RNAs into blood cells in vivo. We have also established a novel knockin mouse with the MECOM mutation as a mouse model of BMFS, which will be a useful tool to demonstrate the efficacy of the HSC-targeting LNPs in repairing the disease-causing mutation.

LNP-based gene therapy is a simple, efficient, cost-effective approach to treat patients with specific mutations, such as BMFS. In the future, we will also be able to develop LNPs to target other types of stem cells in various tissues and organs. Gene therapies using LNPs targeting a variety of stem cells will ultimately help many patients with defective stem cells carrying specific mutations.

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## 一般の皆様へ

血液疾患を治すための新しい方法として、遺伝子治療が注目されています。本研究では、ゲノム編集を行うための Cas9 やガイド RNA を脂質ナノ粒子で包んだ新しい薬物送達システムを用いて、全ての血液細胞の元となる造血幹細胞でゲノム編集を行うための基盤となる技術を確立しました。また、MECOM という遺伝子の変異によって引き起こされる骨髄不全症候群のモデルマウスを作製しました。今後も、この造血幹細胞指向形 LNP と骨髄不全症候群モデルマウスを活用して、生体内遺伝子治療法の開発を進めていきます。

# Elucidation of the molecular mechanism of plant immune response activated by mechanosensory trichomes

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## Summary Abstract

Among the environmental cues that affect the outcome of plant–microbe interactions, rain is a major cause of plant diseases. Recently, we found that trichomes on the epidermis directly sense external mechanical forces, including raindrops, to anticipate pathogen infections through intercellular calcium signaling. To dissect the molecular mechanism of the trichome-mediated mechanosensory system, we established a method to isolate trichomes and performed transcriptome analysis of trichomes obtained from *Arabidopsis* leaves. We then screened mutants that have defects in inducing mechanical stimuli-induced gene expression. Here we demonstrate that the cell walls of trichomes and their modifying enzymes are involved in sensing mechanical forces and transmitting the calcium signaling.
***Key Words*** : Plant Immunity, Calcium Signal, Mechanical Stimuli

## Introduction

Perception of pathogen-derived ligands by corresponding host receptors is a pivotal strategy in eukaryotic innate immunity. In plants, this is complemented by circadian anticipation of infection timing, promoting basal resistance even in the absence of pathogen threat. Rain is known as a major cause of devastating plant diseases as natural raindrops contain infectious fungal spores and bacteria, suggesting that it would be beneficial for plants to recognize rain as an early risk factor. Recently, we found that trichomes, hair-like structures on the epidermis, directly sense raindrops as a mechanical force to anticipate pathogen infections by trichome-mediated calcium signaling[1)]. However, the molecular mechanisms involved in the initiation and propagation of calcium waves remain unclear.

## Results

We have demonstrated that mechanical stimulation of trichomes induces calcium waves propagated away from them and activates immune responses effective against various microbes including biotrophic and necrotrophic pathogens. To identify the regulatory factors involved in calcium waves initiated by trichomes on the leaf surface, we created a profile of genes that are specifically and/or significantly expressed

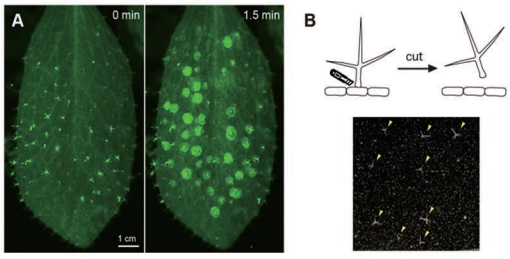


**Figure**
RNA-seq analysis of isolated trichomes, hair-like structures on the epidermis

**A**:The figure shows fluorescent images of the tramsgenic plant leaf surface of a recombinant plant containing the probe GCaMP3, which visualizes changes in intracellular $Ca^{2+}$ concentration, after mechanical stimulation.
**B**: A stereomicroscope photograph of an actual isolated trichome is shown

in trichomes with or without mechanical stimuli. The methods for trichome isolation have already been reported, in which calcium-chelating agents are used to soften the cell walls followed by the physical detachment of trichomes by agitating leaves with metal beads. However, because these methods likely induce profound changes in transcriptomes and cellular metabolisms in the trichomes, we established a rapid and accurate method to specifically isolate trichomes from the surface of four-week-old Arabidopsis leaves (Figure).

Using the established method, trichomes were isolated from leaves with or without mechanical stimuli. Total RNA was extracted and subjected to the library preparation for RNA sequencing. We performed 81 bp sequencing using Illumina NextSeq550 and obtained a set of genes that are highly expressed in trichomes compared to whole leaves. The Gene Ontology analysis revealed that gene categories related to calcium signaling and the cell wall were significantly enriched in the trichome-specific transcriptome. We then collected more than 150 mutant individuals with defects in candidate genes specifically expressed in the trichomes. After obtaining homozygous knockout seeds, we screened all the mutants that exhibited a significant reduction in the expression levels of mechanical stimuli-induced genes. To evaluate the possible role of candidate genes in the mechano-signaling pathway, we are introducing the calcium sensor GCaMP in those mutants. We plan to perform live imaging tests by stimulating the trichomes of mutants to see if the induction and propagation of calcium waves are affected compared to wild-type plants. As preliminary results, we have found some regulatory genes whose malfunction leads to the suppression of calcium waves. Furthermore, we conducted inoculation tests using the virulent biotrophic pathogen, *Pseudomonas syringae* pv. *maculicola* ES4326 to investigate if mechanical stimuli-induced immunity is compromised in the mutants. We inoculated *Psm* ES4326 into 4-week-old leaves and evaluated the pathogen growth 3 days after inoculation, and confirmed that some above-mentioned mutants show enhanced disease susceptibility compared to the wild type. Importantly, those mutants with reduced levels of calcium influx exhibited altered transcriptome profiles, especially in calcium/calmodulin-mediated signaling and mechanical responses, indicating that these genes are involved in the mechano-signaling pathway. To further verify the assumption, we have been creating transgenic lines that can conditionally overexpress any genes regulated under the dexamethasone (DEX)-inducible promoter. The temporal overaccumulation of the candidate genes may facilitate calcium waves and calcium-dependent gene expression to establish enhanced disease resistance. In the future, we will use virulent fungi such as *Alternaria brassicicola* and *Botrytis cinerea* to evaluate the role of candidate genes in mechano-sensitive immune responses.

## Discussion & Conclusion

Our RNA-seq analysis indicated that trichomes express more than 2000 genes distinct from those in whole leaves, and these are significantly categorized into “calcium signaling” and “cell wall”, suggesting that these genes may be involved in the mechanosensitive response in plants. Indeed, some T-DNA knockout mutants of genes in these categories exhibited weakened calcium waves and enhanced disease susceptibility. These results indicate that many trichome-specific genes are involved in sensing mechanical stimuli via trichomes to initiate calcium waves. Because the homologs of the candidate genes are expressed in other cell types such as mesophyll cells, a conserved mechano-perception system may exist.

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## 一般の皆様へ

植物に感染する病原体は、その多くが雨によって媒介されることから、雨の危険性は認識されているが、植物の雨に対する応答は不明であった。私たちは、植物は雨によって負荷される機械刺激を葉面の毛状突起（トライコーム）によって感知し、トライコームを中心としたカルシウムウェーブを発生させることで、免疫を活性化することを示した。現在までに、トライコーム依存的に生じるカルシウムウェーブの起動や伝播を担う、チャネルや酵素などの制御因子を同定し、その機能解析を進めている。本研究成果によって、機械刺激応答性免疫を標的とした分子育種に繋がることが期待される。本手法は全く新しい植物の形質を提供するものであり、減農薬といった持続的な農業の推進や食料の安定確保などの極めて有効な手段の一つとなることが期待できる。

# Elucidation of anti-obesity effect by signal sensing epigenetic modifier-mediated mitochondrial biogenesis

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## Summary Abstract

Environmental factors, alongside genetic factors, play a vital role in the development of lifestyle diseases linked to obesity. Histone demethylase JMJD1A induces the beiging of subcutaneous white adipose tissue (scWAT) upon chronic cold stress through two consecutive steps: cold sensing via phosphorylation (1st step) and epigenetic re-writing (2nd step). Through the analysis of mice with enhanced JMJD1A phosphorylation and a deficiency in JMJD1A demethylation activity, we demonstrate that JMJD1A induces beiging through epigenetic modifications in scWAT, specifically targeting the enhancers for the mitochondrial master regulator genes *Pgc1a/b*, promoting mitochondrial biogenesis and the formation of beige adipocytes.
***Key Words*** : Mitochondrial biogenesis, Beiging, Epigenome,

## Introduction

In addition to genetic factors, environmental factors are crucial for the onset of lifestyle diseases associated with obesity. Histone demethylase JMJD1A is phosphorylated at S265 upon cold exposure (1st step, signal sensing), forming a transcriptional complex that is recruited to thermogenic gene loci, and promotes gene expression via histone demethylation (2nd step, epigenetic re-writing), inducing beiging of scWAT. Beiging of scWAT accompanies enhancement in the quality and quantity of mitochondria, increasing energy expenditure and improving energy metabolism. This study examines how enhancing 1st step JMJD1A phosphorylation through the inhibition of the pS265-JMJD1A phosphatase, MYPT1, and the inhibition of 2$^{nd}$ step demethylation through a knock-in mutation in JMJD1A catalytic core impacts metabolic health through the regulation of beiging and mitochondrial biogenesis

## Results

**Analysis of pre-adipocyte specific *Mypt1* knockout mice:**

To analyze the impact of enhancing 1$^{st}$ step JMJD1A phosphorylation on beiging and metabolic health, we compared the metabolic phenotype of mice with pre-adipocyte specific depletion of *Mypt1*, a regulatory subunit within pS265-JMJD1A phosphatase complex (*Mypt1*$^{+/flox}$*::Pdgfra-Cre,* cKO) with *Mypt1*$^{+/flox}$ mice (Ctrl) and obtained the following results.

1) we regularly measured the body weight of cKO mice before and after high fat diet (HFD) feeding and showed that cKO mice are resistant to HFD-induced obesity compared to Ctrl mice.

2) we conducted glucose tolerance test on HFD-fed cKO mice and demonstrated that glucose tolerance is improved in cKO mice compared to Ctrl mice. In addition, we measured serum insulin levels in HFD-fed cKO mice and showed that serum insulin levels are lower in cKO mice under fasting conditions or after glucose injection compared with control mice.
3) Through histological analysis, we showed that cKO mice had more UCP1-positive multilocular beige adipocytes in scWAT than did Ctrl mice.
4) Though the qPCR analysis of scWAT and scWAT culture of cKO mice, we showed that thermogenic genes including *Pgc1a* are increased compared to those in Ctrl mice.
5) We demonstrate that depletion of *Mypt1* in scWAT by injecting adeno-associated virus expressing Cre recombinase into scWAT of *Mypt1*$^{flox/flox}$ mice increases thermogenic gene expression and NE-induced oxygen consumption rate in scWAT.

**Analysis of *Jmjd1a*$^{HY/HY}$ mice:**

To determine the role of 2$^{nd}$ step histone demethylation by JMJD1A on cold-induced mitochondrial biogenesis during beiging and gain mechanistic insights, we conducted a series of molecular biological experiments using mice with the H1122Y mutation (*Jmjd1a*$^{HY/HY}$) which results in a catalytic dead protein and obtained the following results.

1) Through the measurement of mitochondrial DNA content by qPCR in scWAT of *Jmjd1a*$^{HY/HY}$, we showed that *Jmjd1a*$^{HY/HY}$ mice had significantly lower levels of mitochondrial DNA content compared to *Jmjd1a*$^{+/+}$ mice in scWAT after chronic cold exposure. In addition, we demonstrated that the mRNA expression of nuclear-encoded mitochondrial genes and the levels of mitochondrial proteins were lower in scWAT of *Jmjd1a*$^{HY/HY}$ mice compared to *Jmjd1a*$^{+/+}$ mice.
2) Through RNA-seq analysis using scWAT from *Jmjd1a*$^{HY/HY}$ exposed to chronic cold stress, we showed that oxidative phosphorylation (OXPHOS) and thermogenesis-related genes regulated by the master regulator of mitochondrial biogenesis, *Pgc1* were downregulated in *Jmjd1a*$^{HY/HY}$ compared to *Jmjd1a*$^{+/+}$ mice were related to.
3) By combining the H3K27ac and H3K4me1 ChIP-seq dataset from beige adipocytes in scWAT and Hi-C data in differentiating 3T3-L1 preadipocytes with our ATAC-seq dataset from cultured beige adipocytes, we successfully predicted distal enhancers for *Pgc1* genes. Though ChIP-qPCR analysis, we demonstrated that H3K9me2 levels remained higher in scWAT cultures of *Jmjd1a*$^{HY/HY}$ cells at the predicted enhancers of *Pgc1b* compared to that of *Jmjd1a*$^{+/+}$ mice after beige adipocyte differentiation.

## Discussion & Conclusion

In our current study, we generated pre-adipocyte specific *Mypt1* knockout mice which resulted in the enhanced JMJD1A phosphorylation at Ser265 and demonstrated that enhancing the 1st step JMJD1A phosphorylation (signal sensing mechanism) can induce the expression of thermogenic genes including *Pgc1a* by enhancing the 2$^{nd}$ step epigenetic re-writing in scWAT, leading to beiging and improvement in obesity and energy metabolism[1].

We also generated mice with a single amino acid mutation in the Jumonji domain of JMJD1A that eliminates demethylase activity and showed that the expression of key

mitochondrial regulators including *Pgc1a* and *Pgc1b* in scWAT are regulated by the histone demethylase JMJD1A during cold-induced beiging in mice. Using this *Jmjd1a*$^{HY/HY}$ mouse model, we demonstrate that JMJD1A plays a pivotal role in modulating H3K9me2 levels at enhancers of key mitochondrial genes *Pgc1a/b* to promote their expression, thereby facilitating mitochondrial biogenesis and the development of beige adipocytes during cold exposure[2].

Overall, this present study demonstrated that the function of epigenetic enzymes can be activated to induce fat-burning cellular memory and mitochondrial biogenesis through the modulation of their post-translational modifications. This discovery opens up significant possibilities for groundbreaking therapies for the treatment of lifestyle-related diseases aimed at enhancing the function and number of mitochondria in fat cells.

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## 一般の皆様へ

私たちは、寒冷環境に晒されるとエネルギー貯蔵を担う白色脂肪を、脂肪を燃焼し熱を作るベージュ脂肪細胞に変化させる仕組みを備えています。ベージュ脂肪細胞はエネルギーの燃焼に重要なミトコンドリアを多く持ち、その誘導が生活習慣病の新規治療・予防戦略として注目されています。

本研究では、遺伝情報の後天的修飾であるエピゲノムの書き換えを行う酵素 JMJD1A の活性を制御することで、ベージュ化が誘導され、太りにくくなることを見出し、そのメカニズムとしてベージュ化の過程で、JMJD1A がミトコンドリアの数を増やす遺伝子の働きを調節していることを明らかにしました。肥満や生活習慣病に対する新たな治療法や予防法への応用につながる成果です。

# The molecular mechanism governing STING activation

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## Summary Abstract

Stimulator of interferon genes (STING) is critical for the type I interferon response to pathogen- or self-derived DNA in the cytosol. STING may function as a scaffold to activate TANK-binding kinase 1 (TBK1), but direct cellular evidence remains lacking. Here we show, using single-molecule imaging of STING with enhanced time resolutions down to 5 ms, that STING becomes clustered at the trans-Golgi network (about 20 STING molecules per cluster). The clustering requires STING palmitoylation and the Golgi lipid order defined by cholesterol. Single-molecule imaging of TBK1 reveals that STING clustering enhances the association with TBK1. We thus provide quantitative proof-of-principle for the signaling STING scaffold, reveal the mechanistic role of STING palmitoylation in the STING activation, and resolve the long-standing question of the requirement of STING translocation for triggering the innate immune signaling.
***Key Words*** : cGAS-STING signalling, innate immune signalling, Golgi, type I interferon

## Introduction

The innate immune system, an ancient defense mechanism, detects and eliminates pathogens through pattern recognition receptors (PRRs) such as the Toll-like receptor and STING pathways[1,2]. However, dysregulated innate immune signaling can lead to excessive inflammation and various diseases[3]. STING, a protein located in the endoplasmic reticulum under normal conditions, activates the TBK1 kinase at the trans-Golgi network (TGN) to initiate immune responses against DNA viruses[4]. This study investigates the mechanism of STING activation at the TGN using advanced microscopy techniques, including photoactivated localization microscopy (PALM) and direct stochastic optical reconstruction microscopy (dSTORM)[5].

## Results

The study utilized a live-cell PALM technique to measure STING clusters. Initially, calibration experiments were conducted using mEos4b embedded in polyvinyl alcohol (PVA) to understand blinking and photoactivation kinetics. Positive control experiments with mEos4b-cavin1 in PC3 cells validated the method, successfully quantifying molecular counts in clusters using <Nblink> of mEos4b. Subsequent expression of mEos4b-STING in STING-knockout (KO) MEFs showed an increase in STING clustering after stimulation, confirmed by studying cis-Golgi and TGN markers. Moreover, colocalization with recycling endosomal proteins indicated STING translocation to recycling endosomes after leaving the

TGN. Overall, PALM analysis revealed clustering of STING at the TGN, with some clusters containing over 800 STING molecules.

Previous research highlighted the significance of STING palmitoylation in facilitating STING clustering for TBK1 and IRF3 activation[6]. Live-cell PALM observations of mEos4b-STING with palmitoylation inhibitors demonstrated reduced clustering and suppression of large clusters post-stimulation. Similarly, in STING-KO MEFs expressing a palmitoylation-deficient variant of STING, PALM observations showed significantly reduced clustering compared to wild-type STING, emphasizing the critical role of STING palmitoylation in cluster formation.

COPA syndrome, resulting from heterozygous mutations in the COPA gene, leads to increased expression of type I interferon-stimulated genes[7–9]. Live-cell PALM observation of COPA variants in mEos4b-STING cells revealed significantly increased STING clustering, suggesting a connection between COPA syndrome and STING activation. Surprisingly, the absence of cGAMP, the natural STING ligand, did not impede STING clustering, suggesting that cGAMP binding is not inherently essential for this process.

Beyond STING palmitoylation, Golgi lipid order plays a crucial role in STING signaling. Disrupting Golgi membrane order suppressed STING signaling and reduced cluster formation, while inhibiting oxysterol-binding protein decreased STING signaling and cluster formation without affecting STING translocation from the ER to the Golgi. Additionally, adding cholesterol back into live cells restored STING signaling, highlighting cholesterol's crucial role in STING signaling and clustering.

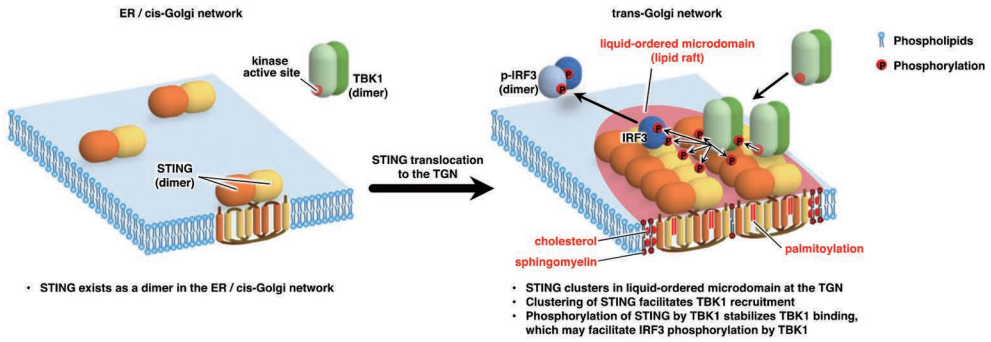
Investigating cholesterol's role in STING signaling and clustering led to an examination of its subcellular localization using the cholesterol biosensor iD4H. Increased accumulation of iD4H at the TGN upon STING stimulation suggested that STING palmitoylation drives cholesterol accumulation at the cytosolic leaflet of TGN membranes. Colocalization of iD4H with TBK1-mScarletI and its accumulation at the TGN correlated with high membrane rigidity, suggesting that lipid order influences STING-associated signaling events at the Golgi.

Further investigation into STING's role as a scaffold for TBK1 recruitment and activation revealed transient colocalization of TBK1-HaloTag7-SF650T molecules with mEos4b-STING clusters upon STING stimulation. Inhibition of STING palmitoylation or TBK1 activity reduced colocalization duration, while STING (L373A), unable to bind TBK1, exhibited short colocalization durations. These data suggest that palmitoylation-dependent STING cluster growth facilitates TBK1 recruitment, while TBK1 phosphorylation by STING stabilizes their binding to these clusters.

## Discussion & Conclusion

Through in vitro and in silico analyses, STING is suggested to oligomerize in the presence of cGAMP, potentially facilitating STING signaling activation[10–12]. However, the mechanism behind TBK/IRF3 activation at the Golgi/TGN instead of the ER, where STING binds cGAMP, remains unclear. Based on our cellular data using single-molecule localization microscopy, we propose a model for STING activation at the TGN. Initially, cGAMP binding to STING induces conformational changes or oligomerization, allowing STING to translocate from the ER to the cis-Golgi network and further to the TGN. At the TGN, STING clusters with the aid of palmitoyl groups on Cys88 and Cys91, cholesterol, and sphingomyelin. This palmitoylation-driven cholesterol accumulation in the cytosolic leaflet in TGN may enhance STING clustering, given palmitoylated proteins' affinity for raft lipids.

Analyzing TBK1 colocalization with STING suggests that STING clustering facilitates TBK1 recruitment by increasing the encounter probability of TBK1 and STING molecules. Reduced Golgi cholesterol levels have a lesser effect on p-TBK1 compared to inhibiting STING palmitoylation, likely due to differences in STING cluster orientations. Our findings highlight the TGN's role in innate immunity signaling via a cholesterol-based system, endorsing the generation of liquid-ordered lipid microdomains where palmitoylated STING clusters activate TBK1. STING activation induces various innate immune and proinflammatory genes, such as CH25H, which regulates STING signaling homeostasis. Targeting STING palmitoylation, like with H-151, may offer a new strategy to treat STING-mediated inflammatory diseases by interfering with cellular cholesterol levels, particularly in the TGN.



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## 一般の皆様へ

自然免疫は、先天的に備わっている、異物に対する応答機構です。自然免疫応答タンパク質 STING は、DNA ウイルスの感染に応答して炎症応答を誘導します。それによって STING 経路は DNA ウイルス感染から身体を守っていますが、その一方で、異常な活性化は自己免疫疾患、神経変性疾患、がんなどの疾患を引き起こします。今回我々は、STING が細胞内で活性化する分子機構を1分子レベルで解析し、DNA 刺激時に STING が平均20分子以上のクラスターを形成して下流分子を活性化することを見出しました。本研究成果は、上記疾期の新規治療戦略の開発につながることが期待されます。

# Elucidation of Novel Regulatory Mechanism of Glucose Metabolism in Liver Type 2 Innate Lymphocytes by Single Cell Analysis and Spatial Transcriptome Analysis

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## Summary Abstract

This study investigates the role of group 2 innate lymphocytes (ILC2s) in the liver's contribution to glucose metabolism and its regulatory mechanism, emphasizing the importance of understanding gluconeogenesis to prevent excessive glucose production. We found that IL-33 significantly promotes IL-13 production by liver ILC2s, reducing fasting blood glucose and suppressing pyruvate-induced gluconeogenesis. Further analyses by scRNA-seq revealed that ILC2-derived IL-13 reduce gluconeogenic enzyme expression in hepatocytes, suggesting liver ILC2s directly inhibit gluconeogenesis via IL-13. Thus, this study characterized novel liver ILC2s in maintaining hepatic glucose metabolism homeostasis.
***Key Words*** : type 2 innate lymphocytes (ILC2), diabetes, single cell analysis

## Introduction

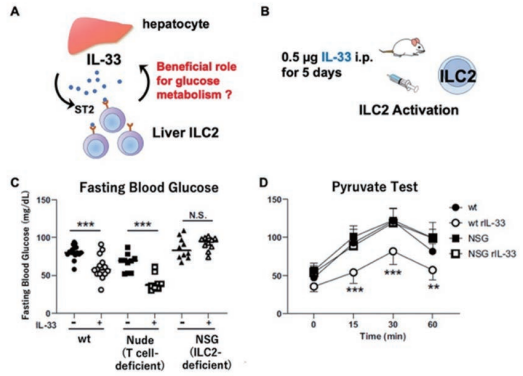
The multifaceted pathophysiological effects of “type 2 innate lymphocytes (ILC2)” have been revealed, and their metabolic improving effects in adipose tissue and atherosclerosis have attracted much attention. Focusing on the liver as an insulin target organ that contains many immune cells and receives influx of external antigens and high concentrations of nutrients (metabolic metabolites) from the intestinal tract, we have demonstrated that ILC2 in the liver has hypoglycemic effects by inhibiting the release of sugar via IL-13. Although the impact of ILC2s on blood glucose levels in adipose and pancreatic tissues is documented, their effect in the liver remains unclear. Therefore, the purpose of this study is to elucidate the mechanism of type 2 spontaneous lymphocyte diversity and organ characteristics using single-cell analysis of insulin target organs, especially liver and single-cell interaction analysis based on anatomical characteristics.

## Results

First, we examined whether activation of ILC2 reduces blood glucose levels and expression of glycogenic enzymes in the liver of obese mice. To determine which cell type is responsible for the hypoglycemic effect of IL-33 (Figure 1A and 1B), we used wild-type mice and nude mice, and NOD/Scid/Il2Rγ-nul(NSG) mice, which lack both ILC2 and T cells. The results showed that rIL-33 reduced fasting blood glucose levels in wild-type and nude mice, but not in NSG mice (Figure 1C). In addition, IL-33 significantly suppressed the rise in blood glucose levels in wild-type mice, but not in NSG mice, when glycogenesis was induced

by loading pyruvate, a glycogenic substrate (Figure 1D). In addition, wild-type mice showed a decrease in fasting insulin and an increase in liver glycogen content, whereas this effect was not observed in NSG mice. Furthermore, IL-33 treatment significantly suppressed the expression of glycogenic enzymes (G6pc, Pck1, and Hnf4a) in both wild-type and nude mice, but this effect was abolished in NSG mice. Collectively, these findings indicate that rIL-33 suppresses gluconeogenesis and lowers blood glucose levels through a mechanism that is dependent on hepatic ILC2.

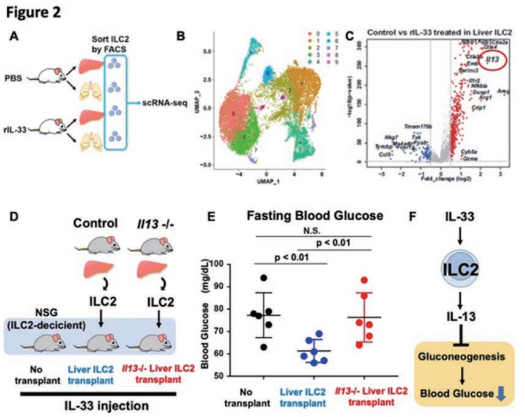
Figure 1




Next, why does the activation of ILC2 by rIL-33 treatment suppress hepatocyte glycogenesis? Hepatocytes are a heterogeneous population with different functions depending on their histological location, and immune cells of the same species are also considered to be heterogeneous. Therefore, to molecularly characterize liver ILC2 activated after rIL-33 administration at the single-cell level, we performed scRNA-seq analysis of an immune cell population enriched with ILC2 by FACS sorting. Lung ILC2, which has been well characterized in previous reports, was used for comparison (Fig. 2A). The results showed that ILC2 was divided into 10 clusters, while liver ILC2 was enriched in specific clusters (1, 2, 4, 6, and 8) and showed different expression patterns of key transcription factors such as GATA3 and Il1 receptor like 1 (Il1rl1) (Figure 2B). The expression patterns of GATA3 downstream genes (*Areg, Il1rl1, and Gzma*) were also different in each cluster among ILC2, reflecting the differences in GATA3 control mechanisms. Then, we compared the differences in gene expression profiles of liver ILC2 with or without IL-33 stimulation. We found that IL-13 expression was strongly induced by IL-33 stimulation (Figure 2C). In addition, comparing the different gene profiles of liver ILC2 and lung ILC2, IL-13 expression was significantly enhanced in liver ILC2 compared to lung ILC2, suggesting that the regulation of GATA3-dependent cytokines is different under different conditions and between different organs.

To examine whether IL-13 derived from liver ILC2 affects glucose metabolism in hepatocytes. we assessed blood glucose lowering effect using Il13 knockout mice. We found that rIL-33 reduced fasting blood glucose and inhibited gluconeogenesis in wild-type mice, but this effect was not observed in Il13-/- mice. Furthermore, we transplanted liver ILC2 from wild-type and IL13-/- mice into ILC2-deficient NSG mice and observed the effects after rIL-33 injection (Figure 2D). Fasting blood glucose was decreased by rIL-33 in the wild-type liver ILC2 transplant group, but not in the Il13-/- liver ILC2 transplant group (Figure 2E). These results suggest that IL-13 derived from ILC2 mediates the hypoglycemic effect of IL-33 administration via gluconeogenesis suppression (Figure 2F).

Figure 2



## Discussion & Conclusion

This research revealed the unique roles of innate lymphoid cells (ILC2s) in different organs, focusing on their novel function in the liver regarding glucose metabolism. We discovered that liver ILC2 shows stronger IL-13 production than lung ILC2. Besides, IL-33 stimulation of liver ILC2s significantly suppresses gluconeogenesis through IL-13 production, which downregulates key gluconeogenic enzymes. This suppression aids in improving glucose metabolism, especially under conditions of obesity and diabetes. The findings also highlight the interorgan heterogeneity of ILC2s and the organ-specific regulation of their effector cytokines, possibly due to differential expression patterns of GATA3-binding partners. Furthermore, the research suggests potential therapeutic targets for managing excessive gluconeogenesis, offering new treatment avenues for obese diabetic patients.

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## 一般の皆様へ

免疫系にはたくさんの種類がありますが、その中の「2型自然リンパ球（ILC2)」は、喘息やアレルギー反応などの病気に関わる関与することが知られています。特に、血糖値や代謝に対して、良い改善効果を持つことが明らかにされており、注目を集めています。 しかしながら、免疫細胞を多く含む肝臓での働きや、血糖を下げる仕組みとの関係については、未だ明らかになっていません。 そこで、本研究では、肥満や糖尿病と関わりの深い肝臓組織に着目して、最先端の技術であるシングルセル解析という手法を用いて、肝臓に存在している2型自然リンパ球がどのようなメカニズムで血糖を下げる役割を果たしたり、糖尿病を改善させるのかを明らかにします。この研究成果は、糖尿病や肥満症に対する全く新しい治療薬の開発につながります。

# Elucidation of the role of alveolar capillary in alveolar formation for clinical application

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## Summary Abstract

Alveologenesis is the final step of lung development aimed at generating alveoli, which create a large surface area for gas exchange between the lungs and blood. Alveolar formation is a spatially coordinated morphogenetic event in which alveolar myofibroblasts play a crucial role. Recent studies have also suggested the potential role of alveolar capillary endothelial cell (EC)s for proper induction of alveolar morphogenesis, although the underlying mechanism is still not fully understood. In this study, we uncover a novel mechanism of EC-mediated organ morphogenesis, in which ECs regulate alveologenesis by constructing basement membranes that act as a scaffold for myofibroblasts.
***Key Words*** : Lung development, Alveologenesis, Myofibroblast, Endothelial cell, Rap1

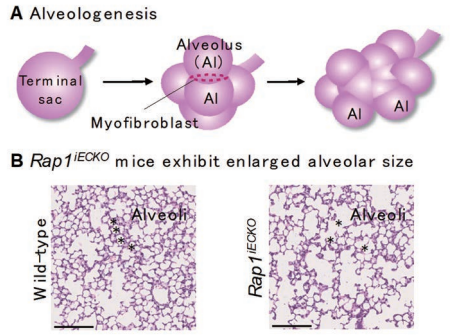
## Introduction

Alveolar formation is a spatially coordinated morphogenetic event regulated by multiple cell types including alveolar epithelial cells, myofibroblasts, and endothelial cells (ECs)[1]. Alveolar myofibroblasts are regarded as a key player in alveologenesis[2, 3]. 3D imaging analysis showed that alveolar myofibroblasts contract to subdivide the terminal sacs generating the new alveoli (Figure 1A). Furthermore, recent studies have shown that the contraction of myofibroblast depends on a mechanical signaling including myosin light chain kinase and Yes-associated protein[4, 5]. On the other hand, previous studies indicate that the pulmonary ECs regulate alveolar morphogenesis independently of myofibroblast differentiation[6, 7]. However, the mechanism underlying EC-mediated alveologenesis is still not fully understood.

## Results

In the present study, we generated mice lacking both *Rap1a* and *Rap1b* encoding small GTPases of the Ras superfamily in ECs (*Rap1*$^{iECKO}$) by crossing double-floxed *Rap1a/b* mice (*Rap1*$^{fl/fl}$) with mice expressing CreERT2 recombinase under the control of the *Cdh5* promoter. After delivering tamoxifen via the lactating mothers from postnatal day 0 to day 2, we found that *Rap1*$^{iECKO}$ mice exhibited postnatal lethality until one month. Identifying the causes of death, we noticed that the lungs of *Rap1*$^{iECKO}$ mice were significantly smaller than those of controls at P21 (Figure 1B). Furthermore, *Rap1*$^{iECKO}$ mice exhibited enlarged alveolar size indicating the impaired postnatal alveologenesis.

Figure 1




A) Schematic representation of alveologenesis in mouse. The contraction of alveolar myofibroblast surrounding terminal sacs results in the generation of new alveoli.
B) Hematoxylin-eosin staining from wild-type and *Rap1*$^{iECKO}$ mice at postnatal 21 days.

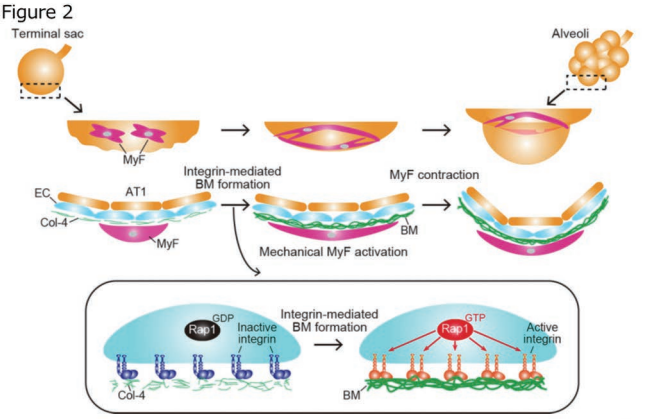
Since alveolar myofibroblasts are required for alveologenesis, we first examined whether the impaired alveolarization is attributable to the defective formation of alveolar myofibroblasts. Although we found the normal emergence of alveolar myofibroblast during alveologenesis in *Rap1*$^{iECKO}$ mice, we noticed that the filaments of α-SMA in myofibroblasts of *Rap1*$^{iECKO}$ mice were significantly thinner than that of controls pointing to a functional impairment of myofibroblasts. Alveolar myofibroblasts generate contractile force through activation of mechanical signaling involved in MLCK-dependent phosphorylation of myosin light chain (MLC) and nuclear localization of YAP. Therefore, we next investigated whether the mechanical signaling in alveolar myofibroblasts is affected in *Rap1*$^{iECKO}$ mice by 3D immunostaining with antibodies against phosphorylated-myosin light chain (pMLC) and YAP. pMLC signal surrounding alveolar ring in myofibroblasts of *Rap1*$^{iECKO}$ was dramatically decreased as compared to that of controls. Furthermore, nuclear localization of YAP in myofibroblast was similarly impaired in *Rap1*$^{iECKO}$ mice. Accordingly, these results suggest that Rap1 regulates mechanical signaling required for alveolar myofibroblast contraction.

To address how ECs regulate mechanical signaling in alveolar myofibroblasts in a Rap1-dependent manner. we first analyzed spatial localizations of ECs and myofibroblasts in alveoli. As a result, capillary ECs surrounded and created intimate contacts with AT1 cells to form alveoli in P9 lungs. Furthermore, myofibroblasts covered the outside of the alveolar space by making tight contacts with ECs. It can reasonably be assumed that basement membranes (BMs) mediate an indirect contact between ECs and myofibroblasts. Staining of Collagen type IV (Col-4), a component of basement membrane, showed the disorganized basement membrane in *Rap1*$^{iECKO}$ mice. Collectively, these findings suggest that ECs generate BMs required for myofibroblasts to Rap1-dependently induce mechanical signal-mediated alveologenesis.

We next investigated the molecular mechanism by which endothelial Rap1 induces BM formation. *Rap1*$^{iECKO}$-derived ECs showed the lower numbers of FAs and FCs as well as the cell size which is one of the readouts of cell spreading activity than in control ECs, indicating that ECs in the lungs of *Rap1*$^{iECKO}$ mice have decreased adhesive activity of integrins. Furthermore, the number of FAs and FCs containing activated form of integrin β1, which

is a common β-subunit of collagen receptors, was significantly reduced in *Rap1*$^{iECKO}$-derived ECs. Therefore, endothelial Rap1 stimulates the adhesive activity of integrin β1 to recruit Col-4, thereby generating BMs required for myofibroblast-mediated septa formation. To uncover the downstream signaling in vivo, we similarly generated *Itgb*$^{iECKO}$ mice and found that the similar phenotypes to that of *Rap1*$^{iECKO}$ mice including hypo-alveolarization, disorganized BMs as well as the defective mechanical signaling in myofibroblasts. Taken together, these results indicate that endothelial Rap1 stimulates integrin β1 to induce the formation of BMs, which act as a scaffold for myofibroblasts to induce mechanical signal-mediated alveologenesis[10] (Figure 2).

Figure 2




Schematic representation of proposed model. During alveologenesis, alveolar ECs generate BMs by stimulating integrin β1-mediated recruitment of Col-4 in a Rap1-dependent manner. BMs formed by ECs act as a scaffold for alveolar myofibroblasts (MyF) to induce mechanical signal-mediated secondary septa formation, ultimately giving rise to alveoli. Excerpt from reference 10.

## Discussion & Conclusion

In summary, our data demonstrate that ECs facilitate alveologenesis in postnatal lungs by constructing BMs through Rap1-mediated activation of integrin β1, which is required for activation of mechanical signaling in myofibroblasts. Our findings are not only important for understanding lung development but may have also implication for lung disease. Surgical removal of lung lobes, i.e., pneumonectomy, induces compensatory growth of the remaining lobes not only through the growth of existing alveoli but also via the formation of new alveolar units by reactivated alveolar myofibroblasts [8, 9]. Stimulating EC-mediated BM formation might be an effective therapeutic strategy to induce realveolarization post pneumectomy but also for lung diseases such as bronchopulmonary dysplasia and chronic obstructive pulmonary disease.

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## 一般の皆様へ

肺は、呼吸における酸素と二酸化炭素の交換を担う生命維持に欠かせない臓器であり、このガス交換を担う場が「肺胞」です。肺胞は小さな袋状の構造をしており、内面を覆う肺胞上皮細胞とそれを裏打ちする血管内皮細胞が密に接着することで、肺胞内の空気と血液の間のガス交換を可能としています。

今回私達は、血管内皮細胞だけで *Rap1* 遺伝子を破壊したマウスの解析から、血管内皮細胞の Rap1 が基底膜の形成を促進することで、肺胞形成を制御していることを発見しました。本研究成果は難治性の呼吸器疾患等において、肺胞の再生を促す新しい治療法を生み出す可能性があります。

# Development of three component reaction for site-selective peptide modification

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## Summary Abstract

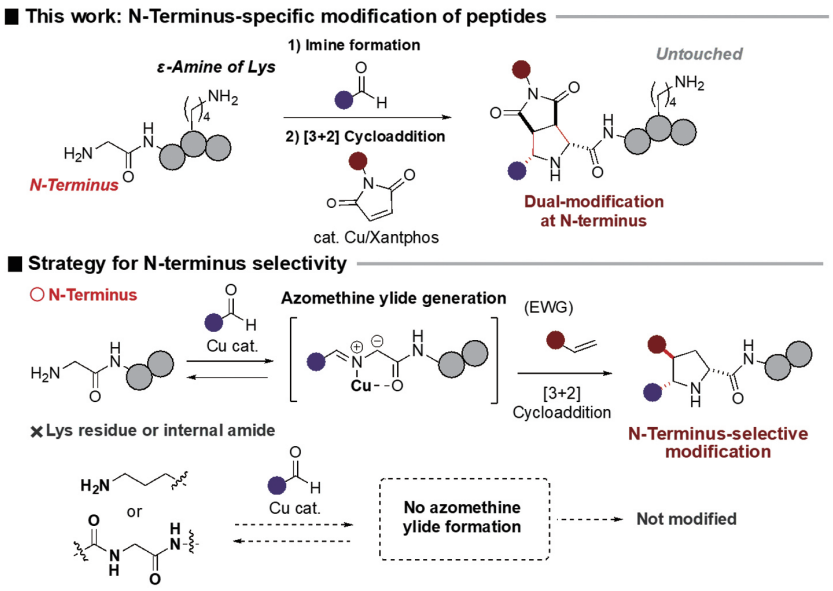
We developed an N-terminal-specific three-component integration method using peptides, aldehydes, and maleimides through copper-catalyzed [3+2] cycloaddition. This reaction enables the dual modification at the glycine N-terminus, regardless of the presence of lysine residues. The reaction tolerated a variety of functional groups, and efficiently applicable to polypeptides containing up to 26 amino-acid residues. Additionally, this method was further extended to the peptide–peptide coupling using dehydroalanines as the dipolarophile.
***Key Words*** : Peptide modification, N-Terminus, Three-component reaction, Cycloaddition

## Introduction

Peptides are essential as therapeutic agents, drug candidates, and chemical biology probes. Thus, there is a great need for methods to modify peptides and introduce functional molecules into them. However, the site-selective modification of peptides to produce their structurally uniform conjugates remains challenging due to the presence of various nucleophilic sites, such as the highly reactive and abundant lysine residues. In this context, the N-terminus has received significant attention as a target of site-selective peptide modification because it represents one unique site in any single-chain peptide.[1]

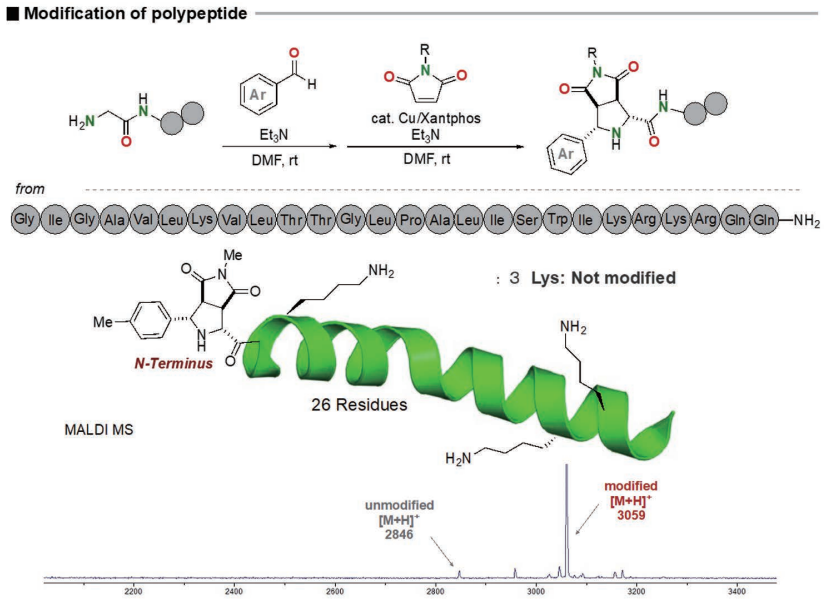
# Results

In this work, we designed a new method for the N-terminal-selective modification of peptides through a three-component [3+2] cycloaddition reaction. This approach capitalizes on the exclusive and efficient generation of metalated azomethine ylides[2-4] at the N-terminus, followed by cycloaddition with olefins. On the other hand, the ε-amine of lysine and internal amide cannot form such a dipolar, leaving these moieties untouched.




After the extensive screening of reaction conditions, we found that a catalyst generated from Cu salt and Xantphos promotes the [3+2] cycloaddition reaction using imino-dipeptide and *N*-methyl maleimide to afford the desired cycloadduct in quantitative yield with exclusive *exo*-diastereoselectivity. The optimized conditions could be applied to a broad range of iminopeptides and aldehydes, affording various combinations of cycloadducts with *exo*-diastereoselectivity. The complete N-terminal selectivity of this cycloaddition was proved by the inter- and intramolecular competition reaction using Lys-containing peptides.

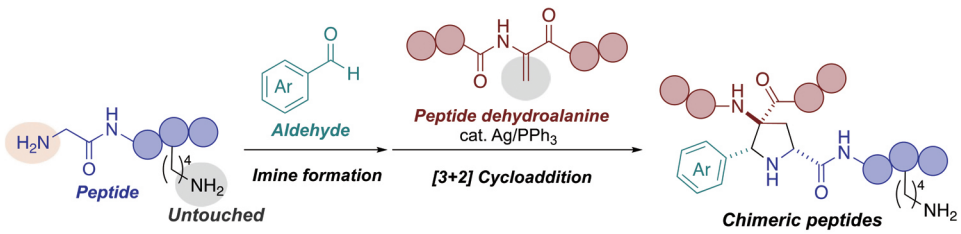
Although efficient and selective, the copper-catalyzed cycloaddition requires the preparation of iminopeptides from peptides and aldehydes in advance. In fact, the isolation and purification of such iminopeptides are often complicated and even fail in some cases. To address this problem, we have developed a one-pot procedure for rapid three-component integration of more complex structures without the isolation of the imine. To our delight, we found that a sequence of the imine formation and the cycloaddition proceed efficiently in a one-pot manner. This protocol



could be applied to various dipeptides and tripeptides to afford the desired cycloadducts in excellent yields. We have also been able to apply the one-pot protocol to the rapid three-component integration of longer peptides, such as Melittin·TFA bearing three lysine ε-amine residues and various types of functional groups from the 26-amino acid residues. The reaction of Melittin proceeded quite efficiently to produce the desired cycloadduct, leaving three lysine ε-amine moieties untouched. This result collectively demonstrate the utility of this one-pot assembly method for the uniform, site-specific, and efficient bifunctionalization of polypeptides.

With the successful implementation of N-terminus-specific modification of peptides using maleimide derivatives as dipolarophile in mind, we turned our attention to peptide-peptide coupling using the N-terminal azomethine ylide with dehydroalanine (Dha)-containing peptides. Although the Cu-based catalyst system failed to promote the desired cycloaddition, we found that the Ag/$PPh_3$ catalyst promotes the reaction, yielding the cycloadduct as a peptide chimera in high yield with complete *endo*-diastereoselectivity. Tolerating a broad range of functional groups, including the ε-amine of a lysine residue, this method offers an opportunity for the expedient and modular assembly of readily accessible aldehyde, N-terminus-unprotected peptides, and peptide-based Dhas into the chemically robust pyrrolidine ring.

■ **Three-component site-specific coupling for chimeric peptides**




While the Ag/$PPh_3$ catalytic system produced the cycloadducts as nearly 1:1 mixture of diastereomers, the use of (*S*)- and (*R*)-DTBM-SEGPHOS as chiral ligands allowed for complete control over the diastereochemical outcome of the [3+2] cycloaddition regardless of the stereochemistry of the adjacent peptide. We anticipate that the present method and its further improvements will enable the expedient assembly of more complex and bioactive peptides and proteins using a diverse set of functional molecule-tagged aldehydes into pyrrolidine rings. Further studies along this line are currently underway.

## Discussion & Conclusion

In summary, we have developed a method for N-terminal-selective dual modification of peptides through a Cu-catalyzed 1,3-dipolar cycloaddition reaction that forms a chemically robust pyrrolidine ring. This method capitalized on the N-terminal-selective generation of metalated azomethine ylides, followed by cycloaddition with olefins. We have also developed a one-pot procedure, which allows for the straightforward assembly of diverse complex peptides with aldehydes and maleimides. This method tolerates the modification of diverse peptides, including the polypeptide bearing up to 26 amino acid residues. In addition, the construction of chimeric peptides was also achieved by using peptide dehydroalanines as the dipolarophile We expect that our method offers an opportunity for the convenient and rapid construction of doubly functionalized peptides.

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## 一般の皆様へ

ペプチドはタンパク質を構成する構造であり，多彩な生命科学研究において，蛍光分子や生物活性分子などの機能性分子の精密な導入が重要である．一方で，様々な反応部位が存在するため，均一な品質でこれらを導入することは容易ではない．本研究課題では，通常1カ所しか存在しないペプチドのN末端に対して，ピンポイントで複数の機能性分子を導入できる三成分反応の開発に成功した．本手法は，完全な選択性と高い変換効率を示し，26残基からなる長鎖のペプチドへの適用にも成功した．これらの結果から，本手法の様々な生命科学研究への応用が期待される．

# Study about a mechanism of cancer-specific transcription termination that produces noncoding RNA

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## Summary Abstract

Transcription termination contributes to a productive cycle of RNA synthesis. Therefore, perturbation of transcription termination generates aberrant transcripts that are mainly noncoding RNAs in intergenic region. In this study, my group dissects mechanism of transcription termination in cancer cells which have epigenetic catastrophe. Additionally, we found a reagent that induces noncoding RNAs caused by premature transcription termination within protein coding genes. We are currently further dissecting the mechanisms and function of the noncoding RNAs.
***Key Words*** : Cancer, Noncoding RNA, Nascent RNA, SETD2, Premature transcription termination

## Introduction

Large-scale transcription start site (TSS) analyses have detected 100,000 types of non-coding RNAs (ncRNAs), far more than the number of protein-coding genes (20,000), and their functions have been actively analyzed. My group recently revealed that ncRNAs are produced not only from independent ncRNA gene units but also from protein-coding genes via transcriptional termination. Transcription termination is disrupted by various cellular stresses (hyperosmotic pressure, heat shock), and aberrant regulation of transcription termination is thought to be involved in the production of a new class of functional ncRNAs. Recent studies reveal diverse mechanisms of transcription termination, however the details remain largely unknown.

## Results

The PolyAdenylation Signal (PAS) is a known RNA cis sequence element required for transcription termination of protein-coding genes. However, several regulatory cis sequences and trans factors that regulate transcription termination independently of PAS have been recently reported. Notably, it is technically difficult to analyse transcription termination since its derived ncRNAs are highly unstable. Therefore, it is necessary to analyze the newly synthesized RNAs that have just been produced from the transcription machinery (nascent RNAs). In order to dissect such fragile ncRNAs, my group developed a nascent RNA sequencing technology, mNET method (Nojima et al., *Cell*, 2015), and has also successfully developed a derivative method, POINT method (Sousa-Luis et al., *Mol Cell*, 2021). Using these state-of-the-art technologies of nascent RNA sequencing, we found that transcription termination of specific protein-coding genes is perturbed by depletion of epigenetic factor and small compounds. The results are described as follows.

SETD2 mediated transcription termination

We coincidentally detected transcriptional termination defect (alternatively extended readthrough ncRNAs) of approximately 10% of protein-coding genes in human cultured cells (U2OS) with CRISPR knockout (KO) of the histone H3 lysine 36th trimethyl (H3K36me3) transferase gene *SETD2*. In addition, we detected transcription termination defect in ccRCC patient-derived cells which have a catalytic mutation in *SETD2* gene. This indicates the diversity of transcription termination mechanisms, but it is unclear how SETD2 regulates gene-specific transcription termination. In this study, we analyzed various histone and DNA modifications in *SETD2* KO cells and found several up- and downregulated epigenetic marks. In addition, we employed Auxin-dependent protein degradation system for SETD2 (SETD2-AID2) in HCT116 cells. Our western blot analysis successfully detected depletion of SETD2 protein and H3K36me3 mark after few days of auxin treatment, but our POINT-seq failed to detect termination defect. This result indicates that SETD2 indirectly regulates transcription termination. We are currently investigating the epigenetic catastrophe which is responsible to transcription termination defect in loss of SETD2.

Premature transcription termination in anti-tumor drug treatment

We previously reported that an RNA splicing inhibitor (Pladienolide B, PlaB) which targets SF3B1 protein in U2 snRNP machinery induces transcription termination in intronic region of protein coding genes, suggesting that RNA splicing promote productive transcription and mRNA synthesis. This event is called as Premature Transcription Termination (PTT). In this study, we coincidentally found that a reagent X which is bound to DNA also strongly induces the PTT and generates PTT-derived ncRNAs (PTT-ncRNAs). Notably, such PTT-ncRNAs are well spliced. Indeed, this PTT mechanism caused by reagent X is distinct from that generated by PlaB. Furthermore our POINT analysis show that both PlaB and reagent X induce intronic PAS usages, but in different subsets of protein coding genes. We are currently identifying the full length of PTT-ncRNAs using a long-reads sequencer and will analyse what proteins bind to the PTT-ncRNAs to reveal the biological function.

## Discussion & Conclusion

In this study, my group investigated mechanisms of transcription termination in cancer cells. We found that loss of SETD2 methyltransferase activity induces transcription termination defect in a subset of protein coding genes and proposed the potential mechanism. However, it remains largely unknown what cis and trans factors (RNA elements and proteins) directly regulate the transcription termination in loss of SETD2. In addiotion, we found a novel mechanism of PTT. We will further dissect the molecular mechanism of PTT and characterize PTT-ncRNAs which are potentially applicable to establish a new approach to conquer cancer cell proliferation.

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## 一般の皆様へ

本研究から、がんと転写終結には繋がりがあることが示唆された。例えば、メチル基転移酵素活性を失った *SETD2* 遺伝子変異を有する腎臓がん患者細胞では、転写終結破綻と DNA 損傷レベルの上昇が確認されている。この DNA 損傷は、新たな体細胞遺伝子変異を引き起こすことが考えられる。そのため今後、転写終結の調節を介した DNA 損傷抑制アプローチの開発にも取り組む。未成熟転写終結がどのように制御されているのか、今後特に、RNA スプライシング依存的・非依存的な分子機構についても明らかにする。さらに、未成熟転写終結によって産生される ncRNA の同定とそれらの機能解析を行うことにより、医学的に応用可能な ncRNA をリスト化することを目指す。

# Diet intervention and chronic obstructive pulmonary disease

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## Summary Abstract

The change in the gut microbial environment was induced in chronic obstructive pulmonary disease (COPD) model mice. Moreover, by diet intervention, the symptoms of COPD were improved. However, these effects were partially abolished in antibiotic-treated mice.

***Key Words*** : Gut microbiota, Diet, Nutrition, COPD

## Introduction

Over the past 30 decades, the research on gut microbiota has developed the relationship between changing gut microbiota and host homeostasis. Furthermore, clinical applications targeting gut microbiota have already begun to be suggested for gastrointestinal tract-related diseases such as inflammatory bowel disease, and it is expected that further research on various pathological conditions and gut microbiota may be conducted vigorously in the future. Recently, it has been suggested that gut microbiota may be involved in chronic obstructive pulmonary disease (COPD), a lifestyle-related disease of the lungs that is now considered a national affliction and is attracting attention as a potential therapeutic target

# Results

In our previous study, we found that COPD model mice, which we had originally created, showed a dramatic decrease in the fecal short-chain fatty acid (SCFA) levels, a gut microbial metabolite. Therefore, 16S rRNA sequencing was performed to focus on the possibility of dramatic changes in gut microbiota in the pathogenesis of COPD. We found that a clear change in the composition of gut microbiota was observed between the healthy and COPD groups (Fig., unpublished data). Additionally, we found that the dietary intervention study on COPD model mice attenuated the pathogenesis of COPD, however, the effect of the dietary intervention study was abolished in antibiotic-treated mice, which simulate germ-free mice that do not have gut microbiota.

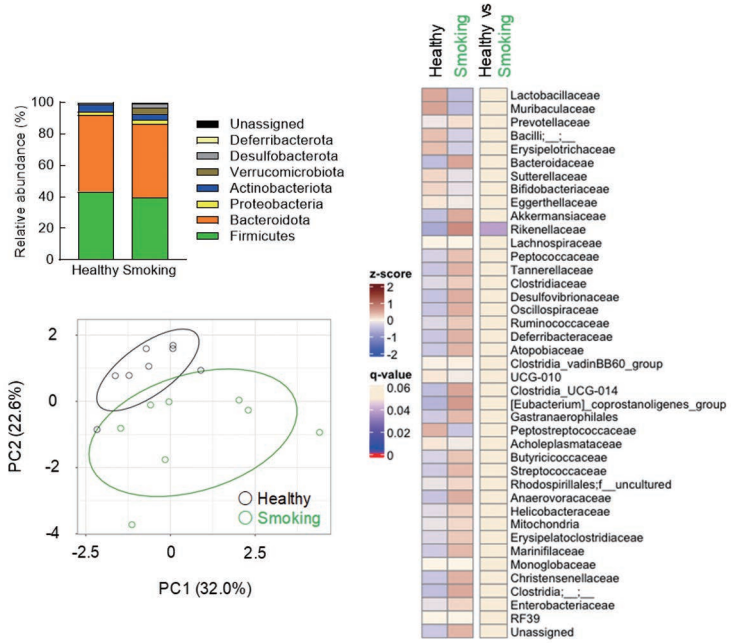


Fig. Gut microbiota in COPD model mice.

## Discussion & Conclusion

Through the analysis of tissue section of human cancer patients, we identified the actual sites where $T_{RM}$-like TIL are generated in the tumor, and local antigen signaling are likely to be a factor that promote differentiation of $T_{RM}$-like TIL. Our preliminary data showed that, moreover, a large number of macrophages are found in both DS and RS while dendritic cells are rarely found in those areas, suggesting that macrophages are the provider of antigen signaling that promote differentiation of $T_{RM}$-like TIL. It is well known that TGFβ is required for up-regulation of CD103, a key molecule for differentiation of $T_{RM}$-like TIL[5]. Since TGFβ signaling suppresses effector functions of $CD8^+$ T cells, we expect that $T_{RM}$-like TIL receive additional signaling that overcome TGFβ-mediated suppression. Macrophages in DS and RS could also be a provider of such signaling. We will investigate the role of these macrophages in the differentiation of $T_{RM}$-like TIL.

In our previous study, we found that COPD model mice, which we had originally created, showed a dramatic decrease in the fecal short-chain fatty acid (SCFA) levels, a gut microbial metabolite. Therefore, 16S rRNA sequencing was performed to focus on the possibility of dramatic changes in gut microbiota in the pathogenesis of COPD. We found that a clear change in the composition of gut microbiota was observed between the healthy and COPD groups (Fig., unpublished data). Additionally, we found that the dietary intervention study on COPD model mice attenuated the pathogenesis of COPD, however, the effect of the dietary intervention study was abolished in antibiotic-treated mice, which simulate germ-free mice that do not have gut microbiota.

COPD is a major cause of death both domestically and internationally, and the treatment is actively being established. Administration of anti-inflammatory drugs, including bronchodilators and inhaled steroids, improves respiratory function and subjective symptoms, however, no improvement in life expectancy has been reported. One possible cause is that emphysema, the main pathology of COPD, is irreversible and these drugs are poorly effective. Therefore, the development of new therapeutic medicines and interventions to prevent the onset and progression of emphysema and COPD is expected. These results are expected to lead to the establishment of a world-leading treatment targeting gut microbiota in COPD since dietary intake contributes to the improvement of COPD pathology through changes in the gut microbial environment.

## 一般の皆様へ

慢性閉塞性肺疾患は国内外で主要な死亡原因の一つであり、治療法の確立が積極的に行われている。気管支拡張薬や吸入ステロイドをはじめとした抗炎症薬の投与は呼吸機能、自覚症状の改善は得られるが生命予後の改善効果は報告されていない。そのことの原因の一つに慢性閉塞性肺疾患の主病態である肺気腫は不可逆的であり、こうした薬剤の効果が乏しいことが考えられる。そこで新規の治療薬の開発及び、肺気腫・慢性閉塞性肺疾患の発症及び進行を予防する介入が望まれている 。本研究成果は、単なる食事の摂取が腸内環境の変化を介して慢性閉塞性肺疾患の病態改善に寄与することから、世界に先駆けた慢性閉塞性肺疾患における腸内細菌を標的とした治療法の確立に繋がることが期待される。

# Immunogenomic analysis to elucidate the mechanism of immune evasion in endometrial carcinoma with high microsatellite instability

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## Summary Abstract

The efficacy of immune checkpoint inhibitors in cancer treatment has recently attracted considerable interest, particularly in tumors exhibiting high microsatellite instability (MSI-H). However, variability in the antitumor immune response across organs, including the colon and uterus, underscores the need for tailored treatment strategies based on organ and tumor histology. Our study conducted comprehensive genomic and transcriptomic analyses of endometrial cancer, revealing disparities in genetic abnormalities and immune response correlated with histological classification. These findings inform the optimization of immunotherapy for endometrial cancer. Additionally, ongoing investigation into transcriptional variants influencing immune responsiveness aims to elucidate organ-specific molecular mechanisms underlying immune and metabolic states.
***Key Words*** : microsatellite instability, endometrial carcinoma, immunotherapy, gene expression profile

## Introduction

Recently, there has been growing interest in the effectiveness of immune checkpoint inhibitors for cancer treatment, particularly in tumors with high microsatellite instability (MSI-H). However, even within MSI-H tumors, the antitumor immune response varies across different organs, including the colon and uterus. This necessitates the development of tailored treatment strategies based on organ and tumor histology. Additionally, various cancers harbor specific transcriptional variants closely associated with their unique characteristics. While these transcriptional variants may influence the phenotypic status of cancer cells, such as their immunological and metabolic profiles, the underlying details remain unclear.

# Results

In our study, we conducted whole exome sequencing and RNA-seq analyses on 81 cases of endometrial carcinoma spanning various histological types (Figure 1). This comprehensive approach revealed disparities in genetic abnormalities and gene expression profiles associated with histological classification. Initial histological diagnosis, performed by a pathologist blinded to genomic analysis results, provided the foundation for our subsequent investigations. Furthermore, we evaluated the antitumor immune activity by staining and counting CD8 positive cells within tumor tissues.

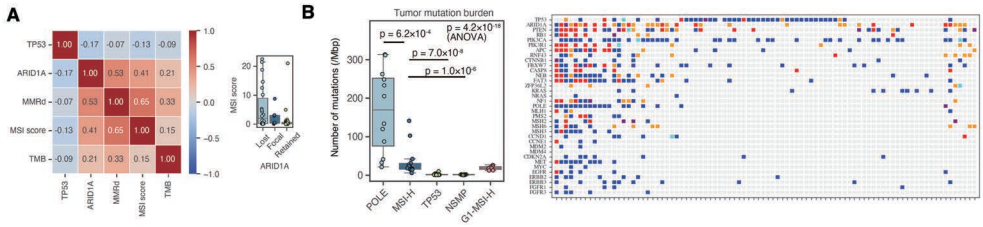


**Figure 1 Whole exome sequencing and gene expression analyses on endometrial carcinomas**

**A** Correlation matrix of characteristics of endometrial carcinomas determined by whole exome sequencing or pathological examination (left). MSI score of tumors based on the ARID1A staining status (right). **B** Results of whole exome sequencing. Tumor mutation burden (TMB) by genomic subtype classification (left). Overview of gene mutations. The x-axis represents each case. Blue indicates missense mutations, red indicates nonsense mutations, orange indicates frameshift mutations, and light blue indicates amino acid deletions/insertions.

Tumors were stratified based on genomic abnormalities using established criteria from the Cancer Genome Atlas (TCGA) Program (ref1). Within our cohort, cases with POLE mutations were classified as the POLE type, while MSI-H type was identified through MSI analysis or MSI scores from the remaining cases. Notably, some POLE type tumors exhibited the MSI-H phenotype, and mutational signature analysis suggested that mutations in genes associated with mismatch repair were acquired due to the hypermutability of POLE deficiency. Moreover, mutations in ARID1A emerged as a recurrent finding, particularly associated with MSI-H tumors, corroborating previous reports (ref2). These mutations were correlated with the immunological status of tumor tissues, as indicated by increased infiltration of CD8 positive T cells, suggesting potential implications for therapeutic targeting.

Expanding our analysis beyond genomic profiling, we aimed to delineate tumor subtypes based on gene expression patterns. We selected 5000 genes with robust expression and significant variability among cases for clustering analysis. Using t-distributed stochastic neighbor embedding (t-SNE) for dimensionality reduction, we identified a cluster comprising normal tissues and three clusters of tumor tissues (data not shown, manuscript under preparation). Cluster 3 exhibited basal-like or stem-like cell characteristics, while Cluster 1 showed heightened activity in the TNF or NF-κB pathways, indicating a more active tumor immune response and potentially greater efficacy of Immune Checkpoint Inhibitor (ICI) therapy.

To validate our findings, we conducted analyses using TCGA data, which confirmed

the presence of three tumor sample clusters and one normal sample cluster, consistent with our results. Furthermore, by comparing the gene expression profiles of endometrial carcinoma with colorectal cancers, we identified disparities that may underlie the distinct responsiveness to anti-tumor immunity between MSI-H endometrial carcinoma and MSI-H colon cancer. The results also indicated the possibility that the immune response of cancer cells is partly determined by the cell type from which the tumor cells originated. We also found that differences in immune status are stratified by the presence or absence of ARID1A mutations, which well align with the previous reports of correlation between ARID1A status and mismatch repair deficiency (Figure 2).

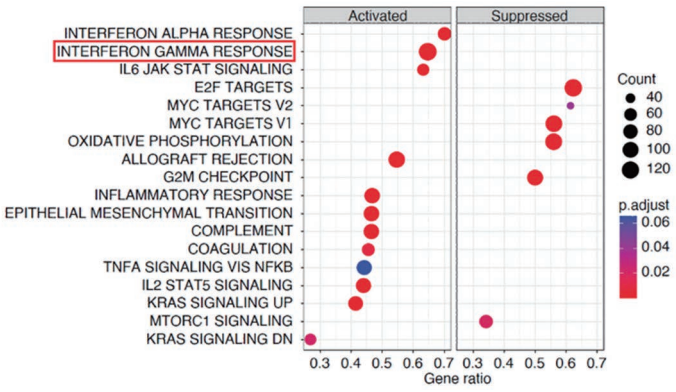


**Figure 2 Gene Set Enrichment Analysis showing the gene ratio for different pathways in Activated and Suppressed states in ARID1A defective tumors**
The x-axis represents the gene ratio, and the size of the dots indicates the count of genes. The color gradient represents the adjusted p-value (p.adjust), with blue indicating higher p-values. Key pathways include INTERFERON ALPHA RESPONSE, INTERFERON GAMMA RESPONSE, and IL6 JAK STAT3 SIGNALING, among others.

Currently, these findings are undergoing manuscript preparation.

## Discussion & Conclusion

In this study, we have clarified the distinctions between endometrial carcinoma and colon cancer, as well as the variations in immune response linked to cluster classification in endometrial carcinoma. These findings are expected to contribute to the refinement of immunotherapy for endometrial carcinoma in the future.

Additionally, we are investigating the transcriptional variants of genes that influence anti-tumor immune responsiveness, aiming to elucidate the molecular mechanisms underlying differences in organ- and histology-specific immune responses. To achieve this, we conducted full-length transcriptomic analysis (ref3) using long-read sequencing for 16 cases. We will continue our detailed analysis to further explore organ-specific transcriptional variants associated with immune and metabolic states.

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## 一般の皆様へ

近年、免疫チェックポイント阻害剤のがん治療における有用性が着目されており、とくにマイクロサテライト不安定性の高い（MSI-H）腫瘍で効果的です。しかし、同じ MSI-H 腫瘍でも、臓器によって抗腫瘍免疫の状態が異なり、臓器に応じた治療戦略を考える必要があります。そこで、さまざまな組織型の81症例の子宮体癌について、組織型による遺伝子異常や遺伝子発現プロファイルの違いを詳しく調べました。その結果、MSI-H 子宮体癌と MSI-H 大腸癌の免疫応答性の違いを見出しました。さらなる研究で子宮体癌における効果的な治療法の開発につながることが期待されます。

# Molecular basis of calcium ion-regulated cellular uptake of nutrients into cancer cells

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## Summary Abstract

Macropinocytosis (MPC) is a type of endocytosis involving actin-driven membrane ruffle formation and closure to produce macropinosomes for fluid-phase cargo uptake. In this study, we found that intracellular and extracellular $Ca^{2+}$ are essential for epidermal growth factor (EGF)-stimulated MPC in A431 human epidermoid carcinoma cells. We identified *ATP2B4* as the $Ca^{2+}$-related regulator of EGF-stimulated MPC. We demonstrated that knockout of *ATP2B4* inhibits membrane ruffle closure and macropinosome formation. We also found that *ATP2B4* is involved in EGF-stimulated $Ca^{2+}$ oscillation near the plasma membrane. These findings suggest that *ATP2B4*-dependent $Ca^{2+}$ regulation is essential for EGF-induced MPC.

***Key Words*** : Macropinocytosis, Calcium ion, Epidermal growth factor, Membrane ruffle

## Introduction

MPC is a conserved fluid-phase endocytosis for the uptake of extracellular cargo. Notably, cancer cells utilize MPC to take up extracellular materials as nutrients, such as amino acids and proteins, facilitating their proliferation [1]. Hence, gaining a comprehensive understanding of MPC should offer promising prospects for the development of therapeutic drugs against cancers.

In our previous study, we showed $Ca^{2+}$-influx through Piezo1 channel activation using its agonist Yoda1 significantly inhibited EGF-stimulated MPC in A431 cells, implying that $Ca^{2+}$ could be involved in EGF-stimulated MPC [2]. However, the role of $Ca^{2+}$ in EGF-stimulated MPC and its responsible genes remain ambiguous.

## Results

We first investigated whether intracellular and extracellular $Ca^{2+}$ are involved in EGF-stimulated MPC in A431 cells expressing high amount of EGF receptors. To this aim, we carried out a dextran uptake assay under depletion of intracellular and extracellular $Ca^{2+}$, and analyzed using confocal microscopy and flow cytometry. Tetramethyl rhodamine-labeled 70 kDa dextran (TMR-dex70) was used as a marker of macropinosomes in this study.

Depletion of intracellular or extracellular $Ca^{2+}$ using BAPTA-AM or EGTA, respectively, led to a significant decrease in TMR-dex70 uptake. This suggests that both intracellular and extracellular $Ca^{2+}$ are essential for EGF-stimulated MPC. MPC includes two important

steps to form macropinosomes: formation of membrane ruffles by actin rearrangement and followed by closure of the ruffles. We thus studied the effect of $Ca^{2+}$-depletion on actin rearrangement upon EGF stimulation. As a result, confocal microscopy analysis showed that actin rearrangement and ruffle formation happened even under $Ca^{2+}$-depleted condition. This suggests that $Ca^{2+}$ in EGF-stimulated MPC could be involved in the process of membrane ruffle closure to form macropinosomes.

We then hypothesized that $Ca^{2+}$-related genes are responsible for EGF-stimulated MPC, especially for membrane ruffle closure. To test this idea and identify the genes, we focused on the coelomocyte uptake defective (CUP) genes and their mammalian homologs [3]. CUP genes are thought to be essential for fluid-phase endocytosis in *C. elegans*, and thus their mammalian homologs can be candidate genes responsible for MPC. We focused on potential $Ca^{2+}$-related genes among these candidates and validated the expression of *ATP2B4* in A431 cells.

We examined whether *ATP2B4* is involved in EGF-stimulated MPC. Knockdown of *ATP2B4* significantly decreased in cellular uptake of TMR-dex70, whereas did not affect transferrin, a representative cargo internalized into cells by clathrin-mediated endocytosis. This result suggested that *ATP2B4* is selectively involved in MPC.

We then established *ATP2B4* knockout (KO) cells using CRISPR-Cas9 system to eliminate possibility of off-target effects of siRNA. Moreover, we established *ATP2B4*-KO cells expressing an EGFP-conjugated wild-type protein encoded by *ATP2B4* as well as EGFP-conjugated mutants. Flow cytometry analysis using these cells showed that *ATP2B4*-KO decreased cellular uptake of TMR-dex70 upon EGF stimulation and that wild-type protein expression in KO cells rescued the cellular uptake of TMR-dex70. These results indicate that *ATP2B4* is $Ca^{2+}$-related gene essential for EGF-stimulated MPC in A431 cells.

We next investigated whether *ATP2B4*-KO affects membrane ruffle formation. Confocal microscopy analysis as well as scanning electron microscopy analysis showed that *ATP2B4*-KO did not influence on ruffle formation upon EGF stimulation. This suggests that *ATP2B4* is involved in macropinosome formation in EGF-stimulated MPC.

We thus performed phase-contrast time-lapse imaging, which can visualize macropinosomes by the difference of contrast, using wild type (WT) cells and *ATP2B4*-KO cells upon EGF stimulation. Both WT and KO cells showed membrane ruffle formation within 5 min after EGF stimulation. While WT cells successfully formed macropinosomes within 10 min, KO cells hardly formed likely due to failure of ruffle fusion.

We furthermore examined $Ca^{2+}$ dynamics at the plasma membrane using a genetically encoded $Ca^{2+}$ indicator in WT and *ATP2B4*-KO cells. The $Ca^{2+}$ imaging and quantification demonstrated that *ATP2B4*-KO significantly inhibited $Ca^{2+}$ oscillation upon EGF stimulation, suggesting that *ATP2B4* could generate $Ca^{2+}$ dynamics at the plasma membrane which could be essential for membrane ruffle closure in EGF-stimulated MPC.

## Discussion & Conclusion

Although the connection between $Ca^{2+}$ and EGF-stimulated MPC remained ambiguous, our study demonstrates the key role of $Ca^{2+}$ in EGF-stimulated MPC, especially in closure of membrane ruffles to form macropinosomes. By focusing on $Ca^{2+}$-related genes among mammalian homologs of CUP genes, we identified *ATP2B4* as an essential regulatory gene in EGF-stimulated MPC. Through the studies using *ATP2B4*-KO cells, we showed that *ATP2B4* is not involved in membrane ruffle formation. However, regulation of $Ca^{2+}$ dynamics by *ATP2B4* is crucial for membrane ruffle closure, leading to the formation of macropinosomes.

In future studies, it will be essential to elucidate detailed molecular mechanisms underlying how the protein encoded by *ATP2B4* and $Ca^{2+}$ dynamics contribute to membrane ruffle closure.

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## 一般の皆様へ

私たちは細胞が外部からものを取り込む経路の一つであるマクロピノサイトーシスという現象について研究しています。私たちの最近の研究によって、マクロピノサイトーシスには細胞内でシグナル伝達物質として重要なカルシウムが関与していることが分かってきました。そこで、本研究ではカルシウムに関連する遺伝子に着目して研究を行ったところ、マクロピノサイトーシスを制御する遺伝子を新たに見つけることができました。マクロピノサイトーシスはがん細胞での栄養取り込み経路としても重要であることから、本研究の成果が、新規がん治療法への応用につながることも期待されます。

# Exploring follicular lymphoma-specific immunity

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## Summary Abstract

We performed multi-omics single-cell analysis of follicular lymphoma (FL), the second most common lymphoma subtype characterized by slow growth and high frequency of disease relapse. We discovered novel T-cell subsets that are transcriptionally and spatially distinct from known T cell types and counterpart cells in different cancer types. These subsets showed unique spatial distribution and robustly predicted favorable prognoses in the Discovery and Validation cohorts of FL. Our findings provide a previously unrecognized concept of immunity that controls the biological and clinical fates of lymphoma.
***Key Words*** : Lymphoma, Microenvironment, T cell, single-cell analysis

## Introduction

Intrinsic immunity has historically been implicated in FL, a prevalent incurable malignancy that originates from proliferative germinal center B cells but paradoxically exhibits a waxing and waning clinical course[1-5]. However, cellular ecosystems in FL have not been completely understood.

## Results

We analyzed single-cell RNA/TCR sequencing data of >500,000 human T cells from FL (obtained from four cohorts) and other 25 cancer types, as well as homeostatic and reactive lymph nodes (LNs), to construct a comprehensive single-T-cell atlas. Using this atlas, we investigated differentially expressed genes, RNA velocity, and TCR clonality. Unsupervised clustering analysis combined with subclustering analysis identified distinct T-cell subsets increased in FL relative to homeostatic LNs. We annotated these cell subsets in lymphoma as “lymphoma T cell subsets”. A subset was observed in various cancer types with remarkable cross-cancer transcriptional heterogeneities. Other subsets were specific to FL and/or diffuse large B cell lymphoma. TCR repertoire analysis revealed T cell types that give rise to these lymphoma T cell subsets. In line with these findings, RNA velocity survey suggested that lymphoma T cell subsets originated from T cell types suggested by the TCR repertoire analysis. Furthermore, phenotypes of these lymphoma T cell subsets suggested that they carry multiple functions that inhibit lymphoma development and progression. To determine the functions of T cell subsets, we performed in vitro cytokine production and co-culture assays in combination with cell activation/suppression, cell division, and apoptosis assays using human FL samples. These functional co-culture assays demonstrated discrete direct or indirect anti-tumor effects of lymphoma T cell subsets. Using the PhenoCycler-Fusion system, we conducted multiplex digital spatial profiling (MDSP) for >25 antibodies

on 242 FL samples from three independent cohorts and performed single-cell spatial and protein expression profiling and retrospective prognostic analysis. Spatial analysis revealed that lymphoma T cell subsets have unique distribution patterns in FL tissue compartments. Distance from each lymphoma T cell subset and neoplastic follicles or malignant B cells confirmed these distribution patterns. Furthermore, cellular neighborhood analysis identified distinct niche formation patterns of each lymphoma T cell subset with known T cell subsets. All of these findings supported the idea that these lymphoma T cell subsets synergistically suppress lymphomagenesis. Prognostic analysis revealed that the proportion of each lymphoma T cell subset correlated with early disease relapse and predicted a significantly longer time-to-relapse in FL. Notably, these findings were confirmed by the analysis of validation cohorts. Furthermore, multivariate analysis showed that the prognostic impact of these two cell subsets was independent of the FL international prognostic index (FLIPI). Subclustering analysis also showed that the prognostic impact of lymphoma T cell subsets were retained irrespective of the patient age, pathological grade, and treatment content. Receiver operating curve analysis revealed that the incorporation of information regarding lymphoma T cell subset proportions significantly increased the area under the curve of the FLIPI for time to relapse prediction. Improvements in continuous net reclassification and integrated discrimination were also statistically significant.

## Discussion & Conclusion

We uncovered the landscape of follicular T cells in human LNs and TMEs, and identified multiple cell subsets as follicular T cell populations. Surprisingly, these subsets are closely linked with each other in their generation, spatial distribution, and biological and clinical significance. They are orchestrated for anti-tumorigenesis and synergistically affect the outcomes of lymphoma. The distinctness of the lymphoma cell subsets was corroborated upon comparison with known T cell components and counterparts in LNs or multiple cancer types. Our spatial analysis, including MDSP, revealed unique spatial features of the lymphoma cell subsets; these features are distinguishable from those of similar cell types. Interestingly, the prognostic impact of the lymphoma T cell subsets was independent of the FLIPI, highlighting their clinical potential in establishing a new risk-adopted lymphoma management strategy. Thus, the lymphoma T cell subsets merit further study to advance our understanding of lymphoma immunity and follicular T cell ecosystems in cancer, and promote their clinical application.

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## 一般の皆様へ

本研究は、頻度の高い悪性リンパ腫の組織を最新の高解像度解析技術を複数用いて調査したものです。この解析により、リンパ腫の組織内に存在する特異的な免疫 T 細胞を複数種類同定することに成功し、そのユニークな組織内での分布パターンや機能、そして臨床的影響を明らかにしました。今後の臨床応用を目指したいと思います。

# Development of novel PFK1 inhibitors for the therapy of mitochondrial diseases

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## Summary Abstract

We have identified series of new PFK1 inhibitors that are more potent than existing PFK1 inhibitors

***Key Words*** : mitochondrial diseases / PFK1 / tryptolinamide (TLAM)

## Introduction

Therapeutic strategies for patients with mitochondrial diseases remain unestablished. We have previously discovered that tryptolinamid (TLAM), an inhibitor of the glycolytic rate-limiting enzyme phosphofructokinase 1 (PFK1), restores mitochondrial function in cells derived from Mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS) patients, and demonstrated that both attenuation of glycolysis and activation of the pentose phosphate pathway are required for the remarkable effect. However, existing PFK1 inhibitors including TLAM exhibit the weak efficacy at micro-order concentrations, making it challenging to achieve potent therapeutic effects. Therefore, in this study, we have tried to develop PFK1 inhibitors that are more potent than existing inhibitors.

## Results

1. Identification of a new PFK1 inhibitor

To identify compounds that inhibit PFK1 in cultured cells, we employed the assay system for detecting the metabolic shift from glycolysis to oxidative phosphorylation, which was established previously[1]. This assay system allows us to detect the activity of compounds that support energy production under glucose-limited conditions due to metabolic modulation, such as pyruvate dehydrogenase kinase (PDK) inhibitors and phosphofructokinase-1 (PFK1) inhibitors. We screened 9600 compounds in the chemical collection, and yielded three compounds that attenuated the cytotoxicity of 2-deoxyglucose (2DG) more than 50% as effectively as 5 mM dichloroacetate, a PDK inhibitor, used as a positive control. Of the three compounds, compound X reproducibly restored cell viability and prevented cell death following 2DG treatment in HeLa cells. Furthermore, we confirmed that compound X acts as a metabolic modulator using an extracellular flux analyzer; compound X increased oxygen consumption rate and decreased extracellular acidification rate, immediately after treatment. In addition, we performed *in vitro* PFK1 enzymatic assay, and demonstrated that compound X directly inhibits PFK1. The $IC_{50}$ value for PFK1 was 1.3 ± 0.38 μM, which is more potent than TLAM ($IC_{50}$ value: 13 ± 5.4 μM), under the same

experimental condition. Subsequent kinetics studies on compound X for PFK1 revealed that compound X inhibits PFK1 in an ATP-uncompetitive and an F6P-noncompetitive manner, while TLAM inhibits in an ATP-uncompetitive and an F6P-competitive manner. Therefore, we succeeded in obtaining a new and more potent PFK1 inhibitor with the distinct inhibitory mechanism compared to TLAM.

2. Synthesis and structure-activity relationship study of stereoisomers of TLAM as PFK1 inhibitors

Because TLAM was a hit compound from high throughput screening[1], the precise stereochemistry has remained unclear. In this part, stereoisomers anticipated from the five chiral carbons in TLAM were individually synthesized *via* asymmetric synthesis, and the most potent stereoisomer was identified. Initially, synthesis of *endo* and *exo* isomers as well as *cis* and *trans* isomers at the characteristic norbornene moiety of TLAM was conducted, followed by comparison of their PFK1 inhibitory activities. As a result, a compound with two carbonyl groups in *trans* configuration showed no PFK1 inhibitory activity, while compounds with *endo* and *exo* configurations exhibited PFK1 inhibitory activity. Additionally, NMR and HPLC analyses revealed that the norbornene moiety of TLAM adopts an *endo* configuration. Subsequently, aiming to enable the design of more potent PFK1 inhibitors, optical resolution of intermediate β-carbolines, X-ray crystallography, and PFK1 enzymatic activity assays were conducted to obtain structure-activity relationship (SAR) information based on the stereochemistry of TLAM. Four stereoisomers were asymmetrically synthesized, and their absolute configurations were determined using X-ray crystallography and HPLC analysis, successfully identifying the most potent stereoisomer.

## Discussion & Conclusion

In this study, to obtain new PFK1 inhibitors, we performed screening for compounds that activate mitochondrial respiration. Consequently, we identified compound X, which actually inhibits PFK1 *in vitro* and in cultured cells. Compound X inhibits PFK1 more potently than TLAM. Furthermore, the kinetics study revealed that compound X inhibits PFK1 in an ATP-uncompetitive and an F6P-noncompetitive manner. The inhibition mechanism is different from that of TLAM, which inhibits PFKP in an ATP-uncompetitive and an F6P-competitive manner. Taken together with the SAR study based on the stereochemistry of TLAM, our data provide information for further development of potent and selective PFK1 inhibitors for therapies for patients with mitochondrial diseases.

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## 一般の皆様へ

指定難病であるミトコンドリア病に対する根本的な治療法は確立されていません。私たちは解糖系律速酵素ホスホフルクトキナーゼ（PFK1）阻害剤が患者由来細胞のミトコンドリア機能を回復させることを見いだしてきました。この発見は従来の概念からは想定できなかったことであり、非常に独創性の高い治療標的であると考えています。本研究をさらに発展させ、ミトコンドリア病治療薬開発を目指します。

# Elucidating the mechanism of Sertoli cell polarity that plays a crucial role in the differentiation of male germ cell lineage

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## Summary Abstract

Our immunostaining analyses suggested that Map7, a microtubule-binding protein, regulates Sertoli cell polarity by aligning microtubule bundles. To this end, we used array tomography to examine Sertoli cells in *Map7* knockout (KO) mice. The results clearly showed increased non-adherent Sertoli cells, abnormal apical-basal arrangement, shorter apical elongation, extended tight junctions, and lost adherens junctions, indicating Map7's role in Sertoli cell polarity establishment. Additionally, single-cell RNA-seq (scRNA-seq) analysis revealed altered gene expression at the pachytene stage and delayed differentiation progression in *Map7* KO mice, indicating that Sertoli cell polarity supports meiotic progression in male germ cells.

***Key Words*** : Spermatogenesis/Sertoli cell polarity/Map7/Male germ cell differentiation

## Introduction

Spermatogenesis dysfunction accounts for 82.4% of male infertility cases. Studies have suggested an association between some forms of spermatogenesis dysfunction and genetic mutations. However, the pathogenesis of many spermatogenesis dysfunctions remains unclear. One reason for this is that the nature of the disease makes it difficult to conduct human genetic analyses using clinical samples, which are powerful tools for disease research. Therefore, the development and analysis of mouse models that mimic human pathology—another powerful tool in disease research—are essential to elucidate the pathogenesis of spermatogenesis dysfunction.

## Results

Our immunostaining analyses revealed that Map7, a microtubule-binding protein, may be involved in Sertoli cell polarity by regulating the alignment of microtubule bundles. To verify this possibility, it is necessary to clarify the morphological changes of Sertoli cells in *Map7* KO mice. However, since it was difficult to visualize the morphology of Sertoli cells by immunostaining, we analyzed the morphology of Sertoli cells using serial-section scanning electron microscopy, known as array tomography. The results showed that in *Map7* KO mice, the number of Sertoli cells that did not adhere to the basement membrane increased, and the arrangement of Sertoli cells along the apical-basal axis of the seminiferous tubules was abnormal. Even when Sertoli cells adhered to the basement membrane, their elongation in the apical direction was shorter than in control mice. Furthermore, the tight

and adherens junctions between Sertoli cells adhering to the basement membrane were altered, with an extension in the area of tight junctions and a loss of adherens junctions in *Map7* KO mice. Therefore, Map7 guides microtubule bundles to control Sertoli cell adhesion to the basement membrane and polarization.

Next, we elucidated how these abnormalities in Sertoli cell polarity affect male germ cell differentiation. To this end, we defined the state of male germ cell lineages at the gene expression level using scRNA-seq analysis and measured and compared the progression of differentiation. We found that in *Map7* KO mice, gene expression is altered at the pachytene stage, and the progression of differentiation is delayed. It has been previously reported that the disruption of Sertoli cell polarity by KO of polarity molecules and actin cytoskeleton remodeling-related molecules arrests male germ cell differentiation at the elongating spermatid stage (Tanwar et al., *Hum. Mol. Genet.*, 2012.; Xiong et al., *Biol. Reprod.*, 2018.; Heinrich et al., *Cell Rep.*, 2020.; Heinrich et al., *Cell Rep.*, 2021.). However, our high-resolution analysis of differentiation progression in male germ cells using scRNA-seq has newly revealed that Sertoli cell polarity may also be involved in meiotic progression.

## Discussion & Conclusion

In general, microtubule orientation is important in the process of cell polarization. However, it is not known how microtubule orientation is regulated during the establishment of Sertoli cell polarity. We found that Map7 guides microtubule bundles to control Sertoli cell adhesion to the basement membrane and polarization, and that abnormalities in this regulation lead to failures in the luminal structure of the seminiferous tubules. However, the molecular mechanism centered on Map7 that regulates microtubule orientation remains unclear and will be elucidated in the future.

Sertoli cell polarity is thought to be essential for the formation of the microenvironment required for male germ cell differentiation (reviewed by O'Donnell et al., Semin. *Cell Dev. Biol.*, 2022.), but its exact nature has remained unclear. We analyzed the progression of differentiation into male germ cells at high resolution using scRNA-seq and found that Sertoli cell polarity may be involved in meiotic progression. However, the molecular mechanism by which Sertoli cell polarity regulates meiosis is still unknown. Therefore, to better understand the substance of the microenvironment, we aim to clarify how the function of Sertoli cells is altered by abnormalities in polarization and to correlate these changes with abnormalities in meiosis in the future.

Since *Map7* KO mice serve as disease model mice mimicking spermatogenesis dysfunction, further analysis will help to better understand the pathophysiology of spermatogenesis dysfunction through a more detailed understanding of the molecular mechanisms.

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## 一般の皆様へ

造精機能障害は精巣における精子生成機能の低下や不全が原因であり、その多くは先天的なものである。これまでの研究で、一部の造精機能障害と遺伝子変異との関連が示唆されているが、多くのケースでは発症メカニズムが不明である。そこで、私共はヒト病態をミミックするような疾患モデルマウスの開発・解析を進めてきた。その中で、精子形成を支える細胞であるセルトリ細胞の形態と機能の連関について、新たな発見につながる研究成果が得られた。今後は、その分子メカニズムの解明を目指したい。

# Mechanistic load-sensing cardiac-specific super-enhancer By Phase-Separated Transcription Factories

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## Summary Abstract

CR9 element is shown in vivo to act as a super-enhancer of the natriuretic peptide genes in the heart and to regulate gene expression in response to pathological conditions. Further detailed mechanistic analysis of CR9 will lead to the elucidation of cardiac-specific transcriptional mechanisms and their application to cardiac gene therapy.
***Key Words*** : phase-separated transcription factory, natriuretic peptides, heart failure-sensitive super-enhancer

## Introduction

The "phase-separated transcription factory model," in which a group of transcriptional regulators efficiently activate transcription of cell type-specific genes by locally and densely aggregating and phase-separating transcription factors in DNA regulatory regions called super-enhancers, has attracted attention. In this study, we will introduce the concept of phase-separated transcription factory to the heart failure-sensitive super-enhancer CR9 element that we have identified, and aim to elucidate the molecular mechanism of cardiac-specific transcriptional responses.

## Results

We confirmed that the expression levels of natriuretic peptide genes (Nppa and Nppb) in the ventricular tissues of CR9 knockout (CR9-KO) mice were markedly reduced compared to wild-type mice. In addition, the expression of Nppa and Nppb genes was significantly suppressed in CR9-KO mice compared to wild-type mice after aortic arch stenosis surgery in a pressure-loaded heart failure model. These results suggest that the enhancer CR9 region is sensitive to pressure loading that induces heart failure. Next, ATAC-sequence analysis was performed to examine changes in chromatin structure. In wild-type ventricular tissue, open chromatin structures in the CR9 region and the Nppa and Nppb gene regions were evident, but in CR9-KO mice, open chromatin structures in the CR9, Nppa, and Nppb regions were lost. ChIP-sequence analysis was further performed to examine changes in chromatin modifications. In wild-type ventricular tissue, a large peak of H3K27ac, a marker of activated enhancers, was observed in and around the CR9 region, but in CR9-KO mice, the CR9 region and the surrounding H3K27ac peak were absent. Furthermore, the enhancer activity of the CR9 region by CRISPR-activation and CRISPR-inhibition using the CRISPR/dCas9 system was examined using cultured cardiomyocytes. In the CRISPR-activation experiment, Nppa and Nppb gene expression increased only when gRNA for the

CR9 region was introduced, while in the CRISPR-inhibition experiment, Nppa and Nppb gene expression was suppressed only when gRNA for the CR9 region was introduced.

Using ventricular tissue from wild-type and CR9-KO mice, the physical interaction of the CR9 region with the promoter regions of the Nppa and Nppb genes was examined by 4C analysis. In the wild type, the CR9 region interacted with the promoter regions of Nppb and Nppa, and the peak at those regions was reduced in CR9-KO.

To visualize and observe changes in size and frequency of Nppa and Nppb transcriptional bursts, we planned to create human iPS cells (Nppa-MS2/MCP-GFP and Nppb PP7/dtTomato-PCP). We are currently in the process of obtaining BACs containing CR9, Nppa, and Nppb regions and introducing genes such as MS2, PP7, MCP-GFP, and dt-Tomato-PCP into them. iPS cells, once completed, can be differentiated into cardiomyocytes to visualize Nppa and Nppb transcriptional bursts in a live environment. We have also successfully attempted to visualize freshly transcribed RNA from the Nppa and Nppb genes using quantitative single-molecule RNA FISH. Finally, using CR9 element DNA column and LC/MS/MS analysis, we identified four transcription factors that bind to the CR9 region and regulate enhancer activity in cultured cardiomyocytes.

## Discussion & Conclusion

We previously identified a potential enhancer region, CR9 element, for natriuretic peptide genes in cardiomyocytes. In the current study, we demonstrated that the CR9 region regulates the expression of Nppa and Nppb genes as an enhancer in hearts in vivo using the knockout mice of CR9 element. In addition, we demonstrated that CR9 element interacts with the promoter regions of the Nppb and Nppa genes in vivo. Furthermore, we successfully attempted to visualize CR9 element and freshly transcribed RNA from the Nppa and Nppb genes using quantitative single-molecule RNA FISH. In the future, we plan to examine the relationship between the CR9 region and the RNAs of Nppa and Nppb, as well as the relationship between the CR9 region and the mediators that make up the super-enhancer under physiological and pathophysiological (heart failure) conditions.

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## 一般の皆様へ

ナトリウム利尿ペプチドは心臓で特異的に発現する遺伝子で、心不全に際してその転写が劇的に誘導されます。この心臓特異的かつ病態特異的な転写誘導のメカニズムにスーパーエンハンサー CR9が関与していると考えられます。この分子メカニズムの解明は、心臓特異的に発現する分子の転写制御機構の解明のヒントになり、さらには心臓遺伝子治療への応用にもつながります。

# Evolution and activation mechanisms of tandemly duplicated NLR immune receptors

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## Summary Abstract

NLR proteins act as immune receptors. Gene duplication amplifies the number of NLR genes, and subsequent mutations occasionally provide modifications to the second gene that benefits immunity. However, evolutionary processes of duplicated NLRs remain largely unclear. Here, we report that the rice NLR protein Pit1 was associated with its paralogue Pit2. The two are required for the resistance to rice blast fungus but have different functions: Pit1 induced cell death, while Pit2 competitively suppressed Pit1-mediated cell death. During evolution, the suppression of Pit1 by Pit2 was probably generated through positive selection on two fate-determining residues in Pit2. Consequently, Pit2 lost its plasma membrane localization but acquired a new function to interfere with Pit1 in the cytosol. These findings illuminate the evolutionary history of tandemly duplicated NLR.
***Key Words*** : NLR immune receptor, rice, evolution

## Introduction

Nucleotide-binding domain and leucine-rich repeats (NLR) family proteins act as intracellular immune receptors to detect pathogen effectors, thereby inducing effector-triggered immunity (ETI). Gene duplication events amplify the number of NLR family genes, and subsequent random mutations occasionally provide modifications to the second gene that benefits immunity. However, **evolutionary trajectory after gene duplication and functional relationships between duplicated NLR genes remain largely unclear**.

## Results

Several groups, including us, were the first in the world to discover "paired NLR proteins" that could explain why plants have a large number of immune receptors (Cesari et al., EMBO J 2014). Paired NLR proteins consists of two different NLRs where one of them plays the role of sensor for "ligand recognition" whole the other has role in "immunity induction". The two NLRs co-operate to function as one large receptor complex. Our group has been leading research on paired NLR proteins by identifying multiple downstream key signaling molecules. We have revealed that the small GTPase OsRac1 and its activator OsSPK1 which is a direct downstream target of the NLR protein Pit1, and induce disease resistance to rice blast fungus factors (Wang et al., Plant Cell Environ. 2022; Akamatsu et al., Plant Cell Physiol. 2021; Wang et al., P PNAS 2018; Kawano et al., J Biol Chem 2014; Kawano et al., Cell Host Microbe 2010). These studies are important milestones in understanding the activation mechanism of pairs of NLR proteins. However, **the mode of action of Pit1 in relation to other NLRs is not known.**

In this project, we report that the NLR-type rice resistance protein Pit1 was associated with its paralogue Pit2 (Li et al., Nat Commun in press). The two are required for the resistance to rice blast fungus but have different functions: Pit1 induces cell death, while Pit2 competitively attenuates Pit1 homocomplexes by forming heteromers with Pit1 to suppress Pit1-mediated cell death (Figure). Pit2 may compete with Pit1 for binding to OsSPK1 and OsRac1, acting in a dominant negative manner. This phenomenon is called paralogue interference.

We assume a possible evolutionary model of *Pit1* and *Pit2* as follows (Figure): the duplication of an ancestral *Pit* gene occurred about 14.69 Mya (Li et al., Nat Commun in press). *Pit2* is considered a new copy. Occasionally, as the redundant, Pit2 accumulated substitutions including the fate-determining mutation between Pit1 and Pit2. The mutations at P300 and F415 in Pit2 have resulted in neo-functionalization, causing the loss of its plasma membrane localization and acquisition of a new function. This new function sequesters Pit1 in the cytosol and regulates the membrane-localized Pit1 functions such as cell death execution.

What is the significance of Pit2 forming a complex with Pit1? One interesting hypothesis is that Pit2 acts as a fine tuner of the membrane-localized Pit1 functions, ensuring that immune activation is carefully regulated to prevent excessive response. It is possible that this regulatory mechanism potentially enhances the yield of domesticated rice.

We found the selection signatures at the C-terminus of Pit2 (Li et al., Nat Commun in press). This result raises the possibility that the polymorphisms in the LRR domain of Pit2 contribute to the specific recognition of pathogen effectors. These findings illuminate the evolutionary history of tandemly duplicated NLR genes after gene duplication and how two NLR proteins orchestrate plant immunity.

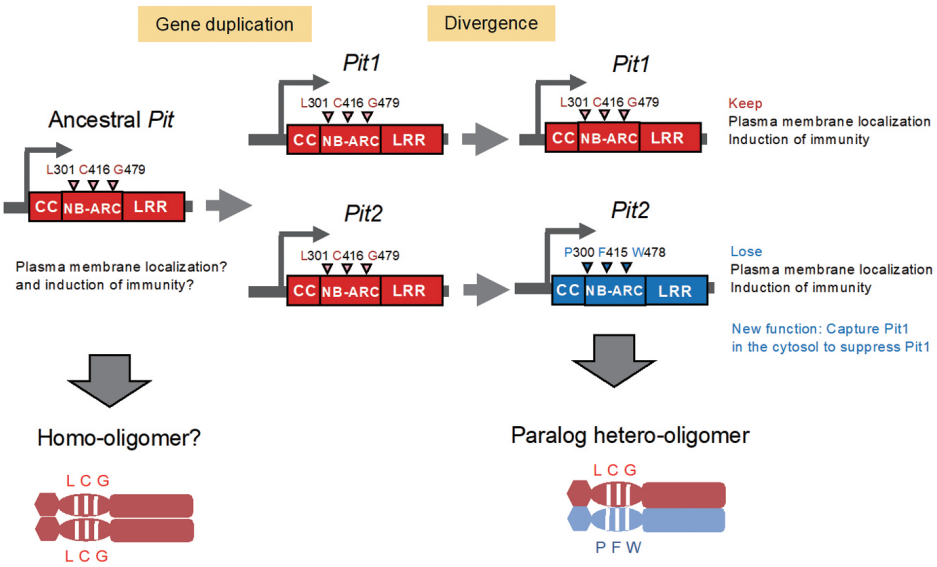


**Figure: Model of the evolution and function of Pit1 and Pit2**

## Discussion & Conclusion

In this project, we expanded upon our initial finding of the rice NLR protein Pit1 interacting with other NLRs, based on previously established research from my group and collaborators (Wang et al., Plant Cell Environ. 2022; Wang et al., Proc Natl Acad Sci U S A 2018; Kawano et al., J Biol Chem 2014; Cesari et al., EMBO J 2014; Kawano et al., Cell Host Microbe 2010). We conduct detailed molecular analyses to define the downstream mode of action and subcellular localization of the newly identified paralog Pit2 and its interplay with Pit1, such as its interaction with OsRac1 (Li et al., Nat Commun, In press). We demonstrated a robust physical interaction between Pit1 and Pit2, followed by a detailed characterization of their cooperative roles in fine-tuning immune responses and their localization differences. The strength of our work lies in the comprehensive characterization of functional differences between these closely related paralogous NLRs, providing their evolutionary history, and delineating the critical two residues contributing to the functional divergence between Pit1 and Pit2. Our results explain how neofunctionalization occurred in Pit2, a duplicate copy of the apparently more ancient Pit1. Our evolutionary analyses with molecular clock dating revealed the origin of this duplication event within the Oryza lineage. Further comparison was made on the two key residues that correspond to the sub-clades of Pit1/Pit2 according to the provided tree, indicating their importance in shaping their evolutionary trajectories.

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## 一般の皆様へ

植物は、非常に多くの NLR 型免疫受容体を持ち、この遺伝子数の多さが免疫の堅牢性に貢献していると推測されている。しかしながら、増加した NLR 遺伝子がどのような進化の過程を経て新しい機能を獲得し、植物免疫に貢献しているかは十分に理解されていない。本研究により、イネいもち病菌に対する NLR 型免疫受容体 Pit1と、そのパラログ Pit2では、「パラログ抑制」と「新機能獲得」と呼ばれる遺伝子重複後に見られる特徴的なイベントが起きたことが示唆された。この2つのイベントにより、Pit1と Pit2は、ペア NLR タンパク質として一つの免疫受容体として働くことが示唆された。本研究により、植物免疫の中心的なシステムの一つであるペア NLR 型免疫受容体が生み出された進化過程の理解が可能となり、その成果により高性能な人工免疫受容体の設計などに繋がる可能性がある。

# Defective mitophagy in pancreatic β-cells leads to diabetes

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## Summary Abstract

Mitochondrial dysfunction in pancreatic β-cells leads to impaired glucose-stimulated insulin secretion (GSIS) and type 2 diabetes (T2D), highlighting the importance of autophagic elimination of dysfunctional mitochondria (mitophagy) in mitochondrial quality control (mQC). In this study, we compared the effects of imeglimin, a new anti-diabetic drug, metformin and insulin on β-cells in diabetic model *db/db* mice. Our results indicated that imeglimin, but not metformin, mitigated the accumulation of dysfunctional mitochondria and led to an increase in the amount of secreted insulin and suppression of apoptosis in pancreatic β-cells from *db/db* mice.

***Key Words*** : Diabetes, Pancreatic β-cells, Mitochondria, Mitophagy

## Introduction

Imeglimin is a new anti-diabetic drug that is structurally related to metformin, a drug widely used to treat T2D. Imeglimin was shown to potentiate GSIS, probably by improving mitochondrial function. In addition, recent studies showed that imeglimin treatment also improved mitochondrial morphology and suppressed apoptosis in β-cells in *db/db* mice. Thus, imeglimin may improve hyperglycemia by enhancing insulin secretion and preserving b-cell mass, likely through the maintenance of mQC in pancreatic β-cells. However, it remains unclear whether imeglimin affects mQC in diabetic β-cells. Therefore, we examined the effects of imeglimin on the maintenance of mQC from the perspective of mitophagy in pancreatic β-cells from *db/db* mice.

## Results

In this study, we compared the effects of imeglimin with the structurally similar metformin and insulin, which maintains functional β-cells in T2D patients. We first examined the effect of these drugs on glycemic control in *db/db* mice. Oral glucose tolerance test and insulin tolerance test were performed following 6 weeks of treatment with these drugs. Insulin treatment reduced blood glucose levels, but did not affect insulin sensitivity, whereas both imeglimin and metformin improved blood glucose levels and insulin sensitivity. However, the amount of serum insulin was increased in imeglimin-treated, but not metformin-treated, *db/db* mice.

Impaired mitochondrial function and morphologically abnormal mitochondria have been detected in pancreatic β-cells from T2D patients. Thus, we next hypothesized that imeglimin may reduce the number of dysfunctional mitochondria in β-cells from db/db mice. To test this hypothesis, we cultured β-cells from control-treated or drug-treated *db/*

*db* mice and visualized functional mitochondria using Mitotracker (MTR), a fluorescent dye that stains functional mitochondria. Compared to non-diabetic *db/m+* control mice, the signal intensity of MTR was markedly decreased in β-cells cultured from *db/db* mice. The signal intensity of MTR was restored in β-cells cultured from insulin-treated *db/db* mice. Meanwhile, the signal intensity of MTR was significantly elevated in β-cells cultured from imeglimin-treated, but not metformin-treated, *db/db* mice. Mitophagy is activated by the accumulation of dysfunctional mitochondria to preserve cellular homeostasis. Given our result using MTR, we hypothesized that the mitophagic activity in β-cells from *db/db* mice would be mitigated by imeglimin and insulin treatment. To assess this possibility in vivo, we used CMMR mice, in which mitophagy signal can be visualized in pancreatic β-cells. We crossed CMMR mice with *db/db* mice, and found that many mitophagy signals were observed in β-cells from *db/db* mice. Insulin treatment dramatically decreased the number of mitophagy signals in *db/db* mice. Correspondingly, imeglimin, but not metformin, also significantly reduced mitophagic activity. Taken together, these results demonstrated that imeglimin and insulin, but not metformin, ameliorated the accumulation of dysfunctional mitochondria in β-cells from *db/db* mice.

Because mitochondrial dysfunction leads to excessive ROS production and oxidative stress, we next examined endogenous ROS levels in β-cells from *db/db* mice. Pancreatic β-cells were cultured on coverslips from control-treated or drug-treated *db/db* mice, and stained with MitoSOX, a mitochondrial superoxide indicator. The MitoSOX signal intensities in β-cells cultured from control-treated *db/db* mice were significantly higher than those from *db/m+* mice. Furthermore, this increase in MitoSOX in *db/db* mice was restored by insulin treatment. Consistently, OxyBlot analysis revealed that the amount of protein oxidized by free radicals, such as ROS, was markedly increased in *db/db* islets compared with that in *db/m+* islets, and was dramatically reduced by insulin treatment. Likewise, treatment with imeglimin, but not metformin, significantly reduced the MitoSOX signal intensity in β-cells and the OxyBlot signal intensity in isolated islets. These results demonstrated that treatment with imeglimin or insulin, but not metformin, ameliorated ROS production and oxidative stress in β-cells from *db/db* mice.

We expected that reduction of dysfunctional mitochondria in *db/db* mice would improve β-cell function. Thus, we examined GSIS and apoptosis in β-cells from *db/db* mice treated with imeglimin, metformin or insulin. The impairment in insulin secretion in islets isolated from *db/db* mice was significantly ameliorated by insulin treatment. Similarly, imeglimin, but not metformin, improved GSIS in *db/db* islets. Apoptosis was assessed by TUNEL assay in pancreatic sections, and the number of TUNEL-positive nuclei in pancreatic β-cells were counted. The numbers of TUNEL-positive β-cells were markedly reduced in both imeglimin- and insulin-treated *db/db* mice compared with those in control-treated *db/db* mice. Metformin treatment also led to lower levels of apoptotic cell death in these mice, but the result did not reach statistical significance.

Taken together, these results demonstrated that imeglimin treatment restored mQC by reducing dysfunctional mitochondria and ROS production in β-cells, which led to the restoration of functional β-cells and amelioration of blood glucose levels in *db/db* mice.

## Discussion & Conclusion

We demonstrated that imeglimin and insulin, but not metformin, mitigated the accumulation of dysfunctional mitochondria and led to an increase in the amount of secreted insulin and suppression of apoptosis in pancreatic β-cells from *db/db* mice.

Insulin treatment suppressed ROS production probably by alleviating the burden of excessive insulin secretion in β-cells, which could lead to preservation of functional β-cells in T2D patients. In contrast, imeglimin likely reduces ROS production through a direct effect on β-cells. Imeglimin reportedly upregulates the expression of NAMPT, a key enzyme in NAD+ biosynthesis pathway in β-cells. This might activate sirtuins and reduce ROS generation, enhance GSIS and inhibit apoptosis in β-cells from *db/db* mice.

Our findings suggest that maintenance of mQC is important for preserving β-cell function and survival, and support the use of imeglimin treatment in T2D patients, especially for the preservation of β-cell function.

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## 一般の皆様へ

2型糖尿病では、インスリンを分泌する膵臓のβ細胞が疲弊して、インスリン分泌量が減少することがきっかけとなり、血糖値が上昇することが知られています。本研究では新規経口糖尿病薬であるイメグリミンが膵β細胞のミトコンドリアに対してどのような影響を及ぼすかについて検討を行いました。その結果、イメグリミンは膵β細胞のミトコンドリアを保護することで、膵β細胞が疲弊することを防いでいることがわかりました。この結果は、膵β細胞におけるミトコンドリア保護の重要性を示しており、今後の糖尿病治療薬開発への新たな視点を提供するものと考えております。

# Differential Regulation of Stem Cell Ability by Calcium Signaling in Normal and Abnormal Hematopoietic Stem Cells and Therapeutic Applications

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## Summary Abstract

Plcl1 KO hematopoietic stem cells (HSCs) showed comparable gene expression to wild type HSCs, but with increased absolute numbers. In particular, platelet-biased HSCs (CD41+HSCs), the apex of a heterogeneous population of HSCs (ref1), were increased. This suggests that Plcl1 function may be involved in platelet production. Indeed, Plcl1KO HSCs showed rapider recovery of platelets after platelet depletion than wild type HSCs. Moreover, myeloid regeneration was accelerated after myeloablation in Plcl1KO mouse. CD41 expression is regulated by increasement of intracellular calcium via Plc signaling. Plcl1 is an inactive Plc, so it is a negative regulator and buffer for Plc signaling. Therefore, Plcl1 may function to maintain HSCs dormant by buffering calcium signal via Plc after proliferative stimuli.

***Key Words*** : Platelet-biased HSC, calcium, Plc signaling,

## Introduction

Intracellular calcium ions are important second messengers, and cytosolic Ca2+ concentration (Ca2+i) is important for various biological phenomena such as cell proliferation, energy metabolism and cell death. We have previously shown that activation of Ca2+ signaling associated with elevated Ca2+i is important for the maintenance of stemness of HSCs (ref2), but the molecular basis for this is not known. We also found that Plcl1, a regulator of Plc signaling involved in Ca2+i regulation, is specifically expressed in HSCs. Thus, Plcl1 has some roles to maintain stemness of HSCs by modulating Ca2+i.

## Results

We examine the mechanisms of stemness regulation by calcium signaling in normal and abnormal hematopoietic stem cells (HSCs). Here we focused on the functional analysis of Plcl1, which acts as a buffer for intracellular calcium signaling in HSCs, and the results suggest that Plcl1 may be important for stem cell maintenance by negatively regulating the activation of platelet-polarized HSCs. Specifically, by using public databases (ref3), we found that Plcl1 is highly expressed in platelet-polarized hematopoietic stem cells (Vwf+) among HSCs (Fig. 1). On

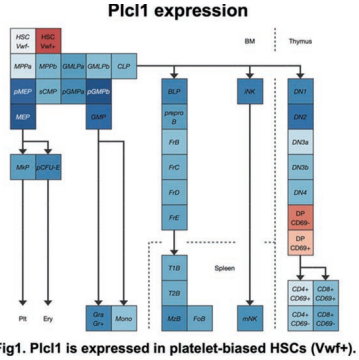


Fig1. Plcl1 is expressed in platelet-biased HSCs (Vwf+).

the other hand, RNA sequencing analysis revealed limited changes in gene expression in Plcl1KO HSCs compared to wild type (data not shown). This suggested that Plcl1 may be important for protein-level signaling rather than gene expression. To verify this, we evaluated the response of Plcl1KO and wild-type HSCs in a model of acute thrombocytopenia using CD42b antibody. Plcl1KO showed a rapid platelet recovery compared to wild-type (Fig.2). Wild-type HSCs were found to express CD41 on the membrane surface at 2 hours after dosing with CD42b antibody (Fig.3). This suggests that CD41 expression in HSCs is regulated at the protein level rather than by gene expression. Correlating with this, we observed an increase in Plcl1KO platelet-biased HSCs compared to wild type after platelet depletion. This suggests that Plcl1 suppresses the rapid proliferation and differentiation of platelet-biased HSCs in response to thrombocytopenia. To confirm that Plcl1 suppresses Plc signaling, we treated wild-type and Plcl1KO HSCs with Plc agonists and found that Plcl1 KO HSCs showed significantly increased CD41 expression compared to wild-type cells (Fig.4). In addition, Plcl1KO showed an early recovery of myeloid cells compared to wild type in a 5FU-induced myelosuppression model. This suggests that Plcl1 plays an important role in the regeneration of myeloid cells as well as platelets.

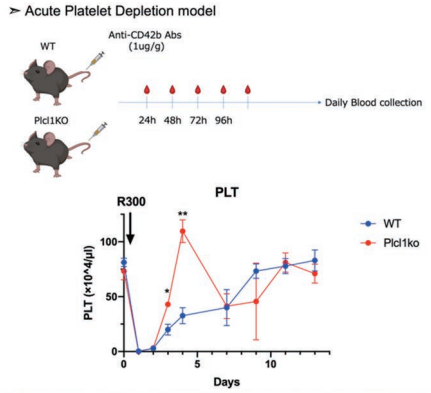


Fig.2 More rapid emergent megakaryopoiesis was observed in Plcl1-KO mouse.

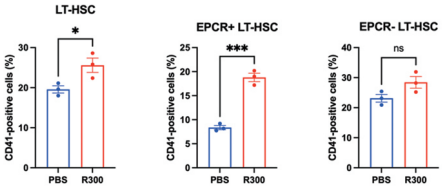


Fig.3 Augmentation of CD41 expression on LT-HSCs, in particular, EPCR+LT-HSC (platelet-biased HSCs are enriched) at 2 hours after platelet depletion

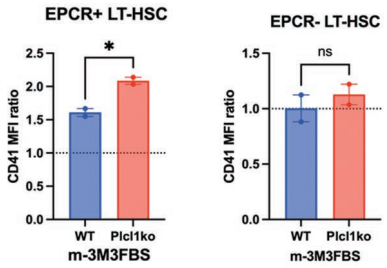


Fig.4 Plcl1 KO HSCs showed significantly increased CD41 expression compared to wild-type cells after treatment of Plc agonist (m-3M3FBS).

## Discussion & Conclusion

In this study, we found Plcl1 as buffer for Plc signaling, which is highly expressed in platelet-biased HSCs. Plcl1KO HSCs showed accelerated proliferation of myeloid cells, in particular, platelets after proliferative stimulation such as acute platelet deletion and 5FU-induced myelosuppression. Therefore, Plcl1 may have functions to suppress proliferation and differentiation of platelet-biased HSCs after stimulation. Plc agonist treatment also induced higher CD41 expression in Plcl1KO HSCs than wild-type HSCs. Here we examined roles of Plcl1 in normal HSCs. So, we will examine role of Plcl1 in leukemic stem cells (LSCs) in the future study. Given that Plcl1 suppress and/or buffered calcium signaling via Plc, probably Plcl1 may be a negative regulator for development of LSCs We will examine it using myeloid leukemia such as AML and CML models. We also performed in vivo calcium imaging using GCaMP6s mice crossed with Vav1-Cre or Hlf-CreERT2, but we could not detect any signals in the skull BM. This experiment showed that a brighter calcium probe is needed.

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## 一般の皆様へ

造血システムにおいて最も重要な機能は止血であると言える。この止血を担っているのが血小板である。血小板は迅速に傷口に集積し、傷口をふさぐ。近年、造血幹細胞は前駆細胞を介さずに巨核球を経由して血小板を作ることが知られてきた。迅速な傷口への対応には前駆細胞を経由していては処置が後手に回ることを考えれば納得である。本研究において急激な血小板減少を人工的に起こすと造血幹細胞が2時間以内に反応することを突き止めた。この迅速な反応には造血幹細胞が枯渇するリスクがあるために厳密に制御される必要がある。これを担っているのが Plcl1というタンパクである。いわば造血幹細胞の増殖・分化のブレーキ的な役割を担っている。したがって、この Plcl1を中心としたブレーキのメカニズムを理解することで、急性・慢性骨髄性白血病幹細胞の増殖・分化のブレーキとして利用する新たな治療戦略への貢献が期待できると考え研究を行なっている。

# A study about a molecular mechanism of regulation of flowering from roots

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## Summary Abstract

It is known that flowering time in plants is controlled by molecular mechanisms that function in shoot apex and leaves. Here, I found that the peptide-encoding genes, that are expressed in roots, negatively regulate flowering time in *Arabidopsis thaliana*. Thus, flowering time is not only controlled by shoots but also by roots.
***Key Words*** : Flowering, Peptide, Long-distance signaling

## Introduction

It is known that flowering time in plants is controlled through long-distance signaling between leaves and shoot meristem, as well as through local signaling at the shoot meristem. The molecular mechanism underlying these signaling has been well studied, so far. In contrast, the regulation of flowering by roots is largely unknown, including whether such a molecular mechanism exists. Recently, in the model plant *Arabidopsis thaliana*, I found the possibility that a peptide, Flowering-related Peptide (FP), and its homologs regulate flowering from roots. I therefore planned a study to explore this possibility.

## Results

So far, I found that flowering time was delayed in *FP*-overexpressing plants, and mutations in *FP* and its homologs (*fps* mutant) resulted in early flowering phenotype. Considering that FP and its homologs mainly expressed in roots, there is a possibility that they regulate flowering from roots. To explore this possibility, I performed grafting experiments to combine the roots and shoots of WT and fps plants (Figure). We confirmed that grafted plants consisting of scions and rootstocks of the same genotype (WT/WT and *fps*/*fps*) showed the flowering times comparable to those of the corresponding intact plants. Interestingly, WT/*fps* grafted plants exhibited early flowering phenotype, and their flowering time was similar to that of *fps/fps* grafted plants. On the other hand, *fps*/WT grafted plants showed normal flowering time as observed in WT/WT grafted plants. This result indicates that FP and its homologs in roots negatively regulate flowering in shoots.

Next, I tried to search for candidate receptors for FP in the regulation of flowering time. So far, I have found that mutant plants of a receptor protein, R1, showed early flowering phenotype. I also examined the flowering time in mutants of genes encoding other receptors and found that mutation in another receptor, R2, also resulted in early flowering. Therefore, we analyzed the flowering time of *r1* and *r2* mutants by comparing with *fps* mutants under several conditions to determine whether R1 or R2 could serve as a receptor for FP. Usually,

*A. thaliana* plants are grown under 23 ℃ , so I first analyzed flowering time under the normal temperature and short day (10 hours light / 14 hours dark) condition. As a result, the flowering time of both *fps* and *r2* mutants was about 30 days after germination (DAG), but that of *r1* mutants was slightly later, about 33 DAG, although that of these three mutants was earlier than that of WT (about 40 DAG). Next, *fps, r1* and *r2* mutant plants were grown under 23℃ for 17 days, then they were grown under low temperature condition (13℃ ). As a result, all of their flowering time became late, but interestingly, the flowering time of *fps* and *r2* mutants (about 46 and 48 DAG, respectively) were significantly earlier than that of *r1* mutants (about 60 DAG). Under this condition, the flowering time of WT was about 67 DAG. Considering these results, R2 is a strong candidate for the receptor of FP peptides. To examine whether R2 is the receptor for FP peptides, I am currently in the process of crossbreeding of *FP*-overexpressing plants into *r2* mutant plants.

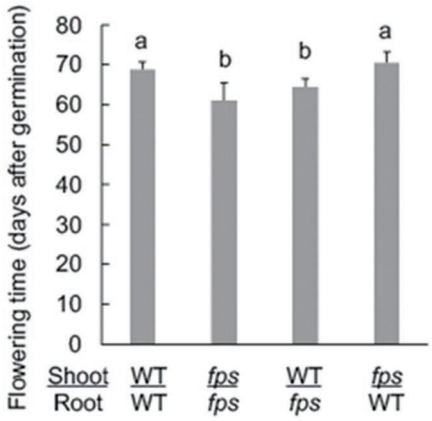


**Figure** Flowering time of grafted plants. Statistical differences were evaluated by Tukey's tests ($P < 0.05$). Each result was obtained from 6 to 11 individual plants.

It has been reported that the flowering time is affected by various environmental stimuli including soil conditions, such as soil temperature and minerals. To explore the conditions under which *FP* and its homologous genes respond, WT plants were grown under low nitrogen, phosphorus or potassium nutrient condition or low temperature (13 ℃ and 4 ℃ ) condition and analyzed the expression levels of *FP* and its homologous genes. However, significant changes in the mRNA level of *FP* genes were not observed under those conditions. So, now I try to examine the mRNA levels of *FP* genes under other conditions that are known to affect flowering time.

## Discussion & Conclusion

In plants, it is known that flowering time is regulated through long-distance signaling between leaves and shoot meristem, as well as through local signaling at the shoot meristem. On the other hand, it has been unclear whether root control flowering through root-to-shoot long-distance signaling. In this study, I found that the peptides, FP and its homolog peptides, negatively regulate flowering from roots.

In general, peptides that move from roots to shoots pass through xylem, but they cannot be translocated to shoot apex directly, because xylem is not well developed in shoot apex. Therefore, it is possible that root-derived FP and its homolog peptides are translocated to leaves through xylem, and they are recognized by the receptor R2 which is expressed in leaves. Then, secondary signal should be translocated from leaves to shoot apex through phloem, such as FT protein. This molecular model needs to be tested in detail in the future.

## 一般の皆様へ

植物にとっていつ花を咲かせるかは、その個体の一生において重要です。また、農作物が花を咲かせる時期は、収穫の時期に大きく影響します。これまでに、植物の花を咲かせる時期は、葉や茎頂が調節することが知られており、それらに関する分子機構も詳細に研究されてきました。その一方で、根が花を咲かせる時期を調節する仕組みは、それが存在するかどうかも含めて、これまで明らかにされていませんでした。これに対して、私は根で発現するペプチド遺伝子 *FP* とそのホモログが花を咲かせる時期を負に調節することを見出しました。

# The analysis on the re-establishment of epigenome in mouse gonocyte

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## Summary Abstract

The study focuses on epigenetic mechanisms in germ cells, particularly H3K27me3, crucial for development and differentiation. In mammals, DNA methylation and histone modifications are vital for both homeostasis and differentiation. H3K27me3, a repressive histone modification, changes during cellular differentiation, affecting gene activity in general. Analysis reveals H3K27me3 re-accumulation in spermatogonial stem cells after elimination in gonocytes. Additionally, H3K4me3 opposes DNA methylation, particularly at transcription start sites. Upregulation of genes in gonocytes occurs upon H3K27me3 elimination, with some activated by nearby transposons. The study provides insights into H3K27me3 dynamics in germ cells, shedding light on chromatin reorganization and gene regulation, vital for understanding male fertility in mammals.
***Key Words*** : Epigenetics, embryonic germ cells, H3K27me3, DNA methylation, transposon

## Introduction

In mammals, epigenomes such as DNA methylation and histone modifications are essential for life. What about other organisms? Bacteria and archaea also have DNA methylation that differs from that of mammals, and a group of proteins that are functionally similar to histone proteins have been discovered that are important for cell growth and adaptation to changes in the external environment. In other words, almost all life requires an epigenome. In this study, we will elucidate how the epigenome is formed from a new mesoscopic viewpoint.

## Results

DNA methylation plays a central role in mammalian homeostasis, development and differentiation. In particular, during cellular differentiation, the regions of DNA methylation introduced into the genome differ according to each cell lineage. Conversely, genome-wide DNA demethylation occurs when cells acquire pluripotency, such as during iPSC establishment. These suggest there is a negative correlation between the differentiation potential of cells and the amount of DNA methylation in the genome. In the male germ cells of mice, which are the focus of this study, genome-wide DNA demethylation and subsequent methylation occur from fetal to neonatal stage. We have focused on specific cells, called gonocytes, during which genome-wide methylation occurs, and have analyzed their chromatin state. We found that H3K27me3 is eliminated from chromatin during the gonocytes (termed “H3K27me3

elimination"), and since H3K27me3 is a histone modification that, along with DNA methylation, affects the state of development and differentiation, it was predicted that this would define the differentiation state of these germ cells.

In this research project, we have studied this H3K27me3 elimination. As a result, H3K27me3, which was eliminated in gonocytes, re-accumulated in the genome in spermatogonial stem cells (SSCs), the next developmental stage of gonocytes. H3K27me3 is a histone modification that correlates with gene repression, whereas H3K4me3 correlates with gene activation. The presence of these two opposing histone modifications on the same gene is called bivalent, and it is known that the number of bivalent genes is higher in undifferentiated cells than in differentiated cells. In this study, we analyzed the number of bivalent genes in gonocytes and SSCs, and found that about 80% of the genes that were bivalent in SSCs were already bivalent in the early stage of gonocytes. This indicates that the epigenetic memory of such genes is maintained before and after H3K27me3 elimination without any significant change in their bivalent status.

To verify how H3K27me3 is re-established during differentiation into sperm stem cells, we performed CUT&TAG on Ezh2, the enzyme responsible for adding H3K27me3. However, this did not yield data, probably due to the nature of the chromatin specific to gonocyte stage, so CUT&RUN was performed instead. The results showed that the localization of Ezh2, which once disappeared in mid-gonocytes, was restored by P3. We plan to examine the localization of Ezh2 at later time points.

We next tested the significance of H3K27me3 elimination occurring at the same time as the establishment of DNA methylation. Although DNA methylation occurs genome-wide in gonocytes, the transcription start site region (TSS) of genes evades methylation. Furthermore, H3K4me3 has previously been shown to work antagonistically with DNA methylation. Our analysis revealed that upon H3K27me elimination, H3K4me3 accumulates in the TSS and inhibits DNA methylation only in the TSS region **(Li et al., in prep.) (Fig. 1)**

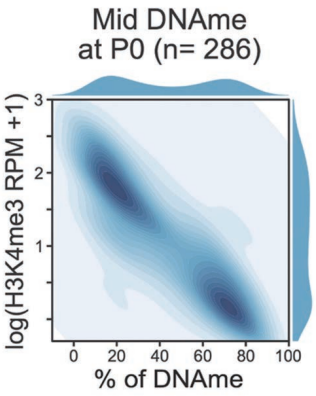


**Fig.1 H3K4me3 level dictates whether genes are coated with DNAme or not**
Density of plot for genic TSSs. H3K4me3 level over TSSs, and their DNAme level are shown. Please note that H3K4me3 has a strong negative correlation with DNAme in gonocytes.

About 3,000 genes are upregulated in gonocytes upon H3K27me3 elimination. On the other hand, certain genes were upregulated in gonocytes despite the absence of H3K27me3 elimination. Interestingly, these genes were upregulated not by their own promoters but upon activation of nearby transposons. Furthermore, a factor called Morc1 was found to be involved in the regulation of this latter group of genes **(Uneme et al., 2024, PNAS) (Fig. 2)**

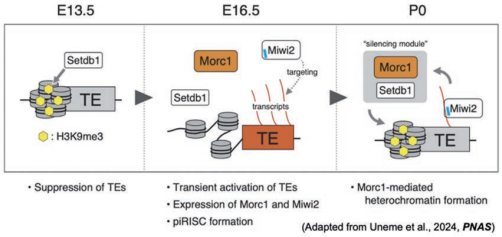


**Fig.2 Morc1 regulates gene expression through suppressing nearby transposons**
During gonocytes, Morc1 deposits H3K9me3, one of repressive histone marks, onto transposons and turns their TSSs into closed state. This suppressive role of Morc1 also affects the expression level of nearby genes.

## Discussion & Conclusion

This study advances our understanding of the dynamic regulation of H3K27me3 in germ cells and the biological role it plays. There have been reports on the dynamics of H3K27me3 in germ cells before gonocytes (primordial germ cells) and in germ cells after gonocytes (SSCs). On the other hand, the analysis had not progressed in gonocytes because of the small number of cells and the need for special transgenic mice for isolation from testis. Our results provide insight into the multifaceted biological role of H3K27me3 in gonocytes. Derivative analyses also revealed new insights into the symbiotic relationship between transposons and genes in gonocytes **(Uneme et al., 2024, PNAS)**. Surprisingly, chromosomes are entirely relaxed in gonocytes **(Yamanaka et al., 2019, Developmental Cell)**. Thus, contrary to expectations, we see a large-scale reorganization of chromatin in gonocytes, which connect primordial germ cells with less sex specificity to SSCs in a committed male germline state. In the future, we will elucidate the reorganization process of H3K27me3 in detail down to the molecular level and examine how it regulates male fertility.

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## 一般の皆様へ

生殖補助医療の需要は年々増加していることから、「不妊症」は現代社会が取り組むべき課題の一つである。不妊症の15% は、その原因が未解明（原因不明不妊）であるなど治療法確立に向けた取り組みは十分でない。

生殖細胞は適切な分化を経ることで生殖能を獲得する。不妊症の原因の中には、成体ではなく胎仔期の生殖細胞で起きるイベントに由来するものがある。このようなイベントの一つに、クロマチン構造変化が挙げられる。胎仔期生殖細胞における特異なクロマチン構造変化を起こさないマウスは不妊の表現型を成体期で示す。さらに、この胎仔期生殖細胞でのクロマチン構造変化に伴ってゲノム中のトランスポゾンが活性化し、生殖細胞の“質”が変化することがマウスで示唆されている（Yamanaka et al., 2019, Developmental Cell; Uneme et al., 2024, PNAS）。本研究は、この胎仔期の生殖細胞のクロマチン状態と、生殖能との関係性を明らかにすることで、ヒトの不妊症の原因を解明することを目指したものである。

# Demonstration of the vertebrate CMP-sialic acid synthetase as a novel regulatory protein of neurogenesis by interacting with particular proteins gonocyte

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## Summary Abstract

CMP-Sialic acid synthetase (CSS) is a key enzyme for the expression of Sia-glycoconjugates. In our study, we showed that CSS not only works as a sialylation-involved enzyme, but also as an apoptosis-related protein using a medaka strain with a point-mutation in the N-domain of CSS. In addition, we also demonstrated that CSS regulates the neurogenesis by interacting with FXR1 protein, which is mRNA-binding protein that is required for neurogenesis.

***Key Words*** : CMP-Sialic acid synthetase, medaka, neurogenesis, FXR1 protein

## Introduction

CMP-Sialic acid synthetase (CSS) is prerequisite for the expression of Sia-containing glycoproteins and glycolipids, because free Sia can be transferred to the terminus of glycan chains of glycoconjugates only after activated to CMP-Sia by CSS. To understand the biological significance of CSS at the organism level, I generated several medaka strains expressing the mutated CSS. Interestingly, a strain with a point-mutation in the N-domain of CSS (MuN) was found to be lethal at early developmental stage due to neural cell apoptosis without affecting the sialylation state. These results indicate that CSS plays a critical role in neurogenesis, not only as a sialylation-involved enzyme, but also as an apoptosis-related protein.

## Results

To understand the underlying mechanism of abnormal apoptosis caused by the MuN mutation, we tried to find interacting proteins of CSS by the proximal protein identification technique Turbo-ID. This technique led us to identify the fragile X mental retardation protein 1 (FXR1), and we could confirm the interaction between FXR1 and CSS using anti-FXR1 antibody. Furthermore, we cloned CSS and FXR1 cDNAs from medaka brain and studied the CSS and FXR1 interaction by co-immunoprecipitation (CO-IP) experiment using the Neuro2A cell line transfected with these proteins' cDNAs. Given that the MuN mutation was in conserved motif V, alanine scanning mutants of motif V of medaka CSS were tested for the interaction with medaka FXR1 by the CO-IP technique. However, the interaction of MuN and alanine mutants with medaka FXR1 did not change significantly.

To evaluate the expression profiles of medaka FXR1 protein in MuN medaka fry at 8 days post-fertilization (dpf), the gene expression and protein levels of medaka FXR1 were

quantified by real-time PCR using specific primers and by western blotting using anti-FXR1 antibody. The gene expression level of medaka FXR1 was not changed in homozygous MuN medaka fry at 8 dpf; however, conformation of FXR1 proteins was markedly altered in homozygous MuN medaka fry. The heart development of MuN medaka fry was also observed from 0 dpf to 8 dpf, and we found that the homozygous MuN medaka showed a significant abnormality in atrium contraction at 8 dpf, which might be the main cause of lethality of MuN medaka fry. These results are consistent with the report that FXR1 protein's reduction causes cardiomyopathy in zebrafish [1].

To identify differentially expressed and/or coregulated genes causing the abnormal apoptosis in MuN medaka, the total RNA from wild-type and homozygous MuN medaka fry at 8 dpf were extracted using Direct-TRI method, and RNA sequencing analysis was performed by the Lasy-Seq, a high-throughput library preparation method for RNA-Seq [2]. A volcano plot revealed that the expression of several interesting genes in certain signaling pathway was obviously decreased in homozygous MuN medaka fry. This suggests that abnormal apoptosis in MuN medaka is induced through this signaling pathway.

## Discussion & Conclusion

This study first revealed that CSS regulates not only sialylation as a Sia-metabolic enzyme, but also non-enzymatic processes by interacting with other proteins. The proximal labeling showed that medaka CSS interacted with FXR1 protein, which was previously reported as a CSS-binding protein [3]. The point-mutation in the conserved motif V of medaka CSS (MuN) impaired the normal conformation of FXR1 protein at the animal level, causing abnormal apoptosis in MuN medaka at 8 dpf. These results indicate that the interaction between CSS and FXR1 is crucial for development of medaka embryos. We observed increased expression of CHOP gene and caspase 3 in MuN medaka at 8 dpf, suggesting that endoplasmic reticulum stress occurred in this medaka [4]. RNA-Seq analysis of wild-type and MuN medaka at 8 dpf revealed significant differences in the expression of neurotransmitter transport-related genes in MuN medaka, consistent with the histological changes in the brain observed using hematoxylin and eosin stain. The decrease of particular protein genes indicates that CSS also regulated apoptosis during neurogenesis through the related pathway.

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# 一般の皆様へ

シアル酸は脊椎動物の細胞表面を覆う糖鎖の末端を修飾する単糖残基です。私達はシアル酸の発現に必須な酵素 CSS の遺伝子の点変異がメダカの発達途上で脳の細胞死を起こし、致死となることを見出しました。この変異体メダカの致死性の原因を調べた結果、CSS が細胞の生存に関与する FXR1と相互作用すること、この CSS 変異が FXR1のコンホメーションを異常化させて致死性が誘導されることが明らかになりました。この変異は酵素活性に影響しないことから、本研究は CSS が細胞の生存維持というシアル酸代謝以外の機能をもつことの初めての証明となりました。

# Interorgan networks of islets with liver, fat, or macrophages to regulate β-cell mass

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## Summary Abstract

In this study, we investigated the regulatory mechanism of pancreatic β-cell mass in terms of the inter-organ networks. We clarified the mechanism by which pancreatic β-cell proliferation is regulated by the interaction between pancreatic islets and macrophages mediated by the inflammatory factor S100A8.
***Key Words*** : Diabetes, pancreatic β-cell proliferation, macrophages, inflammation

## Introduction

Increased number of macrophages exist in the islets of type 2 diabetes patients by a dysregulation of islet derived inflammatory factors, may cause β cell loss. S100 calcium-binding protein A8 (S100A8), a member of damage-associated molecular pattern molecules (DAMPs), reportedly contribute to the islet inflammation by mediating interaction between β-cells and macrophages.

## Results

We first showed that the expression of S100A8 is induced in pancreatic islets under type 2 diabetes condition, which is the activation with glucose, palmitate and macrophages. Next, we established S100A8-floxed mice. By crossing S100A8-floxed mice with Ins1-Cre mice, we generated pancreatic β cell-specific S100A8 knockout (KO) mice. To assess the gene expression in islets and macrophages, we performed co-culture experiments. We put isolated islets from control mice or KO mice in the upper layer and peritoneal macrophages isolated from Floxed mice were cultured in the lower layer. We also checked the effects of palmitate or glucose on the gene expression in this co-culture system. The expression of S100A8 in control islets is synergistically increased in the presence of high glucose and palmitate. However, the increase in the expression of S100A8 was not observed in islets from KO mice.

We evaluated the inflammatory cytokine gene expression in macrophages co-cultured with islets from control mice or KO mice. The expression of TNFα, IL-6, or CCL2 were significantly reduced in macrophages co-cultured with KO islets compared to that co-cultured with control islets.

To investigate the role of S100A8 in β cell proliferation, we fed control mice or KO mice

with high-fat diet for 20 weeks. Body weight gain showed no significant differences between control mice and KO mice both in normal-chow and high fat diet-fed mice. There were no significant differences in glucose tolerance between two genotypes under the normal chow-fed mice. However, glucose tolerance of high-fat diet-fed KO mice was improved compared to control mice. β cell mass expansion induced by high fat diet-feeding was significantly enlarged in KO mice compared with control mice. Knocking out of S100A8 in β cells also significantly enhanced BrdU-incorporated proliferating β cells in diet-induced obese mice.

## Discussion & Conclusion

The main findings of this study are as follows; S100A8 from β cells upregulated the expression of proinflammatory cytokines in co-cultured macrophages. Deletion of S100A8 in β cells increased β cell mass and β cell proliferation. S100A8 may regulate β cell proliferation via GCN2 by reduction in amino acid content in islets. S100A8 suppressed β-cell proliferation via macrophages.

Thus, S100A8 is induced in β cells by diet-induced obesity and S100A8 activates macrophages which suppresses GCN2 expression in β cells. So, S100A8 act as a brake on adaptive β cell proliferation. In β-cell specific S100A8 KO mice, GCN2 is further increased under high-fat diet-feeding, thereby accelerating β cell proliferation.

On the basis of our results, S100A8 might be a central regulator for islets inflammation, interacting with macrophages. GCN2 potentially mediates β cell proliferation in β cell specific S100A8 knock out mice. The suppression of S100A8 could be a therapeutic approach for diabetes by increasing pancreatic β cell mass and β cell proliferation.

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## 一般の皆様へ

インスリンは体内で唯一血糖値を下げることができるホルモンです。血糖値が上がってしまう糖尿病が発症および進展する原因の1つは膵臓の膵島という組織にあるインスリンを産生する膵β細胞が減少することです。我々の研究により、糖尿病状態で膵島の中に入ってくるマクロファージという炎症細胞が、膵β細胞と相互作用することによって、肝臓から血液中に放出されるアミノ酸の取り込みが変化して、結果として膵β細胞が増えにくい状況が起こっていることがわかりました。すなわち、このマクロファージを介した膵島での炎症を抑えることが膵β細胞を増やす新たな糖尿病治療につながることが期待されます。

# Sexual experience and neural mechanisms controlling male sexual function: focusing on the spinal gastrin-releasing peptide receptor neurons

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## Summary Abstract

Several sexual function centers are located in the laminae X of the lumbar spinal cord in male rats. The spinal ejaculation generator (SEG) express gastrin-releasing peptide (GRP) and control ejaculation at the spinal cord. In this project, we found GRP receptor-expressing neurons were distinct from SEG and found that laminae X GRPR neurons in the lumbar spinal cord were activated after ejaculation. In the future, understanding the mechanism of sexual function regulation of laminae X GRPR neurons might lead to the discovery of a new sexual function center and contribute the developing new therapy of erectile disfunction.
***Key Words*** : Gastrin-releasing peptide, male sexual function, spinal cord

## Introduction

The neural networks that control male sexual behavior are widely distributed throughout the central nervous system. Previously, we have reported that the gastrin-releasing peptide (GRP) neurons called as spinal ejaculation generator, which is located in the laminae X of the upper lumbar spinal cord, and regulates male sexual function via spinal autonomic and somatic nucleus expressing GRP receptors (GRPRs) in the lumbosacral spinal cord (1). We recently reported that oxytocin projection originating from paraventricular nucleus of hypothalamus activates the spinal ejaculation generator and facilitates male sexual function (2). Thus, we have identified functional linkages between the brain and spinal cord in the regulation of sexual function. Further, we recently reported that sexual experience increases the GRP and oxytocin receptor expression in spinal ejaculation generator neurons (3). Transcriptome analyses of laminae X in males before and after sexual experience showed that GRPR mRNA are also expressed in the laminae X of the upper lumbar spinal cord. Therefore, we might be able to find novel neurons controlling sexual functions by focusing on sexual experience. However, little is known about the mechanisms of sexual behavior regulation by GRPR neurons in the upper lumbar spinal cord.

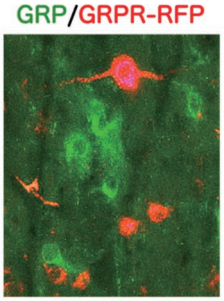
## Results

The localization of GRP receptor (GRPR)-expressing cells in the laminae X of the upper lumbar spinal cord was examined using GRPR promoter-human diphtheria toxin receptor (hDTR)-2A-red fluorescent protein (RFP) transgenic (GRPR-hDTR-RFP Tg) rats. RFP-expressing neurons were observed in the laminae X of lumbar spinal cord. First, double in situ hybridization for *hDTR* mRNA and *Grpr* mRNA was performed in the upper lumbar

spinal cord of GRPR-hDTR-RFP Tg rats to confirm whether RFP-expressing cells express *Grpr* mRNA. The results showed that most hDTR-positive cells expressed *Grpr* mRNA. This suggests that RFP-expressing cells co-express *Grpr* mRNA and can be regarded as GRPR-expressing cells.

Next, immunostaining for GRP was performed in the upper lumbar spinal cord of GRPR-hDTR-RFP Tg rats. The number of RFP-expressing cells was then compared between sexes. In the upper lumbar spinal cord, approximately 600 RFP-expressing cells were present in both sexes, and no sexual differences were observed. GRP-immunoreactive neurons were observed more in males than females. Few RFP-expressing cells (~7%) showed GRP-immunoreactivity in both sexes. RFP-expressing cells were in close apposition to GRP-positive cells. These results suggest that in the upper lumbar spinal cord, GRPRs are not expressed in GRP neurons but in different cell populations (see figure).

Furthermore, to investigate whether GRPR-expressing cells are associated with male sexual behavior, the expression of c-fos, a marker of neuronal activation, was examined after ejaculation of GRPR-hDTR-RFP Tg males. GRPR-hDTR-RFP Tg males were fixed 90 min after ejaculation. Males that did not present females were used as controls. The number of c-fos positive neurons were observed in the laminae X of males after ejaculation. There were no differences in the number of RFP-expressing neurons. RFP-expressing cells in the upper lumbar spinal cord expressed c-fos after ejaculation, and the number of c-fos-positive RFP-expressing cells was significantly higher after ejaculation than in control males. These results suggest that GRPR-expressing neurons are involved in the regulation of sexual function, but their function is not sex-dependent.



Laminae X GRPR neurons and
spinal ejaculation generator neurons.

## Discussion & Conclusion

This study revealed that laminae X GRPR-expressing neurons in the upper lumbar spinal cord were distinct from spinal ejaculation generator neurons and that there were no sex differences in the number of GRPR-expressing neurons. On the other hand, we also found that laminae X GRPR-expressing neurons were activated after sexual behavior including ejaculation. These findings suggest that laminae X GRPR-expressing neurons are sexual function centers distinct from spinal ejaculation generator. The spinal cord contains several centers that control sexual functions. Of those sexual function centers, the Spinal Pattern Generator triggers the urogenital reflex, but its function does not differ by sex. Therefore, laminae X GRPR-expressing neurons might be candidates for Spinal Pattern Generator. In the future, by specifically lesion of laminae X GRPR-expressing neurons and analyzing their sexual behavior, we might clarify the mechanism of sexual function regulation by laminae X GRPR-expressing neurons.

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## 一般の皆様へ

本研究課題では、脊髄に存在する未知の性機能調節ニューロン（神経細胞）の発見を目指しています。今回はガストリン放出ペプチド受容体（GRPR）という遺伝子を発現するニューロンが性機能に関わることを新規に見出しました。ストレス社会の現代日本では、成人男性の4人に1人が性機能障害に悩んでいますが、神経性の勃起障害のメカニズムはよくわかっていません。性機能の神経制御メカニズムを明らかにすることで、将来、心因性の勃起障害をはじめとした性機能障害の病態解明・治療法開発につながると期待しています。

# The mechanism of early placental cell differentiation conferred by virus-derived acquired genes in mammals

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## Summary Abstract

The placenta, which connects the fetus to the mother, is an indispensable tissue for the normal development of the fetus, and it is probable that there are many cases of unexplained abnormalities in embryonic development be caused by placental abnormalities. However, while research on the differentiation and developmental mechanisms of fetal tissues has made great progress, the development of the placenta is still poorly understood. In this study, through the functional analysis of the virus-derived gene PEG10, which is considered to be a key molecule for placental cell differentiation, we aim to elucidate the early differentiation mechanism of placental tissue cells.
***Key Words*** : Virus-derived gene, PEG10, Placenta

## Introduction

*PEG10* is a novel gene that was acquired from a retrovirus infecting the common ancestor of marsupials and eutherians. We have previously reported that *Peg10* knockout (KO) mice show early embryonic lethality due to severe placental dysplasia (Ono et al. 2006 and Shiura et al. 2023). This result indicates that *PEG10*, a virus-derived acquired gene, is essential for differentiation of placental cells necessary to establish a highly functional eutherian placenta. In this study, to elucidate *Peg10*-mediated differentiation mechanisms of placental cells, we performed detailed phenotypic analysis of early placental tissues of *Peg10* KO mice.

## Results

In mice, early placental tissues comprise of ectoplacental cone (EPC) and extraembeyonic ectoderm (ExE) differentiating into cells of spongiotrophoblast (ST) and labyrinth (Lb) layers, respectively. As we have known that placental defects of *Peg10* KO mice is observed from embryonic days 6.5 (E6.5) in EPC and E7.5 in ExE, respectively, we attempted to sample EPC at E6.5 and E7.5 and ExE at E6.5, E7.5 and E8.5 separately and performed gene expression analysis to examine the abnormal gene expression occurring in the KO placental tissues. To achieve this analysis, we first mastered the technique of sampling EPCs and ExEs separately in mouse embryos after E6.5, which enabled us to analyze the differentiation process of each lineage separately, "EPC to ST" and "ExE to Lab", rather than the entire extraembryonic tissue. Additionally, we also performed gene expression analysis of decidua which is the maternal-origin epithelial tissue of endometrium and surrounds conceptus after implantation, because it is very likely that some molecular

signals from decidua induces differentiation of placental cell. In fact, an *in vitro* culture study of E7.5 embryos isolated from the decidua shows that placental tissue development is not advanced (Aguilera-Castrejon et al. Nature. 2021), suggesting that the presence of the decidua is essential for the differentiation and proliferation process of early placental cells. The results showed that upregulation of the trophoblast differentiation marker genes was undetectable in the KO EPC cells; instead, a particular signaling pathway is activated. This signaling pathway is thought to be related to cell mortality, suggesting that this activation may prevent survive and differentiation of KO EPC cells. In addition, comparative gene expression analysis between EPC and decidua suggest that this KO EPC cell-specific activation of the signaling is induced by decidua-derived molecular signals. In ExE lineage, the loss of upregulation of the trophoblast differentiation marker genes and activation of signaling pathways related to cell death were observed as shown in EPC. However, the signaling pathways was different from that seen in EPC. These results indicate that *Peg10* protects EPC and ExE cells during differentiation in different ways. Not only this, as an *in vitro* study has demonstrated that *Peg10* is essential for trophoblast stem cells (TSCs) differentiation (Abed et al. PLOS ONE, 2019), now, we're currently investigating the function of *Peg10* on EPC and ExE differentiation itself.

## Discussion & Conclusion

In this study, we performed comprehensive gene expression analysis of not only trophoblast cells of EPC and ExE lineages but also maternal deciduae samples between E6.5 and E8.5, and demonstrated that *Peg10* regulates signaling pathways associated with cell mortality and acts as a guardian gene enabling differentiation of trophoblast into mature placental cells in both EPC and ExE lineages. However, how *Peg10* engages with the regulation of those pathways and trophoblast differentiation itself remains undetermined. Therefore, we plan to investigate the further underlying molecular mechanism in future studies.

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# 一般の皆様へ

胎児と母親をつなぐ「胎盤」は胎児の正常な発生・成長に欠くことのできない組織であり、原因不明の胎児期の発生・成長異常の中には、胎盤異常が原因となっている例も数多く存在すると考えられます。しかし、胎児組織の分化・発生メカニズム研究が大きく発展しているのに対し、胎盤の発生については理解が進んでいません。本研究では、哺乳類の祖先に感染したウイルスが元となり誕生した新しい遺伝子 PEG10に着目し、この遺伝子の機能解析を軸として哺乳類胎盤の初期発生メカニズムの解明を進めています。これまでの解析から胎盤を形づくるうえでのこの遺伝子の重要性が徐々に明らかとなってきており、本研究をさらに発展させることで、胎盤組織に起こるあらゆる異常・疾患の病因の解明、その治療法の開発に大きく貢献できると考えています。

# Development of novel preventive and therapeutic approaches for hypertensive disease based on elucidation of the immunogenetic mechanisms

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## Summary Abstract

Stroke-prone spontaneously hypertensive rat (SHRSP) is a well-known disease model of essential hypertension and its complications. We recently found that genotypes of stomal interaction molecule 1 (*Stim1*) and mono-ADP-ribosyl transferase 2b (*Art2b*) may strongly affect susceptibility to hypertensive end-organ damage in SHRSP. STIM1 and ART2b can regulate T cell functions. Therefore, this study aimed to explore possible immunogenetic mechanisms mediated by the two genes. In addition, we generated knock-out (KO) or knock-in rats using a CRISPR-Cas9 technique for *in vivo* examination.
***Key Words*** : hypertension, SHRSP, T cells, genome editing

## Introduction

Hypertension is a leading cause of cerebro-cardiovascular and chronic kidney diseases. Genome-wide association study (GWAS) have identified multiple loci associated with high blood pressure in human; however, genetic mechanisms underlying the development of hypertension and its complications are still poorly understood. We recently suggested that genotypes of *Stim1* and *Art2b* affect susceptibility to stroke in a genetically hypertensive rat model, SHRSP (1). STIM1 and ART2b encodes a $Ca^{2+}$-storage sensor in the endoplasmic reticulum and T cell-specific mono-ADP transferase, respectively. According to proposed immunological functions of the two proteins, we explored possible immunogenetic mechanisms responsible for pathogenesis of stroke in SHRSP.

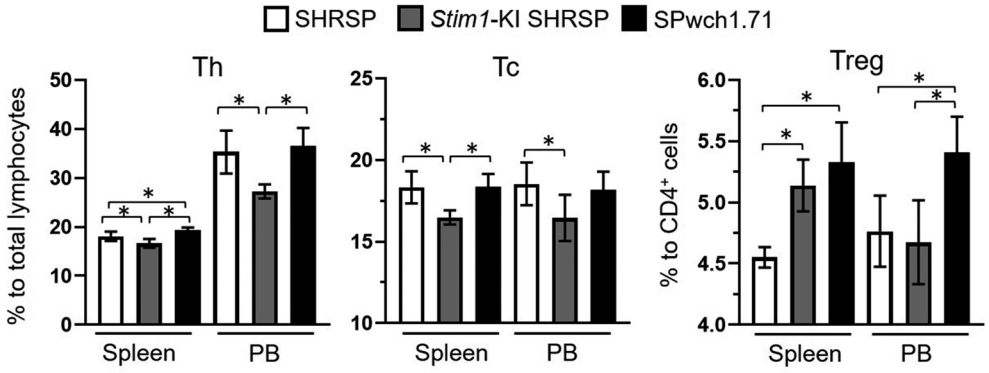
## Results

In the present study, we used three rat strains harboring different alleles for *Stim1* or *Art2b* (ref.1): 1) wild-type SHRSP harboring a nonsense mutation (p.Arg640X) in *Stim1*, 2) *Stim1*-KI SHRSP harboring wild-type *Stim1* allele (ref.2), and 3) SPwch1.71, a SHRSP-based congenic strain harboring a Wistar-Kyoto (WKY)-derived 0.6 Mb fragment of chromosome 1 including *Art2b* locus. In a recent study (ref.1), we reported that *Stim1*-KI SHRSP and SPwch1.71 showed lower and higher susceptibility to stroke than wild-type SHRSP, respectively.

We performed T-cell subset analysis of peripheral blood cells and splenocytes obtained from hypertensive adult animals (12-15 weeks of age). Proportions of helper- (Th; $CD3^+CD4^+$-positive) and cytotoxic-T cells (Tc; $CD3^+$ $CD8^+$-positive) in SHRSP were significantly higher than those in *Stim1*-KI SHRSP in both peripheral blood and spleen.

Except for Tc proportion in peripheral blood, similar results were observed between SHRSP and SPwch1.71. These phenotypes might represent a low-grade inflammatory state in *Stim1*-KI SHRSP when compared with SHRSP and SPwch1.71.

Proportion of regulatory T cells (Treg; $CD4^+CD25^+FOXP3^+$-positive) in SPwch1.71 was significantly higher than those in SHRSP and *Stim1*-KI SHRSP in peripheral blood. Interestingly, splenic Treg proportions in SPwch1.71 did not differ significantly from that in *Stim1*-KI SHRSP. These results imply that increased mobilization of immunosuppressive Treg cells into peripheral tissues in SPwch1.71 but not in *Stim1*-KI SHRSP. We also performed the T-cell subset analysis of peripheral blood cells at a pre-hypertensive stage (5 weeks of age). Proportion of Th cells in SPwch1.71 was significantly higher that those in SHRSP and *Stim1*-KI SHRSP even in the pre-hypertensive age, while no significant differences were found in Tc and Treg proportions among the 3 strains.




T-cell subset analysis by flowcytometry. PB; peripheral blood. n=5 per group. *$p<0.05$.

In order to investigate pathophysiological roles of STIM1 and ART2b on the pathogenesis of hypertensive end-organ damage, we generated *Stim1*-KI SPwch1.71 that harbors wild-type *Stim1* allele and *Art2b*-KO SPwch1.71 or SHRSP by the genome-editing via oviductal nucleic acids delivery (GONAD) method. Backcross or brother-sister mating of heterozygotes to obtain homozygous progenies are in progress.

## Discussion & Conclusion

Present results suggested that genotypes of *Stim1* and/or *Art2b* could affect immunological process mediated by T cells in SHRSP and the inter-strain differences found in T-cell subset proportions were caused by an aging-dependent manner. Further analyses on T cell functions such as activity of cytokine production are warranted to verify these hypotheses. Novel *Stim1*-KI and *Art2b*-KO models generated in this study will be good tools for clarifying genetic and pathophysiological mechanisms of hypertension and its complications in SHRSP both *in vitro* and *in vivo*.

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## 一般の皆様へ

降圧剤は脳卒中などの高血圧性疾患の予防に効果的ですが、既存の薬では十分な降圧が得られない患者さんも多く存在するという事実があります。高血圧性疾患の制圧には、「なぜ高血圧になるのか？」という根本的な問いを解明する必要がありますが、高血圧の発症には多くの遺伝的・環境的要因が関わり、容易ではありません。私は日本で開発された高血圧性疾患のモデルラット（SHRSP）を用いて、その問いに答えるための研究に取り組んでいます。最近、SHRSP における病気の発症や進行に強く関わり得る2つの候補遺伝子を見つけ、ここでは免疫系への影響に着目して研究を行いました。高血圧ラットの健康維持につながる手法の開発を通じて、ヒトを対象とした医療の充実に貢献したいと考えています。

# Elucidation of the proteasome maintenance mechanism and its application to drug discovery

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## Summary Abstract

The ubiquitin-proteasome system is the major protein degradation system in eukaryotic cells. While proteasomes play an essential role in maintaining cellular proteostasis, and although it has become clear that they are involved in aging and the onset of diseases, the mechanism by which proteasomes are regulated remains largely unknown. We recently found that enhanced protein O-GlcNAc modification is important for maintaining proteasome function during proteasome dysfunction. In this study, we identified novel regulators important for maintaining proteasome function and proceeded with research to elucidate the specific molecular mechanism.
***Key Words*** : proteasome, O-GlcNAc, proteostasis

## Introduction

The accelerated expression of the proteasome is known in cancer cells. The proteasome inhibitor bortezomib has been used as a treatment for refractory multiple myeloma and has shown efficacy. However, the detailed mechanism of cancer cell-specific cell death induction by bortezomib is not clear, and no effective approach has been established for the emergence of bortezomib-resistant cells. We have identified that an increase of O-GlcNAcylated protein is important for cell survival by enhancing the proteasome turnover. Depending on this observation, this study focuses on elucidating the molecular mechanism of the maintenance of proteasome function.

## Results

1: Elucidation of specific molecular functions of novel regulators in the proteasome function maintenance mechanism

We have conducted the whole genome siRNA screening to explore the regulators for cell death under bortezomib. We also conducted the RNAseq to clarify the expression profile under the bortezomib treatment. Through the combined analysis of genetic screening and RNAseq analysis, we identified the set of genes that are induced and important for cell viability under proteasome dysfunction. We are supposed to these genes as a candidate for novel proteasome regulators to maintain the proteasome function under proteasome dysfunction. In addition, we conducted the mass spectrometry analysis to identify the O-glcNAcylated proteins under bortezomib by using click chemistry to pull down the O-GlcNAcylated proteins.

We verified whether the novel proteasome regulators identified in the genetic screening or mass spectrometry are involved in the maintenance of proteasome function through O-GlcNAcylation. Since no clear results have been obtained regarding the O-GlcNAcylation of candidates, we plan to establish stable cells or KO cells against each candidate to verify whether they are working to maintain proteasome function. Some genes are revealed to be important for cell viability under bortezomib.

We have confirmed that defective proteasome assembly under treatment with bortezomib in KO cell of gene X which is the most promising novel proteasome regulator. X was associated with the proteasome via their C-terminus under the bortezomib condition. Then, we identified that X associates with proteasome assembly intermediates in vitro pull-down assays and immunoprecipitation. We also revealed that the enzymatic activity of X does not contribute to the association with the proteasome. These results suggest that X associates with newly synthesized proteasome under bortezomib. Furthermore, mass spectrometry analysis suggested post-translational modification of specific proteasome subunits by X, so we investigated the details of the molecular mechanism. Unfortunately, we have not yet successfully demonstrated the importance of the modification of subunits by X for proteasome maintenance. The mechanism of how X recognizes the newly synthesized proteasome should be revealed. We will also examine the promotion of cellular senescence and aggregation-prone protein accumulation in X KO cells, and clarify the effects on aging and neurodegenerative diseases in X KO mice.

2: Elucidation of the physiological function of the proteasome maintenance mechanism in mice

We confirmed that bortezomib enhanced tumor growth suppression in a mouse xenograft model transplanted with X KO cells, demonstrating the potential of X as a new therapeutic target for cancer treatment when used in combination with bortezomib. Therefore, we are conducting similar verifications for enzymatic activity mutants of X. We generated X KO mice and have confirmed that KO mice exhibit normal development and reproduction, suggesting that X would play an important role in proteasome dysfunction conditions, as shown in cell experiments. Thus, we have observed the accumulation of ubiquitinated proteins during bortezomib, heat shock, or oxidative stress conditions in X KO MEFs.

## Discussion & Conclusion

Problems with cancer treatment using bortezomib include serious side effects and the emergence of resistant cells due to the suppression of proteasome activity in normal cells, poor prognosis for refractory multiple myeloma, and low efficacy against solid tumors. known. To overcome this problem, the proteasome function control mechanism, typified by the compensatory transcriptional enhancement of the proteasome through the Nrf1-DDI2 pathway, has begun to attract worldwide attention as a drug discovery target. However, Nrf1 is involved in regulating the expression of a wide range of stress-responsive genes other than the proteasome, and various cellular responses occur in bortezomib-resistant multiple myeloma cells, including changes in metabolic pathways such as glycolysis. Currently, the resistance mechanism cannot be fully explained using only known responses. We hope the progress of our research will establish a novel understanding of proteasome regulation and propose a novel approach to cancer therapy.

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Enhanced O-GlcNAcylation mediates cytoprotection under proteasome impairment by promoting proteasome turnover in cancer cells.

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## 一般の皆様へ

細胞内タンパク質分解を担う真核生物に必須のプロテアソームの機能変化が多様な疾患の発症に関わることが最近の研究から明らかになるとともに、プロテアソーム阻害剤が抗がん治療に使われるようになり、新たな創薬標的として注目されています。しかしながら、正常細胞のプロテアソームも阻害することによる副作用や薬剤耐性細胞の出現など、まだまだ治療アプローチとしては課題が多く基礎研究に立脚した改善が必須です。本研究ではプロテアソーム阻害時の細胞内恒常性維持に O-GlcNAc 化亢進が重要であるという発見を端緒として、新たなプロテアソーム制御因子を同定するとともに、これらの機構を利用したプロテアソーム阻害剤との併用アプローチの有用性について明らかにしつつあります。まだまだ萌芽的な部分を持つ研究ですが、発展性や臨床的な実現性は高いと思われることから、今後も着実に研究を推進していきたいと考えています。

# Elucidation of the disease development caused by disruption of a novel phospholipid transport factor required for mitochondrial homeostasis

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## Summary Abstract

Mitochondria are constructed from phospholipid bilayers, primarily composed of phosphatidylcholine (PC). Therefore, maintaining mitochondrial PC is crucial for mitochondrial functions. StarD7 is essential for the homeostasis of mitochondrial PC [1,2]. This study analyzed mice specifically lacking the protein in skeletal muscle. These mice exhibited a significant reduction in linoleic acid-containing PC and its derivative cardiolipin in mitochondria, indicating mitochondrial abnormalities. Furthermore, pathological analysis revealed several lesions in the skeletal muscle suggestive of the onset of myopathy. These findings suggest that StarD7 is a new gene responsible for mitochondrial myopathy.
***Key Words*** : phosphatidylcholine, mitochondria, myopathy, lipid trafficking

## Introduction

The major phospholipid constructing mitochondrial membranes is PC. However, mitochondria cannot synthesize the lipid on their own and entirely rely on the supply from the endoplasmic reticulum (ER), the PC-synthesizing organelle. The applicant found a novel protein, StarD7, which transports PC from the ER to mitochondria, and has revealed its essential roles in mitochondrial function, morphology, and myoblast differentiation into muscle fibers [1,2,3]. This study investigates the effects of StarD7 deficiency in skeletal muscle-specific mice, focusing on (**A**) mitochondrial abnormalities and (**B**) the development of mitochondrial myopathy.

## Results

**A. Mitochondrial abnormalities in the skeletal muscle-specific StarD7-KO mice**

(1) Disruption of phospholipid homeostasis in mitochondria

Mitochondria were isolated from skeletal muscle using the Percoll-Nycodenz method, the level of phospholipids were analyzed by lipidomics using liquid chromatography tandem mass spectrometry (LC-MS/MS). A significant decrease in the level of cardiolipin was found in the KO mice. Cardiolipin is a unique phospholipid localizing in the inner mitochondrial membrane, and is known to be crucial for the formation of cristae structures and maintenance of respiratory chain complex activity. Cardiolipin contains four fatty acids, and most of them are linoleic acids. Cardiolipin is synthesized through several steps, and the final step is catalyzed by Tafazzin. This enzyme selectively recognizes PC with linoleic acid, and transfers the linoleic acids to monolysocardiolipin to form cardiolipin. The

applicant examined the protein levels of enzymes involved in cardiolipin synthesis including Tafazzin, and found that there was no significant decrease in the level of the enzymes in the StarD7-KO mice. Importantly, in the KO mice, the level of PC was significantly decreased in both the outer and inner mitochondrial membranes. Among them, PC with linoleic acid was most greatly decreased in the mice. These results suggest that the decrease in cardiolipin was probably due to the reduction of linoleic acid-containing PC in mitochondria. To verify whether StarD7 preferentially transports PC with linoleic acid, the applicant examined a lipid binding assay. PC molecular species with several fatty acids were incubated with His-tagged StarD7. After purification of His-tagged StarD7 with affinity resins, PC bound to the protein was analyzed by LC-MS/MS. It was revealed that StarD7 most strongly binds to PC with linoleic acid. These results suggest that StarD7 is necessary for the homeostasis of PC with linoleic acid and plays important role for the synthesis of cardiolipin.

(2) Exploration of biomarkers through omics analysis

As mentioned above, lipidomic analysis of mitochondria revealed a significant decrease in linoleic acid-containing PC and cardiolipin in the StarD7-deficient mice. These phospholipids are considered to be biomarker candidates for diagnosing the mitochondrial myopathy caused by StarD7 deficiency. The applicant plans to screen for other metabolites and proteins for biomarkers in the next study.

(3) Role of StarD7 in ceramide maintenance

The applicant initially reported StarD7 as a PC-specific transport protein [1]. However, a subsequent report suggested that this protein also transports ceramide [4]. To verify this, the level of ceramide in mitochondria was measured by LC-MS/MS. There was no significant difference in the level of ceramide between wild-type and the KO mice, suggesting that StarD7 does not function in ceramide maintenance. Ceramide is known to be transferred from the ER to the Golgi apparatus by StarD11 (or CERT). Because it is unclear how mitochondrial ceramide is maintained, the applicant examined the influence of CERT deficiency on mitochondrial ceramide in HEK293 cells. However, there was no significant changes in the level of ceramide between wild-type and CERT-KO cells. Interestingly, detailed analysis of phospholipids revealed an increase in the level of plasmanylcholine, an ether-type phospholipid, in CERT-KO cells. This result led to the novel finding that CERT transports not only ceramide but also alkylacylglycerol from the ER to the Golgi apparatus. The applicant summarized these findings and published them in a journal [5].

**B. Tissue histological analysis and pathological diagnosis of mitochondrial myopathy in the StarD7-KO mice**

Histological analysis was performed to diagnose the pathology of mitochondrial myopathy in the StarD7-KO mice. Hematoxylin and eosin staining of soleus muscle revealed the presence of chain-like nuclear chains in the KO but not in wild-type mice, suggesting abnormality in the muscle fibers. Further verification with an increased number of samples is planned for the future study. Although observation of muscle fibers using transmission electron microscopy was challenged, no abnormalities were observed in the sarcomere structure. Internal structure of mitochondria was also examined, but due to low resolution, it was difficult to for verification. The applicant plans to conduct mouse exercise tests in the next study.

## Discussion & Conclusion

In this study, the applicant analyzed the skeletal muscle-specific StarD7-KO mice. There was a significant reduction in both linoleic acid-containing PC and cardiolipin, indicating the occurrence of mitochondrial abnormalities. Furthermore, pathological analysis of skeletal muscle revealed several lesions suggestive of myopathy development. From these results, it was suggested that StarD7 was a novel causative gene for mitochondrial myopathy. Further investigation will be necessary to strengthen this conclusion. For example, the applicant plans to examine mitochondrial abnormalities in detail by assessing oxygen consumption rate, mitochondrial membrane potential, and levels of reactive oxygen species. It is also planned to monitor abnormalities in the internal cristae structure of mitochondria using electron microscopy. Muscle motor function will be assessed through grip tests and treadmill exercise tests. In this study, the applicant used mice under one year of age. Because it is presumed that myopathy become worse with aging, it is also necessary to validate aged mice in the future study.

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## 一般の皆様へ

ミトコンドリア膜はホスファチジルコリン（PC）を主とするリン脂質二重層で構築されている。しかし、ミトコンドリア自身にはPC合成能がないため、リン脂質合成器官である小胞体からの供給に依存している。これまで申請者はPCを小胞体からミトコンドリアへ輸送するタンパク質StarD7を先駆けて見出した。本研究では、骨格筋特異的に本タンパク質が欠失したマウスの解析を行なった。骨格筋から単離したミトコンドリアのリン脂質を調べた結果、欠損マウスではリノール含有PCと、それから合成されるカルジオリピンが著減しており、ミトコンドリアの異常が認められた。骨格筋を病理解析した結果、ミオパチーの発症を示唆するいくつかの病変が確認された。以上から、StarD7はミトコンドリアミオパチーの新たな原因遺伝子であることが示唆された。

# Molecular mechanism of dendrite remodeling during the critical period of brain development

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## Summary Abstract

We found that BTBD3 deletion caused significant delay in activity-dependent dendrite pruning in cerebellar granule cells. Unlike the previous report, BTBD3 translocated to the centrosome but not to the nucleus in response to neuronal activity. We demonstrate that the translocation of BTBD3 to the centrosome is critical for activation of dendrite pruning in cerebellar granule cells.
***Key Words*** : BTB/POZ domain–containing 3, critical period, dendrite pruning, cerebellar granule cell, neuronal activity

## Introduction

Neural circuits in the mammalian brain undergo robust remodeling in response to external stimuli during critical periods of postnatal development. BTB/POZ domain–containing 3 (BTBD3) has been identified as a regulator of neuronal activity-dependent remodeling of dendritic branches, but its molecular mechanism remains unclear (Matsui et al., 2013). In this study, we aimed to elucidate the molecular mechanisms of activity-dependent action of BTBD3 in dendrite pruning in cerebellar neurons during postnatal development.

## Results

We have previously found that activity-dependent dendrite pruning, which is seen in the second postnatal week in wildtype mice, was significantly delayed in BTBD3 conditional knockout (cKO) mice (NeuroD1-Cre; Btbd3$^{flox}$ and Delta2-Cre; Btbd3$^{flox}$) which lack BTBD3 in postmitotic cerebellar granule cells (CGCs) and Purkinje cells, respectively. For more detailed molecular analyses, we first established a dissociated culture of CGCs in which we can recapitulate activity-dependent dendrite pruning (Gaudilliere et al., 2004). We confirmed that CGCs differentiate and form ~10 immature dendrites at the third day of culture (3DIV), which gradually decrease and stabilize at around 4 mature dendrites by 6DIV. Pharmacological inhibition of NMDA receptor by AP-5 treatment led to a significant delay in the dendrite pruning. Likewise, cultures from BTBD3 cKO mice exhibited a delay in the dendrite pruning compared to the culture from wildtype littermates, supporting that BTBD3 is involved in activity-dependent dendrite remodeling in cerebellar neurons.

Westernblotting with anti-BTBD3 revealed that two isoforms of BTBD3 are expressed in developing cerebellum, gradually increasing and peaking around 2-3 postnatal weeks.

The BTBD3 protein consists of an N-terminal intrinsically disordered region (IDR), BTB domain required for protein binding, BACK domain for interaction with actin and intermediate filaments, and PHR domain with unknown function. The Isoform2 (BTBD3-iso2) lacks the first half of the IDR domain of the full-length Isoform1 (BTBD3-iso1). We constructed an HA-tagged BTBD3-iso1 and -iso2 and analyzed its subcellular localization in HeLa cells, N2a cells and CGCs. The localization of both isoforms was sensitive to culture conditions such that it forms cytosolic puncta or vesicles depending on the expression level or cellular stresses such as low temperature. This may be partly due to LLPS event mediated by the N-terminal IDR. Unexpectedly, however, neither isoforms translocated to the nucleus by excitatory stimuli such as glutamate treatment in excitable cells (N2a cells and CGCs). In contrast, we observed accumulation of BTBD3-HA in the centrosome in CGCs at 1h after treatment with 10 μm glutamate. We then analyzed dynamics of BTBD3 translocation by time-lapse imaging of cultured CGCs transfected with a construct of BTBD3 tagged with mNeonGreen (BTBD3-mNG). BTBD3-mNG (both iso1 and iso2) evenly distributed in the cytoplasm in resting CGCs. Upon treatment with 10 μm glutamate, it gradually accumulated in the centrosome in 30 min. Both isoforms are excluded from the nucleus and did not translocate to the nucleus even after glutamate treatment. These results questioned the function of BTBD3 as a transcriptional activator in response to neuronal activity.

We made deletion mutants of BTBD3 and examined their distribution in CGCs. ΔIDR construct lacking the IDR domain was found to localize at the centrosome regardless of glutamate treatment. We further identified that the BACK domain is critical for centrosomal localization. These results suggest that the IDR domain masks the centrosome localizing signal in the BACK domain in resting states, but neuronal activity inhibits the IDR domain, allowing centrosomal translocation of the protein.

We then performed gain-of-function assay by overexpressing BTBD3 in developing CGCs. Overexpression of BTBD3-iso1 or BTBD3-iso2 which distributed in the cytoplasm did not show obvious effects on dendrite formation nor pruning in CGCs. In contrast, ΔIDR which localizes at the centrosome regardless of the activity state of neurons accelerated dendritic pruning in cultured CGCs. Taken together, it is suggested that the translocation of BTBD3 to the centrosome is regulated by the IDR domain and is critical for the activity-dependent dendrite pruning in cerebellar neurons.

## Discussion & Conclusion

It has been postulated that BTBD3 acts as a transcription (co)factor that regulates activity-dependent dendrite remodeling through transcriptional activation. Unlike this prevailing view, we found that BTBD3 translocates to the centrosome but not the nucleus upon excitatory stimuli, negating the involvement of transcriptional activation in dendritic pruning. Some BTB domain proteins have been shown to interact with E3 ubiquitin ligase and regulate protein degradation (c.f. Geyer et al., 2003). The centrosome is known to scaffold proteins of the ubiquitin-proteosome system (UPS). We thus surmise that BTBD3 translocates to the centrosome and regulates the UPS-dependent degradation of proteins involved in the formation and pruning of dendritic arbors. Another finding is that the N-terminal IDR domain is responsible for activity-dependent translocation to the centrosome. Protein motif search revealed that the IDR domain contains multiple phosphorylation sites. We surmise that the calcium signals trigger phosphorylation of the

IDR, unmasking the BACK domain and leading to centrosomal translocation. Analyses is ongoing to clarify the molecular basis of activity-dependent dendrite pruning regulated by BTBD3 function.

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## 一般の皆様へ

幼若期の脳神経回路は余計に形成される傾向にありますが、感覚刺激による活動レベルで要不要の選別が起こり、個体の生活環境により最適化される仕組み（臨界期機構）を備えています。本研究では、臨界期のニューロンの樹状突起が神経活動により剪定される過程に関わることが分かっているBTBD3分子の動作機構の解析を行いました。本研究により、BTBD3が神経活動により細胞内での局在を変えることが樹状突起の剪定に必須であることを見出し、この局在変化の分子基盤の一部が明らかになりました。

# Phase separation dynamics of pathogenic RNA granules uncovered with novel visualization and manipulation method of endogenous RNAs

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## Summary Abstract

In this research, by using novel visualization and manipulation method which I developed applying the artificial RNA binding protein, I study the formation and degradation mechanism of pathogenic RNA granules formed by RNA repeat sequences. This will help to future development of treatments for cancer, Huntington's disease, amyotrophic lateral sclerosis (ALS), and other neurodegenerative diseases caused by pathogenic RNA granules.
***Key Words*** : RNA, RNA granule, Live-imaging, Liquid-liquid phase transition

## Introduction

RNA granules are intracellular non-membranous organelle containing RNAs and proteins. It is known that pathogenic RNA granules are involved in some diseases such as cancer and neurodegenerative diseases including Huntington's disease and amyotrophic lateral sclerosis (ALS). Especially in neurodegenerative diseases, the formation of RNA granules is regulated by liquid-liquid phase separation (LLPS), and it has been reported that mutations in RNA granule component proteins cause abnormal LLPS and induce cytotoxicity. On the other hand, little is known about how the RNAs themselves, which constitute these pathogenic RNA granules, are involved in LLPS and cytotoxicity.

## Results

First, I evaluated the performance of an artificial RNA-binding protein which was originally developed by myself. I confirmed that the artificial RNA-binding protein binds to the target RNA with high specificity and affinity in vitro and in cells by using it in combination with a high-intensity luminescent protein Nano-lantern (Takai et al., PNAS 2015), which was also originally developed by myself. Furthermore, by fusing the artificial RNA-binding proteins with fluorescent proteins such as GFP and expressing them in living cells, they were applicable to the visualization of endogenous mRNAs. mRNAs are known to accumulate in RNA granules called stress granules, which are formed by LLPS under stress conditions, such as arsenite treatment. I constructed artificial RNA-binding proteins for mRNAs with poly-A chains and expressed them in living cells to analyze the dynamics of stress granule formation. As a result, it was shown that all of the artificial RNA-binding proteins I constructed were capable of live-imaging of the dynamics of stress granule formation. On the other hand, this phenomenon was not observed for the artificial RNA-binding

proteins that were designed not to bind to mRNA, indicating that the novel endogenous RNA visualization probes developed by the applicant were able to specifically live-image the target RNAs. Using the same method, I also succeeded in visualizing paraspeckles, which are known as nuclear RNA granules formed by LLPS. These results suggest that by applying the artificial RNA-binding protein developed by myself, it is possible to visualize the dynamics of RNA granules via LLPS in living cells.

It has been reported that CAG repeat sequences are often observed in Huntington's disease patients. Furthermore, their transcripts, RNA containing CAG repeat sequences, form RNA granules via LLPS. Next, I designed and constructed an artificial RNA-binding protein that binds to these CAG repeat sequences, expressed RNA with CAG repeats in cells, and examined whether the dynamics of the RNA could be visualized. However, from the preliminary results, any RNA granule-like structures were not observed. To confirm whether RNA containing CAG repeats really form RNA granules, we fused an RNA aptamer sequence 3' downstream of the CAG repeat sequence and visualized the aptamer sequence. As a result, it was shown that the RNA containing the CAG repeat sequence formed RNA granule-like structures. These data suggest that there was a possibility that the artificial RNA-binding protein itself designed to the CAG repeat sequence inhibited the formation of RNA granules.

Finaly, we changed the labeling of the artificial RNA-binding protein to RFP and simultaneously visualized RNA granule by using both of a green fluorescent RNA aptamer and red artificial RNA-binding protein. The results showed that only when the artificial RNA protein was coexpressed, RNA granules visualized by the RNA aptamer were almost completely absent, suggesting that the artificial RNA binding protein itself designed to the CAG repeat inhibits the formation of RNA granules. This suggests that the formation of pathogenic RNA granules may be inhibited simply by expressing a protein binding to the CAG repeat RNA sequence. This would be a major finding that may be useful for the development of future treatments for diseases related to pathogenic RNA granules. In the future, I plan to analyze the regulation dynamics of pathogenic RNA granules by using artificial RNA binding proteins that bind to the 5' or 3' side of the CAG sequence instead of the CAG sequence itself in detail.

## Discussion & Conclusion

In this research, I planned to visualize and control pathogenic RNA granules using an RNA visualization and control method originally developed by myself. Interestingly, the results suggest that the RNA granule visualization method itself may inhibit RNA granule formation. Although further analysis is required to elucidate the detailed mechanism of action, it would be a significant discovery if this method can be applied as a method to inhibit the formation of pathogenic RNA granules. If the effect of this method was confirmed using iPS cells derived from patients with neurodegenerative diseases such as Huntington's disease, it is expected to be applied to the treatment of neurodegenerative diseases. In addition, if a small chemical compound with similar activity can be obtained through drug screening, it could lead to the development of therapeutic drugs for neurodegenerative diseases. On the other hand, since RNA granules have been reported to be associated with other diseases such as cancer, there is a possibility that it may lead to the development of therapeutic methods for various diseases related to RNA granules. It is expected that the results of this research will lead not only to the elucidation of the physiological significance of RNA granules, but also to the development of therapeutic methods for various diseases related to pathological RNA granules.

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## 一般の皆様へ

本研究は、ガンや神経変性疾患などの難治性疾患の発症メカニズムの1つである病原性の RNA 顆粒に注目し、その RNA 顆粒の形成を阻害する方法を探索することで将来的な難治性疾患の治療に役立てようとする基礎研究です。公益財団法人ノバルティス科学振興財団のノバルティス研究助成金のおかげで本研究が飛躍的に推進され、難治性疾患の治療に役立つ可能性を持つ多くの知見が明らかになりました。本研究成果が難治性疾患に苦しむ多くの患者様の治療法へと役立てることを心より祈念いたしております。

# Molecular mechanism of intrinsic resistance to KRAS G12C inhibitor associated with LKB1/KEAP1 mutation

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## Summary Abstract

In this study, we aim to uncover molecular mechanisms regulating the sensitivity to KRAS inhibitors, especially focusing on the loss-of-function of tumor suppressor genes LKB1 and KEAP1. Through the analyses of the response to treatment with KRAS G12C inhibitor sotorasib in NSCLC cells, we have demonstrated that inactivation of KEAP1 confers resistance to sotorasib treatment both *in vitro* and *in vivo*. Additionally, we identified candidate genes that would be responsible for the resistance to sotorasib in cells lacking functional KEAP1 by RNA sequencing. Moreover, through drug screening, we identified candidates that effectively suppress the growth of cells with KEAP1 inactivation when combined with sotorasib.

***Key Words*** : Lung Cancer, KRAS G12C inhibitor, LKB1 mutation, KEAP1 mutation

## Introduction

The molecular mechanisms regulating the sensitivity to KRAS inhibitors are yet to be fully elucidated. In this research, we focus on the loss-of-function of tumor suppressor genes LKB1 and KEAP1 which play a role in the regulation of the tumor microenvironment and are also frequently observed in the refractory tumor treatment with KRAS G12C inhibitor (ref.1). We aim to uncover molecular mechanisms by which LKB1 and KEAP1 regulate drug sensitivity leading to the development of novel combination therapies to overcome the resistance to KRAS inhibitors.

## Results

1. Elucidating the molecular mechanisms of resistance to sotorasib treatment associated with LKB1/KEAP1 mutations.

Recent studies implicated that those various genetic mutations, including mutations in KEAP1 and LKB1 genes, are related to resistance against treatment with sotorasib in non-small cell lung cancer (NSCLC). To explore the role of KEAP1 and LKB1 in the sensitivity to treatment with sotorasib in NSCLC, we first created KEAP1/LKB1 isogenic

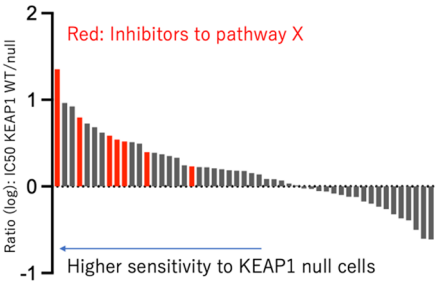


Figure1. Screening to extract the drugs which are especially effective in KEAP1. null cells.

cells by using syngeneic murine KRAS G12C mutant NSCLC cells which we established before, and human KRAS G12C mutant NSCLC cell lines including NCI-H358, NCI-H1373, NCI-2030, and HCC44. Consequently, we found that multiple cell lines inactivated KEAP1 showed resistance to treatment with sotorasib in two-dimensional (2D) culture conditions. Conversely, LKB1 depletion did not markedly affect cell proliferation in 2D cultures or rather sensitized the cells to sotorasib treatment. Consistent with these results, KEAP1 reconstitution restored sensitivity to treatment with sotorasib in KEAP1 mutant NSCLC cells. Next, we analyzed gene expression profiles through RNA sequencing following treatment with sotorasib in KEAP1 wild-type or depleted background and extracted various candidate genes that are potentially responsible for the resistance to sotorasib in cells lacking functional KEAP1. Furthermore, we performed drug screening to identify the candidates that specifically suppress the growth of cells with KEAP1 inactivation when combined with sotorasib (Figure 1). Notably, combination therapy with the candidate drug X and sotorasib significantly inhibited the growth of KEAP1 mutant cells which show intrinsic resistance to sotorasib monotherapy. As a next step, we plan to investigate whether the combination treatment with sotorasib and drug X is effective against KRAS mutant NSCLC harboring concurrent KEAP1 mutations *in vivo*.

2. Elucidating the therapeutic effects of sotorasib through antitumor immunity.

Recent studies have highlighted that concurrent mutations in KEAP1 and LKB1 genes confer resistance against immunotherapy in KRAS mutant NSCLC. As reported previously (ref.2), sotorasib exerts its therapeutic effect not only by directly suppressing the survival and proliferation of cancer cells through inhibiting KRAS downstream signaling but also potentially through the activation of antitumor immunity. Therefore, the loss of function in KEAP1 and LKB1 may affect the efficacy of sotorasib treatment by regulating the immune tumor microenvironment. In this study, utilizing a syngeneic murine KRAS G12C lung cancer model that facilitates in-depth analysis of the cancer-immune cell interactions, we first conducted pharmacodynamic studies to identify the optimal dosing schedule for sotorasib administration. We determined the conditions for administering sotorasib that efficiently inhibited KRAS downstream signaling without causing adverse effects, such as weight loss, in this context. Next, we evaluated the impact of KEAP1 mutations on the efficacy of sotorasib treatment by using this immunocompetent murine model and found that KEAP1 inactivation induces sotorasib resistance *in vivo*. Currently, we are investigating how KEAP1 mutations affect the immune microenvironment and cytokine secretion dynamics in response to sotorasib treatment, through the analysis of sotorasib-treated tumor tissues. Furthermore, to validate the antitumor immune-mediated therapeutic effects of sotorasib, we are also conducting analyses to examine whether the efficacy of sotorasib differs between our immunocompetent and immunodeficient models such as the NSG (NOD/SCIR/IL2r gamma null) model.

## Discussion & Conclusion

Through the analyses of the response to sotorasib treatment in KEAP1/LKB1 isogenic NSCLC cells that we have established, we have demonstrated that inactivation of KEAP1 confers resistance to sotorasib treatment both *in vitro* and *in vivo*. Additionally, we identified several candidate genes that would be responsible for the resistance to sotorasib in cells lacking functional KEAP1 by RNA sequencing. Moreover, through drug screening, we identified candidates that effectively suppress the growth of cells with KEAP1 inactivation when combined with sotorasib. Based on these findings, it is necessary to elucidate the molecular mechanisms by which KEAP1 inactivation induces resistance to sotorasib treatment. Furthermore, we plan to analyze the impact of the double mutation in LKB1 in addition to KEAP1 on the resistance to sotorasib treatment, as well as analyze the effects of sotorasib treatment on the immune tumor microenvironment by utilizing the syngeneic murine KRAS G12C model.

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## 一般の皆様へ

肺がんは世界で最も罹患数の多いがん種であり、肺がんを引き起こす「ドライバー遺伝子変異」を標的とした新薬が次々と開発されてきました。しかし肺がんで最も高頻度に観察されるKRAS遺伝子変異に対する有効な薬がこれまで存在しませんでした。しかし、2022年度より日本国内において初めてKRAS阻害薬の１つであるソトラシブの使用が開始され、生体内での詳細な作用機序や薬剤耐性のメカニズム、有効な患者を選別するための奏功マーカーの開発は今後の重要な研究課題となっています。本研究では、これまで研究代表者が行なってきた肺がん細胞に対する研究成果を基盤としてこれらの課題にアプローチしています。

# Optimization of protein amino (N)-terminal regions of artificial mRNAs by an evolutionary engineering approach

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## Summary Abstract

In this study, we attempted to optimize the protein N-terminal region with the aim of expanding the use of mRNA medicine. We have identified several candidate protein-end sequences that support high protein expression from artificial mRNA. In the future, we will further explore both the N-terminal and the C-terminal candidate sequences, and also validate the use of the identified sequences in the expression of different proteins, different cell types, and animal models.
***Key Words*** : mRNA medicine, protein expression, artificial mRNA

## Introduction

The emergence of mRNA vaccines against COVID19 has brought mRNA therapeutics to the forefront of the world's attention. On the other hand, the low efficiency of protein expression is a major challenge for mRNA medicine to be used not only as mRNA vaccines but also for various other purposes such as gene therapy, cancer immunotherapy, and genome editing. In this study, we focused on the sequences attached to the protein-end, which has not received much attention so far, and first aimed to optimize the N-terminal sequence.

## Results

In this study, we attempted to screen for sequences that ensure high protein expression using EGFP libraries with randomized protein-end sequences. Although the initial plan was to screen the N-terminus sequences, it was decided to proceed with the selection of both the N-terminus and C-terminus sequences in parallel, since there was no significant technical difference. We attempted to select sequences that ensure high protein expression by evolutionary engineering approach by introducing EGFP libraries with randomized terminal sequences into HeLa cells, selecting high EGFP-expressing cells by FACS, reconstructing EGFP libraries from the mRNA recovered from the cells, and repeating this selection cycle. As a preliminary experiment to confirm whether the cycle repetition works effectively for selection, we introduced terminally randomized EGFP library mRNA into HeLa cells and compared it with control EGFP mRNA, and found that the library mRNA showed significantly lower expression efficiency. Although the half-life of artificial mRNA is about 2-3 hours, EGFP mRNA was also detected in the mRNA extracted from cells 24 hours after

introduction of library mRNA. Then, EGFP mRNA collected at 24 hours was re-librarized, mRNA was synthesized, introduced into the cells, and collected 24 hours later, which was repeated 5 times, expecting either silenced or stabilized mRNAs should be enriched at the end of the selection cycles, which perhaps results in either silenced or enhanced EGFP expression. However, no significant change in EGFP expression was observed. This suggests that the diversity of mRNA species introduced per cell was not sufficiently small compared to the library size, and for the same reason it would be difficult to promote selection effectively when using FACS selection.

Therefore, as an alternative method, although less efficient, we switched to isolating individual clones from the protein-end-randomized library, synthesizing mRNA derived from each clone, introducing them into cells individually, and evaluating their protein expression. Using this method, we have evaluated protein expression (EGFP fluorescence) of approximately 200 clones, N-terminally and C-terminally. So far, we have not identified any N-terminal sequences that show a significant (>1.5-fold) increase in expression, but we have obtained several sequences that show increased (1.5-2.5 fold) expression in the C-terminally randomized EGFP clones, which was being analyzed at the same time. Because the number of sequences obtained is still small, no common features can be found among the identified sequences at this time. Individual clones were not characterized by frequency of hydrophobic/hydrophilic, acid/basic, or polar amino acids. It remains to be seen how these sequences contribute to the increased expression, but the fact that they showed higher expression than controls at early time point (8h) after mRNA transfection probably suggests increased translation efficiency. Further analysis is needed to determine the details. In addition, we identified two other sequences that alter the subcellular localization of EGFP, although no significant changes in expression levels were observed for these clones. EGFP is detected not only in the cytoplasm but also in the nucleus, while two sequences showed extreme cytoplasmic localization and thus may function as nuclear export signal. Indeed, one has a sequence that was close to a conserved sequence of a known nuclear export signal, while the other was a novel sequence that was completely different from the conserved sequence.

## Discussion & Conclusion

In this study, we aimed to optimize the protein end sequence in order to improve the expression efficiency of artificial mRNA, which is necessary for the further development of mRNA medicine. Although the originally planned screening has not been successful so far, several sequences that support high protein expression have already been identified by the method of evaluating individual clones, and it is expected that further expansion of this search will likely lead to the identification of even higher expression sequences. As mentioned above, the sequences obtained in this screening seem to increase the efficiency of translation, but the molecular mechanism by which the sequences near the termination codon increase the efficiency of translation remains to be elucidated. Also, it is necessary to carefully examine which steps of gene expression; mRNA stability, translation efficiency, and protein stability the sequences obtained in the future will contribute to. Subcellular localization will also be carefully examined, including not only nuclear and cytoplasmic localization but also organelle localization. We will also need to test the obtained sequences for proteins other than EGFP and verify them in animal models.

## 一般の皆様へ

mRNA 医薬は新型コロナウィルスの mRNA ワクチンの登場で一躍注目を集めましたが、この他にも、遺伝子治療、がん免疫療法など多様な目的での応用が期待されています。しかし、人工 mRNA からのタンパク質合成効率の低さが、その妨げとなっています。本研究ではこの課題に取り組むため、これまであまり注目されてこなかったタンパク質末端配列の最適化により、タンパク質発現効率を上昇させることを目指しています。これまでに複数の発現高効率化配列を同定しており、今後さらにスクリーニングを進め、mRNA 医薬の分子基盤の構築に貢献したいと考えています。

# Search and creation of photo-activated adenylyl cyclases for optogenetics tools

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## Summary Abstract

To explore and create novel photoactivated adenylyl cyclases (PACs), a system was developed to measure cAMP production by PACs with high temporal resolution using cyclic nucleotide-gated (CNG) channels. This system would allow us to investigate the properties of different PACs in response to blue light illumination more accurately.
***Key Words*** : Optogenetics, ion channel

## Introduction

Photoactivated adenylyl cyclase (PAC) is a unique protein that produces cAMP in response to blue light illumination and is used as a tool to non-invasively regulate cellular events (1). Several types of PACs have been identified, but their detailed properties have not been compared. In this study, to investigate these properties in detail, we developed a system to measure cAMP production by PACs with high temporal resolution using cyclic nucleotide-gated (CNG) channels.

## Results

To measure cAMP production from PACs using cyclic nucleotide-gated (CNG) channels, PACs and CNG channels were purified, and the cAMP-dependent activity of the channels was confirmed using one of the electrophysiological methods, a planer bilayer method (2). In addition, a system for measuring CNG channel activity in the presence of PACs whose activity was regulated by blue light illumination was established. Since this method allows us to measure channel activity as a current, changes in CNG channel activity induced by cAMP from PACs can be detected with high temporal resolution.

Purification of PACs

Two types of PACs proteins were purified using an affinity column. Briefly, eight PAC genes with a histidine tag (His-tag) were introduced into pQE30 vectors. Among them, two constructs encoding PAC from *Oscillatoria acuminata* and PAC from *Beggiatoa sp.* genes were transformed into *E.coli* XL1-Blue and overexpressed by adding isopropyl β-D-1-thiogalactopyranoside. The expressed PACs were isolated using a $Co^{2+}$ affinity column that specifically binds the His-tag of the channel.

Purification of CNG channels

Two types of CNG channels with different cAMP sensitivity, KcsA-CNG channel and SthK channel, were extracted from a membrane fraction using surfactants and purified by an affinity column. These channels with His-tags were overexpressed in cell membranes of *E.coli*. Most of the KcsA-CNG channel was extracted from the membrane fraction by solubilization with decyl maltoside (DM) and they could be isolated using the $Co^{2+}$ affinity column. On the other hand, it was difficult to solubilize the SthK channel from the membrane fraction with DM. Therefore, we investigated four surfactants (octyl glucoside, sodium cholate, CHAPS, and MEGA-G) to solubilize it. Among them, only the sodium cholate was able to solubilize it. Solubilization of the SthK channel occurred at 5 times the critical micelle concentration (CMC) of the sodium cholate, and more than half of the SthK channel in the membrane fraction could be solubilized at 20 times the CMC. The solubilized SthK channel was purified using the $Co^{2+}$ affinity column.

cAMP-dependent CNG channel activity measurement with the planer bilayer method

The activities of two purified CNG channels were measured by the planar bilayer method. The purified channels were incorporated into liposomes, and their channel currents of them were measured by fusing the liposomes with the bilayer membrane. The currents could be detected with high temporal resolution, and the open probabilities (Po) of the KcsA-CNG channel and the SthK channel at 100 mV were 0.026 and 0.075, respectively. The Po of the KcsA-CNG channel was increased by the addition of cAMP (Fig.1). Po at 50 mV was 0.004 at 0 μM cAMP, whereas it was 0.588 at 1 μM cAMP. This result indicates that changes in cAMP concentration can be detected by measuring Po of the KcsA-CNG channel.

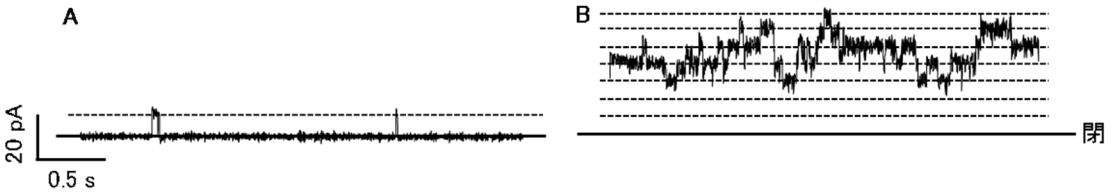


Figure 1 cAMP dependent current of the KcsA-CNG channel
A) A typical current trace of the KcsA-CNG channel in absence of cAMP. B) A current trace at 1μM. cAMP.

System to measure light-dependent PAC activity using CNG channels

A system for measuring PAC activity using the CNG channel was constructed by combining the channel current recording system with a light illumination system (Fig.2). An LED was placed over a chamber in which the lipid bilayer was formed, and the illumination time was regulated by a microcomputer. This system would allow us to measure changes in cAMP concentration induced by PAC in response to blue light illumination with high temporal resolution.

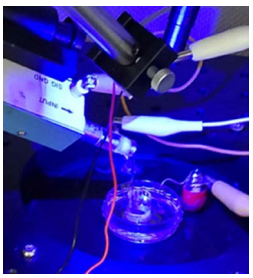

Figure 2 Photograph of the measurement system.

## Discussion & Conclusion

Changes in cAMP concentration could be detected with high temporal resolution by measuring the current of CNG channels: The open probability of one of the CNG channels, KcsA-CNG, was changed depending on cAMP concentration. Since each CNG channel has different cAMP sensitivity, it may be possible to detect a wide range of cAMP concentration by using different types of CNG channels. In addition, we constructed the system to measure channel currents while controlling light illumination. With this system, changes in cAMP produced by PACs in response to blue light illumination would be detected using the CNG channel, which would allow a more accurate comparison of the light responsivity of different PACs.

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## 一般の皆様へ

細胞のはたらきを光で操作する技術の高度化を目指し、その技術で用いるツールの探索と改良を行っている。ツールである光感受性タンパク質の一種である光感受性アデニル酸シクラーゼ（PAC）は生物界に数多く存在している。本研究では、それらの特性、特に光応答性を正確に比較するための測定系を確立した。様々なPACの特性を比較・改変することにより、細胞の光操作ツールとして選択できるPACの種類を増やし、生命現象の機序の理解や疾患の原因解明の進展に貢献したい。

# Novel lipid metabolism essential for the ceramide homeostasis in stratum corneum

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## Summary Abstract

We recently identified SLIP as an essential factor for the skin barrier function. In this study, we established an *in vitro* assay system for the lipid metabolizing enzymatic activity of SLIP and revealed its tissue localization in skin epidermis. The external application of candidate product lipids of SLIP partially restored the impaired skin barrier function in a mouse model of atopic dermatitis. These results will advance our understanding of the molecular mechanisms underlying the establishment of the skin barrier function and will also provide important implications for developing treatments of related skin diseases.
***Key Words*** : Skin, Barrier function, Stratum corneum, Lipids, Ceramides

## Introduction

Homeostasis of ceramides in the stratum corneum of skin is essential for the barrier function. We discovered SLIP (Skin-specific lipase protein) as a novel lipid metabolizing enzyme in skin. Targeted disruption of *Slip* gene in mice resulted in the abnormal composition of ceramides in the stratum corneum and excessive water loss from their skin, suggesting its association with diseases that involve barrier dysfunction. In this study, we aimed to clarify the enzymatic activity of SLIP and establish its molecular function in lipid metabolism as an essential factor for the barrier function.

## Results

In this study, we conducted experiments according to the following three research topics to elucidate the molecular function of SLIP and verify its potential for developing novel therapeutics for related diseases.

**Topic 1: Identification of substrates and reaction products of SLIP.** To clarify the lipid metabolizing process catalyzed by SLIP, which is essential for the skin barrier function, we tried to identify its substrates and reaction products. As the first step, we attempted to detect the enzymatic activity of SLIP using an *in vitro* assay system. Esterified 4-methylumberlliferones (4-MUs) were mixed with purified recombinant SLIP, and the fluorescence of 4-MU (ex: 365 nm, em: 450 nm) released by ester hydrolysis reaction was measured by spectrofluorometer. As a result, we could successfully demonstrate the enzymatic activity of SLIP at the molecular level for the first time, clarifying its substrate

concentration- and pH dependence. In further evaluations using multiple esterified 4-MUs to elucidate the substrate specificity of SLIP, no significant differences were observed between all the tested compounds. We also conducted a competitive inhibition assay of the esterified 4-MU hydrolysis by adding excess concentrations of multiple substrate lipid candidates. However, the substrate specificity of SLIP was not successfully determined since no inhibitory effects on the activity of SLIP were detected with any of them.

**Topic 2: Recovery of the barrier function by reaction product candidates of SLIP.** As described above, we could not identify the SLIP substrate and reaction products. However, the results of comparative lipidomics analysis performed in advance, between the stratum corneum of wild type mice and *Slip* knockout mice, allowed us to narrow down the candidate lipids to a certain extent. We therefore prepared a vaseline-based ointment containing a mixture of candidate lipids and investigated the possibility to restore the abnormal barrier function. In this study, we selected atopic dermatitis as a representative disease of dysregulated barrier function. NC/Nga mice are known to spontaneously develop symptoms resembling atopic dermatitis under conventional breeding conditions. The experiment was performed by externally applying the ointment supplied with candidate lipids or vaseline only (as a negative control) to NC/Nga mice. As a result, we found that several pathology scores, such as transepidermal water loss, skin inflammation, and itching behavior, were significantly recovered by externally applying the candidate lipids compared to control, mostly in a dose-dependent manner. These results suggest that the disruption of ceramide homeostasis due to SLIP-deficiency can be restored by supplementing SLIP reaction products.

**Topic 3: Analysis of the tissue localization of SLIP.** To elucidate the physiological function of SLIP, it is necessary to clarify its tissue localization in detail. We performed immunohistochemistry/fluorescence on mouse skin sections with home-made anti-SLIP monoclonal antibody. We have already confirmed that this antibody successfully detect endogenous SLIP by western blotting of mouse skin lysates. However, immunostaining analyses did not visualize any reliable signals. As an alternative approach, we constructed a strain of knock-in mice, in which an epitope tag was fused to the C-terminus of SLIP. Using an antibody against the epitope tag, we found that SLIP is highly selectively expressed in the granular layer of epidermis. Studies were also conducted using primary culture of mouse epidermal keratinocyte. We found that the SLIP expression shows a time course similar to that of a late differentiation marker of keratinocyte, loricrin, at both the mRNA and protein levels. These results indicate that the expression of SLIP is triggered by the terminal differentiation of keratinocytes, resulting in the granular layer-specific expression *in vivo*. Immunoelectron microscopy of skin tissue was also performed with the knock-in mice. However, no relevant immunoreactive signals were detected successfully due to the high backgrounds raised by dense intracellular keratin cytoskeleton.

## Discussion & Conclusion

In this study, we experimentally demonstrated the lipid metabolizing enzymatic activity of SLIP for the first time and clarified its detailed tissue localization in skin epidermis. SLIP is thought to be involved in lipid metabolizing process at the final step of terminal differentiation of keratinocytes, which contributes to the formation of the functional skin barrier. During this research period, we could not determine the substrate and reaction products of SLIP. This is possibly because the hydrophobic physiological conditions in stratum corneum were not reproduced in our *in vitro* assay system, in which we used relatively hydrophilic 4-MU-derivative compounds as substrates but not the actual endogenous lipids. It will be necessary to establish an assay system that can detect activity under conditions more similar to *in vivo*. Still, the impaired skin barrier function in the mouse model of atopic dermatitis was successfully recovered by the externally applying the candidate product lipids. These results greatly contribute to elucidating the molecular mechanisms of the development of the skin barrier function, establishing SLIP as a novel essential factor in it. Further studies may lead to propose innovative treatments for diseases that involve skin barrier dysfunction.

## 一般の皆様へ

皮膚のバリア機能は、異物の侵入から身を守ったり、体内の水が失われるのを防いだりしています。特に重要なのが、皮膚を覆う角質に存在するセラミドと呼ばれる脂質です。我々は、セラミドを保つ働きをもつ新規酵素を発見し、その分子機能の一端を明らかにしました。この酵素の機能によって生じる脂質は、バリア機能の破綻を回復させる可能性があるため、本研究の成果は、アトピー性皮膚炎等、関連する疾患の治療の提唱にも繋がるものと期待されます。

# Development of multifunctional molecular probes with high sensitivity for detecting precancerous lesions in pancreatic cancer

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## Summary Abstract

We developed diagnostic agents using gold nanoparticles for detecting precancerous lesions in pancreatic cancer. By combining nanobodies and middle molecules with gold nanoparticles, we aimed to develop three multifunctional types: Raman probes for liquid biopsy and photoacoustic and nuclear medicine probes for in vivo imaging. We successfully developed several types of molecules targeting survivin and ADAM8 that express in the precancerous lesions. Novel silica-gold nanoparticles with low fluorescence noise and high Raman signal, advancing liquid biopsy materials. These findings can provide new insight for development of effective molecular probes for detecting precancerous lesions in pancreatic cancer.

***Key Words*** : Precancerous lesions, Raman probes, Nuclear medicine probes, Gold nanoparticles

## Introduction

Pancreatic cancer, with a 5-year survival rate of 7%, is projected to become the second leading cause of cancer deaths by 2030.[1] Imaging methods like CT, FDG-PET, and MR/ERCP are used, but early symptoms are rare, leading to late-stage diagnoses. The disease progresses over 10 years, with precursor lesions like PanIN and IPMN.[2] Early detection is crucial for better outcomes, but current imaging struggles, highlighting the need for sensitive diagnostics. We're developing molecular probes for PanIN and IPMN detection[3,4] and aim to develop two probes: one targeting exosomes in blood using Raman technology and another for photoacoustic and nuclear imaging.

## Results

Development of survivin targeting nanobodies and peptides

Survivin expresses in the PanIN and IPMN of precancerous lesions. Therefore, we aimed to develop survivin binding agents including nanobodies and peptides. For development of nanobodies, after immunizing camels and isolating lymphocytes from blood samples, we conducted screening using phage display, resulting in obtaining clones with Kd values in the range of 10 nM. Since survivin is primarily expressed intracellularly, we subsequently induced the fusion of an excellent membrane-permeable peptide and nanobody complexes.

These nanobody derivatives showed consistent localization with the survivin expression in the pancreatic cancer cells. Currently, we are conducting fundamental investigations on the efficiency of intracellular delivery and the specificity to survivin using fluorescence or radioisotope labeling.

We have developed peptides targeting survivin[3,4], and have proceeded to develop peptides labeled with the radioactive isotope $^{125}$I using these parent compounds. Several molecules have consistently shown binding to the survivin-rich regions of pancreatic cancer cells. Currently, we are optimizing cyclization and membrane-permeable peptide modifications to enhance the metabolic stability and membrane permeability, aiming to further enhance the efficacy of these molecules as delivery agents for gold nanoparticles.

Development of ADAM8 targeting antibodies and nanobodies

We developed and characterized an $^{111}$In-labeled anti-ADAM8 monoclonal antibody for early detection of pancreatic cancer by SPECT imaging. Immunohistochemical staining confirmed ADAM8 expression in pancreatic ductal adenocarcinoma and intraductal papillary mucinous adenoma tissue, suggesting its potential as a target for early pancreatic cancer imaging. Cell binding experiments showed higher accumulation of the labeled antibody in cells overexpressing ADAM8 compared to those with low expression. Biodistribution studies demonstrated increased uptake in ADAM8-positive cells, and SPECT imaging effectively visualized ADAM8-positive tumor tissues. Therefore, ADAM8 shows promise as a target for early pancreatic cancer diagnosis, with the labeled antibody serving as a useful imaging agent for detection. We also developed several ADAM8 targeting new nanobodies which exhibited 10-20 nM levels of Kd values. These nanobodies demonstrated consistent accumulation with the ADAM8 expression in the pancreatic cancer cells. Therefore, these IgG and nanobodies are promising candidate for application to the gold nanoparticle-based diagnostic agents for precancerous lesions.

Development of novel Raman probes

We attempted to develop surface-enhanced Raman scattering (SERS)-based Raman probes using spherical gold nanoparticles as a parent material, capable of low-noise and stable measurements, for the early diagnosis of trace cancer cells and exosomes in blood. To achieve this, we synthesized novel silica-coated gold nanoparticles utilizing extinction molecules as Raman reporters to mitigate fluorescence noise from existing near-infrared fluorescent dyes, and conducted basic evaluations. Detailed shape analysis using techniques like DLS and SEM confirmed that the synthesized silica-coated gold nanoparticles had a structure where 60 nm gold nanoparticles were encapsulated by silica shells of approximately 30-50 nm. It was observed that the shape of the formed silica-coated gold nanoparticles varied depending on the concentration of reagents and reaction temperature. After evaluating various Raman reporter molecules, we introduced cancer-targeting molecules into newly developed gold nanoparticles, which exhibited high signals. Evaluation using cancer cells showed promising accumulation in response to the expression levels of the target protein (HER-2. EGFR, and integrin $\alpha_v\beta_3$), indicating potential for cancer diagnostics.

## Discussion & Conclusion

Our developed IgG, nanobodies and middle molecules targeting survivin and ADAM exhibited high affinity and specificity towards each target. They hold promise for future applications for precancerous lesions of pancreatic cancer diagnostic agents.

We successfully developed novel Raman probes with great cancer targeting and low fluorescence signals. Further exploration of Raman reporters, metal nanoparticles, modified molecules, and target molecules is expected to lead to applications in various cancer diagnostic fields.

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## 一般の皆様へ

膵臓がんは、全がんの中で最も予後が悪く、その治療成績向上は喫緊の課題です。臓がんは、進行性のがんになる前に、特徴的な前癌病変が見られることがあります。これらを早期に発見できれば、治療成績が大幅に改善される可能性があります。本研究では、血液検査から画像診断まで一貫した診断により、迅速かつ高精度に膵臓がんを前癌病変の状態で発見できる画期的な診断薬開発を目指しました。研究期間に、いくつかの前癌病変へ強固に結合する化合物の開発に成功しました。また、血液検査に用いることのできる金ナノ粒子型の新たな素材の開発にも成功し、今後の更なる展開が期待されます。

# Stratification of Alzheimer's Disease by Integrative Multi-Omics Analysis

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## Summary Abstract

This study aims to elucidate the causes of heterogeneity in Alzheimer's disease (AD) at the molecular network level using cutting-edge molecular biological methods. By integrating RNA-seq and microRNA-seq data obtained from post-mortem brain tissues, we identified a network related to ApoE genotype and the presence of argyrophilic grain pathology, which was difficult to stratify by single omics analysis alone. The introduction of epigenome analysis, including histone modification analysis using the CUT&Tag method, has laid the foundation for a multi-layered understanding of AD pathology. These findings deepen our understanding of the complex pathological mechanisms of AD and are expected to lead to the development of personalized therapies tailored to individual patients in the future. Further research progress, such as cell type-specific analyses and examination of pathogenesis in cellular and mouse models, is desired to overcome AD.

***Key Words*** : Alzheimer's disease; heterogeneity; multi-omics integration; molecular networks; personalized medicine

## Introduction

Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β (Aβ) and phosphorylated tau (p-tau) as the final pathology. However, the clinical presentation is highly variable, with a complex interplay of environmental factors, genetic risk factors, and co-occurring complications contributing to a heterogeneous disease course. Despite this diversity, knowledge on the underlying pathology is limited, with the majority of research focused on amyloid-beta and phosphorylated tau. Furthermore, the integration of multiple "omics" data, such as RNA-seq, microRNA-seq, and epigenome data, has yet to yield a comprehensive understanding of the disease, due in part to the differences between patient cohorts and the challenges of data integration. This study aims to address these gaps by employing advanced molecular biological methods to stratify AD pathology at the molecular network level.

## Results

In this study, we integrated RNA-seq and microRNA-seq data obtained from post-mortem brain tissues to stratify AD pathology using similarity network fusion (SNF). The integration of these data revealed a network corresponding to ApoE genotype and the presence of argyrophilic grain pathology, which was difficult to stratify by single omics analysis alone. This network is thought to reflect important molecular mechanisms in the pathogenesis of AD.

To capture the epigenomic changes underlying AD pathology, we introduced histone modification analysis using the CUT&Tag method on post-mortem brains. Although we have confirmed that data acquisition is possible at this point, we have not yet reached a sufficient sample size. In the future, we will proceed with the analysis and integrate the data with RNA-seq and microRNA-seq data to aim for a more detailed stratification of AD pathology.

Furthermore, we plan to perform cell type-specific epigenome analysis on the cell types that are the main loci of the molecular pathology hubs identified by the SNF analysis. Single-cell RNA-seq will also be conducted to reveal the molecular biological relationship between the disease-causing cell types and the cell populations affected by such cell types. Based on these analyses, we will examine the pathogenesis of each subtype in cellular and mouse models to identify the individual pathologies that constitute the heterogeneous pathogenesis of AD and to identify hub pathologies that may serve as potential therapeutic targets.

## Discussion & Conclusion

This study stratified the heterogeneous pathology of AD at the molecular network level and identified a network corresponding to ApoE genotype and the presence of argyrophilic grain pathology. The integration of RNA-seq and microRNA-seq data enabled the stratification of pathology that could not be captured by single omics analysis alone. Furthermore, the introduction of histone modification analysis is expected to provide a multi-layered understanding of the pathology, considering epigenomic changes. These findings will serve as an important foundation for elucidating the pathological mechanisms of AD and developing personalized medicine.

The results of this study provide significant insights into understanding the complex pathological mechanisms of AD and realizing personalized medicine. By integrating multi-omics data and employing advanced molecular biological methods, we have laid the groundwork for a comprehensive understanding of AD pathology at the molecular network level. Future research, including cell type-specific analyses and examination of pathogenesis in cellular and mouse models, will further deepen our understanding of the heterogeneous nature of AD and aid in the development of targeted therapies tailored to individual patients. The findings of this study represent a significant step towards overcoming AD and improving the lives of those affected by this devastating disease.

## 一般の皆様へ

アルツハイマー病は、アミロイドβとリン酸化タウという2つのタンパク質の蓄積が特徴ですが、実際の症状や進行速度は患者さんによって大きく異なります。この研究では、最先端の分子生物学的手法を用いて、アルツハイマー病の多様性の原因を遺伝子やタンパク質のネットワークレベルで解明することを目指しました。

# Research for the assembly of PML nuclear bodies regulated by Neddylation and the promotion of kidney regeneration.

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## Summary Abstract

This study focuses on the role of PML bodies in the early stages of regeneration, using the Xla.Tg(Xtr.pax8:EGFP) transgenic line for live renal imaging. Damage was induced in proximal tubule regions, followed by a 48-hour culture at 18°C. Using Isoform Sequencing (Iso-Seq), the comprehensive transcriptomic analysis identified 82,907 isoforms with differences in expression between damaged and regenerating tissues. These include injury stress/regeneration-specific splicing variants and transcription products from Alternative Transcription Start Sites (ATSS). The study underscores the critical roles of these isoforms in transcriptional and translational regulation, which is crucial for effective regeneration.
***Key Words*** : Kidney, regeneration, enhancer, nuclear structure

## Introduction

This study aims to comprehensively elucidate the role of nuclear structures such as PML bodies in the initial stages of regeneration, starting from damage signals to the decision and progression towards regeneration. This investigation is based on the regenerative gene KLF15 and its target complex, SAP25-Sin3A, which the applicants discovered.

## Results

We were using the Xla.Tg(Xtr.pax8:EGFP) transgenic line capable of live imaging of renal tissue, we inflicted damage to the proximal tubule region at stage 36/37. We cultured the tissue at 18°C for 48 hours to study regeneration. Tissue samples from the regenerating area and undamaged controls cultured under identical conditions were collected. The harvested cells were preserved in CELLBANKER 1 (Takara Bio). Total RNA was extracted from these samples using ISOGEN (Nippon gene Bio), followed by reverse transcription using the SMATbell prep kit 3.0 (Pacio) with cDNA peaks ranging from 1.5 – 4.0 kbp. Comprehensive transcriptomic analysis was performed using these cDNAs through Isoform Sequencing (Iso-Seq).

Further, libraries were prepared and sequenced using the Sequel II/IIIe system. The library preparation and Iso-seq were supported by the Platform for Advanced Genome Science (PAGS) under the JSPS KAKENHI Grant Number 22H04925 (PAGS). Data were mapped and aligned using ESPRESSO (Error Statistics PRomoted Evaluator of Splice Site Options), employing minimap2 embedded in ESPRESSO with the specific parameters

"minimap2 -ax splice -ub --secondary=no ref.fasta combined.fastq > in.sam" against the Xenopus laevis genome sequence (GCF_001663975.1_Xenopus_laevis_v2) [1] [2].

The results unveiled a staggering 65,787 novel isoforms (novel in catalog, NIC) and 44,887 transcription products and their isoforms not previously described in catalogs (novel not in catalog, NNC). Then, isoform expression comparisons between undamaged and regenerating tissues were conducted using rMATS-long, further enriching our understanding [3]. This analysis not only identified injury stress and regeneration-specific splicing variants but also revealed transcription products originating from regeneration-specific alternative transcription start sites (ATSS).

As a result, it was found that there are 82,907 isoforms with expression differences between damaged and regenerating tissues. These isoforms include not only injury stress and regeneration-specific splicing variants but also transcription products originating from regeneration-specific Alternative Transcription Start Sites (ATSS) (see figure). Additionally, it is known that in nuclear stress bodies (nSB), cells increase the amount of intron-retaining precursor mRNAs upon sensing stress, preparing to produce functional proteins rapidly during stress recovery [4]. This analysis also identified transcription products expected to be intron-retaining precursor mRNAs.

## Discussion & Conclusion

These regeneration-specific transcription start sites suggest the presence of regeneration-specific promoters. These promoters likely indicate that the regenerative enhancers we have identified use these specific transcription start sites (ATSS) to express target genes. Furthermore, the isoforms transcribed from these regeneration-specific ATSSs may have significant roles at various levels, including transcriptional regulation (transcription and splicing rates), translational level (translation speed), and protein functionality, which are essential for enabling regeneration.

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# 一般の皆様へ

ヒトは失った組織や器官をまるごと取り替える能力、再生能を持っていないが、動物の中にはそれができるものがあります。この研究は、ヒトと比べると高い再生能を持つカエルを使って、腎組織がどのように再生するのか明らかにすることを目的としています。細胞の中の核には体の設計図と呼ばれるゲノムが存在します。ゲノムの中には、「遺伝子」と「遺伝子以外の領域」があります。我々の体は様々な状況に応答して、「遺伝子以外の領域」にあるオン・オフ・スイッチを利用して、遺伝子を使う・使わないと決めています。カエルが再生する時に使う遺伝子はヒトも持っています。このことは、遺伝子があるだけでは再生できないこと、つまりヒトではオン・スイッチがうまく働かないことを示しています。この研究は、再生を可能とさせるオン・スイッチに着目して、その全容の解明に取り組んでいます。

# A pathological role of a novel receptor for chemokine ELC/CCL19 in psoriasis

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## Summary Abstract

Chemokine ELC/CCL19 is a functional ligand for CCR7, which is mainly expressed by naïve T cells and B cells. In the present study, we found that ELC/CCL19 is a also functional ligand for GPCR#13, which is broadly expressed by effector cells. We revealed that ELC/CCl19 efficiently induced chemotaxis in GPCR#13-expressing cells as well as CCR7-expressing cells. In addition, ELC/CCL19 may play an important role in psoriasis by recruiting GPCR#13-expressing effector cells in mouse psoriasis model.
***Key Words*** : Chemokine, ELC/CCL19, psoriasis, novel receptor

## Introduction

ELC/CCL19 is a ligand for the chemokine receptor CCR7 and is known to be involved in the homing of naïve T cells and B cells, which express CCR7 to lymph nodes. On the other hands, ELC/CCL19 is reported to be upregulated in effector site such as skin lesions of psoriasis patient. CCR7, a known receptor of ELC/CCL19, is expressed by naïve T cells and B cells but not effector cells. Thus, a role of ELC/CCL19 remains unknown in the skin lesion of psoriasis. In the present study, we explored a novel receptor for ELC/CCL19 using our original screening procedure and revealed the role of the ELC/CCL19 on the effector cell recruitment via the novel receptor using psoriasis mouse model.

## Results

**Screening of a novel receptor of ELC/CCL19**

Traditional chemokine receptor screening has been conducted using intracellular calcium influx as an indicator. In the present study we screened over 50 types of GPCR-expressing cells to explore a novel receptor for ELC/CCL19 using chemotaxis activity as an indicator. We tested the ability of ELC/CCL19 to induce cell migration using a panel of mouse L1.2 cell lines stably expressing all the known chemokine receptors and GPCR receptors. Our screening using chemotaxis activity revealed that ELC/CCL19 induced cell migration for GPCR#13 as well as CCR7, known receptor for ELC/CCL19. This suggests that GPCR#13 is a novel receptor for ELC/CCL19 (data not shown).

**ELC/CCL19 but not SLC/CCL21 induces chemotaxis via GPCR#13**

We next examined cell migration via the ELC/CCL19-GPCR#13 axis. As shown in

Figure 1, ELC/CCL19 induced the migration of L1.2 cells expressing GPCR#13, with a peak response at 100 nM. This efficacy was comparable with that observed in cells expressing the known receptor CCR7. Furthermore, ELC/CCL19-induced cell migration was inhibited by PTX, a Gαi inhibitor. The checkerboard-type analysis confirmed that ELC/CCL19 induced chemotaxis, not chemokinesis, in L1.2-GPCR#13 cells (data not shown). On the other hand, SLC/CCL21, another ligand for CCR7, induced migration in L1.2 cells expressing CCR7 but did not induce migration in L1.2 cells expressing GPCR#13.

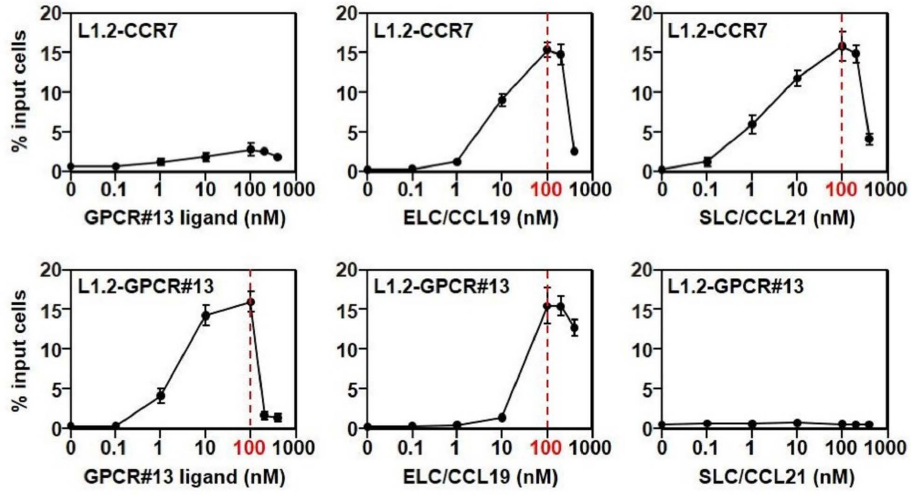


Figure 1. Cell migration activity of ELC/CCL19 via CCR7 and GPCR#13

## Recruitment of effector cells by ELC/CCL19

Naïve T cells and B cells express CCR7 but not GPCR#13. We thus examine the expression of GPCR#13 in other immune cells by flow cytometry. Flow cytometry analysis showed effector cells such as monocytes, NK1.1+NK cell, and CD44+effector T cell expressed GPCR#13 (data not shown).

To examine in vivo recruitment of effector cells, we injected chemokines into the peritoneal cavities of mice. ELC/CCL19 recruited monocytes and NK cells into the peritoneal cavity (data not shown). GPCR#13 inhibitor suppressed cell migration of these effector cells via ELC/CCL19 (data not shown).

## A role of ELC/CCL19 in psoriasis

ELC/CCL19 expression is highly elevated in the skin lesion of psoriasis. Using a psoriasis model in mice, we investigated the involvement of ELC/CCL19 in pathogenesis of psoriasis. We used hydrophilic gel patch as a transdermal administration device. Mice were applied with imiquimod on auricle skin to develop psoriasis. A hydrophilic gel patch containing ELC/CCL19 was applied on the auricle skin of psoriasis mice for last 24 hours. The ear thickness of mice received transdermal administration of ELC/CCL19 increased compared to that of imiquimod alone control (Figure 2). Flow cytometry analysis showed increased infiltration of monocytes and NK cells expressing GPCR#13 in the skin lesions. Their infiltration was suppressed by administration of a GPCR#13 inhibitor (Figure 3).

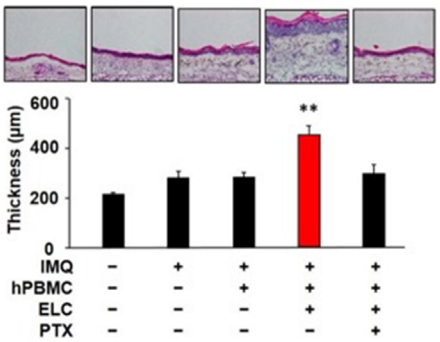


Figure 2. The impact of ELC/CCL19 in psoriasis skin lesions

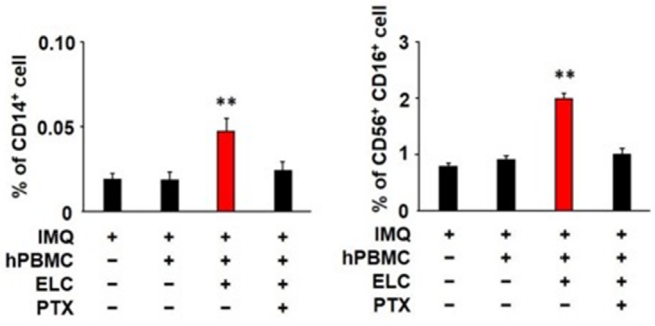


Figure 3. Infiltration of effector cells in skin lesions via ELC/CCL19

## Discussion & Conclusion

ELC/CCL19, formally known as lymphoid tissue homing chemokine is another agonist for GPCR#13 which is expressed on various effector cells. Thus ELC/CCL19 may play a dual role in the naïve immune responses and the effector immune responses. The present results suggest that the ELC/CCL19-GPCR#13 axis may contribute the exacerbation of psoriasis pathology by mediating the infiltration of various effector cells. In addition, we demonstrated that these molecules are highly expressed in the skin lesions of human psoriasis patients. These supports the involvement of the ELC/CCL19-GPCR13 axis in psoriasis.

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## 一般の皆様へ

現在、乾癬などの炎症性疾患に対して効果のある治療薬が多数あります。しかし一部の患者では既存の治療薬が効かない場合もあるのが現状です。この研究では、乾癬が発症する新しいメカニズムを明らかにすることで、既存の治療薬とは異なる作用を示す新しい治療薬を開発することを目的にしています。この研究が進めば、既存薬で効果のない患者にも有効な治療薬ができることが期待されます。

# Development and Application of Stereoselective Synthetic Method for Cyclopropane-fused Heteropolycyclic Compounds

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## Summary Abstract

A stereoselective synthetic route to chiral cyclopropane-fused tetrahydroquinoline was accomplished by preparing hydrazones followed by Ru-catalyzed enantioselective intramolecular cyclopropanation of the electron-deficient alkenes bearing the aryl hydrazone functionality. This sequential one-pot process provides rapid access to a variety of enantioenriched cyclopropane-fused tetrahydroquinolines with up to 95% ee. Synthetic transformations were conducted to demonstrate the synthetic utility of the products and to determine the absolute configuration by X-ray crystal-structure analysis.
***Key Words*** : enantioselective, one-pot process, Ru catalyst, cyclopropanation, electron-deficient alkene

## Introduction

Cyclopropane is a versatile synthetic intermediate and a common structural motif in natural products and pharmaceuticals. Cyclopropane-fused azabicycles are found in many biologically active molecules. Among them, cyclopropane-fused tetrahydroquinolines exhibit potent antiviral activity against HIV-1 non-nucleoside reverse transcriptase.[1] Due to their structural complexity and biological properties, the development of efficient methods to construct such skeletons has received considerable attention in medicinal and synthetic chemistry. However, only a few catalytic enantioselective methods have been established.[2] Herein we report an efficient and facile route to cyclopropane-fused tetrahydroquinolines **1** using a transition metal catalyst.

## Results

Initially, to obtain the enantioenriched cyclopropane-fused tetrahydroquinolines **1**, we screened transition metal catalysts for the enantioselective intramolecular cyclopropanation of an electron-deficient alkene with aryl hydrazone functionality, which is easily prepared from commercially available anthranilic acid. In the presence of 10 mol % of transition metal ($Rh_2(OAc)_2$, $Pd(OAc)_2$, or $AgOCOCF_3$) and 1.5 equiv of NaH in $CH_2Cl_2$ at 20 °C, the reactions could proceed but the yields of *rac*-**1** were low (4–39% yields). Subsequently, chiral metal catalysts were screened. To our delight, the reaction proceeded with chiral Ru catalyst (5 mol %) to give **1** in 59% yield and 97% ee. We then optimized the solvent and temperature. Performing the reaction in 1,2-dichloroethane (DCE) at 60 °C improved

the yield of **1** to 85% and 94% ee. There are a few reports of successful enantioselective intramolecular cyclopropanation reactions of electron-deficient alkenes since transition metal-catalyzed cyclopropanation reactions generally proceed at electron-rich alkenes.[3] Therefore, this enantioselective intramolecular cyclopropanation reaction is particularly noteworthy.

Subsequently, to establish a facile protocol for **1**, a sequential one-pot process for the preparation of the hydrazone followed by Ru-catalyzed enantioselective intramolecular cyclopropanation was examined. $MgSO_4$ most efficiently promoted the formation of the hydrazone from the corresponding anthranilaldehyde derivative and arylsulfonyl hydrazide. Hydrazone formation proceeded in 4 h at 30 °C in DCE, and the synthesis was completed after adding Ru catalyst and NaH to the reaction solution. A comparable yield of **1** was obtained without any erosion of the ee value (83% yield, 94% ee) compared with those obtained in the single-step reaction.

After the successful enantioselective one-pot synthesis of **1**, we investigated the scope and limitations for substrates in this process. A variety of substrates were prepared and the one-pot reactions using these substrates were examined. Although methoxy substituted substrate showed diminished yield and enantioselectivity (31% yield, 81% ee), several substrates substituted on the alkene or the aromatic ring were tolerated to afford products **1** in 64–87% yield with 87–94% ee. Naphthalene- and 1,3-benzodioxole-based products **1** were also obtained with 93% ee and 82% ee, respectively. Furthermore, aryl sulfonyl groups on the nitrogen were well tolerated, and products **1** were obtained with excellent enantioselectivity (92–95% ee). This is the first successful example of enantioselective one-pot synthesis of cyclopropane-fused tetrahydroquinolines **1** via intramolecular cyclopropanation of electron-deficient alkenes.

To demonstrate the synthetic utility, various transformations of the ester functionality in **1** were performed. The corresponding carboxylic acid, amide, and aldehyde were obtained through hydrolysis, amidation, and reduction followed by oxidation, respectively. Furthermore, the aldehyde was converted to its 2,4-dinitrophenyl hydrazone derivative and its absolute configuration was determined by X-ray crystal-structure analysis.

## Discussion & Conclusion

In summary, we have developed an enantioselective one-pot synthesis of cyclopropane-fused tetrahydroquinolines **1** via Ru-catalyzed intramolecular cyclopropanation of electron-deficient alkenes. With optimized reaction conditions, the sequential preparation of the hydrazones and Ru-catalyzed enantioselective intramolecular cyclopropanations were found to yield the desired products **1** in 31–87% yield with 81–95% ee. The absolute configuration of the product was determined by X-ray crystal-structure analysis of the 2,4-dinitrophenyl hydrazone derivative. We are conducting further studies on the applications of the developed one-pot process to complex cyclopropane-fused aza-polycycles, and the results will be presented in due course.

## References

1. Ellis, D.; Kuhen, K. L.; Anaclerio, B.; Wu, B.; Wolff, K.; Yin, H.; Bursulaya, B.; Caldwell, J.; Karanewsky, D.; He, Y. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 4246-4251.
2. (a) Saget, T.; Cramer, N. *Angew. Chem. Int. Ed*. **2012**, *51*, 12842-12845 (b) Zhu, D.; Ma, J.; Luo, K.; Fu, H.; Zhang, L.; Zhu, S. *Angew. Chem. Int. Ed*. **2016**, *55*, 8452-8456 (c) Cao, L.-Y.; Wang, J.-L.; Wang, K.; Wu, J.-B.; Wang, D.-K.; Peng, J.-M.; Bai, J.; Zhuo, C.-X. *J. Am. Chem. Soc*. **2023**, *145*, 2765-2772.
3. Nakagawa, Y.; Chanthamath, S.; Shibatomi, K.; Iwasa, S. *Org. Lett*. **2015**, *17*, 2792-2795.

## 一般の皆様へ

シクロプロパン環は、その特異な反応性や構造的な特徴により、有機合成や医薬化学の中で重要な部分構造です。シクロプロパン環をもつ窒素を含んだ環状化合物は、生物活性天然物や医薬品に多く存在し、その立体構造を制御する合成法の開発が望まれていました。本研究では、簡便な実験操作かつ合成中間体の精製を必要としないワンポット合成にて抗 HIV 活性を示す化合物の骨格構築法を確立しました。本法から得られる生成物は更なる化学変換により様々な誘導体の合成が期待できるため、本研究成果は創薬研究や医薬品の製造に有効であると考えています。

# Publication - FY2022 Grant Recipients

| | |
|---|---|
| Title of the research project | Y Chromosomal Genes Protective against Heart Failure |
| Recipient (Institution) | Soichi Sano (Osaka Metropolitan University, Department of Cardiology, Graduate School of Medicine) |
| Journal article / other material | Nature Cardiovascular Research<br>doi.org/10.1038/s44161-024-00441-z |
| Title of the paper | Disruption of the Uty epigenetic regulator locus in hematopoietic cells phenocopies the profibrotic attributes of Y chromosome loss in heart failure |

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|---|---|
| Title of the research project | Elucidation of Novel Regulatory Mechanism of Glucose Metabolism in Liver Type 2 Innate Lymphocytes by Single Cell Analysis and Spatial Transcriptome Analysis |
| Recipient (Institution) | Tomoaki Tanaka (Chiba University, Department of Molecular Diagnosis) |
| Journal article / other material | Nat Commun . 2022 Sep 15;13(1):5408.<br>doi: 10.1038/s41467-022-33171-6 |
| Title of the paper | Liver group 2 innate lymphoid cells regulate blood glucose levels through IL-13 signaling and suppression of gluconeogenesis |
| Journal article / other material | Commun Biol. 2023; 6: 787.<br>doi: 10.1038/s42003-023-05160-y |
| Title of the paper | Identification of genotype–biochemical phenotype correlations associated with fructose 1,6-bisphosphatase deficiency |

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| Title of the research project | Elucidation of the role of alveolar capillary in alveolar formation for clinical application |
| Recipient (Institution) | Haruko Takano (Nippon Medical School, Department of Molecular Pathophysiology, Institute of Advanced Medical Science) |
| Journal article / other material | Nature communications<br>doi.org/10.1038/s41467-024-45910-y |
| Title of the paper | Endothelial cells regulate alveolar morphogenesis by constructing basement membranes acting as a scaffold for myofibroblasts |

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|---|---|
| Title of the research project | Development of three component reaction for site-selective peptide modification |
| Recipient (Institution) | Kazuya Kanemoto (Tohoku University, Graduate School of Pharmaceutical Sciences) |
| Journal article / other material | Angew. Chem. Int. Ed. 2024,<br>doi.org/10.1002/anie.202320012 |

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|---|---|
| Title of the paper | N-Terminal-Specific Dual Modification of Peptides through Copper-Catalyzed [3+2] Cycloaddition |
| Journal article / other material | J. Am. Chem. Soc. 2024, 146, 3910.3919<br>doi.org/10.1021/jacs.3c11524 |
| Title of the paper | Carboiodanation of Arynes: Organoiodine(III) Compounds as Nucleophilic Organometalloids |
| Journal article / other material | Org. Lett. 2024, 26, 438.443<br>doi.org/10.1021/acs.orglett.3c03419 |
| Title of the paper | Amination of N.(Organodithio)phthalimides for the Modular Synthesis of Aminodisulfides |
| Journal article / other material | Chem. Eur. J. 2024, e202400894<br>doi.org/10.1002/chem.202400894 |
| Title of the paper | Amino- and Alkoxybenziodoxoles: Facile Preparation and Use as Arynophiles |
| Journal article / other material | Orcid.org/0000-0002-3958-7369<br>doi.org/10.26434/chemrxiv-2024-1vp1n |
| Title of the paper | Disulfuration of Azlactones: A Versatile Entry to Unnatural,Disulfide-Linked Amino Acids and Peptides |
| Journal article / other material | Org. Lett. 2024, 26, 1880.1885<br>doi.org/10.1021/acs.orglett.4c00184 |
| Title of the paper | Construction of Diverse Pyrrolidine-Based Skeletons through the Ag-Catalyzed Stereoselective Addition.Elimination Reaction of Azomethine Ylides with Nitroallyl Acetates |
| Journal article / other material | orcid.org/0000-0002-3958-7369<br>doi.org/10.26434/chemrxiv-2024-1917n |
| Title of the paper | Precision Synthesis of Chimeric Peptides through Site-Specific Azomethine Ylide.Dehydroalanine Cycloaddition |

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|---|---|
| Title of the research project | Molecular basis of calcium ion-regulated cellular uptake of nutrients into cancer cells |
| Recipient (Institution) | Hisaaki Hirose (Kyoto University, Institute for Chemical Research) |
| Journal article / other material | Genes Cells. 2024<br>doi: 10.1111/gtc.13118 |
| Title of the paper | ATP2B4 is an essential gene for epidermal growth factor-induced macropinocytosis in A431 cells |

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|---|---|
| Title of the research project | Defective mitophagy in pancreatic beta-cells leads to diabetes |
| Recipient (Institution) | Kyota Aoyagi (Kyorin University School of Medicine, Department of Cellular Biochemistry) |
| Journal article / other material | nature.com/scientificreports/ doi.org/10.1038/s41598-024-56769-w |
| Title of the paper | Imeglimin mitigates mitochondria to restore insulin secretion and suppress apoptosis of pancreatic β.cells from db/db mice |

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|---|---|
| Title of the research project | Interorgan networks of islets with liver, fat, or macrophages to regulate β-cell mass |
| Recipient (Institution) | Jun Shirakawa (Gunma University, Institute for Molecular and Cellular Regulation (IMCR) ) |
| Journal article / other material | Endocrinology, 2023, 164, 1.13 doi.org/10.1210/endocr/bqad095 |
| Title of the paper | Protective Effects of Imeglimin and Metformin Combination Therapy on β-Cells in db/db Male Mice |

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|---|---|
| Title of the research project | The mechanism of early placental development regulated by a virus-derived gene |
| Recipient (Institution) | Hirosuke Shiura (University of Yamanashi, Graduate Faculty of Interdisciplinary Research) |
| Journal article / other material | Frontiers in Cell and Developmental Biology 01 frontiersin.org doi 10.3389/fcell.2023.1273638 |
| Title of the paper | Roles of retrovirus-derived PEG10 and PEG11/RTL1 in mammalian development and evolution and their involvement in human disease |

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| Title of the research project | Elucidation of the disease development caused by disruption of a novel phospholipid transport factor required for mitochondrial homeostasis |
| Recipient (Institution) | Yasuhiro Horibata (Dokkyo Medical University School of Medicine, Department of Biochemistry) |
| Journal article / other material | Archives of Biochemistry and Biophysics 752 (2024) 109871 doi.org/10.1016/j.abb.2023.109871 |
| Title of the paper | The ceramide transport protein CERT is involved in alkylacylglycerol transfer from the ER to the Golgi for the biosynthesis of ether phospholipid |

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|---|---|
| Title of the research project | Development and application of stereoselective synthesis of cyclopropane-fused heteropolycyclic compounds |
| Recipient (Institution) | Tetsuya Tsujihara (Iwate Medical University, Department of Medicinal and Organic Chemistry, School of Pharmacy) |
| Journal article / other material | Org. Lett. 2024, 26, 6502–6506.<br>doi.org/10.1021/acs.orglett.4c02416 |
| Title of the paper | Enantioselective One-Pot Synthesis of Cyclopropane-Fused Tetrahydroquinolines via a Ru-Catalyzed Intramolecular Cyclopropanation |

# ＜論文掲載＞ 2022 年度受賞者

| | |
|---|---|
| 助成タイトル | 心不全の悪化に関連する Y 染色体遺伝子の特定とそのメカニズムの解明 |
| 受賞者 | 佐野 宗一（国立研究開発法国立循環器病研究センター 心血管モザイク研究室） |
| 論文掲載誌・書誌事項 | Nature Cardiovascular Research<br>doi.org/10.1038/s44161-024-00441-z |
| 論文タイトル | Disruption of the Uty epigenetic regulator locus in hematopoietic cells phenocopies the profibrotic attributes of Y chromosome loss in heart failure |

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| 助成タイトル | シングルセル解析と空間的トランスクリプトーム解析から捉える肝臓 2 型自然リンパ球の新たな糖代謝調節機構の解明 |
| 受賞者 | 田中 知明（千葉大学 分子病態解析学講座） |
| 論文掲載誌・書誌事項 | Nat Commun. 2022 Sep 15;13(1):5408.<br>doi: 10.1038/s41467-022-33171-6 |
| 論文タイトル | Liver group 2 innate lymphoid cells regulate blood glucose levels through IL-13 signaling and suppression of gluconeogenesis |
| 論文掲載誌・書誌事項 | Commun Biol. 2023; 6: 787.<br>doi: 10.1038/s42003-023-05160-y |
| 論文タイトル | Identification of genotype-biochemical phenotype correlations associated with fructose 1,6-bisphosphatase deficiency |

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| 助成タイトル | 肺胞形成機構における血管内皮細胞の役割の解明と肺胞再生治療への応用 |
| 受賞者 | 高野 晴子（日本医科大学 先端医学研究所 病態解析学部門） |
| 論文掲載誌・書誌事項 | Nature communications<br>doi.org/10.1038/s41467-024-45910-y |
| 論文タイトル | Endothelial cells regulate alveolar morphogenesis by constructing basement membranes acting as a scaffold for myofibroblasts |

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| 助成タイトル | ペプチドのピンポイント修飾を可能にする三成分連結反応の開発 |
| 受賞者 | 金本 和也（東北大学大学院 薬学研究科） |
| 論文掲載誌・書誌事項 | Angew. Chem. Int. Ed. 2024,<br>doi.org/10.1002/anie.202320012 |

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| 論文タイトル | N-Terminal-Specific Dual Modification of Peptides through Copper-Catalyzed [3+2] Cycloaddition |
| 論文掲載誌・書誌事項 | J. Am. Chem. Soc. 2024, 146, 3910.3919<br>doi.org/10.1021/jacs.3c11524 |
| 論文タイトル | Carboiodanation of Arynes: Organoiodine(III) Compounds as Nucleophilic Organometalloids |
| 論文掲載誌・書誌事項 | Org. Lett. 2024, 26, 438.443<br>doi.org/10.1021/acs.orglett.3c03419 |
| 論文タイトル | Amination of N.(Organodithio)phthalimides for the Modular Synthesis of Aminodisulfides |
| 論文掲載誌・書誌事項 | Chem. Eur. J. 2024, e202400894<br>doi.org/10.1002/chem.202400894 |
| 論文タイトル | Amino- and Alkoxybenziodoxoles: Facile Preparation and Use as Arynophiles |
| 論文掲載誌・書誌事項 | Orcid.org/0000-0002-3958-7369<br>doi.org/10.26434/chemrxiv-2024-1vp1n |
| 論文タイトル | Disulfuration of Azlactones: A Versatile Entry to Unnatural,Disulfide-Linked Amino Acids and Peptides |
| 論文掲載誌・書誌事項 | Org. Lett. 2024, 26, 1880.1885<br>doi.org/10.1021/acs.orglett.4c00184 |
| 論文タイトル | Construction of Diverse Pyrrolidine-Based Skeletons through the Ag-Catalyzed Stereoselective Addition.Elimination Reaction of Azomethine Ylides with Nitroallyl Acetates |
| 論文掲載誌・書誌事項 | orcid.org/0000-0002-3958-7369<br>doi.org/10.26434/chemrxiv-2024-1917n |
| 論文タイトル | Precision Synthesis of Chimeric Peptides through Site-Specific Azomethine Ylide. Dehydroalanine Cycloaddition |

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| 助成タイトル | Ca イオンを基軸とした、がん細胞の栄養取り込み機構の分子基盤 |
| 受賞者 | 広瀬 久昭 ( 京都大学 化学研究所 ) |
| 論文掲載誌・書誌事項 | Genes Cells. 2024<br>doi: 10.1111/gtc.13118 |
| 論文タイトル | ATP2B4 is an essential gene for epidermal growth factor-induced macropinocytosis in A431 cells |

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| 助成タイトル | 膵β細胞におけるマイトファジー不全による糖尿病発症機構 |
| 受賞者 | 青柳 共太（杏林大学 医学部 細胞生化学） |
| 論文掲載誌・書誌事項 | nature.com/scientificreports/<br>doi: 10.1038/s41598-024-56769-w. |
| 論文タイトル | Imeglimin mitigates the accumulation of dysfunctional mitochondria to restore insulin secretion and suppress apoptosis of pancreatic β-cells from db/db mice |

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| タイトル | 膵島と肝臓、脂肪、マクロファージとの相互作用による膵β細胞量調節機構 |
| 受賞者 | 白川 純（群馬大学 生体調節研究所 代謝疾患医科学分野） |
| 論文掲載誌・書誌事項 | Endocrinology, 2023, 164, 1.13<br>doi.org/10.1210/endocr/bqad095 |
| 論文タイトル | Protective Effects of Imeglimin and Metformin Combination Therapy on β-Cells in db/db Male Mice |

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| 助成タイトル | ウイルス由来遺伝子がもたらした哺乳類胎盤初期分化メカニズムの解明 |
| 受賞者 | 志浦 寛相（山梨大学 大学院総合研究部） |
| 論文掲載誌・書誌事項 | Frontiers in Cell and Developmental Biology 01 frontiersin.org<br>doi 10.3389/fcell.2023.1273638 |
| 論文タイトル | Roles of retrovirus-derived PEG10 and PEG11/RTL1 in mammalian development and evolution and their involvement in human disease |

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| 助成タイトル | ミトコンドリア恒常性に必要な新規リン脂質輸送因子の破綻による疾患発症機構の解明 |
| 受賞者 | 堀端 康博（獨協医科大学 医学部生化学講座） |
| 論文掲載誌・書誌事項 | Archives of Biochemistry and Biophysics 752 (2024) 109871<br>doi.org/10.1016/j.abb.2023.109871 |
| 論文タイトル | The ceramide transport protein CERT is involved in alkylacylglycerol transfer from the ER to the Golgi for the biosynthesis of ether phospholipid |

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| 助成タイトル | シクロプロパンが縮環した複素多環式化合物の立体選択的合成法の開発と応用 |
| 受賞者 | 辻原 哲也（岩手医科大学 薬学部 薬科学講座 創薬有機化学分野） |
| 論文掲載誌・書誌事項 | Org. Lett. 2024, 26, 6502-6506.<br>doi.org/10.1021/acs.orglett.4c02416 |
| 論文タイトル | Enantioselective One-Pot Synthesis of Cyclopropane-Fused Tetrahydroquinolines via a Ru-Catalyzed Intramolecular Cyclopropanation |

# Ⅲ.

# Reports from the Recipients of Grants for International Meetings

# Report on Research Meeting

2024/1

1. Name of Research Meeting / Conference
   Asia Pacific Drosophila Neurobiology Conference (APDNC3)

2. Representative
   Adrian MOORE, RIKEN CBS, Chair

3. Opening period and Place
   RIKEN Wako Campus, 2-1 Hirosawa, Wako, Saitama
   2/27/2024–3/1/2024

4. Number of participants
   218

5. Number of participating countries and areas
   154 countries

6. Total cost
   9,394,918 yen

7. Main use of subsidy
   Conference management fees
   Invited Speaker Accommodation

8. Result and Impression

How the brain, which underlies all behavioral and mental activities, is constructed and functions is one of the most mysterious mysteries in the natural sciences. With the development of various molecular biological, genetic, and imaging techniques, the model animal Drosophila melanogaster has provided profound insights into this universal question. Furthermore, in recent years, a series of innovative technologies have been reported, such as the elucidation of the wiring pattern (connectome) between all cells in the brain by electron microscopic image reconstruction and the development of a method to comprehensively label upstream and downstream cells connected by synapses, and we should consider future research prospects utilizing these technologies. We are now at a turning point where we should consider future research prospects utilizing these technologies. Therefore, this international conference aimed to contribute to the elucidation of the connectome and to provide a timely opportunity to discuss the forefront and future of the field by bringing together scientists from various countries in Japan, particularly RIKEN, one of the world's leading centers in the field of neural circuit formation and functional analysis.

The symposium had three major significance. One is to encourage further expansion of the Asia-Pacific research community. Led by Seymour Benzer of the California Institute of Technology, who is considered the father of Drosophila neurobehavioral genetics,

universities and research institutes in the United States and Europe have been leading neuroscience research using Drosophila. On the other hand, researchers from Asian countries such as Japan and Taiwan have contributed greatly to the elucidation of the wiring patterns (connectome) between cells in the brain at meso and micro scales, which has revolutionized research in recent years.

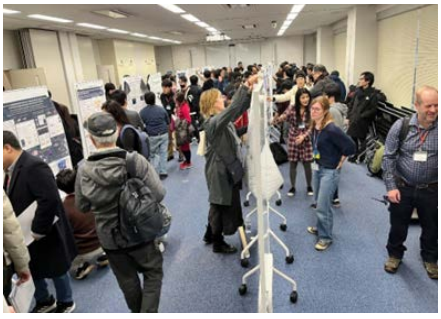
The conference aimed to provide a valuable opportunity for these Asia-Pacific researchers to interact more closely than ever before and to sow the seeds for new collaborations. We were pleased to have successfully organized a wide variety of events, including 13 sessions, 2 plenary talks, 2 poster sessions, 3 career-building seminars, a welcome reception, a reception party, and more.

The second was to give students and young researchers a vivacious experience. By providing young researchers who have been forced to interact online due to the Covid Disaster with an opportunity to present in person in front of a large audience (the main hall of the conference was set up as one single session so that all participants could hear all the talks), meet the world's top scientists, and engage in a great exchange of ideas, The conference was designed to stimulate the next generation of leaders in the field.

Sixty-nine students from Japan and abroad participated, with 46 invited speakers and chairpersons, and were seen interacting greatly with each other at the various events mentioned above. Through the poster awards, we hoped to recognize the students' research and presentation efforts and to motivate them for future research. In addition, three Career Development Lectures were held with researchers, former journal editors, and science editors as lecturers for the purpose of fostering young researchers, and were a great success. The third was to promote the development of an inclusive community and spirit in various aspects, including country and gender. In the end, we invited speakers and chairpersons from 11 countries and regions, with 40% of the speakers being women, a lineup that we believe contributed to the creation of a more balanced community.

9. Additional description

The conference was made possible by grants from seven foundations and organizations and donations and sponsorships from 16 companies and organizations. We, the organizers, would like to express our sincere gratitude to all of them.



# Report on Research Meeting

Date: Oct. 19, 2023

1. Name of Research Meeting / Conference
   International Conference on DNA Computing and Molecular Programming (DNA29)

2. Representative
   Satoshi Murata (Local Organizing Committee Chair of DNA29)

3. Opening period and Place
   Sept. 11-15, 2023,  Katahira Sakura Hall, Tohoku University

4. Number of participants
   181

5. Number of participating countries and areas
   30 Countries

6. Total cost
   11,291,790 JPY

7. Main use of subsidy
   Keynote speaker invitation (Travel/Accommodation cost)
   Venue rental fee

8. Result and Impression

The International Conference on DNA Computing and Molecular Programming has been held annually around the world since 1995 under the International Society for Nanoscale Science, Computation, and Engineering (ISNSCE). The DNA Conference covers a wide range of fields including mathematics, computer science, physics, chemistry, biology, and nanotechnology. During the conference, a variety of presentations will be made on the development of techniques for the analysis, design, and synthesis of informatics-based molecular systems.

The 29th DNA was held for five days from September 11 to 15, 2023, at Sakura Hall in Katahira Campus, Tohoku University.

The main contents of DNA29 are as follows.

Key Note Talks (6)
Tulip Prize Lecture (1)
Oral Session (28 presentations)
Poster Session (121 presentations)
Special Poster Session "Molecular Cybernetics"
Special Session "When Scientists meet Headlines"

In addition to the above,

Conference excursion (Matsushima, Zuiganji Temple)
Conference banquet (including award ceremony)
Technical Tour
(visiting next-generation synchrotron radiation facility "Nanoterasu" at Tohoku University)

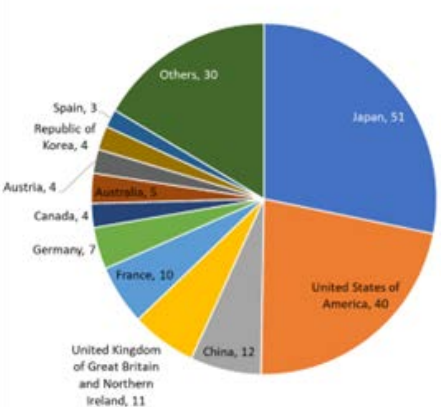
took place.

The community engaged in lively discussions about cutting-edge research in this field and deepened its friendship through several social events.

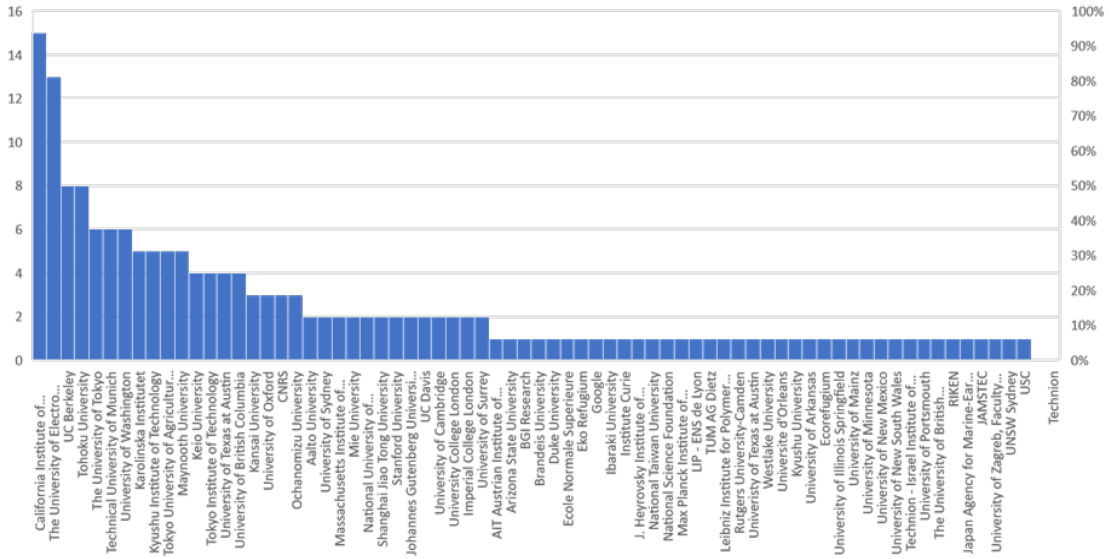
The total number of registered participants (including invited speakers and committee members) was 181.

Many participants have commented that the DNA Conference was a great success.

8. Additional description




Participants by Country




Participants by Institution

# Report on Research Meeting

Date: December 12, 2023

1. Name of Research Meeting / Conference
   "Molecular Movies "International Symposium 2023：Molecular Movies; to be continued

2. Representative
   Minoru Kubo, Department of Life Science, Graduate School of Science,
   University of Hyogo

3. Opening period and Place
   November 30th (Thu) and December 1st (Fri), 2023
   Hybrid (Awaji Yumebutai International Conference Center + Zoom)

4. Number of participants
   137

5. Number of participating countries and areas
   8 (Switzerland, China, England, Romania, Germany, Belgium, France, and Japan)

5. Total cost
   ¥3,684,928

6. Main use of subsidy
   Venue fee: ¥196,800
   Staff fee: ¥93,632
   Equipment fee: ¥101,750
   Service fee: ¥7,818
   (Total: ¥400,000)

7. Result and Impression

We held "Molecular Movies" international symposium 2023. On November 30, we first had the oral session given by 9 speakers from 6 countries. The first 3 speakers were focused on protein chemistry, including chemical modification of proteins. One of the topics included a very hot result (molecular movie observing photo-repair of damaged DNA) that was published in *Science* just on December 1st. The next 3 speakers talked about the latest computational studies on protein structural dynamics, which convinced us that combining molecular movies with computer simulations was a quite powerful option. The final 3 speakers presented molecular movies using state of the art techniques. Two advanced "mixing" techniques enabled us to make movies tracking catalytic reactions of enzymes as a function of time, which has been long-awaited in structural biology. Another technique in the presentation was related to controlling of photo-illumination of a protein, which was used to make a molecular movie capturing an initial event of vision. That movie, reported in *Nature* this year, was breathtaking. After the oral session, we had the

poster session. There were 46 poster presentations, where the scene of active discussion between graduate students and senior researchers were particularly impressive. On December 1, we had the short talk session. There were 32 talks, which were categorized into 4 groups and went on in parallel using 4 rooms. The talks covered structural biology, chemical biology, molecular movie platform design, spectroscopy, and computational chemistry. Finally, we gathered, took a group photo, and closed this symposium. After the symposium, we recapped this international symposium and our molecular movie project with the evaluation committee. By this symposium, we made sure that the molecular movie techniques have been now well established and some technique developed by us has spread to overseas X-ray facilities, contributing to the establishment of “Dynamic Structural Biology”. Through the symposium, we created strong, international bonds between researchers from a wide range of fields that are directly or indirectly associated with molecular movies. We highly appreciate the support from The NOVARTIS Foundation (Japan) for the Promotion of Science.

# Report on Research Meeting

Date:2023.11.2

1. Name of Research Meeting / Conference
   the 10th International MDM2 Workshop

2. Representative
   Rieko Ohki

3. Opening period and Place
   October 15-18, 2023, the auditorium of the National Cancer Center Research Institute (NCCRI) in Tokyo, Japan

4. Number of participants
   160

5. Number of participating countries and areas
   60 (Japanese), USA (59), UK (3), Singapore (10), Germany (5), Belgium (2), Israel (1), Austria (3), France (3), Sweden (5), China (5), Korea (5).

6. Total cost
   10,690,000 yen

7. Main use of subsidy
   inviting overseas researchers
   Domestic transportation
   Accommodation
   facilities and equipment
   Printing

8. Result and Impression

The International MDM2 Workshop is a gathering of prominent national and international cancer research leaders and young investigators. In addition to Dr. David Lane and Dr. Arnold Levine, who discovered p53, about 20 world-renowned researchers have participated from overseas, including Dr. Carol Prives, a member of the prestigious National Academy of Sciences, Dr. Guillermina Lozano, and Dr. Binh Vu, who developed the first MDM2 inhibitor.

One of the features of this workshop is the "MDM2 Special Symposium, Celebrating the Discovery of MDM2," which was held on the first day of the workshop. The symposium invited the researchers who discovered MDM2 and the researchers who first developed MDM2 inhibitors to discuss historical MDM2-related discoveries and future directions of MDM2 research. The symposium provided a fascinating opportunity to hear directly from the actual discoverers of this historic breakthrough in cancer research. To

broaden the scope of the conference, a number of sessions on p53, the primary target of MDM2, were included. Furthermore, on the second day, a special session on "MDM2/p53 and Cancer Immunity" invited prominent experts in cancer immunology from Japan and abroad to discuss the integration of cancer immunotherapy, which has been remarkably advancing in recent years, with therapies targeting the p53/MDM2 pathway, aiming to spark a new movement in p53 and MDM2 research.

In addition to basic biological research, the workshop discussed clinical application research leading to the development of novel cancer-targeted therapies for p53 and MDM2, as well as cancer immunity. It is expected that interactions among researchers of different generations, including young researchers from around the world, and the resulting international collaborations will continue to develop. The MDM2 workshop, held for the first time in Asia, was of great significance for Japanese researchers, demonstrating their presence and leadership in this rapidly evolving field.

9. Additional description

The MDM2 International Workshop served as a platform to assess the current landscape of cancer research, specifically emphasizing the realms of p53 and MDM2 research on a global scale. Its primary objective was to establish a groundwork for the advancement of research among younger generations of scientists, fostering intergenerational dialogue. While the fields of p53 and MDM2 research have gained international recognition, it is regrettable that Japan had not previously hosted a major international conference on these subjects.

With the hosting of the MDM2 workshop in Japan, this landmark event is anticipated to substantially contribute to the growth of Japanese researchers, enabling them to actively participate in and lead the global advancements within this burgeoning field.


# Report on Research Meeting

Date: 4 July 2023

1. Name of Research Meeting / Conference
   International Symposium on Skin Stem Cell Dynamics

2. Representative
   Hironobu Fujiwara (RIKEN Center for Biosystems Dynamics Research)

3. Opening period and Place
   14 – 15 May 2023, University of Tokyo

4. Number of participants /
   105 people (72 Japanese, 33 Foreigners)

5. Number of participating countries and areas
   11 countries and areas (Japan, Singapore, China, Taiwan, South Korea, USA, Australia, Germany, Spain, UK, Denmark)

6. Total cost
   ¥ 8,369,001-

7. Main use of subsidy
   Travel expenses of the invited speakers

8. Result and Impression

On the morning of the first day of the Symposium, spring raindrops cleanse the air, leaving it fresh and invigorating. The 1.5-day Symposium began with an opening remarks by Hironobu Fujiwara, the organizer from RIKEN. The event boasted a total of 105 participants, including 33 individuals from overseas. The programme featured an array of stimulating sessions, as described below:

- Keynote Lecture
- Stem Cell Dynamics in Space and Time
- Stem Cell Microenvironment
- Regeneration and Ageing
- Modelling Development and Diseases
- Poster Session
- Meet the Expert (networking dinner)
- Sponsored luncheon and evening seminars

During the sessions, nine notable invited speakers and seven short talk speakers, all eminent international researchers, gave insightful presentations on the dynamic properties of skin stem cells in development, homeostasis and regeneration. They

also delved into the molecular and cellular disfunctions of stem cells in developmental abnormalities, ageing and cancer. Remarkably, many speakers generously shared unpublished data, provoking vibrant discussions and stimulating participants. Of particular note was Japan's outstanding contribution to skin stem cell research, which has nurtured numerous talented young researchers, including women, who are poised to lead the field on an international level. Consequently, this Symposium served as a timely platform for laying the foundations for future decades of international collaboration. Most of the invited speakers were in their 30s and 40s and the gender ratio was balanced with 6 male speakers and 5 female speakers.

We extend our heartfelt gratitude to the NOVARTIS Foundation (Japan) for the Promotion of Science for their generous support in promoting the symposium.

<Invited speakers>
Kim Jensen (U Copenhagen, Denmark)
Kyogo Kawaguchi (RIKEN, Japan)
Emi Nishimura (U Tokyo, Japan)
Maksim Plikus (UC Irvine, USA)
Fumiko Toyoshima (Kyoto U, Japan)
Masakazu Kurita (U Tokyo, Japan)
Aiko Sada (Kumamoto U, Japan)
Karl Koehler (Harvard U, USA)
Ting Chen (NIBS, China)

<Organizers>
Hironobu Fujiwara (RIKEN, Japan)
Emi Nishimura (U Tokyo, Japan)
Aiko Sada (Kumamoto U, Japan)

# Report on Research Meeting

Date: October 2, 2023

1. Name of Research Meeting / Conference
   2nd Asian Palaeontological Congress

2. Representative
   Professor Tamaki Sato (The Kanagawa University)
   Professor Kazuyoshi Endo (The University of Tokyo)

3. Opening period and Place
   August 3–7, 2023 (field excursions were also held before and after this period)

4. Number of participants /
   441 (including 8 online participants)

5. Number of participating countries and areas
   26

6. Total cost
   ¥16,179,460

7. Main use of subsidy
   To set hybrid system in the conference.

8. Result and Impression

The conference was successfully held at the University of Tokyo as the main venue. We used partially hybrid (onsite and online) system during this conference. The program of the conference is shown below.

August 3. PM: Registration and Ice breaker (at Otemachi Sankei Plaza)
August 4. AM: Opening ceremony, plenary session, poster session; PM: Oral sessions (at the University of Tokyo, UT)
August 5. AM: Plenary session, poster session; PM: Oral session (UT)
August 6. AM: Plenary session (online), online poster session, and online oral session; PM: Oral session (UT)
August 7. AM: Plenary session, poster session; PM: Oral session, general assembly, closing ceremony (UT)

In addition to four plenary sessions, 21 scientific sessions and one open session were proposed for the conference. The number of presentations exceeded our initial expectations, so we tried to increase the conference room and asked some participants to switch to poster presentations. Total number of presentations is 332 (187 oral and 145 poster presentations, excluding those cancelled).

Fruitful discussions, exchange of ideas could be made throughout the conference, which made it successful.

The number of online participants was not as large as we expected, probably due to the improved condition of COVID-19 in Japan and other Asian countries. However, the presence of the online system was essential for us, especially for those who could not attend in person.

Before and after these scientific sessions, we also conducted four field trips shown below:

Jul 31–Aug 3: Chubu Area (Triassic–Jurassic succession in Inuyama, Aichi)
Aug 8–11: Hokuriku Area (Upper Mesozoic Tetori Group)
Hokkaido Area A (Middle–Upper Mesozoic Yezo Group)
Aug 8–10: Hokkaido Area B (Museums, culture and nature in Hokkaido)

9. Additional description

One of the main goals of this conference was to encourage young scientists to communicate with foreign researchers. After four years of lack of on-site communication under the influence of COVID-19, this conference could provide a great opportunity not only for young scientists but also for other participants to communicate with each other.

We are sure that this opportunity could lead to future collaborations with scientists from other countries.

During this conference we tried to set up a nursery to help those who need to take care of their child/children.


1. Group photo at the main venue

2. Discussion during the session

3. Scene at the General Assembly

# 37th Grant Report (FY2023)

The foundation has been conducting public interest activities such as research grant, meeting grant and international exchange programs since its establishment on Sep. 4, 1987 in Japan under authorization of the Ministry of Education, Science, Sports and Culture, followed by a transition to a public interest incorporated foundation on Apr. 1, 2012. The grants conducted in FY 2023 are as follows.

| | | |
|---|---|---|
| 37th Novartis Research Grant: | 41 Researchers (JPY 1.0 mil.), | Subtotal JPY 41.0mil. |
| Research Meeting Grant: | 5 Meetings (JPY 0.4 mil.), | Subtotal JPY 2.0mil. |
| | | Total JPY 43.0mil. |

## 37th Novartis Research Grant (FY2023)

The Grant is to aim supporting creative research in Japan in the field of Bio, life science and relevant chemistry

| # | Name | Institution | Title | Research Project |
|---|---|---|---|---|
| 1 | Kotaro Tsuboyama | Institute of Industrial Science, Tsuboyama Lab, The University of Tokyo | Lecturer | Development of rational design method for de novo proteins that are efficiently delivered into cytoplasm |
| 2 | Masanori Nagatomo | Graduate School of Pharmaceutical Sciences, The Inoue Research Group, The University of Tokyo | Lecturer | Total synthesis of the neurotoxic saxitoxin and development of new functional molecules |
| 3 | Kunihiko Morihiro | Graduate School of Engineering, The University of Tokyo | Research Associate | Development of epigenetics-related proteolytic drugs |
| 4 | Shigeki Kiyonaka | Department of Biomolecular Engineering, Graduate School of Engineering, Nagoya University | Professor | Fluorescent visualization of glutamate receptors with ultra-high resolution |
| 5 | Chao Wang | Synthetic and Medicinal Element Chemistry Laboratory, Kanazawa University | Associate Professor | Development of synthetic transformations based on installing group 13, 14 functional moieties |
| 6 | Rie Kusakabe | Faculty of Chemistry, Materials and Bioengineering, Kansai University | Associate Professor | Functional Analysis of Mesenchymal Stromal Cells in Musculoskeletal Systems |
| 7 | Shuji Wakatsuki | National Institute of Neuroscience, Department of Peripheral Nervous System Research, National Center of Neurology and Psychiatry | Section Chief | Neurophysiological significance of regulation of molecular function by glutathionylation |
| 8 | Nami Haruta | Graduate School of Life Sciences, Tohoku University | Assistant Professor | Molecular mechanism of programed DNA elimination |
| 9 | Yoshikane Kikushige | Center for Cellular and Molecular Medicine, Kyushu University | Lecturer | Elucidation of the human leukemia stem cell-specific nitrogen metabolism pathway |
| 10 | Hideki Hara | Department of Infectious Diseases, Division of Microbiology and Immunochemistry, Asahikawa Medical University | Professor | Elucidating the inflammatory response that aggravates infections of antimicrobial-resistant Staphylococcus aureus |

| # | Name | Institution | Title | Research Project |
|---|---|---|---|---|
| 11 | Nono Tomita | Graduate School of Frontier Sciences, The University of Tokyo | Associate Professor | Mechanism of action of antibiotics targeting the mammalian mitochondrial protein synthesis system |
| 12 | Shohei Takase | Division of Cancer Therapeutics, National Cancer Center Research Institute | Researcher | Establishment of simultaneous paralogous inhibition toward the SMAD4-deficient pancreatic cancer therapy |
| 13 | Sayuri Motani | Institute for advanced studies, Institute for Integrated Cell-Material Sciences, Suzuki group, Kyoto University | Assistant professor | Identification of osteoclast cell fusion factors by expression cloning method |
| 14 | Takayuki Hoshii | Department of Molecular Oncology, Graduate School of Medicine, Chiba University | Senior Lecturer | Identification of transcriptional pause release regulators in leukemia progression |
| 15 | Daichi Inoue | Institute of Biomedical Research and Innovation, Foundation for Biomedical Research and Innovation at Kobe | Professor | Elucidate the roles of SETBP1 mutations in hematopoietic malignancies and congenital Schinzel-Giedion syndrome. |
| 16 | Kyohei Arita | Graduate School of Medical Life Sicence, Structural Biology Laboratory, Yokohama City University | Professor | Structural basis for proteostasis in neuronal cells |
| 17 | Yasunori Toda | Faculty of Engineering, Shinshu University | Associate Professor | Synthesis of Aryl Phosphonates by Green Photocatalysis |
| 18 | Keisuke Nishikawa | Synthetic Organic Laboratory, Department of Chemistry, Graduate School of Science, Osaka Metropolitan University | Lecturer | Creating the Seed Compounds of Small Molecule Drug Discovery for Diabetic Nephropathy Through Establishment of a Unified Synthetic Method of New Skeletal Sesquiterpenoids |
| 19 | Hajime Fukui | Division of Biomechanics and Signaling, Institute of Advanced Medical Sciences, Tokushima University | Associate Professor | Development of the advanced biomechanical approach for the heart morphogenesis |
| 20 | Yujiro Naito | Department of Respiratory Medicine and Clinical Immunology, Graduate School of Medicine, Osaka University | Assistant professor | Exploring the mechanisms of immune semaphorin expression in the tumor microenvironment and its effects on immune cells |
| 21 | Akira Shinohara | Institute for Protein Research, Osaka University | Professor | Mechanism of genome stability in the primordial germ cell |
| 22 | Keiji Mori | Department of applied chemistry, Mori laboratory, Tokyo University of Agriculture and Technology | Associate Professor | Divergent synthesis of nitrogen-containing polyaromatics based on imine-anion mediated ketimine synthesis |
| 23 | Yasuhiro Oishi | Laboratory for Haptic Perception and Cognitive Physiology, RIKEN Center for Brain Science | Researcher | Validation of a recurrent input hypothesis of perception using a mouse model |
| 24 | Masato Yano | Neurobiology and Anatomy, Niigata University | Associate Professor | s of miR125-b involved in common molecular pathways of neurodegenerative diseases |
| 25 | Masaru Enomoto | Laboratory of Applied Bioorganic Chemistry, Tohoku University | Associate Professor | Synthetic Study of Naturally Occurring Cancer Invasion Inhibitors Bearing a Novel Heterocyclic Ring Motif and Their Structurally Simplified Analogues. |

| # | Name | Institution | Title | Research Project |
|---|---|---|---|---|
| 26 | Ryodai Yamamura | Institute for Genetic Medicine, Hokkaido University | Assistant Professor | Developing a novel therapeutic strategy for PDAC utilizing gut microbial metabolites |
| 27 | Joji Nakayama | Cancer Research Institute, Kanazawa University | Assistant Professor | Development of therapeutic strategies targeting HSD11β1 in metastasis of triple-negative breast cancer cells |
| 28 | Takairo Soeta | Graduate School of Natural Science and Technology, Kanazawa University | Associate Professor | Development of regio-and stereoselective functionalization of polyols utilizing organocatalysts |
| 29 | Masahito Inagaki | Bioorganic Chemistry Laboratory, Nagoya University | Project Assistant Professor | Elucidation of Effects of Messenger RNA 5'-Cap Structure Diversity on Protein Translation Activity |
| 30 | Hiroki Kobayashi | Devision of Nephrology, Hypertension, and Endocrinology, Department of Internal Medicine, Nihon University School of Medicine | Assistant Professor | Elucidation of the fibrosis mechanism spanning multiple organs focusing on the novel BMP antagonist |
| 31 | Atsushi Kuhara | Molecular and cellular regulation, Konan University, Graduate school of Natural Science | Professor | Opposite regulation by RNA splicesome factors for high and low temperature tolerance across species |
| 32 | Masaharu Hazawa | Institute for Frontier Science Initiative, Cell-Bionomics Research Unit, Kanazawa University | Associate Professor | Understanding and Control of Nuclear Transport Timer |
| 33 | Keisuke Yaku | Department of Molecular and Medical Pharmacology, University of Toyama | Assistant Professor | Elucidation of the pathogenesis of NASH via adenosine synthesis by BST1 |
| 34 | Ryo Shinnakasu | Advanced Research Support Center, Ehime University | Associate Professor | Establishment of a method for high-function neutralizing antibodies induction against SFTS viruses by strategic activation of germinal center |
| 35 | Miwa Tanaka | Project for Cancer Epigenomics, The Cancer Institute, Japanese Foundation for Cancer Research | Senior Staff Scientist | Application of epigenome editing to advance the targeted therapy for sarcoma |
| 36 | Masayo Kagami | Clinical Endocrine Research Division, Department of Molecular Endocrinology, National Research Institute for Child Health and Development | Chief | Elucidation of mechanism for the risk of the development of imprinting disorders caused by epimutation in assisted reproductive technology using targeted long read sequencing |
| 37 | Seiji Watanabe | Department of Neuroscience and Pathobiology, Research Institute of Environmental Medicine, Nagoya University | Lecturer | Identification of molecular biomarkers representing alternations at the endoplasmic reticulum-mitochondria contacting sites |
| 38 | Hiroyoshi Matsumura | College of Life Sciences, Ritsumeikan University | Professor | Structural and functional analysis of a bacterial divisome using a small synthetic binding protein |
| 39 | Hiromitsu Asashima | Department of Rheumatology, Institute of Medicine, University of Tsukuba | Senior Lecturer | The pathogenesis of Sjogren syndrome focused on T-B cell interactions on inflamed tissues |
| 40 | Kentaro Suzuki | Graduate Faculty of Interdisciplinary Research, Faculty of Life and Environmental Sciences, University of Yamanashi | Professor | Mechanism of sexual differences of pain driven by sex hormone-dependent regulators |
| 41 | Takahiro Chihara | School of Science, Hiroshima University | Professor | Mechanisms of tumor microenvironment formation by normal cells under mechanical stress |

# FY2023 Research Meeting Grant

(JPY 400 thousand x5 = 2.0 million)

| No. | Name | Institution | Title | Place/Date | Meeting |
|---|---|---|---|---|---|
| 1 | Hitoshi Okamoto | Laboratory for Neural Circuit Dynamics of Decision Making, RIKEN Center for Brain Science | Team Leader | Kyoto/ 2024.8.17-21 | 18th International Zebrafish Conference (IZFC2024) |
| 2 | Tsutomu Katayama | Faculty of Pharmaceutical Sciences, Department of Molecular Biology, Kyushu University | Professor | Fukuoka/ 2024.11.18-22 | The 12th 3R (DNA replication, repair, recombination) +3C (chromatin, chromosome, cell cycle) International Symposium |
| 3 | Masafumi Ihara | Neurology, National Cerebral and Cardiovascular Center | Director | Kyoto/ 2024.7.12 | The 4th International CADASIL and VCI Symposium |
| 4 | Kimikazu Matsumoto | Children's Cancer Center, National Center for Child Health and Development | Director | Yokohama/ 2024.6.22-25 | 16th Congress of Asia continental branch of International Society of Paediatric Oncology (SIOP Asia 2024) |
| 5 | Yasushi Okada | Department of Molecular Cell Biology, University of Tokyo, Graduate School of Medicine | Professor | Tsukuba/ 2024.7.17-19 | The 76th Annual Meeting of the Japan Society for Cell Biology |

# 第37期（2023年度）助成事業報告

当財団は、文部大臣の認可を得て1987年9月4日に設立されて以来、研究助成を中心とした公益事業を行って来ました。2012年4月1日には、制度改革に伴い、公益財団法人へ移行しております。2023年度は、下記の総額4,100万円の助成事業を実施しました。

| | | |
|---|---|---|
| 第37回ノバルティス研究奨励金 | 41件（1件100万円） | 4,100万円 |
| 研究集会助成 | 5件（1件 40万円） | 200万円 |
| | | 総額 4,300万円 |

## 第37回ノバルティス研究奨励金（2023年度）

この事業は、生物・生命科学および関連する化学の領域において、我が国で行われる創造的な研究の助成を目的としています。

（受付順、敬称略、所属職位は申請時、贈呈額：1件 100万円）

| No. | 氏　名 | 所　属 | 職位 | 研究課題 |
|---|---|---|---|---|
| 1 | 坪山幸太郎 | 東京大学 生産技術研究所<br>坪山研究室 | 講師 | 細胞質へと効率よく移行する人工タンパク質の合理的設計法の開発 |
| 2 | 長友　優典 | 東京大学 大学院薬学系研究科<br>天然物合成化学教室 | 講師 | 神経毒サキシトキシンの全合成および新機能分子の創製 |
| 3 | 森廣　邦彦 | 東京大学<br>大学院工学系研究科 | 助教 | エピジェネティクス関連タンパク質分解医薬の開発 |
| 4 | 清中　茂樹 | 名古屋大学 大学院工学研究科<br>生命分子工学専攻 | 教授 | グルタミン酸受容体の「超・高解像」可視化および動態解析法の開発 |
| 5 | 王　　超 | 金沢大学 医薬保健研究域<br>薬学系 元素創薬合成化学研究室 | 准教授 | 13、14 族官能基の導入に基づく分子変換反応の開発 |
| 6 | 日下部りえ | 関西大学 化学生命工学部<br>生命生物工学科 | 准教授 | 運動器の形成・維持・老化における間葉系間質細胞の機能 |
| 7 | 若月　修二 | 国立精神・神経医療研究センター<br>神経研究所 疾病研究第五部 | 研究室長 | グルタチオン化による分子機能調節の神経生理学的意義 |
| 8 | 春田　奈美 | 東北大学大学院<br>生命科学研究科 | 助教 | プログラムされた染色体削減機構の解明 |
| 9 | 菊繁　吉謙 | 九州大学病院<br>遺伝子細胞療法部 | 講師 | ヒト白血病幹細胞特異的窒素代謝メカニズムの解明 |
| 10 | 原　　英樹 | 国立大学法人旭川医科大学<br>感染症学講座微生物学分野 | 教授 | 薬剤耐性黄色ブドウ球菌の感染病態を重症化させる炎症応答の解明 |
| 11 | 富田　野乃 | 東京大学<br>大学院新領域創成科学研究科 | 准教授 | 哺乳類ミトコンドリアのタンパク質合成系を標的とした抗生物質の作用機序 |
| 12 | 髙瀬　翔平 | 国立がん研究センター<br>がん治療学研究分野 | 研究員 | がん抑制遺伝子 SMAD4 欠損型膵臓がん治療を指向したパラログ同時阻害法の確立 |
| 13 | 茂谷小百合 | 京都大学 高等研究院<br>物質 - 細胞統合システム拠点<br>鈴木研究室 | 助教 | 発現クローニング法を基にした破骨細胞融合因子の同定 |
| 14 | 星居　孝之 | 千葉大学 大学院医学研究院<br>分子腫瘍学 | 講師 | 白血病進展における転写休止解除制御因子群の同定 |

| No. | 氏　名 | 所　属 | 職位 | 研究課題 |
|---|---|---|---|---|
| 15 | 井上　大地 | 公益財団法人神戸医療産業都市推進機構 先端医療研究センター | 部長 | 造血器腫瘍および先天性 Schinzel-Giedion 症候群における SETBP1 変異の機能解析 |
| 16 | 有田　恭平 | 横浜市立大学 生命医科学研究科 構造生物学研究室 | 教授 | 神経細胞のプロテオスタシスの構造基盤の解明 |
| 17 | 戸田　泰徳 | 信州大学 工学部 | 准教授 | グリーン光触媒を用いた芳香族ホスホン酸エステルの合成 |
| 18 | 西川　慶祐 | 大阪公立大学 大学院理学研究科 化学専攻 合成有機化学研究室 | 講師 | 新骨格セスキテルペノイド天然物群の網羅的合成法を経た、糖尿病性腎症のための「低分子創薬シード分子」創生 |
| 19 | 福井　　一 | 徳島大学 先端酵素学研究所 生体力学シグナル分野 | 准教授 | 心臓管腔形成の理解に向けた新たな力操作法の開発 |
| 20 | 内藤祐二朗 | 大阪大学 大学院医学系研究科 呼吸器免疫内科学 | 助教 | がん微小環境が免疫セマフォリンを発現する機序と免疫細胞に与える影響の解明 |
| 21 | 篠原　　彰 | 大阪大学 蛋白質研究所 | 教授 | 始原生殖細胞のゲノム安定化のメカニズム |
| 22 | 森　　啓二 | 東京農工大学 工学部応用化学科 森研究室 | 准教授 | イミンアニオン型 Smiles 転位によるケチミン合成を基軸とする含窒素多環芳香族化合物の骨格多様化合成 |
| 23 | 大石　康博 | 国立研究開発法人理化学研究所 脳神経科学研究センター 触知覚生理研究チーム | 研究員 | マウスモデルを用いた知覚の反響回路仮説の検証 |
| 24 | 矢野　真人 | 新潟大学 医学部神経解剖学 | 准教授 | 神経変性疾患の分子病態経路に普遍的に関わるマイクロ RNA の解析 |
| 25 | 榎本　　賢 | 東北大学 大学院農学研究科 農芸化学専攻 生物有機化学分野 | 准教授 | 新奇な複素環構造を含む天然由来がん細胞浸潤阻害物質の合成と構造単純化アナログの合成 |
| 26 | 山村　凌大 | 北海道大学 遺伝子病制御研究所 | 助教 | 腸内細菌由来成分を活用した新規膵がん治療法の創出 |
| 27 | 中山　浄二 | 金沢大学 がん進展制御研究所 | 特任助教 | HSD11 β 1 を標的とした転移性トリプルネガティブ乳がんの治療法の開発 |
| 28 | 添田　貴宏 | 金沢大学 理工研究域 物質化学系 | 准教授 | 有機分子触媒を活用するポリオールの位置及び立体選択的官能基化反応の開発 |
| 29 | 稲垣　雅仁 | 名古屋大学 生物有機化学研究室 | 特任助教 | メッセンジャー RNA の 5’キャップ構造多様性が与えるタンパク質翻訳活性への影響解明 |
| 30 | 小林　洋輝 | 日本大学 内科学系腎臓高血圧内分泌内科学分野 | 助教 | 新規 BMP 拮抗分子に着目した複数臓器にまたがる線維化機構の解明 |
| 31 | 久原　　篤 | 甲南大学大学院自然科学研究科 生体調節学研究室 | 教授 | 種間を超越した高温・低温耐性に関わる RNA splicesome 因子よる相反制御 |
| 32 | 羽澤　勝治 | 金沢大学 新学術創成研究機構・セルバイオノミクスユニット | 准教授 | 核輸送タイマーの理解と制御 |
| 33 | 夜久　圭介 | 富山大学 学術研究部医学系 分子医科薬理学講座 | 助教 | BST1 によるアデノシン合成を介した NASH 発症制御機構の解明 |
| 34 | 新中須　亮 | 愛媛大学 学術支援センター | 准教授 | 胚中心の戦略的活性化による抗 SFTS ウイルス高機能中和抗体誘導法の確立 |
| 35 | 田中　美和 | 公益財団法人がん研究会 がん研究所 がんエピゲノムプロジェクト | 主任研究員 | 骨軟部肉腫の病態克服に向けたエピゲノム編集技術の確立 |

| No. | 氏　名 | 所　属 | 職位 | 研究課題 |
|---|---|---|---|---|
| 36 | 鏡　雅代 | 国立成育医療研究センター<br>分子内分泌研究部臨床内分泌研究室 | 室長 | Targeted long read sequencing を用いた生殖補助医療のインプリンティング疾患エピ変異発症リスクの 機序の解明 |
| 37 | 渡邊　征爾 | 名古屋大学 環境医学研究所<br>病態神経科学分野 | 講師 | 小胞体・ミトコンドリア接触領域の変調を反映する新規分子マーカーの同定 |
| 38 | 松村　浩由 | 立命館大学<br>生命科学部 | 教授 | 低分子人工抗体を用いた細胞分裂装置の構造機能解析 |
| 39 | 浅島　弘充 | 筑波大学 医学医療系膠原病<br>リウマチアレルギー内科学 | 講師 | 病変局所の T-B 細胞間相互作用から紐解くシェーグレン症候群の病態解析 |
| 40 | 鈴木堅太郎 | 山梨大学 大学院総合研究部<br>生命環境学域 | 教授 | 性ホルモン依存性痛み抑制因子による疼痛性差形成機構の解明 |
| 41 | 千原　崇裕 | 広島大学 理学部 | 教授 | 機械ストレスを受けた正常細胞によるがん微小環境形成メカニズム |

# 2023年度研究集会助成

この事業は、生物・生命科学および関連する化学の領域において、我が国で開催される国際色豊かな研究集会の助成を目的としています。2023年度は5件の助成を行いました。

（受付順、敬称略、所属・職位は申請時、贈呈額：1件40万円）

| No. | 氏　名 | 所　属 | 職位 | 開催地／開催日 | 研究集会名 |
|---|---|---|---|---|---|
| 1 | 岡本　仁 | 理化学研究所<br>脳神経科学研究センター<br>意思決定回路動態研究チーム | チームリーダー | 京都／<br>2024.8.17-21 | 第18回<br>国際ゼブラフィッシュ会議 |
| 2 | 片山　勉 | 九州大学<br>薬学研究院<br>分子生物薬学分野 | 教　授 | 福岡／<br>2024.11.18-22 | 第12回 DNA 複製・修復・組換え およびクロマチン・染色体・細胞周期に関する国際シンポジウム（略称：第12回 3R+3C 国際シンポジウム） |
| 3 | 猪原　匡史 | 国立循環器病研究センター<br>脳神経内科 | 部　長 | 京都／<br>2024.7.12 | The 4th International CADASIL and VCI Symposium |
| 4 | 松本　公一 | 国立成育医療研究センター<br>小児がんセンター | 小児がんセンター長 | 横浜／<br>2024.6.22-25 | 第16回国際小児がん学会アジア支部学術集会 |
| 5 | 岡田　康志 | 東京大学・大学院<br>医学系研究科<br>分子細胞生物学専攻<br>細胞生物学・解剖学講座<br>細胞生物学 | 教　授 | 筑波／<br>2024.7.17-19 | 第76回 日本細胞生物学会大会 |

# Promotion Results according to the PROGRAM

(Unit : mil yen)

| Year | Research Grants | | Meeting Grants | | Japan-Europe Research Exchange | | Oversea Reseach Trip | |
|---|---|---|---|---|---|---|---|---|
| | # of people | Amount | # of people | Amount | # of people | Amount | # of people | Amount |
| 1987 | 18 | 1,800 | 8 | 400 | 0 | 0 | 0 | 0 |
| 1988 | 39 | 3,900 | 8 | 400 | 7 | 1,740 | 0 | 0 |
| 1989 | 42 | 4,200 | 8 | 400 | 9 | 2,260 | 0 | 0 |
| 1990 | 51 | 7,650 | 10 | 500 | 8 | 2,440 | 0 | 0 |
| 1991 | 55 | 11,000 | 11 | 550 | 9 | 2,710 | 9 | 250 |
| 1992 | 50 | 10,000 | 10 | 500 | 10 | 3,315 | 8 | 265 |
| 1993 | 50 | 10,000 | 10 | 500 | 11 | 3,511 | 9 | 300 |
| 1994 | 50 | 10,000 | 10 | 500 | 8 | 2,530 | 6 | 155 |
| 1995 | 50 | 6,500 | 10 | 500 | 7 | 2,020 | 6 | 170 |
| 1996 | 45 | 5,850 | 10 | 500 | 6 | 1,600 | 4 | 120 |
| 1997 | 41 | 4,920 | 10 | 500 | 6 | 1,610 | 2 | 55 |
| 1998 | 41 | 4,920 | 10 | 500 | 4 | 1,070 | 8 | 160 |
| 1999 | 41 | 4,920 | 10 | 500 | 4 | 710 | 8 | 160 |
| 2000 | 41 | 4,100 | 8 | 400 | 3 | 660 | 0 | 0 |
| 2001 | 41 | 4,100 | 7 | 350 | 2 | 440 | 0 | 0 |
| 2002 | 40 | 4,000 | 8 | 400 | 0 | 0 | 0 | 0 |
| 2003 | 40 | 4,000 | 4 | 200 | 0 | 0 | 0 | 0 |
| 2004 | 45 | 4,500 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2005 | 45 | 4,500 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2006 | 46 | 4,600 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2007 | 50 | 5,000 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2008 | 45 | 4,500 | 7 | 280 | 0 | 0 | 0 | 0 |
| 2009 | 30 | 3,000 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2010 | 38 | 3,800 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2011 | 41 | 4,100 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2012 | 40 | 4,000 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2013 | 42 | 4,200 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2014 | 42 | 4,200 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2015 | 35 | 3,500 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2016 | 35 | 3,500 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2017 | 41 | 4,100 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2018 | 37 | 3,700 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2019 | 37 | 3,700 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2020 | 39 | 3,900 | 9 | 360 | 0 | 0 | 0 | 0 |
| 2021 | 40 | 4,000 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2022 | 39 | 3,900 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2023 | 41 | 4,100 | 5 | 200 | 0 | 0 | 0 | 0 |
| Total | 1,543 | 182,660 | 265 | 12,120 | 94 | 26,616 | 60 | 1,635 |

# Promotion Results according to the PROGRAM

(Unit : mil yen)

| Year | Travel Expense to Japan | | Special Grant | | Total # of people | Total Amount |
|---|---|---|---|---|---|---|
| | # of people | Amount | # of people | Amount | | |
| 1987 | 0 | 0 | 0 | 0 | 26 | 2,200 |
| 1988 | 0 | 0 | 0 | 0 | 54 | 6,040 |
| 1989 | 0 | 0 | 0 | 0 | 59 | 6,860 |
| 1990 | 0 | 0 | 0 | 0 | 69 | 10,590 |
| 1991 | 0 | 0 | 0 | 0 | 84 | 14,510 |
| 1992 | 0 | 0 | 0 | 0 | 78 | 14,080 |
| 1993 | 0 | 0 | 0 | 0 | 80 | 14,311 |
| 1994 | 0 | 0 | 2 | 110 | 76 | 13,295 |
| 1995 | 0 | 0 | 1 | 50 | 74 | 9,240 |
| 1996 | 0 | 0 | 0 | 0 | 65 | 8,070 |
| 1997 | 0 | 0 | 1 | 30 | 60 | 7,115 |
| 1998 | 0 | 0 | 0 | 0 | 63 | 6,650 |
| 1999 | 0 | 0 | 4 | 130 | 67 | 6,420 |
| 2000 | 0 | 0 | 3 | 142 | 55 | 5,302 |
| 2001 | 0 | 0 | 3 | 120 | 53 | 5,010 |
| 2002 | 0 | 0 | 0 | 0 | 48 | 4,400 |
| 2003 | 0 | 0 | 0 | 0 | 44 | 4,200 |
| 2004 | 0 | 0 | 0 | 0 | 50 | 4,700 |
| 2005 | 0 | 0 | 0 | 0 | 50 | 4,700 |
| 2006 | 0 | 0 | 0 | 0 | 52 | 4,840 |
| 2007 | 5 | 1,000 | 0 | 0 | 61 | 6,240 |
| 2008 | 5 | 1,000 | 0 | 0 | 57 | 5,780 |
| 2009 | 3 | 600 | 0 | 0 | 39 | 3,840 |
| 2010 | 0 | 0 | 0 | 0 | 43 | 4,000 |
| 2011 | 0 | 0 | 0 | 0 | 47 | 4,340 |
| 2012 | 0 | 0 | 0 | 0 | 46 | 4,240 |
| 2013 | 0 | 0 | 0 | 0 | 47 | 4,400 |
| 2014 | 0 | 0 | 0 | 0 | 48 | 4,440 |
| 2015 | 0 | 0 | 0 | 0 | 41 | 3,740 |
| 2016 | 0 | 0 | 0 | 0 | 40 | 3,700 |
| 2017 | 0 | 0 | 0 | 0 | 46 | 4,300 |
| 2018 | 0 | 0 | 0 | 0 | 42 | 3,900 |
| 2019 | 0 | 0 | 0 | 0 | 42 | 3,900 |
| 2020 | 0 | 0 | 0 | 0 | 48 | 4,260 |
| 2021 | 0 | 0 | 0 | 0 | 45 | 4,200 |
| 2022 | 0 | 0 | 0 | 0 | 44 | 4,100 |
| 2023 | 0 | 0 | 0 | 0 | 46 | 4,300 |
| Total | 13 | 2,600 | 14 | 582 | 1,989 | 226,213 |

# 助成金実績一覧表

（単位：万円）

| 年号 | 研究奨励金 | | 研究集会 | | 日欧研究交流 | | 海外出張助成 | |
|---|---|---|---|---|---|---|---|---|
| | 人数 | 助成額 | 人数 | 助成額 | 人数 | 助成金額 | 人数 | 助成金額 |
| 1987 | 18 | 1,800 | 8 | 400 | 0 | 0 | 0 | 0 |
| 1988 | 39 | 3,900 | 8 | 400 | 7 | 1,740 | 0 | 0 |
| 1989 | 42 | 4,200 | 8 | 400 | 9 | 2,260 | 0 | 0 |
| 1990 | 51 | 7,650 | 10 | 500 | 8 | 2,440 | 0 | 0 |
| 1991 | 55 | 11,000 | 11 | 550 | 9 | 2,710 | 9 | 250 |
| 1992 | 50 | 10,000 | 10 | 500 | 10 | 3,315 | 8 | 265 |
| 1993 | 50 | 10,000 | 10 | 500 | 11 | 3,511 | 9 | 300 |
| 1994 | 50 | 10,000 | 10 | 500 | 8 | 2,530 | 6 | 155 |
| 1995 | 50 | 6,500 | 10 | 500 | 7 | 2,020 | 6 | 170 |
| 1996 | 45 | 5,850 | 10 | 500 | 6 | 1,600 | 4 | 120 |
| 1997 | 41 | 4,920 | 10 | 500 | 6 | 1,610 | 2 | 55 |
| 1998 | 41 | 4,920 | 10 | 500 | 4 | 1,070 | 8 | 160 |
| 1999 | 41 | 4,920 | 10 | 500 | 4 | 710 | 8 | 160 |
| 2000 | 41 | 4,100 | 8 | 400 | 3 | 660 | 0 | 0 |
| 2001 | 41 | 4,100 | 7 | 350 | 2 | 440 | 0 | 0 |
| 2002 | 40 | 4,000 | 8 | 400 | 0 | 0 | 0 | 0 |
| 2003 | 40 | 4,000 | 4 | 200 | 0 | 0 | 0 | 0 |
| 2004 | 45 | 4,500 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2005 | 45 | 4,500 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2006 | 46 | 4,600 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2007 | 50 | 5,000 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2008 | 45 | 4,500 | 7 | 280 | 0 | 0 | 0 | 0 |
| 2009 | 30 | 3,000 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2010 | 38 | 3,800 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2011 | 41 | 4,100 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2012 | 40 | 4,000 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2013 | 42 | 4,200 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2014 | 42 | 4,200 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2015 | 35 | 3,500 | 6 | 240 | 0 | 0 | 0 | 0 |
| 2016 | 35 | 3,500 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2017 | 41 | 4,100 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2018 | 37 | 3,700 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2019 | 37 | 3,700 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2020 | 39 | 3,900 | 9 | 360 | 0 | 0 | 0 | 0 |
| 2021 | 40 | 4,000 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2022 | 39 | 3,900 | 5 | 200 | 0 | 0 | 0 | 0 |
| 2023 | 41 | 4,100 | 5 | 200 | 0 | 0 | 0 | 0 |
| Total | 1,543 | 182,660 | 265 | 12,120 | 94 | 26,616 | 60 | 1,635 |

# 助成金実績一覧表

（単位：万円）

| 年号 | 海外受入 | | 特別助成 | | 人数計 | 金額合計 |
|---|---|---|---|---|---|---|
| | 助成人数 | 助成金額 | 人数 | 助成金額 | | |
| 1987 | 0 | 0 | 0 | 0 | 26 | 2,200 |
| 1988 | 0 | 0 | 0 | 0 | 54 | 6,040 |
| 1989 | 0 | 0 | 0 | 0 | 59 | 6,860 |
| 1990 | 0 | 0 | 0 | 0 | 69 | 10,590 |
| 1991 | 0 | 0 | 0 | 0 | 84 | 14,510 |
| 1992 | 0 | 0 | 0 | 0 | 78 | 14,080 |
| 1993 | 0 | 0 | 0 | 0 | 80 | 14,311 |
| 1994 | 0 | 0 | 2 | 110 | 76 | 13,295 |
| 1995 | 0 | 0 | 1 | 50 | 74 | 9,240 |
| 1996 | 0 | 0 | 0 | 0 | 65 | 8,070 |
| 1997 | 0 | 0 | 1 | 30 | 60 | 7,115 |
| 1998 | 0 | 0 | 0 | 0 | 63 | 6,650 |
| 1999 | 0 | 0 | 4 | 130 | 67 | 6,420 |
| 2000 | 0 | 0 | 3 | 142 | 55 | 5,302 |
| 2001 | 0 | 0 | 3 | 120 | 53 | 5,010 |
| 2002 | 0 | 0 | 0 | 0 | 48 | 4,400 |
| 2003 | 0 | 0 | 0 | 0 | 44 | 4,200 |
| 2004 | 0 | 0 | 0 | 0 | 50 | 4,700 |
| 2005 | 0 | 0 | 0 | 0 | 50 | 4,700 |
| 2006 | 0 | 0 | 0 | 0 | 52 | 4,840 |
| 2007 | 5 | 1,000 | 0 | 0 | 61 | 6,240 |
| 2008 | 5 | 1,000 | 0 | 0 | 57 | 5,780 |
| 2009 | 3 | 600 | 0 | 0 | 39 | 3,840 |
| 2010 | 0 | 0 | 0 | 0 | 43 | 4,000 |
| 2011 | 0 | 0 | 0 | 0 | 47 | 4,340 |
| 2012 | 0 | 0 | 0 | 0 | 46 | 4,240 |
| 2013 | 0 | 0 | 0 | 0 | 47 | 4,400 |
| 2014 | 0 | 0 | 0 | 0 | 48 | 4,440 |
| 2015 | 0 | 0 | 0 | 0 | 41 | 3,740 |
| 2016 | 0 | 0 | 0 | 0 | 40 | 3,700 |
| 2017 | 0 | 0 | 0 | 0 | 46 | 4,300 |
| 2018 | 0 | 0 | 0 | 0 | 42 | 3,900 |
| 2019 | 0 | 0 | 0 | 0 | 42 | 3,900 |
| 2020 | 0 | 0 | 0 | 0 | 48 | 4,260 |
| 2021 | 0 | 0 | 0 | 0 | 45 | 4,200 |
| 2022 | 0 | 0 | 0 | 0 | 44 | 4,100 |
| 2023 | 0 | 0 | 0 | 0 | 46 | 4,300 |
| Total | 13 | 2,600 | 14 | 582 | 1,989 | 226,213 |

# 37th Financial Report

## Balance Sheet

As of March 31, 2024

(Unit：JP Yen)

| Account | Amount |
|---|---|
| **Ⅰ Assets** | |
| 1. Current Assets | |
| Current Assets Total | 27,127,033 |
| 2. Fixed Assets | |
| (1) Basic Fund | |
| Basic Fund Total | 1,100,000,000 |
| (2) Specific Assets | |
| Specific Assets Total | 0 |
| (3) Other Long - term Assets | |
| Other Long - term Assets Total | 85,037,535 |
| Fixed Assets Total | 1,185,037,535 |
| **Assets Total** | 1,212,164,568 |
| **Ⅱ Liabilities** | |
| 1. Current Liabilities | |
| Current Liabilities Total | 43,126,430 |
| **Liabilities Total** | 43,126,430 |
| **Ⅲ Equity（Net Assets）** | |
| 1. Designated Net Assets | |
| Designated Net Assets Total | 1,000,000,000 |
| （Amount Appropriating to basic Fund） | (1,000,000,000) |
| (Amount Appropriating to specific assets) | 0 |
| 2. General Net Assets | 169,038,138 |
| （Amount Appropriating to） | (100,000,000) |
| **Equity Total（Net Assets）** | 1,169,038,138 |
| **Liabilities & Equity Total** | 1,212,164,568 |

## Statement of Net Assets

From April 1 st, 2023 to March 31, 2024

(Unit：JP Yen)

| Account | Amount |
|---|---|
| **Ⅰ General Net Assets Changes** | |
| 1. Ordinary income & Expenditure | |
| (1) Ordinary income | |
| Interest from basic fund | 21,040,599 |
| Donation | 40,000,000 |
| Other Income | 680,554 |
| Ordinary Income Total | 61,721,153 |
| (2) Ordinaty Expenditure | |
| Project Expense | 10,223,161 |
| Grant Expense | 43,000,000 |
| Novartis Research Grant | 41,000,000 |
| Research Meeting Grant | 2,000,000 |
| Administrative Expense | 4,343,790 |
| Ordinary Expenditure Total | 57,566,951 |
| Ordinary Balance without Appraisal Profit or Loss | 4,154,202 |
| 2. Nonrecurring Profit & Loss | |
| Nonrecurring Balance of Current Period | 0 |
| General Net Assets Ending Balance | 169,038,138 |
| **Ⅱ Designated Net Assets Changes** | |
| Designated Net Assets Change | (0) |
| Designated Net Assets Ending Balance | 1,000,000,000 |
| **Ⅲ Net Assets Balance Ending Balance** | 1,169,038,138 |

# 第37期（2023年度）財務報告

## 貸借対照表

2024年3月31日現在

（単位：円）

| 科　　目 | 金　額 |
|---|---|
| Ⅰ資産の部 | |
| 1．流動資産 | |
| 流動資産合計 | 27,127,033 |
| 2．固定資産 | |
| ⑴ 基本財産 | |
| 基本財産合計 | 1,100,000,000 |
| ⑵ 特定資産 | |
| 特定資産合計 | 0 |
| ⑶ その他固定資産 | |
| その他固定資産合計 | 85,037,535 |
| 固定資産合計 | 1,185,037,535 |
| 資産合計 | 1,212,164,568 |
| Ⅱ負債の部 | |
| 1．流動負債 | |
| 流動負債合計 | 43,126,430 |
| 負債合計 | 43,126,430 |
| Ⅲ正味財産の部 | |
| 1．指定正味財産 | |
| 指定正味財産合計 | 1,000,000,000 |
| （うち基本財産への充当額） | (1,000,000,000) |
| （うち特定資産への充当額） | (0) |
| 2．一般正味財産 | 169,038,138 |
| （うち基本財産への充当額） | (100,000,000) |
| 正味財産合計 | 1,169,038,138 |
| 負債及び正味財産合計 | 1,212,164,568 |

## 正味財産増減計算書

2023年4月1日から2024年3月31日まで

（単位：円）

| 科　　目 | 金　額 |
|---|---|
| Ⅰ一般正味財産増減の部 | |
| 1．経常増減の部 | |
| ⑴ 経常収益 | |
| 基本財産運用益 | 21,040,599 |
| 受取寄付金 | 40,000,000 |
| 雑収益 | 680,554 |
| 経常収益　計 | 61,721,153 |
| ⑵ 経常費用 | |
| 事業費 | 10,223,161 |
| 支払助成金 | 43,000,000 |
| ノバルティス研究奨励金 | 41,000,000 |
| 研究集会助成金 | 2,000,000 |
| 管理費 | 4,343,790 |
| 経常費用　計 | 57,566,951 |
| 当期経常増減額 | 4,154,202 |
| 2．経常外増減の部 | |
| 当期経常外増減額 | 0 |
| 一般正味財産期末残高 | 169,038,138 |
| Ⅱ指定正味財産増減の部 | |
| 当期指定正味財産増減額 | (0) |
| 指定正味財産期末残高 | 1,000,000,000 |
| Ⅲ正味財産期末残高 | 1,169,038,138 |

**[Board of Trustees] 5 trustees, 2 auditors** As of July 1, 2024

| Post | Name | Title |
|---|---|---|
| Chairman | Kuniaki Takata, Ph.D. | President, Gunma Prefectural Public University Corporation<br>Professor Emeritus, Gunma University |
| Trustee | Sadayoshi Ito, M.D., Ph.D. | Special Administrator, Katta General Hospital Professor Emeritus, Tohoku University |
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| | Leo Lee | President, Novartis Pharma K.K. |
| Auditor | Naoki Haruyama, CPA | Representative, Naoki Haruyama CPA Firm |
| | Katsuki Fukuda | Head, Corporate Finance Dept., Novartis Pharma K.K. |

**[Board of Councilors] 10 councilors** **As of July 1, 2024**

| Post | Name | Title |
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| Chairman | Takao Shimizu, M.D., Ph.D. | Director, Institute of Microbial Chemistry, Microbial Chemistry Research Foundation<br>Sr. Fellow, National Center for Global Health and Medicine<br>Professor Emeritus, University of Tokyo |
| Councilor | Masamitsu Iino, M.D., Ph.D. | Special Advisor, International Research Center for Neurointelligence, University of Tokyo<br>Professor Emeritus, University of Tokyo |
| | Masakatsu Shibasaki, Ph.D. | President, Microbial Chemistry Research Foundation<br>Professor Emeritus, University of Tokyo |
| | Mikiko Sodeoka, Ph.D. | Deputy Director, RIKEN Center for Sustainable Resource Science |
| | Hiroyuki Takeda, Ph.D. | Professor, Kyoto Sangyo University Faculty of Life Sciences,<br>Professor Emeritus, University of Tokyo |
| | Akihiko Nakano, Ph.D. | Senior Advisor/Deputy Director, RIKEN Center for Advanced Photonics<br>Professor Emeritus, University of Tokyo |
| | Yoichi Nabeshima, M.D., Ph.D. | Special Appointed Professor, Kyoto University Graduate School of Medicine<br>Professor Emeritus, Kyoto University |
| | Toyoshi Fujimoto, M.D., Ph.D. | Research Professor, Juntendo University<br>Professor Emeritus, Nagoya University |
| | Mayumi Mochizuki, Ph.D. | Executive Advisor, International Medical Information Center<br>Professor Emeritus, Keio University |
| | Tohru Hirose, Ph.D. | Managing Director, Novartis Pharma K.K.<br>Development Japan Head, Novartis Pharma K.K. |

**[Grantee Selection Committee] 20 members** As of July 1, 2024

| Post | Name | Title |
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| Chairman | Tetsuya Higashiyama, Ph.D. | Professor, University of Tokyo Graduate School of Science |
| Member | Fumitoshi Kakiuchi, Ph.D. | Professor, Keio University School of Fundamental Science and Technology |
| | Hiroshi Kawasaki, M.D., Ph.D. | Professor, Kanazawa University Faculty of Medicine |
| | Shoen Kume, Ph.D. | Professor, Tokyo Institute of Technology School of Life Science and Technology |
| | Kiyoshi Takeda, M.D., Ph.D. | Professor, Osaka University Graduate School of Medicine |
| | Kazuhiro Nakamura, Ph.D. | Professor, Nagoya University Graduate School of Medicine |
| | Sachiko Miyake, M.D., Ph.D. | Professor, Juntendo University Graduate School of Medicine |
| | Hozumi Motohashi, M.D., Ph.D. | Professor, Tohoku University Institute of Development, Aging and Cancer |
| | Yoshihiro Sato, Ph.D | Professor, Hokkaido University Graduate School of Pharmaceutical Sciences |
| | Yutaka Takahashi M.D., Ph.D. | Professor, Nara Medical University |
| | Yasushi Sakata, M.D., Ph.D. | Professor, Osaka University Graduate School of Medicine |
| | Osamu Takeuchi, M.D., Ph.D. | Professor, Kyoto University Graduate School of Medicine |
| | Osamu Nureki, Ph.D. | Professor, University of Tokyo Graduate School of Science |
| | Akiko Hayashi-Takagi, M.D., Ph.D. | Team leader, RIKEN Center for Brain Science |
| | Yu Hayashi, Ph.D. | Professor, University of Tokyo Graduate School of Science |
| | Yasuyuki Fujita, M.D., Ph.D | Professor, Kyoto University Graduate School of Medicine |
| | Mamiko Sakata-Yanagimoto, M.D., Ph.D. | Professor, University of Tsukuba Institute of Medicine |
| | Moritoshi Sato, Ph.D. | Professor, University of Tokyo Graduate School of Arts and Science |
| | Norihiko Takeda, M.D., Ph.D. | Professor, University of Tokyo Graduate School of Medicine |
| | Shosei Yoshida, Ph.D. | Professor, National Institute for Basic Biology |

# 公益財団法人ノバルティス科学振興財団

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任期2024年6月19日～ 2026年6月　　2024年7月1日現在（敬称略）

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| 理 事 | 伊藤　貞嘉 | 公立刈田綜合病院 特別管理者<br>東北大学 名誉教授 |
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| | 村上富士夫 | 大阪大学 名誉教授 |
| | レオ　リー | ノバルティス ファーマ株式会社 代表取締役社長 |

任期2024年6月19日～ 2028年6月

| 職 名 | 氏 名 | 現　　職 |
|---|---|---|
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| | 福田　勝紀 | ノバルティス ファーマ株式会社　企画管理本部 コーポレートファイナンス部長 |

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任期2024年6月19日～ 2028年6月　　2024年7月1日現在（敬称略）

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| | 袖岡　幹子 | 理化学研究所　環境資源科学研究センター　副センター長 |
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2024年7月1日現在（敬称略）

| 職 名 | 氏 名 | 現　　職 |
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| | 坂田（柳元）麻実子 | 筑波大学 医学医療系 教授 |
| | 佐藤　守俊 | 東京大学 大学院総合文化研究科 教授 |
| | 武田　憲彦 | 東京大学 大学院医学系研究科 教授 |
| | 吉田　松生 | 自然科学研究機構 基礎生物学研究所 教授 |

# 事 務 局 便 り

## ご寄付のお願い

当財団は、自然科学における創造的な研究の奨励等を行うことにより、学術の振興を図り、国民の健康と福祉の向上に寄与することを目的に公益事業を行っております。

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ご寄付は、随時受付けております。詳しくは、財団事務局までお問合せください。
（E-メール：foundation.japan@novartis.com）

## 事務局より

2024年度も、おかげさまで財団年報を発行する運びとなりました。2020年に世界中に大きな影響を及ぼした新型コロナウイルス感染症は、ようやくあまり意識しなくてもよい生活に戻った気がいたします。これもmRNAワクチンの画期的な発見によるもので、社会に対して非常に大きな役割を果たしました。改めて科学技術の偉大さ、それを支えた基礎研究の重要性を認識させられます。

当財団の研究奨励金助成や研究集会助成事業については、今年度も多くのご応募がありました。基礎研究に向けた研究者の方々の熱意を感じております。

当財団は1987年9月の財団設立以来、助成件数は**総数で1,989件、総額で 約22.6億円**となりました。当財団は、自然科学の創造的研究への助成によって、日本の学術発展に寄与することを目指しており、助成を受けられた研究成果がすぐに応用につながらなくとも、将来、新分野につながることを夢見て、この事業を継続して参ります。

今まで助成事業が継続できていることも、偏に、助成事業にご理解・ご支援をいただいた方々、助成を受けられて研究を継続された皆様、および財団関係者のお力添えの賜物であると、心より感謝申し上げます。引き続きご指導、ご支援の程よろしくお願い申し上げます。

事務局長　原　健記
2024年10月13日

公益財団法人 ノバルティス科学振興財団
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