Annual Report (2025)

No.37
The NOVARTIS Foundation (Japan) for the Promotion of Science

2025年度

財団年報 第37号

公益財団法人 ノバルティス科学振興財団

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Introduction

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

This annual report compiles the research reports from recipients of the 37th Novartis Foundation for the Promotion of Science Research Grants (research conducted from April 2024 to March 2025: 41 research grants and 5 research meeting grants).

Recent advancements in science and technology have been remarkable, encompassing medical biology fields like neuroscience, immunology, and molecular and cellular biology, as well as computer science, epitomized by AI. Tracing these back to their origins reveals dramatic stories of discovery and invention, driven by scientists' curiosity. Small ideas and discoveries, much like seeds germinating and growing, have developed in various ways to support modern society. Research funding, which promotes the advancement of science and technology, acts as the water and nutrients that support this growth. In Japan, the allocation of research funds has shifted from "uniform distribution" to "selection and concentration." However, extreme concentration could risk a situation where there are no subjects left to select. Research projects that involve massive investments tend to be risk-averse, meaning they often aim for predictable outcomes, which limits the potential for truly novel results. Diverse research grants for diverse approaches will pave the way for groundbreaking breakthroughs. We believe that the fact that various private foundations provide research grants from their unique perspectives holds significant meaning, even beyond the mere amount of the grants.

The Novartis Foundation (Japan) for the Promotion of Science was established in 1987 with a generous donation of 1 billion yen from Ciba-Geigy AG (now Novartis AG) in Switzerland. The Foundation's objective, as clearly stated in its Articles of Incorporation, is to "promote academic advancement and contribute to the improvement of health and welfare by encouraging creative research in natural sciences." Furthermore, a document titled "Intent of the Foundation's Establishment," written in the year of its founding, expresses the hope that the Foundation could "contribute to human welfare by promoting and assisting creative research in natural sciences, which will become the axis of science in the coming 21st century," and advocates for "financial assistance for research, as well as providing opportunities for cross-border exchange." Under this policy, over 38 years, the Foundation has provided a total of 2,030 grants, amounting to approximately 2.3 billion yen. In this era of rapid and constant change, the Foundation aims to return to its original principles and continue supporting outstanding research that will open up the next era, particularly for those who are nurturing unique ideas and planning new projects.

This annual report summarizes the excellent research outcomes supported by this Foundation. These are remarkable achievements accomplished within the limited timeframe of one year. The list of past recipients of our Foundation's grants includes numerous leading researchers in various 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 sincerely hope that the current grant recipients will use these research achievements as a springboard for even greater leaps forward. We also extend our deepest gratitude to the selection committee members who meticulously reviewed and selected these outstanding research projects, and to all stakeholders, including Novartis Pharma K.K., the original donor, who support the Foundation's activities.

はじめに

代表理事　高田　邦昭

本年報には、第 37 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（2024 年 4 月～ 2025 年 3 月の研究：研究奨励金 41 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 AG 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、38 年間で総計 2030 件、金額にして約 23 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

Development of rational design method for de novo proteins that are efficiently delivered into cytoplasm

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Summary Abstract

We aim to establish method for designing de novo proteins that are efficiently delivered into cytoplasm to expand the potential of de novo protein application in clinical medicine
Key Words : De novo proteins, cytoplasmic delivery, endosomal escape

Introduction

Intracellular protein-protein interactions are promising therapeutic targets. However, protein interaction surfaces are often flat and lack the “pockets” necessary for binding of small molecule drugs. Antibodies and other agents can effectively inhibit protein-protein interactions, but they are difficult to translocate into the cell. De novo mini-proteins that have stable structures and bind to target proteins are promising therapeutic platforms, but the lack of a method for designing de novo proteins that can be effectively delivered into cells has been a major hurdle for their clinical application. Therefore, we aim to establish a rational design method for de novo proteins that can be efficiently transferred into the cytoplasm.

Results

In this study, the basic strategy to explore the features of proteins that determine the efficiency of cytoplasmic delivery is to (1) design de novo protein library based on the hypothesis, (2) accurately measure the rate of cytoplasmic delivery at a large scale, (3) analyze the large amount of data from the screening, and (4) redesign the libraries based on the results. We tried to repeat this cycle of design-measurement-analysis-redesign to identify the factor(s) that determines the efficiency of the cytosolic delivery of de novo proteins.

First, we designed about 5,000-10,000 mini proteins with diverse structures using a computational design method (#1-3). We tested two strategies for de novo protein design: hypothesis-driven design and ‘random’ design that covers the vast amino acid sequence space.

Hypothesis-driven design: Positive charge, especially arginine, has been suggested in the past literature to be important for cytoplasmic delivery (#4). Based on this finding, we designed 2500-5000 de novo proteins by replacing the surface amino acids with lysine or arginine for about 500 de novo mini-proteins. This will allow us to check if the number of

surface charges, especially arginine, affects the cytosolic delivery efficiency.

'Random' design: We also designed de novo mini-proteins with diverse features and properties. We tried to cover the space of sequence and structural features such as (a) amino acid length, (b) helix and strand arrangement, (c) charge, (d) charge dispersion (densely or sparsely distributed), and (e) number of hydrophobic amino acids on the surface. This allows for broad coverage of the amino acid sequence space even with a limited number of sequences in the library.

Although several miniproteins that can be delivered into the cytoplasm are known, it is difficult to identify the properties/features that determine the efficiency of the cytoplasmic delivery. Therefore, we screened de novo proteins that can reach the cytoplasm using cDNA display method. The Split-GFP system (#5) is used to distinguish between those that remain in endosomes and those that can reach the cytoplasm. GFP1-10 is expressed in the cytoplasm, while GFP11 is tagged to the de novo protein. Then, full-length GFP can be reconstituted only when de novo protein reaches the cytoplasm, and by pulling down the GFP on GFP-Trap beads, only the de novo protein-cDNA complexes that were able to reach the cytoplasm can be separated. Then the cDNA was amplified by PCR and sequenced in NGS to quantify cytosolic delivery efficiency of each of the de novo proteins in the library.

We completed the large-scale screening using this method for de novo protein library as described above and started to analyze the large dataset.

Discussion & Conclusion

De novo proteins are a field that has attracted much attention, including the Nobel Prize in Chemistry awarded to Dr. David Baker, a leading researcher in the field, and are expected to be applied in clinical medicine in the future. However, its application is still limited to therapeutics by targeting biomolecules on the cell surface like receptors. Therefore, it is important to expand such applications, and I believe that this research will greatly expand the potential of de novo proteins by extending the targets to biomolecules in the cytoplasm. I hope this research will serve as a steppingstone to further accelerate the clinical application of de novo proteins.

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一般の皆様へ

わたしたちの体を構成するタンパク質は、運動や視覚・聴覚、また生体防御などあらゆる生物学的機能に貢献する分子です。これらのタンパク質を改良することで、がんや感染症などに対する様々特効薬が開発され、臨床応用されています。しかし、天然タンパク質を基礎としたときには、医薬品として最適なものを開発できるとは限りません。そこで、医薬品として最適な性質、つまり安価に大量に製造可能で、室温でも輸送・保管できるようなタンパク質を人工的に設計することが試みられています。そのような人工タンパク質の臨床応用を更に加速させることを本研究の目的としています。

Studies on the Total Synthesis of Saxitoxin and the Design of New Functional Molecules

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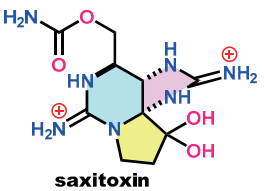
Summary Abstract

The decarbonylative radical coupling condition, applied in a unique manner to an oxime derived from Garner's aldehyde and an acyl telluride obtained from hydroxyproline, resulted in the successful introduction of the entire carbon chain of saxitoxin. This process also led to the stereoselective construction of the vicinal diamine structure.
Key Words : saxitoxin, total synthesis, voltage-gated sodium channel, radical

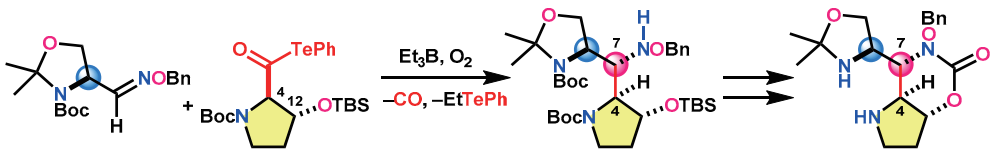
Introduction

Dysfunction of ion channels, which are fundamental to vital biological processes (channelopathies), leads to various diseases. Therefore, the rational development of pharmaceuticals that target these channels is an urgent challenge in modern science. Natural products densely substituted with polar functional groups such as oxygen and nitrogen enable highly selective inhibition or activation of ion channels—something that conventional drugs and other natural compounds cannot achieve.

Results

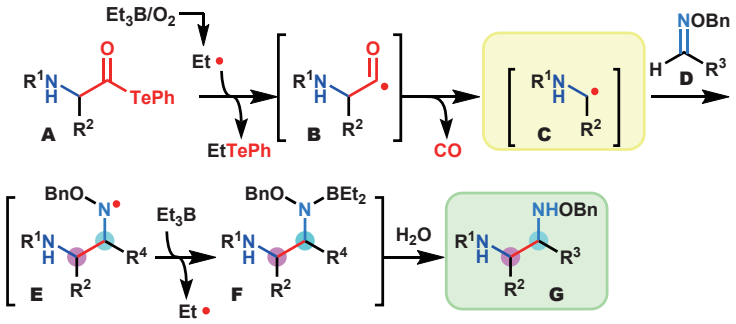


In 2024, we embarked on a total synthesis research project on saxitoxin with fourth-year students. By applying a unique decarbonylative radical coupling condition to an oxime derived from Garner's aldehyde and an acyl telluride derived from hydroxyproline, we successfully introduced the entire carbon chain of saxitoxin and stereoselectively constructed the vicinal diamine structure. Currently, we are pursuing total synthesis and exploring the introduction of bisguanidino groups.

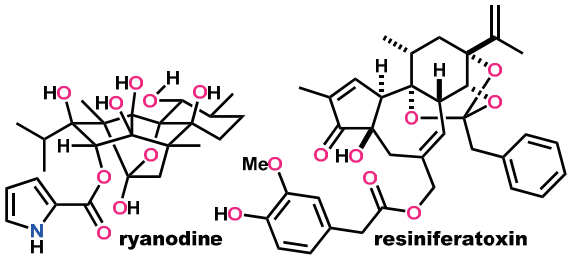


The key reaction mechanism is shown below. Under Et_3B/O_2 conditions, the acyl telluride **A** undergoes a transformation via the acyl radical **B**, leading to the decarbonylation of the intermediate **C**. This intermediate is captured at the C=N bond of the benzyl oxime **D**, forming a C-C bond and subsequently yielding **G**. The reaction proceeds under mildly neutral conditions, preserving polar functional groups.

Because this reaction proceeds under mildly neutral conditions, it preserves polar functional groups and is well suited for the construction of highly oxidized carbon skeletons with vicinal amino groups.



Discussion & Conclusion



We have developed innovative synthetic reactions, strategies and methodologies for biologically active natural products such as ryanodine, a regulator of the ryanodine receptor calcium ion channel, and resiniferatoxin, a modulator of TRPV1. In addition to numerous first-of-a-kind total syntheses, we have also generated both natural and artificial analogs with remarkable results.

So now, we have identified saxitoxin, a guanidine alkaloid neurotoxin densely substituted with polar functional groups, as a fundamental molecule for NaVCh research. Due to its numerous polar functional groups, this natural compound enters into multi-point interactions with NaVCh, either inhibiting or enhancing its function. This allows for powerful and selective recognition and functional regulation of NaVCh - something that cannot be achieved with conventional drugs.

We have begun to develop a novel approach to the analysis and regulation of channel function based on the total synthesis of complex channel modulators.

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Nagatomo, M.; Nishiyama, H.; Fujino, H.; Inoue, M. *Angew. Chem. Int. Ed.* **2015**, *54*, 1537.

一般の皆様へ

生命現象の根幹をなすイオンチャネルの機能不全は、多くの疾患を引き起こすため、チャネルに作用する医薬品の合理的な開発は、現代科学における緊急課題です。酸素や窒素などの極性官能基が稠密に置換した天然物は、一般的な医薬品や天然物では実現不可能な、チャネルの高選択的阻害・活性化を可能にします。

我々は、電位依存性ナトリウムチャネルの研究基盤分子として、極性官能基が稠密に置換したグアニジンアルカロイドである神経毒**サキシトキシン**を設定し、その化学合成研究を開始しました。現在までに、**サキシトキシン**の全炭素鎖の導入とビシナルジアミン構造の立体選択的構築に成功しました。

Development of epigenetics-related proteolytic drugs

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Summary Abstract

In this study, we developed a chimeric molecule, EpiTAC, consisting of a DNA duplex containing an artificial nucleic acid and a ubiquitin ligase ligand linked via an appropriate linker. EpiTAC irreversibly degrades the DNA methyltransferase DNMT1 and exhibits anticancer activity. Furthermore, we found that DNMT1 degradation activity can be modulated by altering the artificial nucleic acid incorporated into the DNA duplex, suggesting the potential for precise control of DNA epigenetics through chemical modifications.
Key Words : Epigenetics / Nucleic Acids / PROTACs / Anticancer Drugs / DNMT

Introduction

Gene expression is a highly sophisticated component of the broader system that sustains biological functions. For instance, methylation at the 5-position of the cytosine base in DNA is a crucial regulatory mechanism of gene expressionand has been shown to play a significant role in various biological processes, from development to the regulation of viral infections and cancer. 5-Methylcytosine, which is abundant in CG sequences, is generated when DNA methyltransferase (DNMT) recognizes duplex DNA sequences in which only one strand is methylated. Epigenetics-related proteins such as DNMT1 have garnered considerable attention as drug targets.

Results

In this study, we developed a novel modality drug, Epigenetics Targeting Chimera (EpiTAC), which enables the degradation of epigenetic enzymes such as DNMT, TET, and TDG by fusing nucleic acid molecules with small-molecule compounds. EpiTAC consists of a dumbbell-shaped DNA incorporating various epigenetic cytosine bases, conjugated to a ubiquitin ligase ligand (e.g., VH032) via a linker. This structure is expected to induce the proteasomal degradation of the corresponding epigenetic proteins. Dumbbell-shaped DNA exhibits greater resistance to nuclease degradation compared to conventional double-stranded DNA, allowing for a more sustained pharmacological effect in vivo.[1] Furthermore, we developed covalent drug-type EpiTACs containing artificial nucleobases for DNMT and TDG. These inhibitors form strong covalent bonds with target proteins within the DNA sequence, making them potentially more potent epigenetic regulators.[2]

Synthesis of EpiTACs

As an initial step, we synthesized a DNA ligand capable of tightly capturing DNMT1, a DNA methyltransferase. To enhance DNMT1 binding efficiency, we synthesized two

phosphoramidite derivatives containing artificial nucleobases: 5-fluorocytosine (FC) and 2-hydroxypyrimidine (Z). These phosphoramidites were synthesized in a few steps from commercially available materials with high yields, making them suitable for future large-scale synthesis. Using these artificial phosphoramidites, we chemically synthesized hairpin DNA duplexes incorporating FC and Z via an automated nucleic acid synthesizer. Two types of duplexes, 16 nucleotides and 20 nucleotides in length, were prepared. The synthesis yield was comparable to that of natural DNA synthesis, enabling the production of sufficient amounts of FC- and Z-modified DNA. Subsequently, the DNA duplexes were conjugated to the ubiquitin ligase ligand VH032 via PEG or alkyl linkers of varying lengths, generating multiple EpiTAC variants. The synthesized EpiTACs were purified using reverse-phase column chromatography (HPLC) and structurally confirmed by mass spectrometry (MALDI-TOF MS).

Intracellular DNMT1 Degradation by EpiTAC

To evaluate DNMT1 degradation, we transfected human breast cancer cells (MDA-MB-231) with various EpiTACs and quantified intracellular DNMT1 levels using Western blot analysis after incubation. Significant DNMT1 degradation was observed with certain EpiTAC variants. Notably, 20-nucleotide-long DNA duplexes were more effective than 16-nucleotide duplexes, and alkyl linkers were superior to PEG linkers in promoting DNMT1 degradation. Furthermore, Z-modified DNA was more efficient in inducing DNMT1 degradation than FC-modified DNA. To confirm the degradation mechanism, we repeated the experiment in the presence of the proteasome inhibitor Bortezomib. The inhibition of DNMT1 degradation under this condition indicated that EpiTAC functions as designed, inducing DNMT1 degradation via the proteasomal pathway. Pull-down assays further demonstrated that hairpin DNA containing artificial nucleobases tightly bound DNMT1, confirming its role as a highly effective DNMT1 ligand.

EpiTAC-Induced Cancer Cell Death

We next assessed the viability of EpiTAC-transfected cells and found that the most effective EpiTAC variant induced significant cancer cell death. Notably, MDA-MB-468 cells, which express higher levels of DNMT1, exhibited a greater susceptibility to EpiTAC-induced cell death. These findings demonstrate that EpiTAC efficiently captures and induces the proteasomal degradation of DNMT1, ultimately leading to cancer cell death.

Future Perspectives

Moving forward, we plan to investigate the effects of EpiTAC administration on genome-wide DNA methylation levels and evaluate its anticancer efficacy in tumor-bearing mouse models. These studies will facilitate the further development of EpiTAC as a novel epigenetic drug.

Discussion & Conclusion

In this study, we developed a novel chimeric molecule, EpiTAC, which induces the proteasomal degradation of the DNA methyltransferase DNMT1. The structure of EpiTAC was optimized by introducing modified variants into human cells and evaluating DNMT1 degradation efficiency via Western blotting. Experiments with proteasome inhibitors confirmed that EpiTAC captures DNMT1 and induces its degradation as designed. The optimized EpiTAC exhibited strong cytotoxicity against human cancer cells with high DNMT1 expression, highlighting its potential as an anticancer drug. In future studies, we plan to comprehensively analyze changes in intracellular genomic DNA methylation and further evaluate its efficacy and side effects in a carcinoma-bearing mouse model.

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一般の皆様へ

エピジェネティクスとは塩基配列に依存しない遺伝子発現調節機構の１つであり、発生や分化といった基本的な生命現象のみならず、その異常が様々な疾患と関連していることが明らかにされつつあります。本研究では、代表的なエピジェネティクスであるDNAメチル化を触媒するDNMT1を分解するEpiTACの開発を行いました。化学合成したEpiTACはヒト細胞内のDNMT1を効率的に分解し、さらにがん細胞を細胞死に誘導することができました。この結果は、EpiTACが抗がん剤として有望であることを示唆しており、今後動物実験などより実践的な創薬研究に展開していく予定です。

Development of visualization technique for glutamate receptors with ultra-high resolution

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Summary Abstract

We developed a new fluorescent probe, PFQX-AF488, that enables reversible visualization of AMPA-type glutamate receptors (AMPA receptors) in cultured neurons. This probe allows for rapid and instantaneous labeling of the receptors and can be easily washed out by simple medium exchange. By taking advantage of these properties and combining PFQX-AF488 with the covalent labeling probe for AMPA receptors that we previously reported, we successfully visualized the mechanism of AMPA receptor accumulation at synapses in cultured hippocampal neurons.
Key Words : AMPA receptor, fluorescent probe, synaptic plasticity

Introduction

During memory and learning, it is believed that the expression level of AMPA-type glutamate receptors (AMPA receptors) on the synaptic membrane changes. To elucidate the underlying mechanisms in detail, it is necessary to analyze the distribution and dynamics of AMPA receptors within synaptic microdomains, which are approximately 200–500 nm in diameter. We previously reported methods for fluorescent visualization of endogenously expressed AMPA receptors in neurons(ref.1, 2). However, even with super-resolution microscopy, it has remained challenging to distinguish intracellular and surface-localized AMPA receptors within the synapse. In this study, we developed a fluorescent probe that enables reversible visualization of AMPA receptors.

Results

We previously developed a two-step labeling technique that combines click chemistry with CAM2-based ligand-directed chemical labeling, which enabled us to achieve fluorescent visualization of surface-expressed AMPA receptors within five minutes (ref. 2). Using this approach, we successfully analyzed both the lifetime and the dynamic behavior of AMPA receptors localized on the cell surface in cultured neurons. However, a limitation of this method is that only AMPA receptors already present on the surface at the time of labeling can be visualized, making it impossible to capture receptor populations that are newly inserted into the membrane via exocytosis from intracellular compartments.

To overcome this limitation, we developed a new method that allows for rapid and temporally flexible fluorescent visualization of AMPA receptors. In terms of molecular design, we synthesized a series of fluorescent probes by covalently conjugating the AMPA receptor-selective antagonist PFQX to various fluorophores. Among these, the

probe in which PFQX is directly conjugated to Alexa Fluor 488 (PFQX-AF488) exhibited the highest affinity and was found to robustly visualize AMPA receptors independently of the extracellular environment. Importantly, X-ray crystallographic analysis of the AMPA receptor in complex with PFQX-AF488 revealed that not only the ligand moiety but also the Alexa Fluor 488 portion contributes to the high-affinity binding by directly engaging with the receptor's ligand-binding domain.

Subsequent binding assays in mammalian HEK293 cells expressing AMPA receptors demonstrated that PFQX-AF488 enables visualization of surface-localized AMPA receptors within tens of seconds after probe application. Furthermore, the probe could be easily removed by simple medium exchange, and re-application of the probe restored fluorescence intensity comparable to the initial labeling. These findings confirm that PFQX-AF488 can function as a reversible "snapshot" imaging probe for AMPA receptors in living cells.

We then applied PFQX-AF488 to cultured hippocampal neurons to assess its applicability in a more physiologically relevant context. Similar to the results in HEK293 cells, we found that the probe allows for temporally controlled and reversible fluorescent labeling of endogenous AMPA receptors in neurons. Notably, upon induction of chemical long-term potentiation (chemical LTP), a widely used model for synaptic plasticity, we observed a significant increase in fluorescence intensity at synaptic sites by live-cell confocal imaging. This fluorescence increase was abolished when inhibitors of key molecules (e.g. NMDA receptors and CaMKII) required for LTP induction were applied, indicating that the observed accumulation of AMPA receptors on the synaptic membrane indeed reflects a physiologically relevant process associated with synaptic strengthening.

To further dissect the mechanism underlying AMPA receptor accumulation during synaptic plasticity, we performed a dual-labeling experiment by combining PFQX-AF488 with our previously established chemical labeling method. Specifically, we first labeled pre-existing surface AMPA receptors with Alexa Fluor 647 via CAM2-mediated labeling, and then used PFQX-AF488 to visualize the newly accumulated receptor population. Quantitative analysis of the spatial and temporal dynamics revealed that the majority of AMPA receptors accumulated during chemical LTP are delivered via exocytosis from intracellular compartments, rather than through lateral diffusion from extrasynaptic regions of dendrites. This finding provides direct experimental evidence resolving a longstanding debate in the field of neuroscience regarding the primary mechanism responsible for AMPA receptor trafficking during synaptic plasticity.

Discussion & Conclusion

In the present study, we developed a novel fluorescent probe, PFQX-AF488, for the visualization of AMPA receptors in cultured hippocampal neurons, and successfully visualized the postsynaptic accumulation of AMPA receptors during synaptic plasticity. Furthermore, by combining this fluorescent probe with our previously established chemical labeling technique, we were able to elucidate the underlying mechanism of AMPA receptor accumulation at synapses. Although PFQX-AF488 itself does not surpass the spatial resolution of conventional fluorescence microscopy, the integration of this probe with a chemically distinct labeling method enabled us to achieve fluorescence live-cell imaging results that are comparable in quality and informational content to those obtained using super-resolution microscopy techniques.

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一般の皆様へ

脳内において、記憶・学習時に神経細胞膜のAMPA受容体と呼ばれるタンパク質の発現量が増加し、その増加が私たちの記憶や学習と直接相関することが知られています。しかし、AMPA受容体が存在するシナプスという場所は非常に微小な環境であり、その増加を機構を詳細に解析することはこれまで非常に困難でした。今回の研究で、AMPA受容体のシナプスへの集積機構を明らかにでき、今後の記憶・学習の分子メカニズムの理解に大きな知見を与えたと考えられます。

Development of synthetic transformations based on installing group 13, 14 functional moieties.

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Summary Abstract

New protocols for the generation and transformation of boryl, silyl, and/or germyl radicals have been established, enabling the efficient and selective installation of B, Si, and/or Ge functional groups into organic molecules under mild conditions. These methods show high applicability for the synthesis of group 13/14 element-containing functional molecules.
Key Words : Boron, Silicon, Germanium, Radical, Molecular Transformation

Introduction

Boryl, silyl, and/or germyl radicals serve as powerful intermediates for building a wide range of group 13/14 element-containing functional molecules and polymers. Their synthetic transformations and applications are therefore of significant interest. However, methods for generating these radical species are still very limited, scarce, which restrict their synthetic applicability. In this project, we set out to develop new, practically useful reactions that enable the efficient generation and selective transformation of group 13/14 element radicals.

Results

1) New Protocol for Generations and Transformations of Group 14 Element Radicals.

[1] Thermally induced hydrogen atom transfer (HAT) reactions of silicon hydrides (R_3SiH) were reported as early as the 1940s, leading to the discovery of the silyl radicals ($R_3Si•$). Since then, such reactions have been predominantly employed for the generation of Group 14 element radicals ($R_3Si•$, $R_3Ge•$), enabling numerous innovative transformations. More recently, photoredox-assisted HAT processes have been developed to generate $R_3Si•$ and $R_3Ge•$ from R_3SiH and R_3GeH, respectively. While these approaches eliminate the need for highly reactive peroxides (as hydrogen abstractors), transition metal catalyst, and high temperature, the synthetic utility remains limited due to the complex structures, high cost, and air and moisture sensitivity of many photoredox catalysts.

In line with our research interests, we aimed to explore mechanistically novel and operationally simple methods for the generation and utilization of silyl and/or germyl radicals. In this research project, we developed a unique catalytic protocol that harnesses photoexcited electron transfer (ET) within an electron donor–acceptor (EDA) complex to drive the HAT of silicon/germanium hydrides (Figure 1). This system employs catalytic

amounts of thiol (serving as both electron donor and HAT catalyst) and benzophenone (as the electron acceptor and ET catalyst), both of which are structurally simple, bench-stable, and commercially available at low cost.

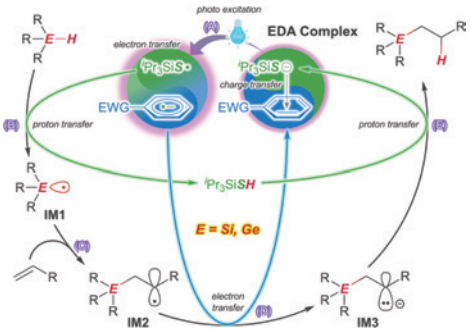

Figure 1

The reaction proceeds smoothly upon visible-light (blue LED) irradiation at room temperature, without the need for any transition metal or dedicated photocatalyst. Silyl and/or germyl radicals generated by this method can be efficiently trapped by a variety of alkenes, providing a mild, selective, and transition-metal-free approach to hydrosilylation and hydrogermylation (Figure 2).

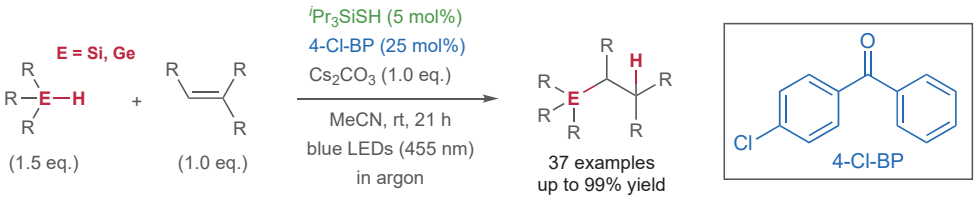

Figure 2

2) New Protocol for Generation and Transformation of Boryl Radicals.[2] Organoboron compounds find broad applications as synthetic reagents and functional materials owing to their unique chemical reactivity and physical properties. In recent years, several boron-containing pharmaceuticals, such as bortezomib (Velcade), tavaborole (Kerydin), and crisaborole (Eucrisa), have also been approved by the FDA. As a result, the development of new and practically useful protocols for introducing boron moieties into organic molecules has attracted increasing interest in synthetic chemistry, materials science, and drug discovery.

Traditionally, ionic reactions (including transition metal-catalyzed reactions) involving trivalent boron compounds are widely employed due to the presence of an empty p orbital on boron. On the other hand, the electron-deficient nature of boron leads to the low stability of boron-centered radicals, making their generation and synthetic application rather limited.

In this study, we established a transition-metal-free, chemo-, regio-, and stereoselective thioboration reaction of various terminal alkynes using triaryl thioborates, based on a new strategy for generating ligated boryl radicals (Figure 3). Triaryl thioborates can be easily prepared by the reaction of trichloroborane with thiophenol. In the presence of a catalytic amount (1 mol%) of sodium thiophenolate, the reactions between alkynes and triaryl thioborates proceeded smoothly and selectively without the need for any solvents, affording cis-(β-(arylthio)vinyl)boranes in good yields. The cis products can be selectively converted into their trans isomers simply by treatment with water in air. Both cis and trans products can be functionalized at either the boron or sulfur site, enabling the diversified synthesis of multi-substituted alkenes with high selectivity and yields. Mechanistic studies based on both experiments and DFT calculations revealed that the formation of a $(ArS)_4B^-$ ate complex facilitates B–S bond homolysis, leading to the efficient generation of boryl radicals (Figure 4, next page).

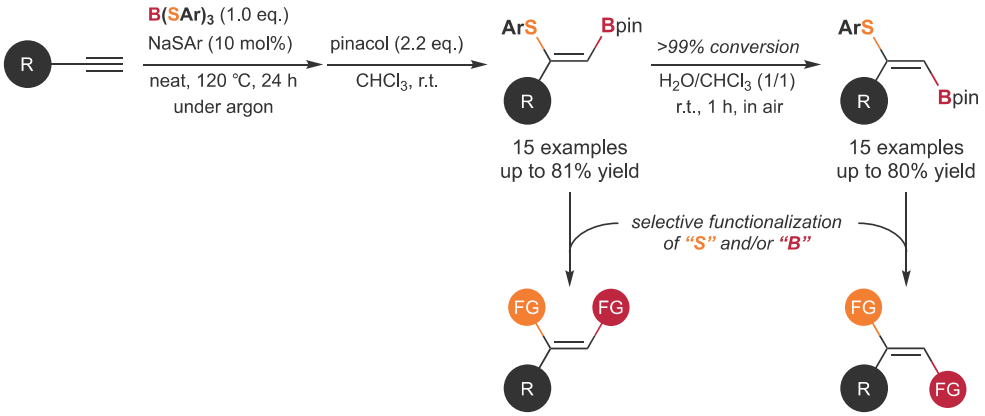

Figure 3

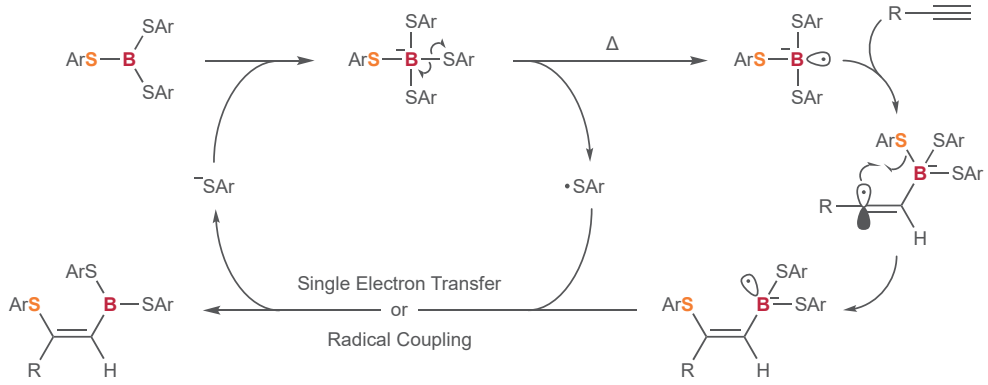

Figure 4

Discussion & Conclusion

The new HAT reaction developed in this research project is highly efficient and easy to handle, offering a practically useful protocol for the generation of silyl and germyl radicals. As such, it is expected to be broadly applicable to the synthesis of a wide range of organosilicon and organogermanium compounds. This method also provides a novel and straightforward strategy for the development of molecular transformations driven by photo-induced electron transfer, circumventing the challenges associated with designing specific photocatalysts by harmonizing the photosensitivity (absorption and excitation) and chemical reactivity (oxidation/reduction potential, ET ability, etc.) of donor–acceptor systems.

In parallel, the first thioboration reaction of terminal alkynes developed in this project not only established a novel protocol for the generation of boryl radicals but also provided a simple and direct approach to regioselective synthesis of multi-substituted vinyl boron and/or vinyl sulfide compounds. Applications of this reaction to the synthesis of biologically active and functional molecules are currently underway.

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DOI: https://doi.org/10.1021/jacsau.4c00907

一般の皆様へ

既存の医薬品にはない体内動態や生理活性を有する候補化合物を創出するためには、生体内に存在しない元素の活用が有望なアプローチの一つです。中でも、13・14 族ヘテロ元素（B、Si、Ge など）は、分子に新たな物性や機能を付与できる特性を有しており、近年ではこれらの元素を含む分子骨格に基づく医薬品の開発が大きな注目を集めています。そこで、我々は、ホウ素やケイ素の導入において、従来法では困難なラジカル種の発生とその制御に着目した新規分子変換手法（化学の“新起点”）を開発するとともに、それによって得られる新規ホウ素・ケイ素含有医薬品候補化合物（薬学の“新目的地”）を創製することを目指し、革新的な合成化学、元素化学、医薬化学研究に取り組んだ。

Functional Analysis of Mesenchymal Stromal Cells in Musculoskeletal Systems

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Summary Abstract

We studied mesenchymal stromal cells (MSCs) in medaka embryos using in situ hybridization and transgenic fish with GFP-labeling in MSCs. MSCs appeared in various tissues, notably in the heart. Crosses with cardiomyocyte-RFP fish showed MSCs near cardiac valves, suggesting a role in heart development. To analyze functional importance of MSCs during developments, mutations were introduced into the MSC marker gene using CRISPR/Cas9 method, affecting 30% of larvae. Mutated individuals were identified via tail biopsy, and the production of heterozygous mutant is underway. We aim to shed new light on the roles of MSCs as in tissue homeostasis, regeneration and aging.
Key Words : Mesenchymal stromal cells, muscle, medaka, embryo, connective tissue

Introduction

The musculoskeletal system contains mesenchymal stromal cells (MSCs) with a variety of characteristics, but their developmental origin and cellular function are largely unknown. In this study, we analyzed the function of MSCs in the medaka fish, whose transparent embryos can be easily observed. Compared to mouse and human embryos, medaka embryos have a simpler muscle morphology, making it easier to follow the behavior of cells in a living state. In this study, the distribution and properties of MSCs was observed using transgenic medaka with fluorescent labels in different cell types.

Results

We first analyzed the expression of a marker gene for MSCs by whole mount in situ hybridization on medaka embryos using an antisense RNA probe for this gene. This gene was expressed in the anterior lateral plate mesoderm and neural crest cells in 1-day embryos. In 2-day embryos, expression was observed in the pharyngeal arches and pectoral fin primordia in 2-day embryos. In 3-day embryos, expression was observed in the pectoral fin and in the cells located near boundaries of the somites. We also visualized MSCs in the embryos of GFP- labeled medaka fish strain. In this transgenic medaka, MSCs are labeled with GFP expression driven by the upstream regulatory region of the MSC marker gene. Fluorescence was observed weakly in the whole body of 1-day embryos. In 2-day embryos, fluorescence was observed specifically in the heart, gill arch and somites. In 3-day embryos, lens of the eyeball showed stable expression that lasts into larval stage. Also the expression in the heart became stronger than other tissues. Combination of these results showed that MSCs appear in various tissues in the medaka early embryos,

suggesting their potential contribution to multiple connective tissues. Next, as an attempt to visualize spatial organization of MSCs in differentiated tissues, we focused on MSCs' contribution to the cells associated with the developing heart, since this was the organ with the most prominent expression of GFP. To visualize the localization of MSCs in the heart, we crossed MSC-GFP strain with another strain associated with cardiomyocyte-specific RFP expression. The embryos obtained from this cross allow us to observe the spatial relationship of MSC and cardiac myofibers. Our preliminary results using a confocal microscope showed that many of the MSCs in the heart were located near the cardiac valves in the internal wall of the heart. It is known that cardiac valves contain connective tissues secondarily formed within cardiac contractile tissues. Identification of cellular status of these GFP-positive cells would lead us to further understanding of developmental process of cardiac valves. We also conducted the genome-editing experiment using CRISPR/Cas9 method. We synthesized two different guide RNAs, tar1 and tar2, both of which are targeting the coding sequence of the MSC marker gene. Guide RNA molecules were mixed with Cas9 protein and injected into fertilized eggs of medaka. Injected eggs were incubated until they reach early larval stage. Genomic DNA was extracted from each individual and the targeted genomic region was amplified by PCR. Amplified fragments were analyzed by polyacrylamide gel electrophoresis. About 30% of medaka larvae injected with tar1/Cas9 were found to be associated with indels in the targeted region of the MSC marker gene. However, experiments using tar2 did not produce mutation. We also succeeded in tail biopsy, in which DNA extracted from a small fragment of tail fin of juvenile medaka is used to identify the mutated individuals (F0). Approximately 100 F0 individuals have been obtained so far. Currently we are attempting to produce F1 generation of the mutants of the MSC marker gene by crossing these F0 with wild type medaka.

Discussion & Conclusion

In summary, we have developed the experimental methods to observe genetically labeled MSCs in medaka. We also established the protocol for genome editing and produced the mutant individuals of the MSC marker gene. One concern would be that, since this MSC marker gene is expressed in a wide variety of tissues starting early embryogenesis, homozygous deletion might cause embryonic lethality. In the future analysis, we will carefully observe the effect of gene disruption by comparing heterozygous and wild type individuals. Concurrently, we are analyzing adipose tissue generation in the medaka, to which MSCs is supposed to serve as the developmental origin. We will search for factors that inhibit fibrosis and adipogenesis of MSCs in muscle regeneration and sarcopenia. We aim to elucidate the role of MSCs in locomotor homeostasis and pathology by utilizing the convenience of fish models and shed light on their role as part of the maintenance mechanism of locomotive systems.

一般の皆様へ

私たちの身体に備わる筋骨格系は、歩行などの明らかな運動に加え、呼吸、嚥下、発生など、生命の維持に不可欠である。そこでは筋繊維、骨、そして筋と骨をつなぐ腱の細胞だけでなく、その隙間を埋めるように、不定形の間葉系間質細胞が存在する。本研究ではこのような細胞の、運動器の恒常性や老化、疾患、再生でのふるまいに迫るため、優れた小型魚類モデル・メダカを用いて、遺伝子レベルの解析を行なっている。本研究により、間葉系間質細胞のメダカ胚での分布を明らかにし、遺伝子変異が起こす異常を観察する手法も確立した。この実験系を活用し、発生過程や老化のステージで、筋肉の再生やサルコペニア、あるいはその抑制における役割を解明することを目指す。

Neurophysiological significance of regulation of molecular function by glutathionylation

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Summary Abstract

Post-translational modifications (PTMs) regulate protein function, yet S-glutathionylation remains poorly understood. Our research highlights the significance of S-glutathionylation in regulating key molecular events during synaptogenesis. It establishes Aurora-A as a direct substrate of the Glutathione S-transferase (GST)-mediated glutathionylation and demonstrates the broader involvement of this PTM in shaping the synaptic proteome. These insights contribute to a growing body of knowledge regarding redox signaling in the nervous system and open new avenues for exploring how oxidative modifications influence brain development and function.
Key Words : Glutathionylation, Synaptogenesis, protein kinase

Introduction

PTMs regulate protein function, yet S-glutathionylation remains poorly understood. Recent findings by our team reveal that S-glutathionylation activates Aurora-A kinase, enhancing local protein synthesis at postsynaptic sites and promoting synapse formation and maintenance in the brain. This suggests a novel mechanism linking glutathionylation to synaptic development. To explore this further, the current study aims to identify new regulatory pathways dependent on glutathionylation through comprehensive proteomic analyses and clarify their roles in neurophysiology.

Results

This study explores the regulatory mechanism of synapse formation through the PTM known as S-glutathionylation, focusing on its role in activating Aurora-A kinase, a process essential for synaptogenesis. Aurora-A is a serine/threonine kinase previously known for its functions in cell cycle regulation. However, recent findings have demonstrated a novel function for Aurora-A in neurons, promoting local protein synthesis and synaptic development through glutathionylation, but the mechanism leading to this site-specific modification remained unclear.

To address this, we expressed HA-tagged wild-type Aurora-A and a glutathionylation-deficient mutant in cultured neuronal cells. Immunoprecipitation with anti-HA antibodies followed by immunoblotting with anti-glutathione antibodies showed that glutathionylation occurs at specific cysteine residues. Notably, this modification was temporally regulated, occurring prominently during the synaptogenesis phase and persisting thereafter. This result highlights the specificity of glutathionylation and suggests its functional importance in

neuronal maturation and network formation.

Next, the researchers sought to identify the enzyme responsible for catalyzing the addition of glutathione to cysteine. GSTs are known to mediate such reactions, and a systematic screen was performed using a cultured synapse model. A glutathione transferase was identified as a key enzyme facilitating this modification among the GST family. When this enzyme expression was knocked down via RNA interference, there was a significant decrease in Aurora-A glutathionylation. This suppression was accompanied by a marked reduction in synapse formation, underscoring the essential role of the enzyme in regulating synaptic development via Aurora-A activation.

To broaden the understanding of how glutathionylation might influence synaptic signaling more generally, we conducted a comprehensive proteomic analysis of glutathionylated proteins. This was done at different stages of neuronal network formation using a modified version of a published protocol (3). The proteomic approach allowed for isolating and identifying a wide array of glutathionylated proteins. Aurora-A emerged as a central player among these, but many other synaptic structural proteins were also identified. Interestingly, the proteomic data revealed enzymes such as lipid kinases, which are not traditionally associated with synapse formation. This suggests that S-glutathionylation may regulate previously unrecognized pathways involved in neuronal development.These findings point to a complex and dynamic landscape of redox-based regulation in neuronal cells, where glutathionylation is a critical synaptic architecture and function modulator. The study provides new insights into how redox biology intersects with neurodevelopmental processes by identifying a GST as the enzyme catalyzing Aurora-A activation and uncovering a network of other glutathionylated synaptic proteins. The results shed light on a novel activation mechanism for a well-known kinase and raise the possibility that manipulating protein glutathionylation could represent a therapeutic strategy for disorders characterized by impaired synapse formation or maintenance.

Discussion & Conclusion

This study expands on our discovery that Aurora-A signaling promotes synaptogenesis (1, 2). Since abnormal synapse formation is linked to neurodevelopmental disorders such as intellectual disability and autism spectrum disorder, which affect about 2% of the global population, understanding these processes is critical for developing new treatments. However, even among well-known PTMs, key regulatory mechanisms remain unclear. To address this, we will combine molecular biology, structural analysis, and computational modeling to investigate how glutathionylation modulates protein function during synapse formation. In addition, we will validate these findings in vivo using genetically engineered animal models targeting glutathionylation pathways. Through this cross-disciplinary approach, we aim to clarify the physiological roles of glutathionylation in brain development and contribute to identifying novel therapeutic targets for neurodevelopmental disorders.

In summary, the research highlights the significance of S-glutathionylation in regulating key molecular events during synaptogenesis. It establishes Aurora-A as a direct substrate of the GST-mediated glutathionylation and demonstrates the broader involvement of this PTM in shaping the synaptic proteome. These insights contribute to a growing body of knowledge regarding redox signaling in the nervous system and open new avenues for exploring how oxidative modifications influence brain development and function.

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一般の皆様へ

この研究では、酵素のはたらきをコントロールする「S-グルタチオニル化」という特別な仕組みが、脳の神経細胞同士がつながって情報をやり取りする「シナプス」という構造をつくる上で大切な役割をしていることに注目しました。シナプスの異常な形成は、自閉スペクトラム症などの神経の発達に関わる病気とも関係があると考えられているため、こうした仕組みを詳しく理解することは、新しい治療法を見つける手がかりになります。今後はコンピュータを使った解析なども取り入れながら、この仕組みがどのようにして酵素のはたらきを変えるのか、さらに詳しく調べていく予定です。

Molecular mechanism of programed DNA elimination

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Summary Abstract

Programmed DNA elimination (PDE) is a phenomenon, in which specific regions of chromosomes are selectively removed in somatic cells during particular developmental stages. *Caenorhabditis auriculariae* undergoes PDE during 8-cell stage embryos and its karyotypes is changed. To understand the physiological meaning and the molecular mechanism underlying PDE in nematode, we used genetical, cytological and biochemical approaches. Since the knockdown of several candidate proteins resulted in the inhibition of PDE, we are characterizing these proteins as a clue of unveiling the molecular mechanism of PDE.

Key Words : Programmed DNA elimination, nematode

Introduction

Programmed DNA elimination (PDE) is an atypical developmental process in which specific chromosomal regions are selectively removed from somatic cells. In *Caenorhabditis auriculariae*, PDE occurs at the 8-cell stage, leading to a distinct change in karyotype. To investigate the physiological role and molecular mechanism of PDE, we applied genetic, cytological, and biochemical approaches. Knockdown of several candidate proteins inhibited PDE, indicating their potential involvement. We are currently characterizing these proteins to elucidate their roles and uncover the molecular basis of PDE in nematodes.

Results

Although *Caenorhabditis auriculariae* belongs to the same genus as the model organism *C. elegans,* WGS and Hi-C analysis suggest that it undergoes PDE. The karyotype of *C. auriculariae* in germ cells is 2n = 12, which is similar to most other *Caenorhabditis* species. After PDE, it changes to 2n = 26 in somatic cells after PDE with the elimination of the terminal and internal regions of each chromosome. A total of 2% of the genome (2.4 Mb), including approximately 300 genes and repetitive sequences, is eliminated and new telomeres are added to the breakpoints of each chromosome.

To elucidate the molecular mechanisms underlying PDE in *C. auriculariae,* we developed the genomic tools based on the methods established for *C. elegans*, including RNA interreference (RNAi), genome editing by CRISPR/Cas9 methods, and construction of transgene expressing strains. Using fluorescence in situ hybridization (FISH) to detect telomeric and repetitive sequences at the eliminated regions, we first observed that PDE in *C. auriculariae* occurs in the somatic blastomeres during the 8-cell stage embryos. Although the telomeric signals rapidly disappeared during the interphase of 8-cell stage blastomeres

before nuclear envelope breakdown (NEBD), signals of the eliminated regions remained after chromosome cleavage and were excluded from nucleus in the cytosol. To observe the dynamics of chromosomes, we generated a worm strain expressing GFP::Histone. Before NEBD at the 8-cell stage embryo, the chromosomes fragment by their condensation, and the fragmented DNA moves toward the cell cortex during prometaphase and metaphase. It is excluded from the nucleus in subsequent cell stages.

Next, we examined the *C. auriculariae* homologs of several candidate proteins in *C. elegans*, based on the observations of the FISH experiments. The RNAi knockdowns of four candidate genes were resulted in the highly embryonic lethality and PDE inhibition. These results suggest that these genes are directly or indirectly involved in the PDE process. Furthermore, these results suggest that PDE is an essential process in *C. auriculariae* embryogenesis that may eliminates the unnecessary genes to prevent abnormal expression and/or other issues. These proteins localize at the telomeric regions, as demonstrated by immunostaining and immuno-FISH. Using a strain with direct tagging if the endogenous gene by CRISPR-Cas9, we found that two of these proteins are co-localize at the telomere region. After PDE at the 8-cell stage, the signals of these proteins were detected with the eliminated DNA fragments in the cytosol. This indicates that these proteins are also excluded with eliminated DNA fragments from the nucleus after PDE.

To characterize these candidate proteins, we attempted to purify them for in vitro assays. Since expressing each protein with a His-tag resulted in the insoluble, we fused the proteins with a maltose binding protein (MBP)-tag to improve the solubility and expressed them in the insect cells. After the cells expressing protein were lysed, one MBP-fused candidate protein was found in the precipitated fraction with genomic DNA, while they were found in the supernatants fraction when the cellular lysate was sonicated. These results suggest that this protein have high capacity to bind DNA stably. Currently, we are working to determine the conditions necessary for high-quality purification after His-tag affinity chromatography, to analyze the DNA-binding activity of these proteins with specific sequences, including telomere DNA, and interactions among these proteins.

Discussion & Conclusion

Although programmed DNA elimination (PDE) was first discovered approximately 140 years ago, research on this phenomenon has progressed slowly due to its atypical nature and its occurrence in a limited number of species, such as parasitic nematodes. However, recent WGS analyses have identified PDE in several species, suggesting that this process has evolved independently multiple times across the phylogenetic tree.

C. auriculariae is a valuable model system for studying PDE, as it belongs to the same genus as well-established model organism *C. elegans*. This allow us to use extensive existing knowledge on gene annotation, characterized various molecular pathways, and advanced genetic tools. Our current focus is on the several candidate proteins whose knockdown by RNAi results in the inhibition of PDE and severe embryonic lethality. The cellular localization of these proteins also strongly suggests their roles involving in PDE. We are characterizing these proteins using both in vivo and in vitro approaches to elucidate their roles and gain insight to the molecular mechanism underlying PDE.

一般の皆様へ

生物の設計図であるゲノムは、生物の個体を作り上げるすべての細胞で同じである。一方、一部の生物では、体細胞でゲノム DNA の情報が一部削減される PDE（染色体削減）という現象を示すが、その生物学的な意義や分子機構はわかっていない。我々は、センチュウの一種で PDE が起きていることを見出し、多角的なアプローチでその分子メカニズムを明らかにしようとしている。これまで、受精卵の 8 細胞期の体細胞系列の細胞核内で PDE がおこり、染色体の断片化と排除される DNA 断片が細胞質に移動する様子を観察している。さらに複数の因子をノックダウンすると、PDE 反応が停止し受精卵が孵化できなかった。今後、これらの因子の役割を詳しく調べることで、この染色体を断片化するという非常に特殊な生命現象の分子機構の解明を目指していきたい。

Identification of the ammonia detoxication pathway as a crucial metabolic mechanism involved in acute myeloid leukemia propagation

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Summary Abstract

Acute myeloid leukemia (AML) is a highly aggressive hematological malignancy characterized by the clonal expansion of immature myeloid blasts. Recent research highlights the importance of AML-specific metabolic activities, particularly amino acid catabolism, which supports leukemia progression. However, this process generates high mitochondrial ammonia levels, potentially leading to toxicity. Our study explored how AML cells manage increased amino acid catabolism and mitigate ammonia accumulation. We identified a unique, cell-intrinsic ammonia-detoxification mechanism in AML, involving an ectopically activated urea cycle and an ammonia-recycling pathway. AML cells utilize the urea cycle to prevent ammonia accumulation and convert excess ammonia into non-essential and essential amino acids via the ammonia-recycling pathway.
Key Words : Acute myeloid leukemia, NH3, OXPHOS, CPS1, metabolism

Introduction

Recent studies have highlighted the importance of metabolic characteristics specific to AML including the pronounced reliance on amino acids catabolism. The enhanced activity of amino acid catabolism, however, inevitably produces high levels of ammonia in mitochondria, which can be associated with cellular toxicity via inducing mitochondrial dysfunction and ROS production. In humans, at the organismal level, ammonia detoxification occurs through the urea cycle in hepatocytes. The full activity of the urea cycle is mainly confined to hepatocytes; non-hepatocytes lack the mechanisms for active detoxification of ammonia except for secretion. AML cells produce profound cellular ammonia which severely impairs the mitochondrial function; however, the molecular mechanisms by which AML cells mitigate the cellular toxicity arising from excessive ammonia accumulation remain largely unexplored.

Results

1. AML cells ectopically utilize the urea cycle to avoid cytotoxicity of ammonia

Based on our metabolome database[1], we found that AML cells contained higher levels of AAs and catabolize AAs, leading to the active produce ammonia in vitro and in vivo. Furthermore, the intermediate metabolites related to urea cycle were significantly elevated in primary $CD34^+$ AML cells as compared to those in normal $CD34^+$ hematopoietic stem progenitor cells (HSPCs). We, therefore, hypothesized that AML cells ectopically utilized

the urea cycle activity to avoid the accumulation of cellular ammonia which damaged mitochondria. To test this, we inhibited the urea cycle activity by using specific inhibitor for CPS1, a rate limiting enzyme of the urea cycle, and found that inhibiting CPS1 significantly increased the cellular ammonia and metabolites related to the urea cycle.

2. AML cells activate the ammonia-recycling pathway to avoid excessive accumulation of cellular ammonia

A recent study revealed a cancer-specific ammonia-recycling pathway to maintain biomass by consuming alpha ketoglutarate (α-KG), an important intermediate metabolite of the TCA cycle. In the ammonia-recycling pathway, cancer cells synthesize Glu from ammonia and α-KG through glutamate dehydrogenase 1 (GDH). To evaluate the activity of ammonia-recycling pathway in AML, we performed stable-isotope tracing experiments using [$^{15}N_1$] NH_4Cl, and found that AML cells utilized the ammonia-recycling pathway to reduce cellular ammonia level. Thus, the ectopically activated urea cycle and the ammonia-recycling pathway coordinately reduce cellular ammonia level to avoid its cellular toxicity.

Furthermore, inhibiting CPS1 which resulted in the increment of cellular ammonia via suppressing urea cycle activity significantly promoted the alternative ammonia detoxification pathway: the ammonia-recycling pathway, leading to the consumption of α-KG. Consistent with the consumption of α-KG, the accumulation of cellular ammonia in AML significantly suppressed OXPHOS in mitochondria.

3. CPS1 inhibition represents an effective therapeutic approach against AML, while sparing normal human hematopoiesis

Considering that human AML cells, especially immature LSCs, heavily rely on OXPHOS for their energy production, we investigated whether inhibiting CPS1 would have an anti-AML effect. We investigated the anti-AML effect of a CPS1-specific inhibitor in vivo. Primary AML cells were transplanted into the irradiated NSG mice. Four to six weeks after transplantation when the reconstitution of human AML was confirmed by blood sampling, we started the daily intraperitoneal injection of CPS1 inhibitor. After the completion of treatment, the recipient mice were euthanized for evaluation. We observed the significant reduction of human AML cells in BM of the recipient mice.

Discussion & Conclusion

In this study, we show that human AML cells actively utilize the urea cycle and ammonia-recycling pathway to avoid excessive accumulation of cellular ammonia, which can be inevitably produced by high metabolic activity of multiples AAs and nucleotides. We further demonstrate that CPS1, a rate-limiting enzyme of the urea cycle, represents an effective therapeutic target for human AML via suppressing OXPHOS activity.

一般の皆様へ

本研究により、AML 細胞の代謝特性であるアミノ酸異化反応により細胞内で生じるアンモニア処理機構を明らかにすることができた。AML 細胞は本来肝細胞が用いる尿素サイクルを異所性に活性化させ利用することでアンモニアの毒性を軽減していることを明らかにした。また、この異所性に活性化した尿素サイクル経路の阻害が AML 治療に応用できる可能性を示すことができた。

The elucidation of the inflammatory response that exacerbates the infection pathogenesis of drug-resistant Staphylococcus aureus

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Summary Abstract

Excessive activation of the inflammasome can worsen infectious diseases. Although Staphylococcus aureus is known to activate the inflammasome, the molecular mechanisms by which the pathogen induces inflammasome activation remain poorly understood. In this study, we found that Staphylococcus aureus activates the serine/threonine Aurora kinase A, which directly phosphorylates the inflammasome adaptor protein to promote caspase-1 activation. Notably, administration of Aurora inhibitor reduced bacterial burdens in organs of Staphylococcus aureus-infected mice and improved mouse survival, even at lethal doses of antibiotic-resistant Staphylococcus aureus. These results reveal an unrecognized function of Aurora A in the regulation of the inflammasome and suggest an alternative therapeutic strategy for bacterial infection.

Key Words : Staphylococcus aureus, Inflammatory response

Introduction

Drug resistance in bacteria such as Staphylococcus aureus is progressing on a global scale[1)]. When these bacteria infect the host, various inflammatory responses are triggered by detecting microbial ligands. While some of these inflammatory responses contribute to the host defense, we have found that pathogens such as Listeria monocytogenes exacerbate infection by inducing an inflammatory response known as the inflammasome[2)]. As virulence factors produced by pathogens exacerbate infections by enhancing the inflammasome response, activation of the inflammasome is a critical factor that affects infection severity. In this study, we aim to elucidate the mechanisms by which drug-resistant pathogens activate the inflammasome, and how the inflammasome response worsens infection pathogenesis.

Results

The inflammasome, an innate immune system, is a protein complex consisting of an intracellular receptor, an adaptor molecule apoptosis-associated speck-like protein containing caspase recruitment domain (ASC), and a cysteine protease pro-caspase-1. Inflammasome mediates the conversion of pro-caspase-1 into the bio-active form, which triggers subsequent maturation of proinflammatory cytokines, interleukin-1β (IL-1β) and IL-18, and activation of pyroptosis through the cleavage of the pore-forming protein gasdermin D by recognizing pathogen-associated molecular patterns (PAMPs) and damage-

associated molecular patterns (DAMPs)[3,4]. Seven types of molecules have been identified as intracellular receptors that activate the inflammasome, and we have reported that Listeria monocytogenes exacerbates infection through the inflammasome response[2].

Staphylococcus aureus is a major human pathogen that causes bacteremia, pneumonia, dermatitis, and food-borne infections[1]. Staphylococcus aureus can display high morbidity which is associated with the development of resistance to anti-microbials including methicillin-resistant S. aureus (MRSA) and vancomycin-resistant S. aureus in both community and hospital settings. To know what intracellular receptors are activated during Staphylococcus aureus infection, we differentiated bone-marrow cells to macrophages, and infected with MRSA. Previous studies have been revealed that Staphylococcus aureus activates inflammasomes through NLR family pyrin domain-containing protein 3 (NLRP3) and NLRP6 in infected macrophages[2,5,6]. In addition to these intracellular receptors, we found that absent in melanoma 2 (AIM2), an intracellular sensor for double-strand DNA, is also activated in macrophages infected with Staphylococcus aureus, suggesting that bacterial DNA of Staphylococcus aureus is released into the cytoplasm from the phagosome. To investigate the downstream signaling pathways of AIM2, macrophages were treated with various inhibitors after infection. As a result, it was found that MRSA activates the inflammasome via JNK, a serine/threonine kinase, leading to the phosphorylation of Aurora A also called serine/threonine kinase 6. Activation of Aurora A promoted inflammasome activation by phosphorylation of ASC, an adaptor molecule of inflammasome complex. To test an impact of inflammasome activation in mice infected with MRSA, we intravenously infected mice with MRSA. Notably, bacterial loads in the liver of ASC-deficient mice were less that of wild-type mice. Furthermore, ASC-deficient mice showed more resistant to MRSA infection than wild-type mice. These results indicate that activation of inflammasome exacerbates MRSA infection. Because JNK-Aurora A signaling is indispensable for inflammasome activation after Staphylococcus aureus infection, we next evaluated the effect of inhibiting the signaling pathway in vivo. The mice infected with a lethal dose of methicillin-sensitive Staphylococcus aureus were rescued by administration of β-lactam antibiotic piperacillin, a semisynthetic penicillin with a wide spectrum of antimicrobial activity, however, all mice succumbed to an infection with MRSA regardless of piperacillin administration. Notably, 80% of Aurora kinase inhibitor Tozasertib-treated mice survived after MRSA infection, which was untreatable with piperacillin, whereas all control mice succumbed. Bacterial burden in the liver was clearly reduced on day 2 by Tozasertib administration. Consistent with these results, the amounts of IL-1β and IL-18 were greatly reduced in the serum of mice treated with Tozasertib compared to control mice. These results suggest that MRSA infection can be controlled independently of antibiotics by blocking the JNK-Aurora A axis through the inhibition of inflammasome responses.

Discussion & Conclusion

In this study, we found that JNK acts upstream of Aurora A to phosphorylate ASC. Because JNK has been reported to promote inflammasome activation via ASC in the context of other pattern-recognition receptors including NLRP3, it is possible that Aurora A is also activated in response to other triggers in addition to Staphylococcus aureus infection. Because the roles of Aurora kinase have been mainly studied as a therapeutic target for

cancer, there is little information about the roles of Aurora kinase in the pathophysiology of infection. However, recent studies showed that Aurora kinase promotes inflammation and Aurora kinase inhibition was suggested as a therapeutic candidate for inflammatory disease. Our studies suggest Aurora-targeted therapy can be an alternative approach to treat certain infections such as MRSA that cannot be treated effectively due to resistance to antimicrobial agents.

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一般の皆様へ

薬剤耐性菌の世界的蔓延が大きな医療問題に発展しており、なかでも国内の黄色ブドウ球菌臨床検体の80％以上がすでに薬剤耐性を獲得していることから緊急性が高い。抗生物質などの菌体を標的とする治療法は新たな耐性菌の出現を誘発する危険性が高いことから、本研究では感染症における炎症応答に着目して新たな治療標的を探索した。その結果、インフラマソームとよばれる自然免疫システムが活性化することで感染病態が悪化することを見出した。また、インフラマソーム応答を抑制する阻害剤を投与することで薬剤耐性黄色ブドウ球菌感染を改善できたことから、既存の抗菌薬に代わる新たな治療標的を見出すことができた。

Mechanism of action of antibiotics targeting the mammalian mitochondrial protein synthesis system

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Summary Abstract

In this study, we investigated the mechanisms by which antibiotics inhibit mitochondrial translation. Through mitoribosome profiling, cryo-EM, and a reconstituted mtPURE system, we demonstrate that CHL and LZD induce ribosomal stalling in a nascent peptide sequence-dependent manner, particularly when alanine, serine, or threonine occupy the penultimate position. Cryo-EM revealed distinct LZD-binding features unique to the mitoribosome. These findings highlight the context-specific nature of mitochondrial toxicity and provide insight into the selective cytotoxicity of these drugs. The mtPURE system was essential for mechanistic dissection and may guide rational antibiotic design with reduced side effects.
Key Words : Mammalian mitochondria, translation, antibiotics

Introduction

Mitochondria are organelles that produce ATP via oxidative phosphorylation and have their own genome and translation system. Due to their bacterial origin, many antibiotics also target mitochondrial ribosomes, sometimes causing side effects like hearing loss. Recently, some antibiotics have been shown to selectively eliminate cancer cells by inhibiting mitochondrial protein synthesis, while having minimal toxicity to normal cells. Structural analyses suggest that mitochondrial ribosomes have distinct antibiotic binding modes compared to bacterial ones. This study aims to elucidate the molecular mechanisms of four antibiotics (TIG, LZD, CHL, Q/D) using a reconstituted mitochondrial translation system (mtPURE system).

Results

To investigate how the antibiotics TIG, LZD, CHL, and Q/D inhibit mitochondrial translation, we employed a combination of mitoribosome profiling, in vitro translation assays, and cryo-electron microscopy (cryo-EM). This study was conducted in collaboration with the Fujimori Lab at the University of California. While their team focused on ribosome profiling and structural analysis, we performed the in vitro translation experiments using a reconstituted mitochondrial system

1. Context-Specific Inhibition Patterns

A major finding of this study is that chloramphenicol (CHL) and linezolid (LZD) induce mitoribosomal stalling in a peptide-sequence-dependent manner. Mitoribosome profiling experiments showed that these antibiotics caused pronounced stalling at specific codons

during mitochondrial translation, with the frequency of stalling closely tied to the amino acid occupying the penultimate (P–1) position in the nascent peptide. Codons encoding alanine, serine, or threonine in the P−1 position were particularly associated with ribosome stalling. This phenomenon parallels context-specific inhibition previously observed in bacterial systems, confirming that sequence-context dependency is a conserved feature in translation inhibition, even within the evolutionarily divergent mitochondrial ribosome.

2. Validation via In Vitro Translation Assays

To validate the context-specific stalling observed in mitoribosome profiling, we employed an in vitro mitochondrial translation assay using the mtPURE system, a fully reconstituted protein synthesis system derived from mammalian mitochondria (Lee, 2021). This system, which we previously established, consists of purified mitochondrial ribosomes, translation factors, tRNAs, and aminoacylation enzymes, and uniquely allows for precise control over both mRNA sequences and nascent peptide compositions. As such, it is currently the only platform capable of dissecting antibiotic effects at single-codon resolution in a mitochondrial context.

Using this system, we synthesized peptide chains from designed mRNAs containing codons that encode residues of interest in the penultimate (P−1) position—specifically alanine, serine, and threonine, which had been implicated in profiling as stalling-prone. Upon exposure to CHL or LZD, the mtPURE system faithfully reproduced the context-specific inhibition patterns: peptides with susceptible P−1 residues exhibited marked reductions in synthesis efficiency, confirming that the primary sequence of the nascent chain directly influences susceptibility to translational arrest. In contrast, sequences lacking these residues were translated more efficiently even in the presence of the antibiotics.

These results not only validate the mitoribosome profiling data in a highly controlled biochemical setting but also emphasize the critical role of nascent peptide identity in determining antibiotic sensitivity. Because the mtPURE system excludes other cellular variables and permits systematic manipulation of codon usage and peptide context, it serves as an indispensable tool for mechanistic dissection of ribosomal inhibitors acting on human mitochondria.

Moreover, these findings have broader implications: they suggest that mitochondrial toxicity and therapeutic selectivity of antibiotics such as CHL and LZD may stem from their sequence-biased inhibition. In therapeutic scenarios, proteins encoded by mitochondrial genes with vulnerable codon contexts may be disproportionately affected, potentially contributing to cytotoxicity in sensitive tissues or selective efficacy in cancer cells.

3. Structural Basis of LZD Inhibition Revealed by Cryo-EM

To gain insight into the molecular interactions underlying this selectivity, we used cryo-EM to visualize the binding of LZD to the human mitoribosome. The resulting structures show that LZD binds at the peptidyl transferase center (PTC)—a central site of catalytic activity in the ribosome, and a common antibiotic target. While the overall binding site aligns with that in bacterial ribosomes, several key differences were observed. Notably, LZD adopts a distinct binding conformation, and the human mitoribosome exhibits a unique hydration shell—a network of water molecules surrounding the binding pocket—which helps stabilize LZD in a configuration distinct from that seen in bacteria.

Discussion & Conclusion

Implications for Antibiotic Toxicity and Drug Design

Because CHL and LZD can inhibit mitochondrial protein synthesis, their clinical use is associated with side effects such as myelosuppression or lactic acidosis, especially with prolonged exposure. However, the context-specific nature of inhibition suggests that not all mitochondrial translation is equally affected—only specific nascent chain contexts are vulnerable. This insight opens possibilities for developing antibiotics with reduced mitochondrial toxicity by avoiding interaction with critical human mitoribosome contexts or modifying the drug's structure to selectively bind bacterial but not mitochondrial ribosomes.

Broader Relevance

The study highlights the importance of sequence-specific interactions between nascent peptides and ribosome-bound antibiotics, emphasizing the dynamic and context-sensitive nature of translation inhibition. It also demonstrates the utility of mitoribosome profiling, a technique adapted from cytosolic ribosome profiling, in uncovering mechanistic insights at high resolution.

Ongoing Work and Future Directions

In this project, we have elucidated the mechanisms of CHL and LZD in mitochondrial translation, contributing to a recent publication (Bibel et al., 2025) in collaboration with the Fujimori Lab. These findings closely reflect the objectives of the supported research. We are currently extending our study to the remaining antibiotics, tigecycline (TIG) and quinupristin/dalfopristin (Q/D), using the same mtPURE platform. Results from this ongoing work will be submitted for publication with explicit acknowledgment of the foundation's support.

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一般の皆様へ

薬剤耐性菌の増加により、新しい抗生物質の合理的な設計開発は喫緊の課題となっています。本研究では、抗生物質がミトコンドリアのタンパク質合成をどのように阻害するかを、試験管内で再構築した系（mtPURE system）を使って詳しく調べました。その結果、特定のアミノ酸配列で翻訳が停止する仕組みを明らかにし、薬の副作用の一因であることを突き止めました。これにより、副作用を抑えた抗生物質の開発だけでなく、ミトコンドリアタンパク質合成系を標的とした有効な抗がん剤の開発にも道が拓けると期待されます。

Establishment of simultaneous paralogous inhibition toward the SMAD4-deficient pancreatic cancer therapy

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Summary Abstract

In order to develop therapeutic agents in pancreatic cancer cells deficient in the cancer suppressor gene SMAD4, this study aimed to search for paralogous molecules in a synthetic lethal relationship with SMAD4. First, we aimed to establish a screening method. The commercially available pgPEN library, which consists of 1030 paralog pairs, can be simultaneously knocked out paralog pair with the CRISPR/Cas9 system. In validation of this screening system in HEK293T cells, the gRNAs for growth essential genes were likely to decrease. Also, genetic isogenic cell lines of pancreatic cancer cells were generated to examine the dependence of SMAD4 expression.
Key Words : SMAD4, synthetic lethal, paralog, pancreatic cancer, screening

Introduction

Pancreatic cancer deficient in SMAD4, one of the cancer suppressor genes, is classified as a refractory cancer and therapeutic agents are insufficient. In such defective cancers, therapeutic agents based on synthetic lethality are promising. Synthetic lethality is a phenomenon in which significant cell death is induced only when two factors are simultaneously suppressed. To date, several synthetic lethal relationships have been reported, involving frequently between paralogues [1-4]. In this study, we aimed to establish a genetic screening system that simultaneously inhibits paralogs and to identify the integral synthetic lethality consisting of three factors in pancreatic cancer deficient in the cancer suppressor gene SMAD4.

Results

Recent advance in genetic technologies have made possible to perform the comprehensive genetic screening, and assay systems that can simultaneously suppress two genes, such as the pgPEN library (#171172, addgene), are now available [5]. In this study, we first examined the pgPEN library screening using HEK293T cells. Referring to similar previous studies, the multiplicity of infection (MOI) was determined to be 0.3, and the concentration of puromycin used to select for the lentiviral library infection in HEK293T cells was evaluated. As a result, 1 μg/ml puromycin was likely to be optimal in HEK293 cells. Screening with pgPEN library was then performed on HEK293T cells, in culture for 14 days after puromycin selection. Genomic DNA was extracted from cells after drug selection and from cells cultured for 14 days. The number of viable cells transduced with each gRNA was calculated as the number of reads using a next-generation sequencer. The pgPEN library

used in this study contains gRNAs against essential genes for cell growth. It was expected that the growth of cells transduced with gRNAs against these genes would be suppressed. In fact, these transduced cells appeared to be enriched in a side of decrease after 14 days of culture. In addition, it is commonly recommended that the number of reads from next-generation sequencing analysis in a genetic screening such as our screening is 500 times of the library size. However, the results showed that the total number of reads per sample was not sufficient to reach the recommended number of reads. Therefore, we are currently considering improvements to the sequencing analysis method and library downsizing.

In order to identify the synthetic lethal factors with SMAD4 in pancreatic cancer cells, we attempted to generate the isogenic clonal cell lines using patient-derived pancreatic cancer cell lines. Approximately 40 strains of patient-derived pancreatic cancer cells are held at the National Cancer Center Research Institute. Of these, 22 pancreatic cancer cell lines were evaluated for SMAD4 expression at the protein level and ability of cell proliferation. Western blotting analysis showed that 10 of the 22 cell lines did not express SMAD4 endogenously due to genetic mutation, and all of the remaining cell lines were endogenous SMAD4-expressing pancreatic cancer cells. Among these SMAD4-deficient or SMAD4-expressing pancreatic cancer cell lines, isogenic clones were generated for each of the two cell lines that exerted relatively high proliferative potential. The desired clonal cell lines were selected by limiting dilution method with lentiviral vectors. As a result, several clones stably expressing SMAD4 exogenously were obtained for pancreatic cancer cell lines that delete SMAD4 endogenously. On the other hand, for the endogenous SMAD4-expressing pancreatic cancer cell lines, we also succeeded in selecting several genetically deficient clones in SMAD4 with the CRISPR/Cas9 system. Using these isogenic lines, we are performing a library screening of the CRISPR/Cas9 system for paralog pairs that are different from pgPEN, and attempting to identify paralog pairs that are synthetic lethal factors with SMAD4 in pancreatic cancer cells.

Discussion & Conclusion

It is easily expected that the number of knockout cells for growth essential genes will be reduced. It is also suggested that knockout cells for synthetic lethal genes will be reduced. Therefore, in this study, it is necessary to accurately count the small number of cells. The depth of sequencing analysis is recommended to cover the screening library size of 500 times the number of reads. However, our condition was not fully covered. The improvements in this result include increasing the depth of sequence analysis, increasing the number of analyses of the same sample, and reducing the size of the library itself. We are currently improving this analysis method to avoid high cost and low throughput. In addition, we have succeeded in obtaining isogenic clone lines with genetically modified SMAD4 expression in several pancreatic cancer cell lines. It is suggested that these cell lines will be useful not only for the identification of synthetic lethal factors with SMAD4 in pancreatic cancer cells, but also for the analysis of SMAD4 molecular functions.

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一般の皆様へ

本研究は、がん抑制遺伝子 SMAD4 の機能消失が見られる膵臓がんに対する新規治療薬開発を指向したものである。このような機能欠損型のがんは、難治性がんとして分類され、治療薬がまだまだ満足できるほどに至ってはいない。そこで、本研究では、合成致死という概念を利用して、この難治性がんの治療薬開発に取り組む。合成致死とは、2 つの因子が機能抑制されることによって初めて顕著な細胞死が誘導される現象のことである。つまり、SMAD4 と合成致死の関係にある因子を見つけ出し、その機能阻害をする治療薬は、SMAD4 欠損型がんに対して特異的に効果を発揮する抗がん剤になることが期待される。

Identification of osteoclast cell fusion factors by expression cloning method

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Summary Abstract

To identify osteoclast cell fusion factors, I am establishing cDNA library screening strategy. I have confirmed that these factors are likely expressed downstream of the RANK-RANKL signaling pathway. Based on this finding, I constructed cDNA library from the messenger RNAs of differentiating RAW264 cells, which will be introduced into RAW264 cells via lentivirus infection. While these achievements are significant, I also encountered difficulties in manipulating large, multinucleated cells *in vitro*. Once this challenge is overcome, functional screening can be initiated.

Key Words : Cell fusion
Osteoclast
Unbiassed screening

Introduction

Osteoclastogenesis is a well-known physiological process involving cell-cell fusion. Osteoclasts are derived from macrophages and monocytes in mammalian species. When the cytokine RANKL binds to its receptor RANK on their cell surface, precursor cells become activated, proliferate and undergo cell-cell fusion to form multinucleated osteoclast. However, **the molecular mechanisms underlying osteoclast cell-cell fusion are not yet fully understood.**

In my project, I aim to identify osteoclast cell fusion factors using an expression cloning strategy with a cDNA library.

Results

【1】Osteoclast cell fusion is dependent on RANK-RANKL signaling.

Mouse macrophage cell line, RAW264, can be induced to differentiate into osteoclast by stimulating with RANKL protein. Four days after RANKL addition, RANKL binds to its receptor RANK on RAW264 cell surface, then the activated RAW264 cells fuse with each other and form multinucleated osteoclast. In other words, RANK-RANKL signaling pathway is essential for RAW264 cell fusion.

To test this hypothesis, I established Rank-knockout ($Rank^{-/-}$) RAW264 cell clones by CRISPR-Cas9 system. mCherry-expressing wildtype or $Rank^{-/-}$ RAW264 cells were co-cultured with EGFP-expressing wildtype cells and stimulated with RANKL. Wildtype cells could fuse with each other and differentiated into EGFP and mCherry double positive

osteoclast (**Fig 1(left)**). However, Rank$^{-/-}$ RAW264 cells did not participate in cell fusion, so mCherry-expressing cells stayed mononucleated while EGFP-expressing cells were differentiated to osteoclast (**Fig 1(right)**). Therefore, it is suggested that osteoclast cell fusion factor gene(s) are upregulated downstream of RANK-RANKL signaling pathway.

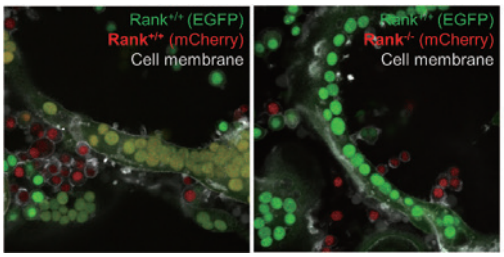

Fig 1. RAW264 cell fusion depends on RANK-RANKL signaling. EGFP or mCherry expressing Rank$^{+/+}$ or Rank$^{-/-}$ RAW264 cells were co-cultured. After stimulating with RANKL for 4 days, the colocalization of EGFP and mCherry was examined by confocal microscopy.

【2】RAW264 cells can be transduced by lentivirus infection

Based on the results in【1】, I hypothesized that RANKL-stimulated, differentiating RAW264 cells express cell fusion factor genes that are not expressed in undifferentiated state. To identify the cell fusion factor genes, I am designing an expression cloning strategy using cDNA library of differentiating RAW264 cells.

In our laboratory, retroviral vectors have been used for cDNA library construction due to their relatively small size and high transduction efficiency across a wide range of cell lines. However, retroviral infection is not recommended for RAW264 cells because of helper virus production (Hartley et al, 2008, Retrovirology). To overcome this issue, I tried lentivirus infection to express several genes ranging from 0.7 to 4 kb in length. The lentiviral system succeeded regardless of gene length and achieved an infection rate of over 30% (**Fig 2**). Based on the results, I decided to use a lentiviral vector to construct the cDNA library.

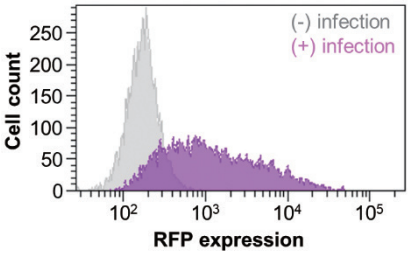

Fig 2. RAW264 cell fusion depends on RANK-RANKL signaling. RAW264 cells were transduced by lentivirus infection to express the gene tagged with RFP. 3 days after infection, infection efficiency was quantified by flow cytometry.

【3】cDNA library construction was completed.

Since lentivirus is more suitable for RAW264 cells than retrovirus (as shown in【2】), I constructed a cDNA library using a lentiviral vector. I extracted messenger RNAs from RANKL-stimulated, differentiating RAW264 cells, then reverse-transcribed into cDNAs. The cDNAs were inserted into a lentiviral vector and introduced into *E.coli* competent cells. As a result, I successfully obtained more than one million clones of a cDNA library.

【4】Manipulating multinucleated RAW264 cells is challenging.

My screening system is designed based on the hypothesis that *"if cell fusion factor gene is expressed in Rank$^{-/-}$ cells, they should acquire the capacity for cell fusion"*. After introducing cDNA library into Rank$^{-/-}$ RAW264 cells, I plan to sort osteoclast differentiated from cDNA library-expressing Rank$^{-/-}$ cells by flow cytometry. Before starting screening, I tested whether osteoclast is compatible with flow cytometry and the target osteoclast can be identified by detecting fluorescent proteins and analyzing DNA contents.

A suspension of RANKL-stimulated cells was applied to flow cytometer and it was confirmed that DNA content could be quantified by using DNA staining dye, so multinucleated cells can be discriminated from mononucleated ones. On the other hand, fluorescent protein signals were not detectable. To improve the sensitivity of fluorescent proteins, I tried to fix and stain with antibody, but, neither approach resolved the issue.

Discussion & Conclusion

To date, many candidate genes have been reported as osteoclast cell fusion factors (Sterling et al, 1998, JCB, Yagi et al, 2005, JEM etc.). However, none of them has been proven as a direct regulator of cell-cell fusion. These genes were primarily found by candidate approaches. I believe that I need to take a different approach from previous studies. In my project, to identify previously unreported candidate genes, I decided to take a functional screening approach, in other words, selecting RAW264 cells that acquired cell fusion capacity after cDNA library introduction.

Through the experiments conducted during the supported year, I encountered difficulties in manipulation of multinucleated cells. Osteoclasts were extremely large and fragile, and were easily destroyed during handling. I understand that these unique characteristics made it challenging to apply the multinucleated cells to *in vitro* experiments. The issue is not limited to osteoclast, but also applied to other fused cells such as myotubes and trophoblast. Once I establish a method to handle osteoclasts without compromising their structure, it will enable me to perform the functional screening of osteoclast cell fusion factors. I hope such techniques can be applied to other multinucleated cell research in the future.

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一般の皆様へ

破骨細胞は、とても小さな前駆細胞から発生します。小さな細胞が互いに融合することでとても大きな、複数の核を持つ破骨細胞になります。破骨細胞の発生過程について長らく研究されていますが、「どうやって細胞融合するのか」はもちろん、「破骨細胞が持つ複数の核はどのように連携するのか」、「細胞分化の開始に必要な因子はわかっていますが、終了する際は何がきっかけになるのか」など多くの謎が残されています。本研究を継続し、細胞融合因子スクリーニングの実験系を作り上げた暁には、その技術を生かして上述のような他の未解明な機構解明につなげたいと思います。

BOD1L mediates chromatin binding and non-canonical function of H3K4 methyltransferase SETD1A

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Summary Abstract

The H3K4 methyltransferase SETD1A plays an essential role in both development and cancer. Here, we discovered that BOD1L exhibits the highest correlated SETD1A co-dependency in human cancer cell lines. BOD1L knockout reduces leukemia cells in vitro and in vivo, and mimics the transcriptional profiles observed in SETD1A knockout cells. The loss of BOD1L immediately reduced SETD1A distribution at transcriptional start sites (TSS), induced transcriptional elongation defect, and increased the RNA polymerase II content at TSS; however, it did not reduce H3K4me3. These results reveal that BOD1L mediates chromatin and SETD1A, and regulates the non-canonical function of SETD1A in transcription.

Key Words : Epigenetics, Transcription, Leukemia, Non-canonical, BOD1L

Introduction

MLL/SET/COMPASS complexes exhibit H3K4 methyltransferase activity, and the catalytic domain is encoded by KMT2 family proteins. These enzymes have redundant and non-redundant functions during tumor development. SETD1A and SETD1B are homolog of Drosophila Set1. Previously, we showed that the specific role of SETD1A is determined by the internal FLOS domain via its binding ability with cyclin K. Cyclin K binds to the N-terminal conserved region of the FLOS domain; however, it is unclear how the C-terminal region of the FLOS domain contributes SETD1A functions.

Results

In the DepMap database (DepMap 22Q1 Public+Score, Chronos), we found that BOD1L was the top-ranked SETD1A co-dependent gene. To evaluate BOD1L expression levels in different cancer types, we compared the mRNA expression levels of BOD1L in GEPIA2 and TARGET datasets and found the highest expression in AML samples with MLL mutations. To investigate the region sensitive to sgRNA and the functional domain of BOD1L, we performed a CRISPR-tiling screen against BOD1L in the doxycycline inducible Cas9-expressing MOLM-13 (iCas9-MOLM-13) and MV4-11 (iCas9-MV4-11) human leukemia cell line, and identified a strong dependency on the Shg1 domain at the N-terminus, but not on the intrinsically disordered regions (IDR) and AT-hook at the C-terminus. Two *BOD1L* sgRNAs strongly reduced the expression of BOD1L itself, and significantly increased the proportion of cells undergoing apoptosis and G1 cell cycle arrest. The strong BOD1L

dependency on AML cells was also confirmed using a mouse MLL-r leukemia model *in vitro* and *in vivo.* These results suggest that BOD1L is an essential co-dependent factor with SETD1A for leukemia and cancer cell survival.

The expressions of downstream targets of SETD1A in AML cells, including *COX15* and *FANCD2*, were significantly downregulated in BOD1L knockout cells. To examine the roles of COMPASS complex and H3K4me3, we also performed RNA-seq analysis of *RBBP5* knockout leukemia cells. As expected, H3K4me3 was suppressed in *RBBP5* knockout leukemia cells. In contrast, *RBBP5* knockout cells showed a different gene expression profile compared with *BOD1L* knockout and *SETD1A* knockout cells. Collectively, these results show that BOD1L is an essential component of the non-canonical SETD1A function in transcriptional regulation. Notably, *BOD1L* knockout significantly reduced SETD1A signals in chromatin. NELFE, NTD, and Ser5P signals were strongly increased by BOD1L knockout, especially at the TSS of SETD1A-target (DR) genes. these results show that BOD1L is an essential component of the non-canonical SETD1A function in transcriptional regulation.

Discussion & Conclusion

Although BOD1L promotes RIF1-dependent NHEJ via the methyltransferase activity of SETD1A, we demonstrated the indispensable role of BOD1L in a non-canonical SETD1A-dependent transcriptional regulation in AML cells. While we did not detect the induction of DNA damage immediately after SETD1A degradation in leukemia cells within 24 h, there is a possibility that BOD1L is required for both DNA damage repair and transcriptional regulation. We also found that the BOD1L-SETD1A complex is adjacent to a group of transcription factors, which might contribute to the BOD1L recruitment at TSS along with the DNA binding ability of BOD1L. E2F family members which were found in this study are all classified as transcriptional repressors. These data indicate that the BOD1L-SETD1A complex function associates with release of transcriptional repression during cell cycle progression. However, the hierarchical transcriptional cascade of these molecules remains unclear. Further studies of the BOD1L-SETD1A complex will provide new insight into a relationship between DNA damage repair, transcription and replication.

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一般の皆様へ

ヒストン修飾酵素 SETD1A は白血病やがんの進行に関わる分子であるが、SETD1A の働きを抑制する薬や手法の開発には至っていない。本研究から SETD1A 結合分子である BOD1L 側を治療標的とすることで、SETD1A の働きを効率的に抑制できることが明らかとなった。SETD1A に依存性の強い白血病やがんの細胞に対して有効となる、効果的な治療法の開発につながることを期待している。

Elucidate the roles of SETBP1 mutations in hematopoietic malignancies and congenital Schinzel-Giedion syndrome.

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Summary Abstract

Gain-of-function SETBP1 mutations are linked to hematological malignancies and Schinzel-Giedion syndrome (SGS). While overexpression studies suggest a role in MDS, physiological functions are unclear. We generated a Setbp1-D868N knock-in mouse model. Hematopoietic-specific KI didn't show changes, but a double Asxl1 KI model developed monocytosis and AML with RAS mutations, mirroring human AML. The exporter of cargo was identified as a therapeutic target; its inhibition induced differentiation and apoptosis. SGS models (systemic and NCC-specific KI) replicated human features, with scRNA-seq revealing impaired osteogenesis in NCCs. These models will clarify SETBP1's roles in disease and development.

Key Words : SETBP1, disease model mouse, hematopoietic malignancies, congenital disorder, neural crest cells

Introduction

Gain-of-function *SETBP1* mutations are identified in hematological malignancies like myelodysplastic syndrome, juvenile myelomonocytic leukemia, and acute myeloid leukemia, and also in the congenital disorder Schinzel-Giedion syndrome (SGS). SETBP1 protein, normally degraded through phosphorylation-dependent ubiquitination, becomes stabilized due to mutations at phosphorylation sites. Prior overexpression studies linked *SETBP1* mutants to MDS via PP2A inhibition and HOXA9 induction. However, the physiological function of wild-type and mutant SETBP1 remains unexplored. Given potential artifacts in overexpression systems, we aim to create a new mouse model with a conditional knock-in of the prevalent *Setbp1*-D868N mutation at the endogenous locus to clarify SETBP1's role in transformation, hematopoiesis, development, and differentiation under physiological levels.

Results

While a hematopoietic-specific *Setbp1* knock-in (KI) mouse model did not alter hematopoietic stem cell properties in vivo, a double mutant model incorporating an *Asxl1* KI demonstrated pronounced monocytosis. This monocytosis was characterized by a distinct transcriptional program featuring *HoxA* and *Setbp1* expression. Notably, this double mutant model spontaneously acquired RAS pathway activating hotspot mutations in *Nras*, *Flt3*,

and *Ptpn11*. The emergence of these mutations led to the development of monocytic acute myeloid leukemia (AML) with serial transplantability, faithfully recapitulating the clinically observed co-occurrence of *ASXL1*, *SETBP1*, and *RAS* mutations in human AML.

To identify potential therapeutic vulnerabilities in *Setbp1*-mutated AML, we performed integrated pan-cancer drug screening and whole-genome CRISPR-Cas9 screens. These unbiased approaches revealed an unexpected dependence on the gene involved in exporting cargo proteins. Pharmacological of genetic inhibition of the protein induced monocytic differentiation followed by apoptosis in AML cells harboring *SETBP1* mutations. In vivo studies demonstrated that the inhibitor of the exporter of cargo achieved sustained remission in *Setbp1*-mutated AML xenograft models by downregulating key SETBP1 target genes, including *Hoxa9* and *Myb*.

Mechanistically, we investigated the role of the exporter of cargo in *SETBP1*-mutated leukemia. We found that the proteins physically interact with the SETBP1 protein. Beyond its well-established role in nuclear export, the protein was discovered to function as a chromatin regulator, facilitating leukemogenic transcriptional programs driven by SETBP1. This non-canonical role of the exporter of cargo highlights a novel therapeutic vulnerability in this aggressive leukemia subtype.

To model the congenital disorder Schinzel-Giedion syndrome (SGS), characterized by multiple malformations, increased cancer predisposition, and distinctive facial features resulting from germline *SETBP1* mutations, we generated a conditional KI mouse model utilizing the CAG-Cre driver to achieve systemic expression of a SETBP1 mutant. The CAG-Cre: *Setbp1* flox-KI mice exhibited key phenotypic features observed in human SGS, including craniosynostosis, skull base hypoplasia, and hydronephrosis, demonstrating a faithful recapitulation of the human disease. Given that many of the skeletal and craniofacial abnormalities observed in SGS are attributed to defects in neural crest cell (NCC) derivatives, we also generated a KI model using the NCC-specific *Wnt1*-Cre driver. The *Wnt1*-Cre: *Setbp1* flox-KI mice displayed a phenotype similar to the CAG-Cre model, suggesting a crucial role for *SETBP1* mutations within the neural crest lineage in the pathogenesis of SGS-related malformations. To further elucidate the downstream transcriptional consequences of SETBP1 mutations in SGS, we utilized a reporter mouse line to isolate NCC-derived cells from the developing skull. We performed bulk RNA-sequencing and single-cell RNA-sequencing (scRNA-seq) on these isolated cells. Our analysis revealed that the *Setbp1* mutation leads to a significant downregulation of osteogenic processes within cranial NCC-derived cells. This finding provides a molecular basis for the observed skull hypoplasia phenotype in our mouse models and contributes to understanding the pathogenesis of craniofacial abnormalities in SGS. These results are currently being compiled for manuscript submission.

Discussion & Conclusion

Our integrated analyses provide critical insights into the clonal evolution and therapeutic vulnerabilities of SETBP1-mutated leukemia. The faithful recapitulation of the *ASXL1*/*SETBP1*/*RAS* co-mutation AML in our novel mouse model underscores its utility for preclinical investigations. The unexpected identification of the gene involved in exporting cargo proteins as a key dependency, with inihibitor demonstrating in vivo efficacy, presents

a promising therapeutic avenue for this challenging disease. The discovery of the exporter of cargo's non-canonical role as a SETBP1-interacting chromatin regulator expands our understanding of leukemogenesis driven by this mutation.

Furthermore, our SGS mouse models, particularly the NCC-specific KI, accurately mirror key aspects of the human congenital disorder, providing valuable tools for dissecting its developmental pathogenesis. The identification of impaired osteogenesis in cranial NCCs as a consequence of the *Setbp1* mutation offers a mechanistic link to the observed skull abnormalities. These findings highlight the pleiotropic roles of SETBP1 in both hematological malignancies and developmental processes, emphasizing the importance of studying its function across different cellular contexts and disease states. The ongoing characterization of these models holds significant potential for informing future therapeutic strategies for both leukemia and SGS.

一般の皆様へ

私たちは、血液のがん（骨髄異形成症候群、白血病など）や先天性の多発奇形を伴うSchinzel-Giedion syndromeの発症に深く関わる「SETBP1」遺伝子の機能異常に着目しています。この遺伝子の変異が、細胞の増殖や分化を制御する重要な仕組みを変化させることが分かっていますが、その詳細な分子メカニズムは未解明です。

本研究では、遺伝子改変マウスモデルを用いて、SETBP1変異が造血細胞や神経堤細胞の特性に及ぼす影響を解析し、疾患発症の機序を明らかにすることを目指しています。さらに、薬剤スクリーニングや遺伝子解析を通じて、SETBP1変異によるがん細胞の脆弱性を探索し、新たな治療戦略の開発に繋げることを展望しています。この研究成果は、難治性の血液がんや先天性疾患に対する理解を深め、革新的な治療法の開発に貢献することが期待されます。

Structural basis for proteostasis of neuronal cells

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Summary Abstract

We investigated how LONRF proteins specifically recognize misfolded proteins. Using structural and biochemical approaches, we showed selective binding to misfolded substrates, highlighting its role in neuronal protein quality control.

Key Words : Proteostasis, Structural biology, LONRF proteins

Introduction

We aim to elucidate the molecular mechanism by which LONRF proteins specifically recognize misfolded proteins and the structural basis underlying its ubiquitination of the misfolded proteins using an integrated structural biology approach combining single-particle cryo-electron microscopy (cryo-EM) and small angle X-ray scattering (SAXS). Based on structural information, we will identify potential regulatory sites that modulate LONRF proteins activity, thereby providing foundational insights for drug development.

Results

Many age-related diseases are associated with the formation of protein aggregates, such as amyloids, which result from protein misfolding. To prevent such cytotoxic aggregation, cells have developed intricate protein quality control (PQC) systems, including translational regulation, molecular chaperones, and protein degradation pathways such as the ubiquitin-proteasome system (UPS) and autophagy. In post-mitotic neurons, where cell division does not occur, the accumulation of misfolded proteins is particularly problematic and is a hallmark of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Recently, members of the LONRF protein family have been identified as neuron-specific ubiquitin E3 ligases that play a key role in neuronal PQC. These proteins contain a substrate-recognition domain homologous to that of LON protease and specifically target misfolded proteins for degradation via the UPS. Notably, neurons from LONRF-deficient mice exhibit ALS-like phenotypes. These findings suggest that LONRF proteins play a central role in the clearance of aberrant proteins in neurons and is essential for maintaining protein homeostasis in the nervous system.

In this study, we focused on one member of the LONRF family, referred to as LONRF-A. We successfully established a protocol for the recombinant expression of LONRF-A using an *E. coli* expression system. Several expression constructs were prepared for downstream biochemical assays and structural studies. The protein was purified to homogeneity, as confirmed by SDS-PAGE and analytical size-exclusion chromatography (SEC). To examine

the structural characteristics of LONRF-A in solution, we performed small-angle X-ray scattering (SAXS) in line with SEC at BL10C of the Photon Factory (Tsukuba, Japan). The data revealed that LONRF-A adopts an extended conformation while remaining fully folded along its entire length. Furthermore, SEC profiles indicated that LONRF-A behaves as a monomer in solution.

Next, we sought to establish a method for inducing misfolding of a substrate protein, termed Protein-B, which would serve as a ligand for LONRF-A. We tested various stress conditions, including changes in pH and temperature, and eventually found that incubation under specific conditions successfully induced misfolding. Circular dichroism (CD) spectroscopy showed a shift in secondary structure content.

To determine whether LONRF-A selectively recognizes the misfolded form of Protein-B, we carried out *in vitro* binding assays using GST-tagged LONRF-A immobilized on magnetic GST beads. When incubated with native or misfolded Protein-B, only the misfolded form exhibited specific binding to LONRF-A. These findings indicate that our *in vitro* system successfully recapitulates the selective recognition of misfolded proteins observed in neuronal cells.

Finally, we attempted to determine the atomic-resolution structure of the LONRF-A:Protein-B complex using single-particle cryo-electron microscopy. The complex was purified via SEC and used to prepare cryo-EM grids. Data collection was performed using a Tecnai Arctica (200 keV) and a Titan Krios G4 (300 keV) at RIKEN Yokohama. The micrographs showed well-dispersed particles, indicating suitable sample quality. However, despite our efforts, we were unable to reconstruct an atomic-resolution structure due to the conformational flexibility of the complex.

Discussion & Conclusion

We aimed to elucidate the molecular mechanisms by which LONRF proteins specifically recognize misfolded proteins and ubiquitinate them for degradation via the ubiquitin–proteasome system. To this end, we successfully prepared recombinant LONRF proteins and their misfolded protein ligands. Although we attempted structural analysis of the LONRF–substrate complex using cryo-electron microscopy, atomic-resolution structures could not be obtained, likely due to the conformational flexibility of the complex in solution. To overcome this limitation, strategies such as chemical cross-linking, introduction of stabilizing mutations, or co-binding with auxiliary interacting proteins could help to reduce the structural heterogeneity. These approaches may facilitate the acquisition of atomic-resolution structures, thereby providing detailed insights into the molecular basis of specific recognition and ubiquitination of misfolded proteins by LONRF family members.

References

Not applicable

一般の皆様へ

本研究では、筋萎縮性側索硬化症（amyotrophic lateral sclerosis：ALS）の発症のメカニズムの解明を目指しました。この病気では、神経細胞で働くタンパク質の形が、何らかの原因でおかしくなり、それがアミロイド線維と呼ばれる構造体を作ることが原因となります。おかしくなったタンパク質＝ゴミと考えると、それが神経細胞の中に蓄積して、いいことがないことがわかると思います。私たちの神経細胞には、このおかしくなったタンパク質（ゴミ）を識別して分解してくれる、いわば掃除機の役割をするタンパク質がいます。この掃除機が働く仕組みを詳しく知ることができれば、より高機能の掃除機を作ることが可能となり、それが神経変性疾患の新しい治療方法となる可能性があります。これからも、この掃除機の仕組みを詳しく調べ、世の中に貢献できる研究を展開していきたいと思います。

Synthesis of Aryl Phosphonates by Green Photocatalysis

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Summary Abstract

A phosphonium ylide-based visible-light organophotoredox catalyst has been successfully applied to the synthesis of aryl phosphonates using aryl halides and trialkylphosphites. The excited state of the photoredox catalyst is capable of undergoing direct photoinduced electron transfer for reductive aryl radical generation.

Key Words : Phosphonium ylide, Organophotoredox catalyst, Aryl radical

Introduction

Photocatalysis development is a rapidly growing field of research in synthetic organic chemistry aimed at providing productive and sustainable systems. Organophotoredox catalysts have gained increasing attention as a powerful tool for radical-mediated transformations due to their tunable catalytic ability by substituent effects.[1] We have established an oxidative quenching cycle by utilizing the potential function of phosphonium ylides for visible-light absorption.[2,3] We focused on aryl halides having a very negative reduction potential, where it is assumed that the PET process would be challenging.

Results

In principle, thermodynamically favorable PET occurs when E^*_{ox} is more negative than the reduction potential of aryl halides. Advanced catalysis, involving electrophotocatalysis, by excited radicals, radical anions, and anions provides elegant reducing power for aryl halides, enabling photoinduced aryl radical generation. Meanwhile, Dietzek-Ivanšić, Wagenknecht et al. recently achieved borylation and phosphorylation of aryl chlorides, where an electron-rich *N*-phenylphenothiazine derivative simply undergoes PET with aryl chlorides under irradiation with a 365 nm LED.[4] This report motivated us to invoke visible-light ylide photoredox catalysis, although blue light energy (ca. 3 eV) is not sufficiently high for some inert substrates (e.g., electron-rich aryl chlorides).

We initially attempted the phosphorylation reaction of *p*-iodoanisole in the presence of a phosphonium ylide (10 mol%) under blue LED irradiation (40 W kessil lamp). The reaction was carried out using triethylphosphite (10 equiv) and DBU (2.0 equiv) in DMSO (0.1 M) at 25 °C for 24 h. To our delight, the desired aryl phosphonate was obtained in 87% isolated yield after purification by silica gel column chromatography, and 5% of anisole was observed in the reaction mixture. Control experiments without the phosphonium ylide, visible-light irradiation, or DBU led to lower yields, which revealed the importance of these three elements. The present reaction is considered to proceed through aryl radical formation, followed by C-P bond formation

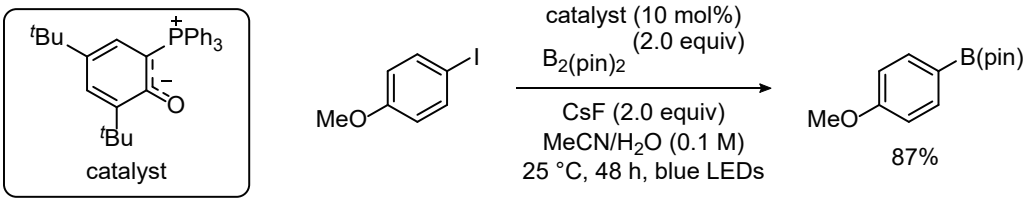
to provide aryl phosphonates.

To gain insight into the mechanism, we constructed Stern-Volmer plots to assess the initial rate of the photoinduced reaction. The emission of the phosphonium ylide was quenched by *p*-iodoanisole more effectively than triethylphosphite, suggesting that radical precursor would receive an electron from the photoexcited catalyst. The Gibbs free energy change in the PET (G_{PET} = +65.3 kcal mol^{-1}). was estimated to be −3.0 kcal mol^{-1}, which clearly indicated that the process could take place only when in the photoexcited state (G_{ET} = +65.3 kcal mol^{-1}). These results supported generation of an aryl radical from the corresponding aryl halide. Based on the experimental evidence, we proposed a mechanism for the phosphorylation of aryl halides as follows. First, visible light irradiation causes excitation of the catalyst, leading to generation of an aryl radical from the aryl halide. The generated radical reacts with triethylphosphite to provide a phosphoranyl radical intermediate, which is oxidized by phosphonium ylide-drived radical cation to result in regeneration of the catalyst and formation of the phosphonium ion intermediate that undergoes an ionic Arbuzov-type reaction to afford the corresponding aryl phosphonate. It is noted that a small activation energy was identified by DFT calculations ($\Delta G^{\ddagger}$ = +4.6 kcal mol^{-1}) in the aryl radical addition step. We hence reasoned that the aryl radical can be smoothly trapped by trialkylphosphites.

To expand the applicability of our methodology, visible-light-driven phosphorylation was demonstrated. The reactions of aryl halides with triethylphosphite were performed by using phosphonium ylide organophotoredox catalysts. Not only *p*-iodoanisole, but also *p*-bromoanisole and *p*-chloroanisole afforded the desired product, albeit in low yield. Phenyl, 2-naphthyl, and 1-naphthyl phosphonates were obtained in good to high yields except for the reaction of bromobenzene. Moreover, 4-chlorobiphenyl was also converted to the corresponding products. Among the screening, the formation of *N*-alkylated DBU with an ethyl group (Et-DBU^{+}) was observed by ^{1}H NMR analysis on unpurified reaction mixture. Although both ionic and radical pathways may be possible for the Arbuzov-type reaction is more likely to occur on the basis of the above experimental results.

Discussion & Conclusion

In summary, we have developed visible-light-driven and phosphorylation of aryl halides by a phosphonium ylide organophotoredox catalyst. This protocol enables reductive aryl radical generation through PET from electron donor (phosphonium ylides) to electron acceptor (aryl halides). Owing to the reducing power of our organophotoredox catalyst, the phosphorylation reactions of a series of aryl iodides, bromides, and chlorides were allowed to proceed effectively under the influence of the photoexcited catalyst. The reduction potential of aryl halides mostly affects the scope and limitation of substrates, which implies that a significant process is initiation of the radical species. Further investigations on fascinating aryl radical transformation are currently underway in our laboratory.



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一般の皆様へ

持続可能な社会の構築に向けて、太陽光のエネルギーを利用する環境調和型有機合成反応の開発が望まれています。無尽蔵にある太陽光の中で、可視光は太陽光の約 4 割を占めることから、可視光に応答して駆動する光触媒を用いる反応が特に注目されています。本研究では、有機物を光触媒として用いることにより、医薬中間体としての利用を期待できる化合物を効率的に合成する手法の開発を目指しました。

Creating the Seed Compounds of Small Molecule Drug Discovery for Diabetic Nephropathy Through Establishment of a Unified Synthetic Method of New Skeletal Sesquiterpenoids

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Summary Abstract

The discovery of seed molecules effective against diabetic nephropathy, along with the identification of key chemical scaffolds suitable for pharmaceutical development, remains an urgent research priority in drug discovery. Focusing on toxicodenanes, sesquiterpenoids derived from the dried resin of the lacquer tree, as potential seed compounds, our group previously established a synthetic route to toxicodenane A, enabling detailed evaluation of its biological activity. In the present study, we aimed to develop a synthetic route to toxicodenane C and successfully constructed its challenging oxygen-bridged eight-membered ring.

Key Words : Asymmetric Synthesis, Sesquiterpenoid, Toxicodenanes, Diabetic Nephropathy, Eight-Membered Ring

Introduction

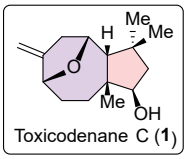
Diabetic nephropathy is a chronic complication in which persistently high blood glucose levels associated with diabetes gradually impair kidney function, potentially leading to end-stage renal failure. Consequently, the discovery of effective seed molecules for diabetic nephropathy and the identification of key chemical scaffolds suitable for its drug development remain urgent research priorities in the field of pharmaceutical science. To address this, we aim to establish divergent and comprehensive synthetic method for toxicodenane-type sesquiterpenoids, which exhibit promising biological activity against diabetic nephropathy. By developing reliable method for their quantitative supply for biological evaluation, we seek to elucidate a wide range of structure–activity relationships and create lead compounds for small-molecule drug discovery targeting diabetic nephropathy.

Results

Among the toxicodenanes,[1] toxicodenanes A, C, and E feature a tricyclic framework comprising a fused bicyclic skeleton and an additional oxygen bridge. Their structures represent a novel and distinctive skeleton not found in other sesquiterpenoids, making these compounds highly promising seed compounds for small-molecule drug discovery. However, only a few synthetic examples have been reported. In fact, only two total

syntheses of toxicodenane A have been published. Our research group has also successfully constructed the tricyclic natural product skeleton of toxicodenane A through a unique strategy we developed.[2] This strategy involves a cyclization reaction using samarium iodide, known as an effective radical reagent for constructing seven-membered carbocycles, followed by an ether ring formation through acid treatment after a site-selective allylic oxidation. As a result, we achieved the first enantioselective total synthesis of both enantiomers of toxicodenane A.

Toxicodenane C (**1**, Fig 1) is a unique oxygen-bridged tricyclic sesquiterpenoid which was isolated from the dried resin of the lacquer tree by Cheng et al.[1] Natural product **1** can significantly inhibit the overproduction of fibronectin, collagen IV, and interleukin-6 in high-glucose-induced mesangial cells in a dose- and time-dependent manner, showing its potential in treating diabetic nephropathy. Since only trace amounts of **1** have been isolated from the resin and its absolute configuration has not been determined, our research group decided to synthesize **1** to seek to elucidate structure–activity relationships and create lead compounds for small-molecule drug discovery targeting diabetic nephropathy. Development of a synthetic method for the challenging oxygen-bridged eight-membered ring is a key step in the total synthesis of compound **1**.



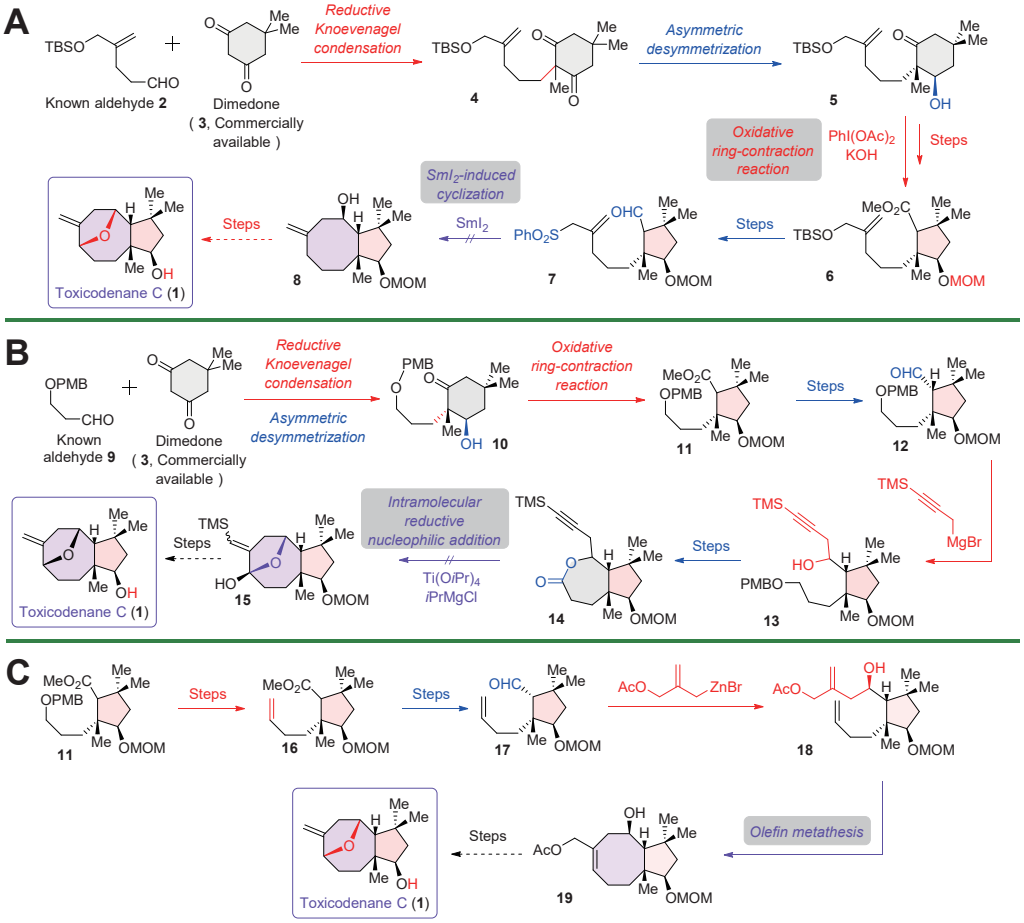
Toxicodenane C (**1**)

Fig. 1

Our asymmetric total synthesis of natural product **1** commenced with the known aldehyde **2**, derived from the commercially available 1,3-propanediol (Scheme 1A).[2] The reductive Knoevenagel condensation between **2** and the commercially available dimedone (**3**) with Hantzsch's ester, followed by methylation, afforded diketone **4**. The asymmetric desymmetrization of **4** by Corey−Bakshi−Shibata reduction using a chiraloxazaborolidine catalyst,[3] provided the desired alcohol **5** with high enantiomeric excess. We constructed a five-membered ring of **1** by an oxidative ring-contraction reaction using iodobenzene diacetate.[4] After the conversion of substituents in **6** provided aldehyde **7**, the attempt was made to construct the oxygen-bridged eight-membered ring in **1**. Unfortunately, we could not obtain the desired **8** with the eight-membered ring.

The next examination of 8-membered ring construction was carried out, as shown in Scheme 1B. According to the same synthetic pathway, as shown in Scheme 1A, ester **11** was prepared through the reductive Knoevenagel condensation between the known aldehyde **9** and **3**, the asymmetric desymmetrization,[3] and the oxidative ring-contraction reaction.[4] After the ester group in **11** was converted into the aldehyde group, the cyclic precursor **14** was generated from aldehyde **12** through an nucleophilic addition using Grignard reagent to install a propargyl group and oxidative lactonization. The treatment of **14** with low-valence titanium species generated *in situ* from $Ti(O\textit{i}Pr)_4$ and *i*PrMgCl was examined,[5] but the desired 8-membered ring **15** was not detected.

Olefin metathesis was examined as the third approach for the 8-membered ring construction in natural product **1** (Scheme 1C). The synthesized **11** (Scheme 1B) was converted into ester **16** through the deprotection of the PMB protecting group, the oxidation, and the olefination. After the reduction of the ester group in **16**, the Barbier-type allylation reaction of aldehyde **17** using the zinc reagent afforded the cyclization precursor **18**. The olefin metathesis of **18** afforded the desired product **19** with the eight-membered ring. We are currently investigating the final steps required to convert **19** into toxicodenane C (**1**).



Scheme 1

Discussion & Conclusion

In this synthetic study, we successfully constructed the challenging eight-membered ring moiety of toxicodenane C (**1**) via olefin metathesis. With only one step remaining, the complete synthetic route is nearly established. Moving forward, we aim to develop a reliable method for the quantitative production of compound **1** based on this synthetic approach. This will enable comprehensive structure–activity relationship studies, including those involving synthetic intermediates. Ultimately, we seek to elucidate the biological functions of these natural products in diabetic nephropathy and apply our findings to the development of small-molecule therapeutics.

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一般の皆様へ

糖尿病性腎症は、糖尿病に起因する高血糖状態により腎機能が徐々に低下し、最終的には腎不全に至る合併症です。糖尿病性腎症に対する創薬研究では、医薬品候補となり得るシード化合物の探索や重要な化学構造の特定が重要です。このような背景から、天然の化合物を対象とした探索研究は必須であり、その中でも植物由来のトキシコデナン類は、従来の類縁体にはない新規骨格をもつことから、有望な候補分子として期待できます。化学合成によるアプローチによって本化合物群の安定な供給を可能とし、その化学構造と生物活性との関係性を明らかにすることで、新規低分子創薬への応用が期待できます。我々はこの観点から、トキシコデナン類の合成研究を進めています。

Development of an advanced biomechanical approach for the heart morphogenesis

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Summary Abstract

In this research, we aim to elucidate how the structured cardiac lumen is organized for functional blood circulation, focusing on the "forces generated from blood flow" and "biological signals" during the lumen formation, and how cells discriminate the characteristics of forces. Specifically, we developed a technique that directly manipulates physical forces in zebrafish embryos and evaluated the biological signal outputs by quantitative approaches.

Key Words : zebrafish, heart morphogenesis, force manipulation

Introduction

Developing cardiovascular systems use mechanical forces, such as fluid shear force, pressure, and contractile forces, to shape tissue form and function. Although any EdCs receive mechanical forces from the beginning of heartbeat, the mechanosensitive mechanisms by which the continuous forces control the signaling during valve morphogenesis remain unclear. Based on our recent advances to artificially introduce mechanical stimuli into living organisms in vivo analysis, this project seeks to isolate the biological functions of each component of the external forces generated in the beating heart.

Results

On my previous research, we found that Ca^{2+}–Nfat signaling occurred in response to forces. To label the calcium dynamics and Nfat activity, we applied GCaMP7a under the control of endocardial promoter and fluorescent expression under the control of Nfat responsive element activation, respectively. I note that endocardial cells constitute the cardiac lumen that face with the blood flow. In contrast, weak-beating and heart-arrested condition never happened Ca2+ influx. While the cells sense continuous forces because of the constant beating, the force-dependent calcium signaling is spatially and temporally regulated until the functional valve formation. By comparing the blood flow and the forces-dependent reporter activity, we noticed that transition of the flow direction associates with the signaling. At each stage, the reporter expression is correlated with the region in which bidirectional flow occurs. Because bidirectional flow happened during the period of valve tissue organization, so we assumed this flow properties govern the force-dependent signaling. We next investigated how to distinguish these external forces—which have

traditionally been treated collectively under the broad term “mechanical stimuli.”

Under physiological conditions, cardiac pulsation and blood flow are tightly coupled, making it difficult to separate pressure from shear stress. To overcome this, we developed a novel magnetic manipulation method using magnetic fluids—a material with unique properties that deform in response to magnetic fields. By injecting this fluid into the cardiac lumen, we successfully introduced controllable blood flow directionality via magnetic manipulation and we found that stress applied perpendicular to the luminal surface induced minimal calcium influx, whereas sustained shear stress applied tangentially led to significant calcium influx, indicating activation of mechanical signaling pathways. These results highlight the importance of distinguishing tangential shear stress from other mechanical inputs.

Currently, magnetic tweezers have been successfully implemented in our laboratory, enabling further manipulation of magnetic forces at the cellular level. Although both bidirectional and unidirectional blood flows generate shear stress, it remains unclear whether they activate biological signaling through different mechanical parameters, or whether cells themselves possess mechanisms to discriminate directional flow information.

Next, we originally planned to employ an ultrasound manipulation technique using microbubbles, but this was suspended due to the discontinuation of the required commercial device. Instead, we shifted our focus to directly measuring intracardiac pressure in zebrafish as a way to segregate the roles of different mechanical forces. In a collaborative research, we established a pressure measurement system based on the insertion of micropipette glass capillaries into the lumen, enabling pressure-to-electrical signal conversion. We now obtained physiological pressure data from various regions of the developing heart, such as atrium, ventricle and cardiac cavity. By inducing altered flow conditions such as flow occlusion using microbeads, we plan to examine how pressure fluctuations affect biological signaling.

Discussion & Conclusion

By manipulating magnetic fluids within the lumen, we observed that calcium influx was rarely induced by stress applied perpendicular to the luminal surface, whereas periodic shear stress led to significant calcium influx. These findings are consistent with pilot studies and suggest the presence of a biological regulatory mechanism that specifically responds to bidirectional shear stress.

Why can cells distinguish directional shear stress? One possible explanation is the involvement of primary cilia, which are organelles protruding from the apical cell membrane that may function as antennas for sensing directional cues. Another potential mechanism is the physical effect of directional flow-induced energy. Blood, being a viscous fluid, allows mechanical energy from shear stress to be converted into thermal energy through friction.

Future investigations focusing on these two possibilities will be instrumental in advancing a comprehensive understanding of the mechanosensory response system within the cardiac lumen.

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一般の皆様へ

心臓では形づくりの初期より拍動を開始し、常に物理的ストレス：「力」に晒されます。実は「力」といってもさまざまな種類が存在するなかで、私たちはそれらを区別して捉えています。複雑に「力」が継続して生じる心臓管腔内で、生体が「力」を適切に調節する機構は未だに不明です。私たちは、細胞はどのような「力」の特性を認識しているのか、小型魚類であるゼブラフィッシュ胚に対して、「力」を直接操作する手法を開発することで、応答原理を解明したいと考えて研究を行っています。

Exploring the mechanisms of immune semaphorin expression in the tumor microenvironment and its effects on immune cells

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Summary Abstract

We show that semaphorin 6D (Sema6D) forward signaling, which is reportedly involved in coordinating the orientation of cell development and migration as a guidance factor, impaired the infiltration and activation of tumor-specific CD8+ T cells in the tumor microenvironment. From these results, we conclude that targeting Sema6D forward signaling is a promising option for increasing ICI efficacy.

Key Words : Cancer immunotherapy, Semaphorin, T cell

Introduction

The neural guidance factor semaphorin has been identified as a repulsive guidance factor for the elongation of nerve axons during development (1). However, semaphorins have been reported to have various functions in vivo, not only in the nervous system but also in blood vessel formation (2)(3), bone homeostasis (4)(5), the cytoskeleton (6), cell migration (7) and induction of immune cell differentiation (8).

We found that semaphorin 4A (Sema4A), a type of immune semaphorin with reported effects on the immune system, activates CD8-positive T cells and its expression in tumours correlated with the therapeutic efficacy of anti-PD-1 antibodies, independent of PD-L1 expression (9). However, the role of Sema6D in antitumor immune responses and sensitivity to ICIs in the TME is still unknown.

Results

Analysis of public clinical datasets revealed a negative correlation between *SEMA6D* expression and several key immune markers: *CD8A* (indicative of $CD8^+$ T cells), *PDCD1* (a marker of T cell activation), and *IFNG* (markers of cytotoxic activity) (Figure 1A). To further investigate this, we analyzed publicly available single-cell RNA sequencing data from head and neck cancer and found that SEMA6D is expressed primarily by tumor cells, fibroblasts, and endothelial cells in the tumor microenvironment. Multiplex immunohistochemistry of clinical head and neck cancer specimens demonstrated that tumors with high Sema6D expression exhibited a reduced number of activated $CD8^+$ T cells within the tumor microenvironment. These findings suggest that Sema6D exerts an immunosuppressive effect that impairs antitumor immunity.

Next, to explore the underlying mechanisms, we used a murine oral carcinoma

model. Overexpression of Sema6D in MOC2 cells (a head and neck cancer cell line) led to increased tumor growth and a reduction in activated CD8$^+$ T cells in the tumor microenvironment (Figure 1B, C). Conversely, when MOC2 cells were implanted into *Sema6d* knockout mice, tumor size was significantly reduced, and infiltration of activated CD8$^+$ T cells was enhanced compared to wild-type mice (Figure 1D, E). In addition, to identify the cellular compartment responsible for Sema6D's effects, we generated bone marrow chimeric mice using wild-type and *Sema6d*-deficient mice. Tumor suppression was observed only when the recipient (stromal compartment) lacked Sema6D, suggesting that Sema6D derived from the tumor stroma plays a critical role in modulating antitumor immunity. This finding aligns with the single-cell sequencing data (Figure 1F). Furthermore, in vitro experiments showed that stimulation of CD8$^+$ T cells (isolated from tumor-bearing mice) with anti-CD3 and anti-CD28 antibodies in the presence of recombinant Sema6D protein significantly suppressed their activation and proliferation (Figure 1G).

Collectively, these results demonstrate that stromal-derived Sema6D suppresses the infiltration, activation, and expansion of CD8$^+$ T cells within the tumor microenvironment, thereby inhibiting antitumor immune responses.

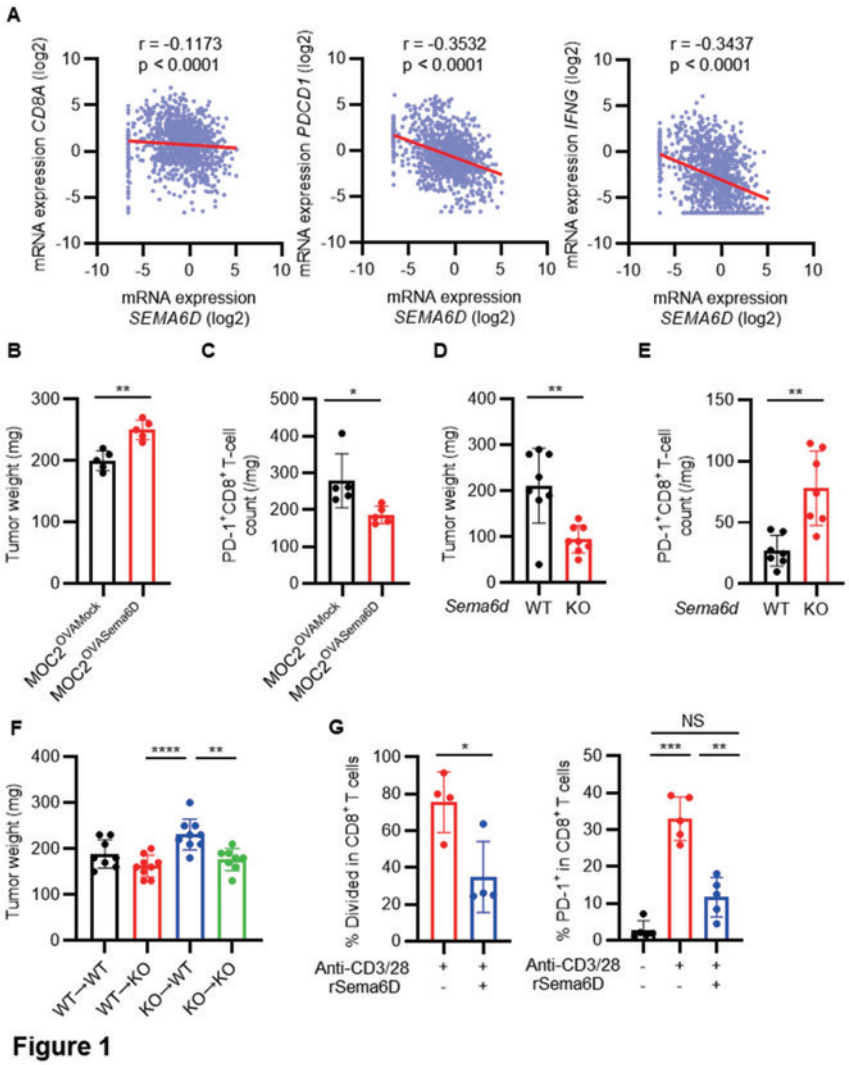

Figure 1

To better understand the regulation of Sema6D expression, we initiated a drug screening project aimed at identifying compounds that modulate Sema6D levels in cancer cells. While previous studies have used qPCR to detect Sema6D expression, we sought a high-throughput method suitable for compound library screening. Flow cytometry using a commercially available anti-Sema6D antibody was performed on cell lines confirmed to express Sema6D by qPCR; however, surface expression was not initially detectable. Therefore, anti-Sema6D antibodies were generated using *Sema6d* knockout mice. The antibody was validated to specifically bind recombinant Sema6D protein (Figure 2A). Subsequent flow cytometry analysis on cell lines predicted to express Sema6D, based on Human Protein Atlas data, confirmed detectable Sema6D expression (Figure 2B). The antibody was also applicable for immunostaining of human small cell carcinoma tissues (Figure 2C). These results establish a reliable experimental platform for high-throughput screening of compounds that regulate Sema6D expression in cell lines.

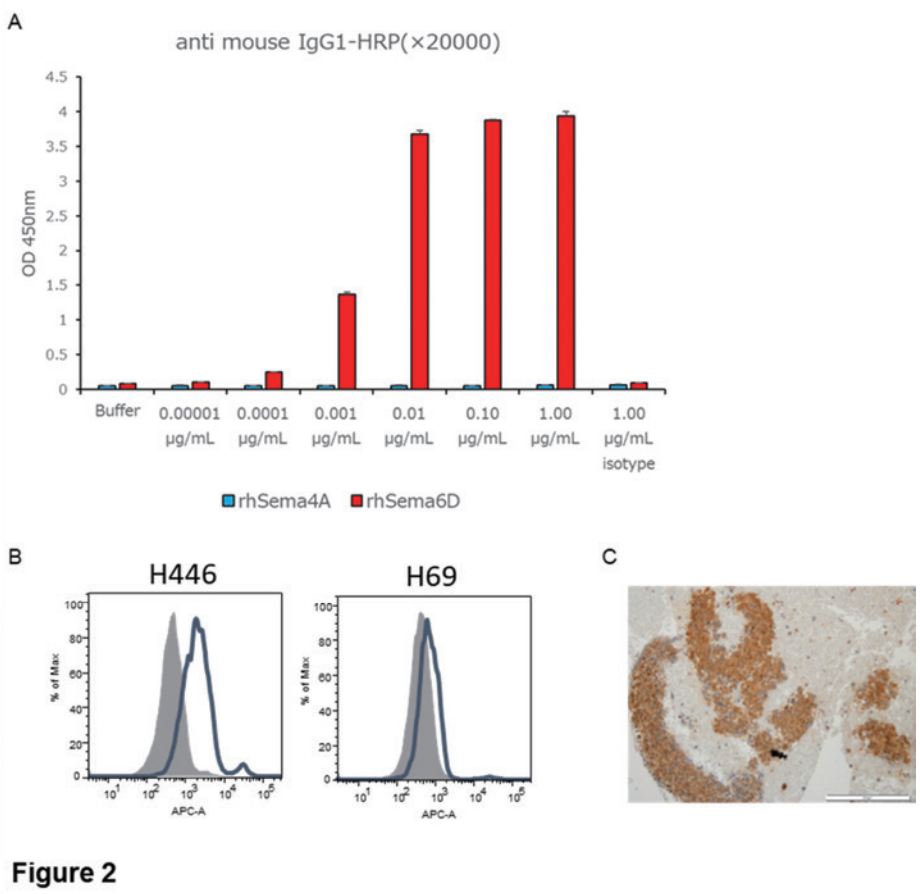

Figure 2

Discussion & Conclusion

We previously reported that Sema4A expressed in the cancer microenvironment enhances proliferative and cytotoxic activity of CD8-positive T cells in tumours by increasing mTORC1 activity and promoting polyamine biosynthesis. On the other hand, Sema6D expressed in the cancer microenvironment was shown to suppressively regulate the infiltration, activation and proliferation of CD8-positive T cells into the tumor and attenuate

anti-tumor immunity, as described above. The usefulness of Sema4A and Sema6D as biomarkers is currently being validated. In addition, these results suggest that elucidating the mechanisms that regulate the expression of immune semaphorin molecules in the cancer microenvironment may lead to the development of novel therapies, and screening using a compound library was also considered in this study. However, a high-throughput identification system is essential for a comprehensive analysis, and we were unable to find a human solid tumor cell line that routinely expresses Sema4A. For Sema6D, the lack of antibodies to identify its expression by flow cytometry was a problem. In this study, we developed an antibody to identify Sema6D, which enabled us to identify Sema6D expression in a high-throughput manner using cell lines. This identification system will enable screening using compound libraries and the discovery of drugs that alter Sema6D expression, which is currently being analysed.

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一般の皆様へ

がん環境において、免疫を調整する分子を同定し、その機序や臨床上の有用性を調べる事は非常に重要である。今回の研究では、セマフォリン 6D 蛋白が抗腫瘍免疫を抑制していることを見出した。この分子の発現機序を解明することで、がん免疫療法の治療効果を予測するバイオマーカーや新規治療法の開発につながる可能性がある。発現機序を解明するためのスクリーニング検査に使用できる実験系を確立し、現在解析を続けている。

Mechanism of genome stability in the primordial germ cell

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Summary Abstract

Germ cells are essential for the reproduction of life. During mammalian development, germ cells are generated from a stem-like cell called the primordial germ cells in the reproductive organs, such as the testis and ovary. How the genome stability of the primordial germ cells is maintained, whose defects clearly affect the fate of next generations, remains elusive. We showed that, in addition to the established roles in meiotic recombination, the genes involved in homologous recombination play a critical role in the genomic stability of the primordial germ cells.

Key Words : Meiosis, germ cell, primordial germ cell

Introduction

Most eukaryotic organisms generate their progeny through the engagement of two gametes, sperm and egg(oocyte), which are produced in reproductive organs, the testis and ovary, respectively. In the testis and ovary, stem cell-like cells, primordial germ cells (PGCs), proliferate and differentiate into progenitor diploid cells, which carries out meiosis to reduce chromosome number by half in gametes. To minimize the risk of the spontaneous mutations generated through the PGC proliferation, which increase the congenital diseases, the PGC is considered to be equipped with highly elaborated mechanisms to keep the genome very stable. However, because of the technical difficulties in handling germ cells such as the PGC, our knowledge on genome stability in cells is poorly described.

Results

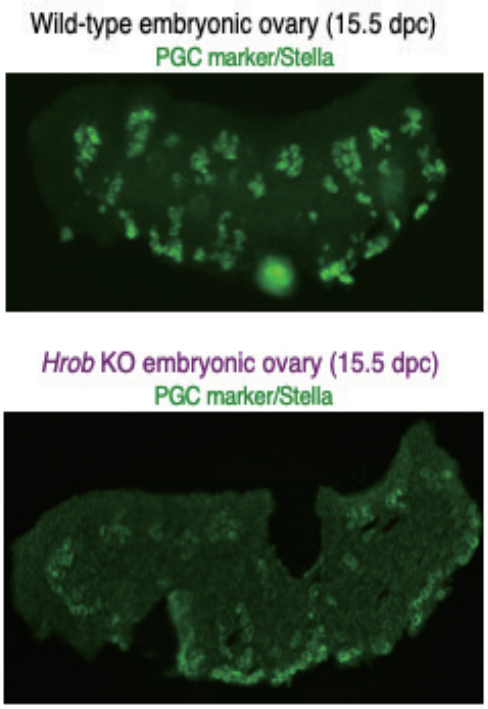
Homologous recombination (HR), DNA exchange between two DNAs, plays a critical role in genomic stability in somatic cells. HR promotes the repair of DNA double-strand breaks, the repair of stalled DNA replication forks and also in the maintenance of telomeres in the absence of telomerase. HR is comprised of several distinct biochemical reactions. HR is initiated by the formation of DNA double-strand breaks (DSBs) which follows the processing of the DSB ends to generate the single-stranded DNA (ssDNA). The ssDNA is used for homology search and strand exchange with a homologous double-stranded DNA (dsDNA), leading to the formation of a recombination intermediate called the displacement (D)-loop. With further processing and DNA synthesis, the second intermediate with two Holliday structures is formed and resolved into two product DNAs.

Homology search and strand exchange between ssDNA and dsDNA, key HR reactions are mediated by the assembly of protein complexes on the ssDNA. RAD51 protein binds to

ssDNAs to form a helical filament for the homology search. The assembly and disassembly of RAD51 filament are regulated by both positive and negative regulators. Indeed, mutations in one of the positive regulators, BRCA2, induce genomic instability, thus with an increased risk of hereditary breast and ovarian cancers. However, studies on the role of HR genes in germ cells are hampered by the fact that HR genes such as RAD51 and BRCA2 are embryonic lethal in knockout (KO) mice. Recently, we created several knockout (KO) mice of the HR genes and found that some develop into adult mice with reduced fertility. This enables us to elucidate the role of HR genes during the development of germ cells into gametes, including the primordial germ cells (PGCs) and meiosis.

We focused on several RAD51 regulators: RAD51 mediator, SWS1-SWSAP1 complex, which promotes the assembly of the RAD51 filament on the ssDNA, and the other regulator HROB-MCM8-MCM9 complex, which facilitates the strand exchange after the RAD51 filament assembly, and a negative regulator, FINGL1, which antagonizes SWS1-SWSAP1 and BRCA2, whose mutations predispose familial breast/ovarian cancers: e.g. the FIGNL1 KO suppresses the HR defect in *swsap1* and/or *brca2* mutant cells. More interestingly, SWSAP binds to FIGNL1 directly, and HROB-MCM8-MCM9 binds to a FIGNL1 partner, FIRRM. Both the *swsap1* and *hrob* KO mice are viable but infertile in both males and females.

The *swsap1* KO is defective in meiotic recombination, which induces loss of gametes by apoptosis in the ovary and testis (Matsuzaki et al. 2019). The germ-line specific depletion of Fignl1 revealed a sexually dimorphic defect in germ cell development. The testis from the Fignl1 conditional knockout (cKO) accumulated a defect in meiotic recombination with the accumulation of RAD51 on meiotic chromosomes, leading to the loss of spermatocytes (Ito et al., 2023). On the other hand, Fignl1 cKO ovary contained oocytes that finished meiosis I without any loss of oocytes. However, these oocytes in Fignl1 cKO ovary accumulated RAD51 on the chromosomes, resulting in the arrest in meiosis I cell division (unpublished results).



Interestingly, immuno-staining analysis of a PGC marker protein, Stella (green in Figure), showed that the early embryonic ovary (15.5 dpc) as well as embryonic testis in the *hrob* KO mice contained a reduced number of PGCs (unpublished results). This shows a critical role of HROB in the maintenance of PGC in reproductive glands.

Discussion & Conclusion

Our results showed the critical role of HR genes in the development of germ cells, including the maintenance of the PGS as well as meiotic recombination and meiosis I cell divisions. These HR defects in germ cells are clearly associated with infertility, birth defects as well as congenital diseases. More recently, some of the HR genes are associated with premature ovarian inefficiency, a type of menopause at earlier ages of women. Our understanding the functions of the HR genes in germ cells provides more information on molecular pathology of human diseases in germ cells as well as the development of the cure.

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一般の皆様へ

生殖細胞は生物の再生に必要な卵子や精子と行った配偶子を作る細胞で、生殖細胞の遺伝情報ではあるDNAは体の細胞の中で唯一、子孫に伝承されることが知られています。子孫に遺伝情報を正確に伝えることは、種の保存にとても大切です。今回の研究から、2つのDNAの交換反応である相同組換えに関わる遺伝子が、配偶子形成、中でも、生殖細胞の幹細胞と言われる始原生殖細胞の維持に大切であることを見つけることが出来ました。

Divergent synthesis of nitrogen-containing polyaromatics based on imine-anion mediated ketimine synthesis

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Summary Abstract

Global access to the nitrogen-containing aromatic compounds such as quinolines and indoles were accomplished using an ortho-hydroxyphenyl ketimines, obtained by imine anion-mediated Smiles rearrangement we recently developed, as a platform molecule. Developed method would be a useful tool for accessing the various nitrogen-containing aromatic compounds and would open the door of discovery of novel drug candidates.

Key Words : imine-anion, Smiles rearrangement, ketimine, quinoline, indole

Introduction

Nitrogen-containing aromatic compounds such as quinolines and indoles could be found in various pharmaceuticals and biologically active compounds. Because of their utility, various synthetic method leading this class of skeletons have been developed. One of the limitations of them is that only same type of skeleton could be constructed in each method, in other word, another original method is required to access the another skeleton. Based on such circumstance, development of novel reactions and tactics for the target structure is strongly desired.

Results

For the global access to the nitrogen-containing aromatic compounds, an appropriate platform compound is required. We envisioned that the compounds, ortho-hydroxyphenyl ketimines, obtained by imine anion-mediated Smiles rearrangement (namely, IASR) we recently developed, would be a good platform molecule. Importantly, these compounds have multiple-reacting points such as hydroxy group and halogen atom. Quinoline skeleton could be constructed by intramolecular coupling reaction between aromatic group on imine-carbon atom and aromatic group on imine-nitrogen atom. Indole skeleton is also accessible by the reaction between aromatic group on imine-nitrogen atom and enamine derived from ketimines under basic conditions. The hydroxy group could be used as a nucleophilic point for further functionalization such as intramolecular nucleophilic addition reaction and coupling reaction after the conversion to triflate, and as a tool for the formation of metal complex.

[Quinoline synthesis]

For the quinoline synthesis, we planned to use a photo-induced electrocyclic reaction (PIECR). In the previous publications, excess amount of some acid catalysts was mostly required to achieve the PIECR from imine for the suppression of undesired n-π^* transition. In addition, their chemical yield remained moderate level (less than 50%) in most cases. We anticipated that planned PIECR would effectively proceed from our ketimines because the intramolecular hydrogen bonding between hydrogen of OH group and imine-nitrogen atom would work as an alternative tool to acid catalyst.

As a model substrate, the ketimine with a 2-naphthyl group on the imine-carbon atom and a 2-naphthyl group on imine-nitrogen atom was selected. Gratifyingly, the PIECR proceeded smoothly under mercury lump irradiation conditions. As expected, addition of external acid catalyst was not required in this case, and corresponding quinoline derivative was obtained in excellent chemical yield (93%). The structure of the product was unambiguously established by X-ray analysis, which indicates that this compound has helical chirality.

Having the promising result in hand, substrate scope of the PIECR was investigated. Although the addition of stoichiometric amount of weak Lewis acid, boron species, was essential to achieve the reaction on the contrary to model substrate described above, various complicated quinoline-based nitrogen containing polycyclic aromatic hydrocarbons, which are otherwise difficult to access by the conventional methods, were obtained in good chemical yields (over 70%). It is to note that both the hydroxy group and the 2-naphthyl group are indispensable structural units for the progression of PIECR. The reaction from the substrates with a methoxy group and a 1-naphthyl group instead of each part did not proceed at all, and each ketimines were recovered in almost quantitatively.

[Indole synthesis]

As for indole synthesis, we firstly examined the aromatic nucleophilic substitution reaction, namely, S_NAr reaction, from the ketimine with a n-butyl group on the imine-carbon atom and 2-fluorophenyl group on imine-nitrogen atom. Simple treatment of inorganic bases such as K_2CO_3, Na_2CO_3, and K_3PO_4 in DMSO at 70 °C were not the suitable reaction conditions, and recovery of starting material was observed in most cases. Versatile organic bases such as Et_3N, DBU, and DBN were also ineffective. The situation was dramatically changed when LDA, strong base, was employed. The desired reaction proceeded in this case (refluxing in THF), and corresponding indole was obtained in good chemical yield (65%). Now we are concentrating on the establishment of reaction conditions which satisfy the better chemical yields and milder reaction conditions.

Discussion & Conclusion

In summary, we showed the high synthetic utility of ortho-hydroxyphenyl ketimines, which were obtained by IASR we recently developed, as a platform molecule for various nitrogen-containing skeleton such as quinolines and indoles. Among the results obtained in this study, the achievement of PIECR for the construction of quinoline-based nitrogen containing polycyclic aromatic hydrocarbons is worthy to emphasize because of the difficulty of progression of PICER from ketimines. Another notable point is that all of the obtained compounds have hydroxy group, which means further structural modification and diversification would be realized from this unit. Present method would be a useful tool for rapid access to various nitrogen-containing aromatic compounds and would open the door of discovery of novel compounds having characteristic biological activity.

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一般の皆様へ

本研究は、独自に開発したケチミン合成法で得られる生成物をプラットフォーム分子として、様々な含窒素多環芳香族化合物の合成を目的としたものです。重要な点としては、得られるケチミンが持つ官能基（化学反応を起こしうる部分）を余すことなく活用しているところです。一見すると特殊な構造を持ち、ある意味では“使い物にならなそうな”ものも、俯瞰してみると活用できそうに見えてきます。そこに着眼点を置けたこと、またそれを上手く利用して様々な有用化合物の合成に繋げられたことが本研究の特徴です。今後は、本研究を基盤とした、新規医薬品の創出につながる独創的な分子を作っていきたいと思います。

Validation of a recurrent input hypothesis of perception using a mouse model

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Summary Abstract

The hypothesis that recurrent input between the sensory cortex and the prefrontal cortex contributes to perception has attracted considerable attention in recent years. In this study, we established an experimental framework to test this hypothesis by combining a newly developed somatosensory perception task in mice with electrophysiological recordings and optogenetic manipulation of neural circuits. We found that, at the onset of perception, pyramidal cells in the frontal cortex (FC) exhibited ignition-like activity, while the primary somatosensory cortex (S1) showed amplified late-phase neural responses. These results suggest that recurrent input between FC and S1 plays a critical role in generating somatosensory perception, thereby supporting the recurrent input hypothesis. This finding may advance our understanding of the neural mechanisms underlying conscious perception and contribute to the development of treatments for hallucinations associated with neurological disorders.

Key Words : Perception, somatosensory cortex, recurrent input, optogenetics

Introduction

A hypothesis suggests that perception is generated through recurrent processing, where sensory information initially activates the sensory cortex and is then sent to the prefrontal cortex, triggering an ignition-like process that feeds back to the sensory cortex and amplifies neural activity [1]. Clinical studies have shown that damage not only to S1 but also to FC significantly impairs somatosensory perception [2]. Additionally, perceptual experiences have been reported to be elicited by electrical stimulation or epileptic seizures in both regions [3]. However, it remains challenging to elucidate these phenomena at the neural circuit level using non-invasive methods in humans [1]. Therefore, in this study, we combined a newly developed somatosensory perception task in mice with electrophysiological recordings and optogenetic manipulation of neural circuits to test the recurrent input hypothesis.

Results

Development of a Novel Behavioral Task to Separate Perception and Response in Mice: Cue-Guided Delayed Detection Task (CG-DDT)

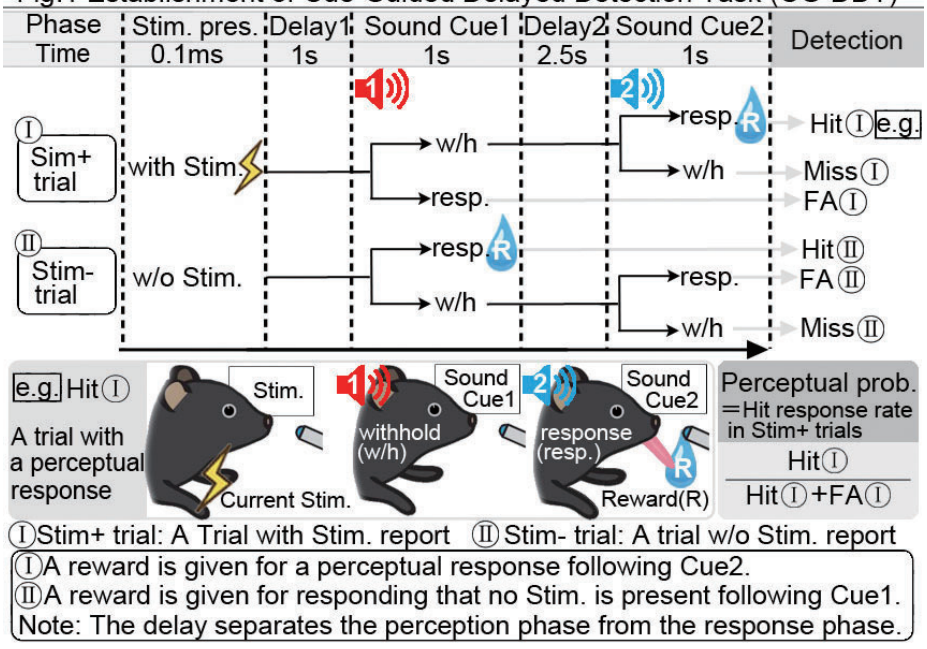
Recent perceptual studies using mice have commonly employed GO tasks in which animals respond immediately to stimuli [4]. However, recent findings have revealed that motor-related neural activity propagates widely across the cerebral cortex, highlighting the

need for a task that can dissociate neural activity related to perception itself from response-related activity [5]. To address this, we developed a novel task called the **Cue-Guided Delayed Detection Task (CG-DDT)**, which introduces a temporal separation between stimulus perception and response selection (Fig.1).

In this task, two sequential cues (Cue 1 and Cue 2) are presented after the stimulus period to temporally dissociate the phases of perception and response. Because mice are required to respond selectively to the appropriate cue, neural activities related to perception and response can be independently analyzed. Specifically, in stimulus-present trials (Stim+), mice withhold their response at Cue 1 and respond at Cue 2 to receive a reward (Fig.1: Hit Ⅰ). In contrast, in stimulus-absent trials (Stim−), responding to Cue 1 is rewarded (Fig.1: Hit Ⅱ). Responding to the inappropriate cue in either condition results in no reward being delivered (Fig.1: FA Ⅰ and FA Ⅱ). Failure to respond to either cue may reflect a failure to perceive the stimulus, insufficient attention, or reduced motivation to perform the task (Fig.1: Miss Ⅰ and Miss Ⅱ).

To train the mice to perform this task, a three-step learning process was conducted over approximately two months: (1) training to respond to an auditory cue, (2) training to suppress responses during Cue 1 immediately following limb stimulation (200 ms), and (3) gradually increasing the delay between limb stimulation and the presentation of Cue 1, up to a maximum of 1 second. This protocol enabled the isolation and analysis of neural activity related to perception.

Fig.1 Establishment of Cue-Guided Delayed Detection Task (CG-DDT)



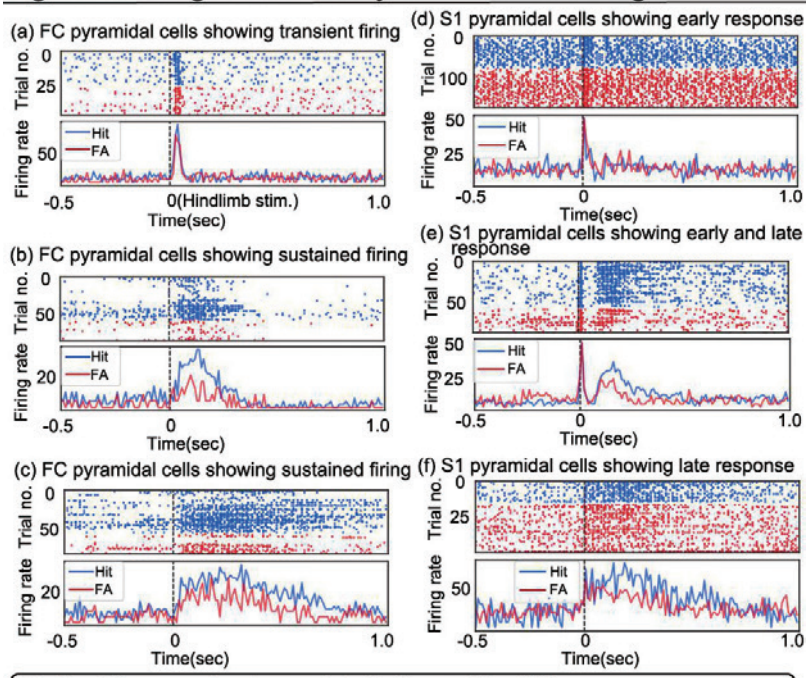
Neural Activity Patterns of Recurrent Input in a Mouse Perceptual Task

Next, during the execution of the CG-DDT in mice, neural activity in the primary somatosensory cortex (S1) and the frontal cortex (FC) was recorded in response to limb stimulation (Fig. 2: 0 seconds). Stimuli near the perceptual threshold, with intensities yielding approximately 30–70% perceptual probability in mice (see Fig. 1 e.g.), were used. Neural activity in each region was recorded using silicon probes during the task, and spike sorting was performed to isolate single units.

For each recorded neuron, activity during perceived trials (blue) and unperceived trials (red) was compared. In the FC, cells exhibiting transient and sustained firing patterns were identified (Fig. 2a–c). Among these, sustained-type cells showed a marked increase in firing during perceived trials. Furthermore, using optogenetic tagging, FC neurons projecting to S1 ($FC_{FC \rightarrow S1}$ neurons) were identified, and approximately 30% of these cells were classified as sustained type. In S1, neurons exhibiting early responses immediately following stimulation and late responses observed after 60 ms were identified. While there was no significant difference in early responses (originating from the primary thalamus) regardless of perception, late responses (presumably arising from corticocortical inputs, particularly feedback from FC to S1) were significantly amplified during perceived trials.

Previous studies by the applicant have shown that optogenetic suppression of FC → S1 feedback (FB) input reduces perception, whereas activation induces perception. Taken together, these results suggest that FC → S1 FB input drives the late neural activity in S1 and plays a critical role in generating perception.

Fig.2 Recording Neural activity in S1 and FC during tasks



Discussion & Conclusion

In this study, we recorded neural activity from the S1 and FC in mice performing a newly developed perceptual task, and labeled the recurrent circuit using optogenetic techniques. Our findings suggest that recurrent input between FC and S1 may contribute to the generation of perception. To further test this hypothesis, we are currently examining the causal role of FC → S1 FB input by recording S1 activity while optogenetically inhibiting this pathway. We aim to determine whether the late component of S1 activity (Figure 2e, f) is selectively reduced during inhibition of FB.

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一般の皆様へ

意識の研究では、「私たちはどのようにして知覚しているのか？」という問いが、過去 25 年間にわたって重要なテーマとして研究されてきました。臨床現場では、患者に意識があるかどうかを判断する際、刺激に対する「言葉での反応」が主な手がかりになります。しかし、発話や運動が困難な患者も多く、意識の状態は連続的で明確な境界がないため、応答の有無だけで意識を正確に判断するのは難しいのが現実です。本研究では、知覚がどのように生じるのかという神経回路のしくみを明らかにすることで、「知覚はあるが応答できない」状態と「そもそも知覚がない」状態を見分ける手がかりを探ります。今後は、マウスで得られた成果を霊長類モデルへ広げ、最終的にはヒトにおける知覚と意識の理解を深めていきたいと考えています。

Roles of miR125-b involved in common molecular pathways of neurodegenerative diseases

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Summary Abstract

We have previously identified several pathological hub molecules from iPS-derived neuron based Bayesian regulatory network analysis (iBRN) using an iPS cell model in FUS-ALS. In this study, we focused on one such a Hub molecule, miR-125b and explored its mRNA targets and downstream gene pathway. Among its downstream genes, we identified a novel target that might be involved in common pathway across multiple neurodegenerative disease states.

Key Words : miRNA, iBRN analysis, neurodegenerative disease

Introduction

RNA-binding proteins and miRNAs bind to many target RNAs including ncRNAs and fine-tunes quality and quantity of RNA and proteins at the genome wide manner. Therefore, it is extremely difficult to identify accurate downstream molecular pathways such as physiological functions and molecular functions. In this study, we focused on miR125b, which has a high impact on quantitative transcriptome and proteome information in ALS disease models. We pursue roles of miR125b.

Results

① Identification of the hub factor miR125b

We previously established an analysis, iBRN method (Non- biased" Bayesian gene regulatory network analysis based on induced pluripotent stem cell (iPSC)-derived cell model) using FUS mutant iPS cell models to predict molecular etiology based on their transcriptome information (Ichiyanagi et al. *Stem Cell Rep.* 2016, Nogami et al. *Neurobiol. Dis* 2021). Among various RNA-binding proteins and miRNAs that have a high impact on transcriptome information in diseased cell models, miR-125b was selected as a candidate factor that display super-hierarchy among several neurodegenerative diseases. It has been reported that miR-125b had an activity to enhance phosphorylation of tau, which is involved in amyloid formation, in dementia reported by other research groups (Ma X, et al. *EMBO J* 2017). And miR125b has also been known to activate microglial NF-kB signaling and induce cytotoxicity in proximal motor neurons via inflammatory response function in ALS (Parisi C et al. *Cell Death Dif.* 2016). Our analysis also observed an increase in γH2AX-positive cells, a DNA damage marker, indicating its involvement with DNA damage in neuronal cells.

② Exploration of miR125b target genes

Next we conducted a research to explore new target genes of miR125b and aim to comprehensively search the molecular pathways common to neurodegenerative diseases. We performed overexpression experiment of miR125b in neuroblastoma SH-SY5Y cells and motoneuron-like NSC-34 cells and RNAseq analysis using RNAs obtained from these cells. We have used the bioinformatics analysis to predict molecular etiology on the molecular pathways and molecular functions influenced by miR125b expression using Gene Ontlogy (GO analysis) and KEGG pathaway analyses.

These results suggest the involvement of molecular pathways common to neurodegenerative diseases, related to inflammatory systems, and proteolytic systems. Furthermore, we performed cumulative frequency differences analysis (CFD analysis) to see the effect of the mRNA expression levels compared to miR125b target genes with perfect seed matching sequences. We confirmed that the expression levels of the miR125b target genes were predominantly decreased relative to the overall variation in gene expression. This suggests that the direct target genes are negatively regulated indicating a role that miR125b down-regulates target mRNAs. In addition, we found that X, which is a gene related to neurodegenerative diseases and important for lysosomal degradation system, is a novel target gene with one bulge in the miR125b seed sequence.

③ Establishment of a cell model for roles of a novel downstream gene X of miR125b

To understand the contribution of gene X in common of neurodegeneration through miR125b, we established a cell model to quantitatively monitor the endogenous expression of this gene X by genome editing. Using this cell model, we found that gene X exhibits a neuroprotective action by increasing its expression in response to lysosomal inhibition through a translational control, whereas miR125b acts as an inhibitor in this process. This result suggests that the neuroprotective function of gene X is weakened when miR125b is highly expressed thereby increasing a risk and the vulnerability of neurons. Furthermore, we confirmed that the accumulation of miR125b dependent p62 and phosphorylated Tau by Western blot analysis. Future work will examine the involvement of gene X in common neurodegeneration.

Discussion & Conclusion

In this study, we identified miR125b as an RNA regulator that displays commonality in neurodegenerative diseases and investigated its role in neurodegenerative diseases. We explored several miR125b target genes that show proteolytic and neuroprotective activities. Furthermore, we established a cellular reporter system to monitor the expression level of one of the target genes, X. This model enables us to monitor the expression of endogenous target genes at the protein level in a very simple manner and to accurately understand their responsiveness to lysosomal damage at the several time points. This model is expected to provide a detailed understanding of the molecular mechanisms induced in neurodegenerative diseases in the future.

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一般の皆様へ

ALS(筋萎縮性側索硬化症) や FTLD(前頭側頭葉変性症) など多くの神経変性疾患は、病態の原因に対して明確な分子的区分ではなく疾患スペクトラムというグラデーションが形成されていると考えられている。私たちは、1つの ALS 細胞モデルと AI ツールを用いて、疾患スペクトラムに内在する共通するハブ因子の同定を試みてきました。その中の候補分子として、多数の標的遺伝子を介した統合的 RNA 制御を担う miRNA の1つである miR125b に着目し、本解析を実施しました。その結果、神経変性疾患に共通性を示しうる新規の miR125b の標的遺伝子を見出しつつあります。

Synthetic study of a naturally occurring tumor cell invasion inhibitory compound

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Summary Abstract

Alchivemycin A is a polycyclic polyketide exhibiting a significant inhibitory effect on tumor cell invasion without cytotoxicity. In this study, we have synthesized its polyol moiety bearing the 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine ring, which is an unprecedented structural motif in natural products, in a highly stereoselective manner.

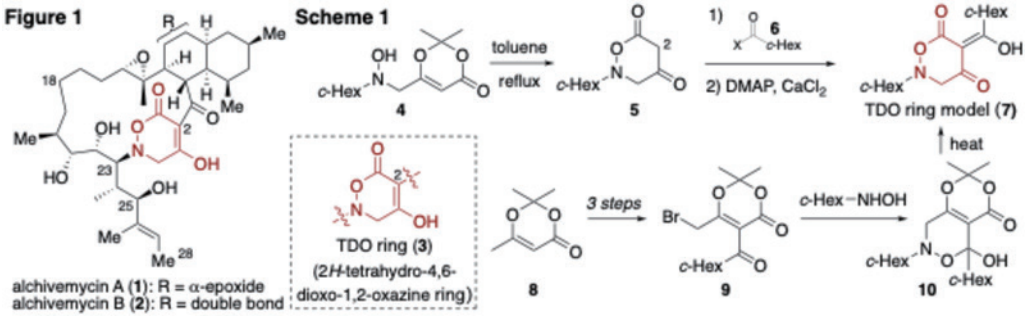
Key Words : cancer, tumor, alchivemycin, natural products synthesis

Introduction

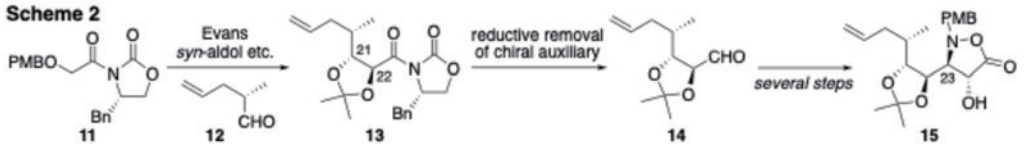
Alchivemycins A (**1**) and B (**2**) are secondary metabolites, isolated from the culture extract of a plant-derived actinomycete.[1)] Their structures, elucidated on the basis of extensive spectroscopic analysis, feature a polycyclic skeleton incorporating a 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine (TDO) ring system (**3**), an unprecedented structural motif in natural products. Since **1** is reported to exhibit a significant inhibitory effect on the invasion of murine colon carcinoma cells into Matrigel without showing cytotoxicity, it could be a promising lead compound for therapeutic cancer treatment. These biological and structural features of **1** prompted us to embark on the total synthesis of **1** and **2**, and the development of their structurally simplified analogs.

Results

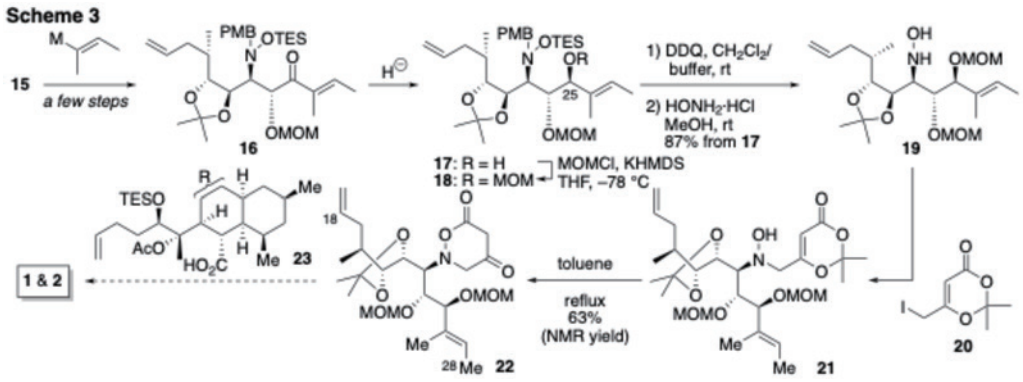
Among the substructures embedded in **1** and **2** (Figure 1), the TDO ring system is believed to be the most difficult one to be synthesized because only a few precedents regarding syntheses of analogous compounds had been reported. As part of their efforts towards the synthesis of alchivemycins, Lei and co-workers reported an efficient method to construct the TDO ring with various functional groups on the N-alkyl chain in 2016.[2)] However, they did not report installation of an acyl group at the C2 position of the TDO ring although developing a synthetic method for forming the C2-acylated ring portion is essential for the total synthesis for alchivemycins. To solve this task, we developed two approaches for the formation of the C2-acylated TDO ring system.[3)] One involves cyclization of an acylketene intermediate, which was generated by heating **4**, followed the C2-acylation of the resulting unstable compound **5** with **6** via an *O*-acylated intermediate, furnishing the TDO ring model of alchivemycins (**7**) in 29% overall yield from **4**. Model compound **7** could also be converted via ‘pre-acylated’ compound **9** and hemiacetal **10** albeit in a modest overall yield of 8% from **8**. These methods are expected to be applicable to the synthesis of alchivemycins. Thus, we began the synthesis of the actual structures of **1** and **2**.



We addressed the synthesis of the polyol moiety (*i.e.*, C18-C28 moiety) of alchivemycins and formation of the TDO ring using the C23 hydroxyamino group. Scheme 2 shows the progress of the research at the start of the grant. To construct the C21/C22 contiguous stereocenters, known acyl oxazolidinone **11** was subjected to the Evans *syn*-aldol reaction using known aldehyde **12**. The resulting aldol was converted to **13** in some steps including deprotection of the PMB group followed by protection of the resulting diol as its acetonide. After removal of the chiral auxiliary, aldehyde **14** was diastereoselectively transformed to isoxazolidin-5-one **15** in several steps. Stereoselective installation of the *N*-hydroxyamino group to the C23 position is considered to be one of the most challenging tasks for the synthesis of alchivemycins. Although we could find a solution for it, we would like to refrain from describing that here since that has not yet been published.



Having succeeded in stereoselective construction of five of the six stereocenters of the polyol moiety of alchivemycins, we turned our attention to the chain elongation of **15** and diastereoselective formation of the C25 hydroxy group. After protection of the C24 hydroxy group as its MOM ether, the resulting compound was transformed to **16** in a few steps. Diastereoselective reduction of ketone **16** was realized by treating **16** with a hydride reagent at –78 °C, providing **17**, which bears all carbon atoms and functionalities of the polyol moiety, in 81% yield (*dr* > 20:1). Protection of the C25 hydroxy group of **17** with MOMCl and KHMDS proceeded smoothly to afford bis MOM ether **18**. With the intention of deprotection of the PMB and TES groups, **18** was exposed to DDQ in CH_2Cl_2/phosphate buffer (pH 7). The TES group was expectedly deprotected under the reaction conditions, but the product obtained was a nitrone. Therefore, the nitrone was subjected to transoximation reaction conditions using $HONH_2$·HCl to give hydroxyamine **19** in 87% yield from **17**, which set the stage for the construction of the TDO ring. According to the procedure we secured in the model study, N-alkylation of **19** with **20** was performed to give **21**. Finally, refluxing the solution of **21** in toluene furnished **22**, the C18-C28 moiety bearing the TDO ring at the C23 position, in 63% yield, based on NMR analysis. Once we complete the preparation of the decalin unit **23**, we will combine **22** with **23** and convert the resulting compound to alchivemycins A (**1**) and B (**2**).



Discussion & Conclusion

In this study, we have achieved the synthesis of **22**, the polyol moiety of alchivemycins A (**1**) and B (**2**) and the construction of the TDO ring at the C23 hydroxy amino group of **22**. The first total synthesis of **1** was accomplished by Lei and co-workers in 2024.[4] However, they constructed the TDO ring system by treating a chemically synthesized biosynthetic intermediate of **1** with enzymes, which were obtained through biosynthetic gene expression of alchivemycins, at the final stage of the synthesis. Due to the specificities of the enzymes, the types of derivatives that can be synthesized through enzymatic synthesis would be limited. Therefore, our efforts toward the first chemical total synthesis of alchivemycins and the development of their structurally simplified analogs will be continued.

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一般の皆様へ

我が国の死因第一位はがんであり，死因の約3割を占めると厚生労働省から報告されております。がんの治療を難しくしている要因の一つに転移が挙げられますが，転移が起こる際にがん細胞は原発巣から周囲の脈管に浸潤を起こします。この浸潤を阻害するがん治療薬を開発することができれば有効ながん治療薬の一つとなることが期待されます。本研究ではがん細胞に対する浸潤阻害活性が報告された天然由来の有機化合物の化学的合成法を確立し，合成品そのものやその類縁体を創薬のために役立てることを最終的な目標として研究を行っております。

Developing a novel therapeutic strategy for PDAC utilizing gut microbial metabolites

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Summary Abstract

We identified a significant reduction of specific gut bacteria and their metabolites in pancreatic cancer patients compared to healthy controls. In a mouse model, supplementation with the key metabolite enhanced the efficacy of an existing anticancer drug without added toxicity. Furthermore, pharmacological activation of the relevant metabolic pathway showed even greater therapeutic effects. These findings suggest a novel microbiome-based strategy to improve pancreatic cancer treatment.

Key Words : Pancreatic cancer, Gut microbiome, Metabolites, Drug discovery

Introduction

Pancreatic cancer remains one of the most intractable malignancies, with rising incidence and mortality and no effective standard therapies. We have recently developed *4-hit* genetically engineered fruit fly models that mimic four key gene alterations commonly observed in the most aggressive pancreatic cancer patients, enabling efficient identification of therapeutic targets. Using this platform, we discovered that gut bacteria A and its metabolite B are markedly reduced in *4-hit* flies, and that supplementation with B enhances the efficacy of drug D via activation of target C. This study aims to validate this combination therapy in mouse models and investigate its mechanism and clinical relevance in patient samples.

Results

To investigate the clinical relevance of our findings in fruit fly models, we first conducted a clinical study at Hokkaido University Hospital. We collected fecal samples from 28 treatment-naïve pancreatic cancer patients and 40 age-, sex-, and ethnicity-matched healthy controls. Using 16S rRNA gene sequencing, we comprehensively analyzed the gut microbiome composition of these individuals. Our analysis revealed that bacterium *E* was significantly depleted in pancreatic cancer patients compared to healthy controls (Figure 1A). We next quantified the levels of metabolite B, a representative metabolic product of bacterium *E*, in the fecal samples using gas chromatography-mass spectrometry (GC-MS). Consistent with the reduction in bacterium *E*, fecal concentrations of metabolite B were significantly lower in pancreatic cancer patients than in healthy individuals (Figure 1B). These findings suggest a possible link between the loss of bacterium *E*, reduced metabolite B production, and pancreatic cancer progression. To evaluate the therapeutic potential of

metabolite B, we employed an orthotopic xenograft model using immunodeficient mice. Luciferase-labeled human pancreatic cancer cells (AsPC-1) were surgically implanted into the pancreas of the mice. The animals were treated with a clinically used anticancer drug, drug D, either alone or in combination with metabolite B. Bioluminescence imaging revealed that co-administration of metabolite B significantly enhanced the antitumor efficacy of drug D without inducing observable toxicity or weight loss (Figure 2). These results suggest that metabolite B can potentiate the therapeutic effect of drug D *in vivo*. To further explore the underlying mechanism, we hypothesized that metabolite B exerts its tumor-suppressive effects through the activation of target C, a key metabolic and tumor-suppressive signaling pathway. We tested this by treating tumor-bearing mice with activator F, a known pharmacological activator of target C. Remarkably, treatment with activator F in combination with drug D produced a much greater antitumor effect than the combination of metabolite B and drug D in the same mouse model (Figure 2). These results support our hypothesis that activation of target C is a key mediator of the enhanced therapeutic response observed with metabolite B, and suggest that direct pharmacological activation of target C may be an even more effective strategy to enhance the efficacy of drug D. Together, these findings demonstrate that bacterium *E* and its metabolite B are significantly reduced in the gut of pancreatic cancer patients, and that metabolite B can improve the response to existing therapy. Furthermore, activation of target C may represent a promising approach to further boost treatment efficacy. These results provide a strong rationale for pursuing microbiome- and metabolism-based combination therapies in pancreatic cancer.

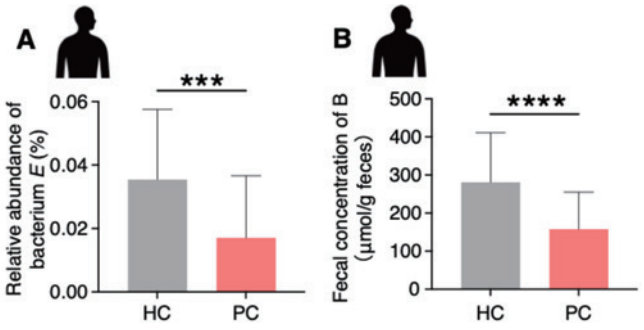

Figure 1. Reduced capacity for metabolite B production in pancreatic cancer patients. A. Decrease in bacterium *E* in pancreatic cancer patients. Comparison of the gut microbiome between 40 healthy controls and 28 pancreatic cancer patients. Bacterium *E*, a member of the human gut microbiota, was significantly reduced in pancreatic cancer patients compared to healthy individuals. **B. Decreased fecal levels of metabolite B in pancreatic cancer patients.** Fecal concentrations of metabolite B were measured by gas chromatography-mass spectrometry (GC-MS). HC, healthy controls, PC, pancreatic cancer patients. ***$P < 0.001$, ****$P < 0.0001$ in Student's *t*-test.

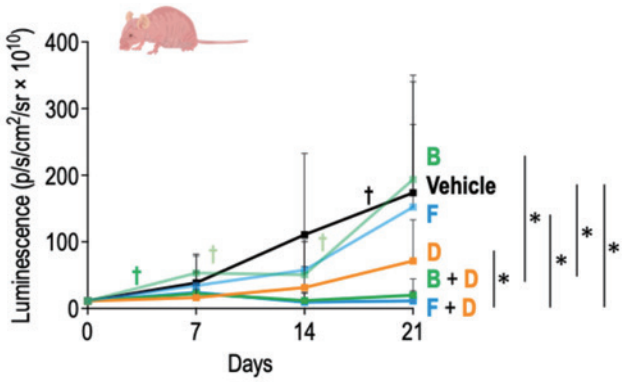

Figure 2. Metabolite B and activator F of target C enhance the antitumor effect of drug D in a pancreatic cancer mouse model. Luciferase-labeled human pancreatic cancer cells (AsPC-1) were orthotopically implanted into the pancreas of mice, which were then treated with B, F, or D. *$P < 0.0001$ in Student's *t*-test.

Discussion & Conclusion

Our study highlights a previously underappreciated link between gut microbiome alterations and therapeutic responses in pancreatic cancer. We identified a significant depletion of bacterium E and its metabolite B in pancreatic cancer patients, suggesting a potential microbiome-metabolite axis involved in disease progression. Functional studies in an orthotopic mouse model demonstrated that metabolite B enhances the efficacy of drug D without additional toxicity, and that direct activation of target C by compound F yields even stronger therapeutic effects. These findings suggest that both microbiome-derived metabolites and pharmacological activation of metabolic pathways may represent promising strategies to improve outcomes in pancreatic cancer. Future studies should focus on elucidating the precise molecular mechanisms of target C activation and validating these findings in larger clinical cohorts. Overall, our work provides a novel rationale for developing microbiome-based or metabolism-enhancing combination therapies to overcome the therapeutic resistance of pancreatic cancer.

一般の皆様へ

本研究では、膵がん患者さんの腸内環境を詳しく調べたところ、健常者と比較して特定の腸内細菌とその細菌が作り出す代謝産物が大きく減少していることが分かりました。また、この代謝産物にはがん治療薬の効果を高める働きがあることも動物実験で明らかになりました。さらにその働きを薬剤によって高める方法も見つかり、より強力ながん治療法につながる可能性があります。今後はより多くの患者さんでの検証を進め、治療に役立てることを目指しています。膵がんの新たな治療法の開発に貢献できることが期待されます。

Development of therapeutic strategies targeting HSD11β1 in metastasis of triple-negative breast cancer cells

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Summary Abstract

11-Beta Hydroxysteroid Dehydrogenase 1 (HSD11β1) expression was observed in 71% of triple-negative breast tumors. Mice models of metastasis demonstrated HSD11β1 inhibitor suppressed metastasis of triple-negative breast cancer cells (TNBC). However, the suppressor effect was limited, and the inhibitor failed to eliminate the TNBC. Therefore, therapeutic strategies using HSD11β1 inhibitor need to be modified. Through elucidating the molecular mechanism of how HSD11β1 promote metastasis of TNBC, this project aims to modify the strategies.

Key Words : Triple negative breast cancer, Metastasis, HSD11β1, EMT,

Introduction

HSD11β1 expression was observed in 71% of triple-negative breast tumors, and was correlated with shorter overall survival times in the sub-type. HSD11β1 expression induced EMT in human mammary epithelial cells and conferred metastatic activities on non-metastatic breast cancer cells; conversely, pharmacologic and genetic inhibition of HSD11β1 suppressed metastatic progression of breast cancer cells through induction of mesenchymal to epithelial transition (MET). Metabolic analyses revealed that HSD11β1 expressing breast cancer cells catalyzed 11-keto-progesterone as a substrate and yielded 11-hydroxyprogesterone (11β-OHP) which induced EMT on human mammary epithelial cells.

Results

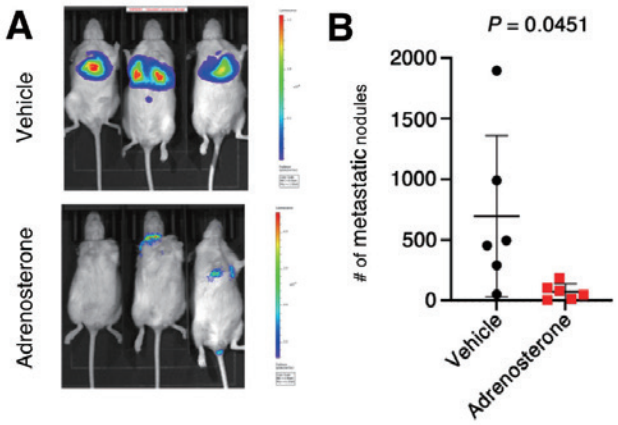
Elucidation of the molecular mechanism of how 11β-OHP regulate EMT.

Gene silence screen targeting 47 nuclear receptors revealed that Peroxisome proliferator-activated receptor alpha (PPARα) was essential for both HSD11β1 and 11β-OHP-driven EMT. In contrast, genetic inhibition of PPARα induced MET in HSD11β1-positive TNBC. These results suggest PPARα may play a critical role in regulating HSD11β1-driven EMT. Chromatin IP analysis revealed that PPARα bound to the promoter region of SNAIL1 in HSD11β1-positive TNBC lines and HSD11β1-expressing sub-clone of MCF7 cells. In contrast, PPARα failed to bind to the promoter region of SNAIL1 in HSD11β1-negative breast cancer cells including MCF7. The analysis also showed PPARα bound to the promoter region of SNAIL1 in 11β-OHP-treated human mammary epithelial cells MCF10A but not in vehicle-treated ones. These results indicate that PPARα which is activated by HSD11β1 or 11β-OHP, would bind to the promoter region of SNAIL1, and then induce EMT.

Examination of whether HSD11β1 inhibitor can suppress the proliferation of TNBC in the lung

Previous study demonstrated that HSD11β1 promoted EMT. Recent studies reported that EMT contributes to late steps of metastasis. Therefore, we examined whether HSD11β1 inhibitor can suppress the proliferation of TNBC in the lung. Metastatic tumor formations in the lung of HSD11β1 inhibitor-treated mice were dramatically decreased compared with those of vehicle-treated mice. This result indicates that HSD11β1-driven EMT would play a critical role in regulating the proliferation of TNBC in the lung.

Figure 1. HSD11β1 inhibitor suppressed the proliferation of TNBC in the lung.



A. Lucigferase-expressing TNBC were inoculated into immunedeficient mice, and then treated with either vehicle (upper) or HSD11β1 inhibitor Adrenosterone (lower).

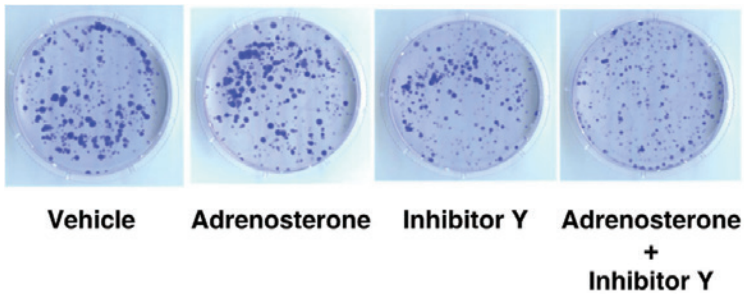
B. The number of metastatic tumor nodules were counted in the surface of the lungs of vehicle (●) or Adrenosterone (■)-treated mice.

Identification of target molecule X which induces synthetic lethal effects associated with loss of HSD11β1 function in metastatic TNBC

Previous study demonstrated that HSD11β1 inhibitor suppressed metastasis of TNBC in a mice model of metastasis. However, the suppressor effect was limited, and the inhibitor failed to eliminate the TNBC. Therefore, we conducted genetic screen to identify target molecule X which induces synthetic lethal effects associated with loss of HSD11β1 function in metastatic TNBC. Although genetic inhibition of HSD11β1 did not affect the proliferation of TNBC, genetic inhibition of X and HSD11β1 dramatically decreased the proliferation of TNBC. And also, inhibition Y targeting the protein that encoded by X gene, and HSD11β1 dramatically decreased the proliferation of TNBC.

Figure 2. HSD11β1 and inhibitor Y suppressed the proliferation of TNBC.

The effects of either vehicle, adrenosterone, Inhibitor Y or combination of adrenosterone and Inhibitor Y on the proliferation of TNBC.



Discussion & Conclusion

Our data showed 11β-OHP-activated PPARα bound to the promotor region of SNAIL1. Normally, PPARα heterodimerizes to RXRα and the complex shows transcriptional activity. However, genetic inhibition of RXRα did not affect HSD11b1-driven EMT. This result suggests that PPARα may heterodimerize to one of other nuclear receptors and transcribe SNAIL1.

Our data also showed HSD11β1 inhibitor suppressed the proliferation of TNBC in the lung. This result indicates HSD11b1-driven EMT promote not only the invasion and migration of TNBC but also the proliferation of TNBC in the lung. We need to elucidate the molecular mechanism of how HSD11b1-driven EMT promote the proliferation of TNBC in the lung

Our data identified target molecule X which induces growth inhibition effects associated with loss of HSD11β1 function in TNBC. In vitro, through genetic inhibition of HSD11β1 did not affect the proliferation of TNBC, genetic inhibition of X and HSD11β1 dramatically decreased the proliferation of TNBC.

Taken together, dual inhibitions of X and HSD11β1 function would dramatically suppress the proliferation of TNBC in the lung.

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Joji Nakayama*, Ami Maruyama, Takamasa Ishikawa, Noriko Gotoh, and Hideki Makinoshima. HSD11β1 promotes EMT-mediated breast cancer metastasis. bioRxiv: DOI: https://doi.org/10.1101/2021.09.27.461934. *corresponding author.

一般の皆様へ

本研究はトリプルネガティブ乳がん（以下 TNBC）の転移を抑制する治療戦略を開発することを目的としています。本邦では年間約1．3万人が乳がんにより死亡していおり、TNBC は転移・再発する確率が高く、非常に難治です。申請者の前研究は TNBC 臨床検体の 70% 以上でストレスホルモン産生酵素である HSD11 β 1 の発現が亢進しており、HSD11 β 1 阻害剤は TNBC の転移を抑制することを動物実験で証明しました。しかしながら、HSD11 β 1 阻害剤による転移抑制効果は寛解には至らず、HSD11 β 1 の転移亢進機構に基づいた治療戦略の改良が必要です。本研究が考案する治療戦略は転移性 TNBC の駆逐に繋がり、その恩恵は TNBC に罹る人の70％に行き渡ると期待されます。

Development of regio-and stereoselective functionalization of polyols utilizing organocatalysts

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Summary Abstract

We developed multifunctional *N*-heterocyclic carbene (NHC) catalysts bearing pyridine recognition sites for the enantioselective desymmetrization of meso-1,2-diols. Using α-benzoyloxyaldehydes as acyl donors, we achieved high enantioselectivity (up to 95% ee) and good yield (77%) for monoacylated diols. This approach enables the efficient synthesis of chiral 1,2-diol building blocks.
Key Words : *N*-heterocyclic carbene, meso-diol, desymmetrization, catalytic asymmetric reaction

Introduction

N-Heterocyclic carbenes (NHCs) are powerful organocatalysts known for their tunable structures and strong nucleophilicity. We aimed to develop multifunctional NHC catalysts that mimic enzyme-like recognition to achieve site- and enantioselective functionalization of polyols, a long-standing challenge due to the similar reactivity of hydroxyl groups. Our strategy focuses on precise catalyst-substrate interactions to control selectivity.

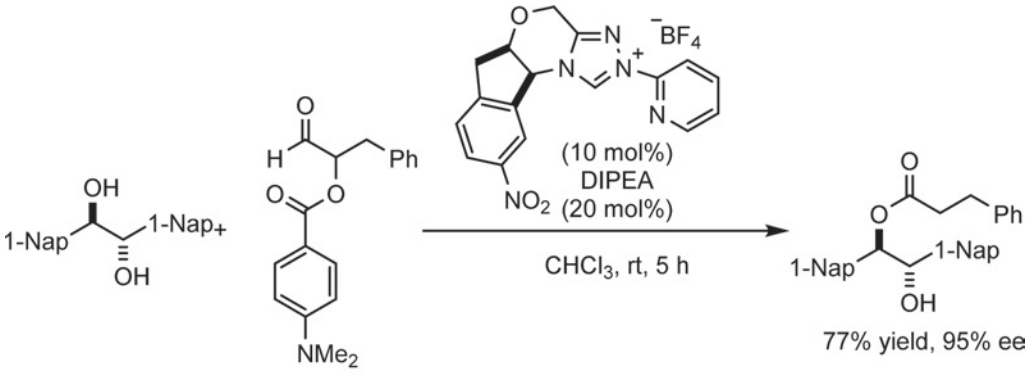
Results

In this study, we developed multifunctional *N*-heterocyclic carbene (NHC) catalysts that feature a pyridine ring to enable selective recognition of polyol substrates. These chiral triazolium salts, derived from aminoindanol, were designed to interact with substrates through both the carbene center and the pyridine nitrogen, thereby achieving site- and enantioselective functionalization. The introduction of the pyridine ring was intended to mimic enzymatic molecular recognition by establishing a dual-point binding interaction with the substrate, improving control over regio- and stereoselectivity.

We selected α-benzoyloxyaldehydes bearing a dimethylamino group at the para position as the acylating agents, which had previously demonstrated good reactivity and selectivity in related NHC-catalyzed processes. As a model substrate, *meso*-1,2-di-1-naphthyl-1,2-ethanediol was chosen due to its sterically demanding structure and symmetric hydroxyl groups, posing a challenge for selective mono-functionalization.

Reaction condition screening was performed to optimize base, solvent, catalyst loading, and temperature. Among various combinations tested, the use of DIPEA as a base and

chloroform ($CHCl_3$) as a solvent at room temperature provided the best results. Under these optimized conditions, the asymmetric monoacylation of the diol proceeded smoothly, affording the monoacylated product in 77% isolated yield with an enantiomeric excess (ee) of 95%. The product was characterized by NMR and chiral HPLC, confirming its structure and enantiopurity. Notably, over-acylation to produce the diacylated byproduct was negligible, indicating a high level of chemoselectivity under the applied catalytic system.



We further evaluated the substrate scope of the catalytic system. Both cyclic and acyclic *meso*-1,2-diols were tested to assess the generality of the transformation. The reaction exhibited good to excellent selectivities across various substrates. In general, substrates with increased steric bulk near the hydroxyl groups led to improved enantioselectivity, supporting the hypothesis that steric complementarity between the substrate and catalyst contributes significantly to the observed selectivity. In contrast, less hindered diols showed moderate selectivities, which suggests that the molecular recognition properties of the catalyst are more effective when there are steric cues to guide the approach of the substrate.

Additionally, control experiments without the pyridine moiety in the catalyst resulted in decreased enantioselectivity and increased byproduct formation, further validating the role of the pyridine ring in substrate orientation and selective activation. This finding underscores the importance of multifunctional catalyst design in complex molecular recognition tasks, especially for polyols, where multiple hydroxyl groups often exhibit similar reactivity.

Overall, the newly developed chiral NHC catalysts demonstrated a high degree of control over both chemoselectivity and enantioselectivity in the desymmetrization of meso-1,2-diols. The use of α-benzoyloxyaldehydes as acyl donors proved effective, and the optimized conditions (DIPEA/$CHCl_3$) allowed for reliable and reproducible results. The catalyst system offers broad substrate applicability and holds promise for future applications in the synthesis of enantiomerically pure 1,2-diols, which are valuable building blocks in medicinal chemistry and materials science.

Discussion & Conclusion

The successful desymmetrization of meso-1,2-diols using our newly developed multifunctional NHC catalysts highlights the power of rational catalyst design based on enzyme-inspired recognition. The dual interaction strategy—through the NHC center and the pyridine ring—proved effective in guiding both site- and enantioselective monoacylation, even in challenging substrates bearing two equivalent hydroxyl groups. The high levels of chemoselectivity and enantioselectivity achieved across various substrates underscore the versatility of the system.

These findings not only provide a practical method for the preparation of chiral 1,2-diols but also suggest broader applications of multifunctional NHCs in the selective functionalization of polyols and related compounds. Future work will focus on expanding the substrate scope, exploring other electrophilic partners, and applying this approach to more complex molecules relevant to natural product synthesis and pharmaceutical development.

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一般の皆様へ

本研究では，糖や薬のもとになる「ジオール」と呼ばれる化合物の一部だけを選んで反応させる，新しい触媒を開発しました。この触媒は，酵素のように分子の形や性質を見分ける仕組みを持ち，左右の区別（不斉）にも優れた選択性を発揮します。これにより，医薬品などに使われる光学活性体を効率よく合成できる可能性があります。今後は，より複雑な化合物への応用や，の種類の反応にも展開していく予定です。分子を「正確に，選んで，つなぐ」技術の発展は，創薬や材料開発にもつながると期待されます。

Elucidation of Effects of Messenger RNA 5′-Cap Structure Diversity on Protein Translation Activity

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Summary Abstract

By using our original PureCap method, we can synthesize completely capped mRNA. The method makes it possible to produce completely capped mRNA for the cap structure chemical modification-activity relationship study. Evaluation of the translational activity of the synthesized mRNA revealed that activity varied depending on the type and number of chemical modifications. While this study focused on 2′-*O*-methyl and 2′-deoxy modifications, we plan to investigate a broader range of chemical modifications in future studies.

Key Words : Messenger RNA (mRNA), Cap Structure, Chemical Modifications, Protein Translation

Introduction

mRNA therapeutics require a 5′-cap structure for initiating protein synthesis. Conventional *in vitro* co-transcription methods yield a mixture of capped and uncapped RNAs due to inefficient cap analog incorporation efficiency. We developed the PureCap method, combining co-transcription and following RP-HPLC purification, to produce fully capped mRNA. This enables precise evaluation of how cap structure modifications affect activity, supporting detailed structure-activity relationship studies crucial for advancing mRNA therapeutics.

Results

We utilized the PureCap method to investigate how chemical modifications in the mRNA cap structure affect translational activity. This method allows the introduction of an o-nitrobenzyl group into the cap via *in vitro* co-transcriptional synthesis using PureCap analogs. The high hydrophobicity of the *o*-nitrobenzyl group

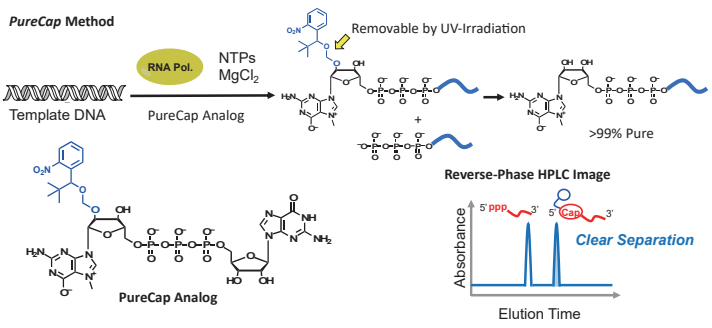

Figure 1. PureCap Method To Realize Synthesis of Completely Capped mRNA

enables complete separation of capped mRNA from the mixture through reverse-phase HPLC. The o-nitrobenzyl group can be removed by UV irradiation (365 nm), resulting in fully capped mRNA (**Figure 1**). Using a dinucleotide PureCap analog (Di-PureCap), we synthesized Cap0-mRNA. Tri-PureCap and Tetra-PureCap analogs enabled the production of Cap1 and Cap2-mRNA, respectively. While Cap2 synthesis is generally difficult, the PureCap method enables efficient high-purity production.[1] We designed and synthesized a variety of chemically modified PureCap analogs and introduced them into the mRNA cap structure *via* co-transcriptional reactions. The translational activity of the resulting modified mRNAs was tested in cultured cells.

Using an automated DNA/RNA synthesizer and HPLC system, we synthesized various di- and trinucleotide analogs with modifications including 2′-fluoro, locked nucleic acid, 2′-O-methyl, 2′-O-methoxyethyl, 2′-deoxy, and phosphorothioate. These analogs were reacted with 7-methylguanosine diphosphate phosphoroimidazolide bearing a 2′-o-nitrobenzyl group, and purified via HPLC. In total, we successfully synthesized over 13 chemically modified cap analogs with yields of 10–30%. Using the synthesized cap analogs, we performed ***i***n vitro co-transcriptional mRNA synthesis. We focused on the effects of the number of 2′-O-methyl modifications, which are abundant in endogenous mRNA, as well as the effect of 2′-deoxy modifications, structurally similar to DNA (**Figure 2**).

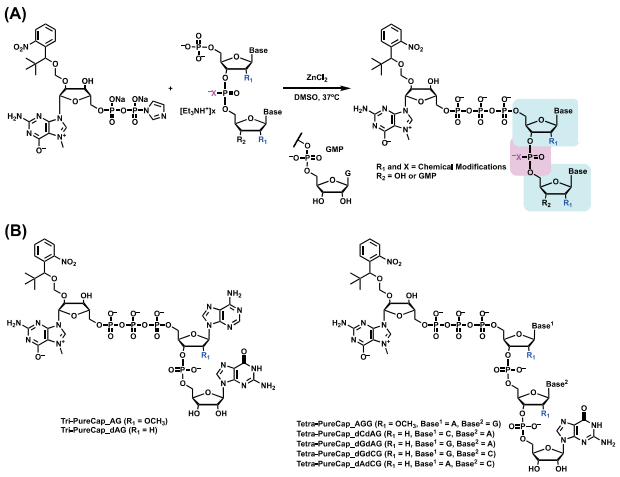

Figure 2. (A) Synthetic Scheme of Chemically Modified PureCap Analogs, (B) Synthesized PureCap Analogs Introduced Into the mRNA Cap Structure in This Work.

For efficient incorporation of cap analogs, we prepared template DNA containing the T7 Φ6.5 promoter and the NanoLuc luciferase coding region (**Figure 3A**). Additionally, we designed templates with A- or C-inserted promoter sequences to enhance incorporation efficiency. Transcripts synthesized using these templates were purified using reverse-phase HPLC, separating capped from uncapped mRNA (**Figure 3B**). Cap incorporation efficiency was evaluated via peak ratios in HPLC. With Φ6.5 and PureCap_dAG, efficiency was 60%, and remained similar with the A-inserted promoter. PureCap_dCdAG and PureCap_dGdAG showed efficiencies ≤27%. In contrast, PureCap_dGdCG and PureCap_dAdCG, which had low efficiency with Φ6.5, reached 70–88% with C-inserted templates **(Figure 3C)**.[2]

We evaluated translational activity using HeLa and JAWS II cells. mRNAs synthesized with Cap0 and Cap1 via PureCap showed 1.1–1.3 times higher activity than those made with CleanCap AG. Notably, Cap2-mRNA demonstrated up to a 3-fold increase, highlighting the positive effect of 2′-*O*-methyl modifications.

We also examined 2′-deoxy modifications. A comparison between Tri-PureCap_AG (Cap1) and Tri-PureCap_dAG showed a 3-fold enhancement in translational activity with

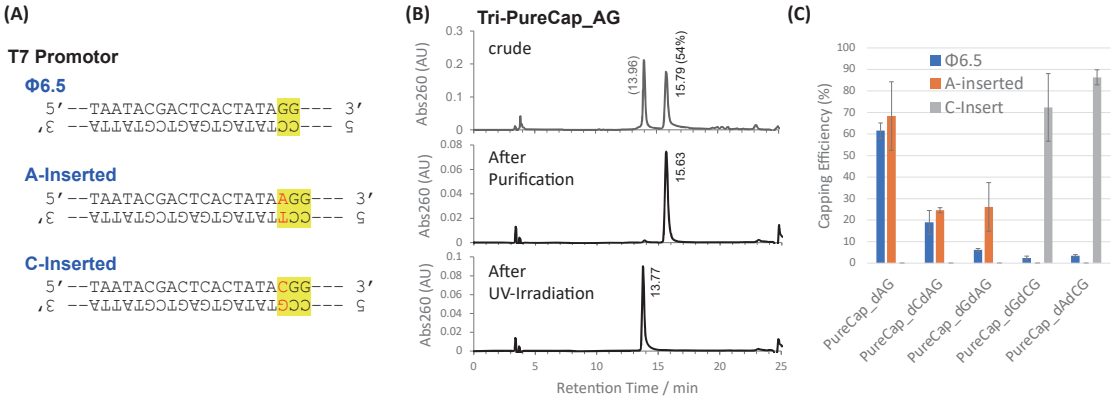

Figure 3. (A) T7 Promotor Sequences Used for mRNA Synthesis, (B) Reverse-Phase HPLC Purification Result to Produce Completely Capped Cap1-mRNA by PureCap Method, (C) PureCap Analog Incorporation Efficiency as the mRNA Cap Structure.

the 2′-deoxy analogs (**Figure 4A**). Furthermore, in mRNA with two 2′-deoxy modifications, translational efficiency varied with sequence context, suggesting that both modification type and sequence affect translation (Figure 4B).[2)]

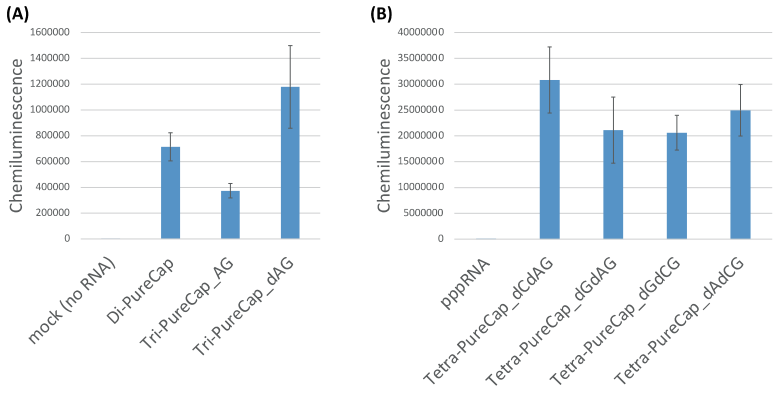

Figure 4. Translational Activities of Completely Capped mRNAs: (A) Comparison of Different Chemical Modifications 2'-*O*-Methyl and 2'-Deoxy, (B) Effect of Nucleobase Sequence Difference on the 2'-Deoxy Modified mRNA.

Discussion & Conclusion

We designed and synthesized novel cap analogs bearing various chemical modifications using a semi-automated synthesis and purification system. *In vitro* co-transcriptional reactions with these analogs showed that introducing a single-nucleotide-inserted Φ6.5 promoter enhanced cap incorporation efficiency. Subsequent evaluation of the translational activity of the synthesized mRNAs revealed that the activity was influenced by the type and number of chemical modifications, as well as the nucleobase sequences. This study primarily focused on 2′-*O*-methyl and 2′-deoxy modifications. In future work, we plan to expand our investigation to include a wider variety of chemical modifications. Furthermore, we aim to perform a more comprehensive analysis of how nucleotide sequence, in combination with different chemical modifications, affects translational efficiency.

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一般の皆様へ

mRNA 医薬は、新型コロナウイルス感染症ワクチンとしての実用化をきっかけに、がんワクチンなどさらなる応用が期待されています。mRNA 医薬の活性発現には、タンパク質合成を開始するために必要な 5’ キャップ構造の存在が重要です。従来の合成法では、キャップされた RNA とキャップされていない RNA が混在した状態になります。私たちは、PureCap 法と名付けた完全にキャップされた mRNA を合成できる方法を開発しました。この方法を用いることにより、キャップ構造への修飾導入とその活性への影響を正確に評価できるようになり、より優れた mRNA 医薬の開発に役立ちます。

Elucidation of the fibrotic mechanism spanning multiple organs through novel BMP antagonist

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Summary Abstract

This study aims to elucidate a novel fibrotic mechanism across multiple organs mediated by NBL1, a BMP-7 antagonist. We demonstrate that NBL1 promotes endothelial-to-mesenchymal transition in vitro and exacerbates cardiac and renal fibrosis in diabetic mice. Although circulating NBL1 was not predictive of cardiovascular events in dialysis patients, proteomic screening identified other candidate biomarkers.
Key Words : NBL1, BMP7 antagonist

Introduction

Through comprehensive plasma proteomic profiling in patients with diabetic nephropathy, we identified NBL1—a known antagonist of the renoprotective cytokine BMP-7—as a potent biomarker predictive of progression to end-stage kidney disease (ESKD). In vivo studies using NBL1 knockout mice demonstrated that circulating NBL1 contributes to kidney fibrosis. Given the shared antifibrotic mechanisms mediated by BMP-7 in the kidney and heart, elevated plasma NBL1 may also play a pathological role in myocardial injury.

Results

(1) In vitro analysis: NBL1-induced endothelial-to-mesenchymal transition (EndMT) in HUVECs

To investigate the role of NBL1 in endothelial plasticity, human umbilical vein endothelial cells (HUVECs) were cultured for three days in medium supplemented with recombinant NBL1 (1000 ng/mL). Quantitative real-time PCR demonstrated a significant upregulation of fibronectin, a canonical mesenchymal marker, compared to untreated controls. Additionally, immunofluorescence staining for VE-cadherin—a key adherens junction protein specific to endothelial cells—was performed following a 7-day exposure to recombinant NBL1. In control HUVECs, VE-cadherin was consistently expressed along intercellular junctions, whereas its expression was markedly reduced in NBL1-treated cells. These findings suggest that NBL1 promotes EndMT-like phenotypic changes, characterized by both molecular marker alteration and morphological transition. Ongoing investigations aim to elucidate the precise molecular pathways through which NBL1 mediates this response.

(2) In vivo study: Diabetic mouse models using NBL1-deficient mice

To elucidate the pathological role of NBL1 in diabetic complications, we generated diabetic mouse models using NBL1 knockout (NBL1 -/-) and wild-type (WT, +/+) mice. Diabetes was induced via streptozotocin (STZ) administration, and animals were observed for 20 weeks. No significant differences in body weight or glycemic control were observed between diabetic and control groups. Notably, plasma NBL1 concentrations were significantly elevated in diabetic WT mice compared to non-diabetic controls ($p < 0.05$). Furthermore, myocardial NBL1 mRNA expression was significantly upregulated in diabetic WT mice ($p < 0.05$). Expression of profibrotic genes—including fibronectin, collagen IV, and CTGF—was markedly increased in diabetic WT mice relative to their NBL1-deficient counterparts ($p < 0.05$). These results implicate NBL1 as a key mediator of fibrosis in both renal and cardiac tissues under diabetic conditions. Ongoing analyses include protein-level validation and histopathological assessment of fibrotic remodeling.

(3) Clinical proteomics: Association of plasma proteins with cardiovascular events in hemodialysis patients

To assess the clinical relevance of NBL1 in humans, we analyzed archived plasma samples from maintenance hemodialysis patients enrolled at six dialysis centers affiliated with Nihon University Itabashi Hospital. Plasma levels of NBL1 and other proteins were quantified using Olink proximity extension assay technology. Associations between protein expression levels and subsequent cardiovascular disease (CVD) events were evaluated using fold change analysis. The study was approved by the Institutional Review Board of Nihon University Itabashi Hospital (approval ID: RK-220308-1), and informed consent was obtained from all participants. Contrary to our initial hypothesis, circulating NBL1 levels were not significantly associated with CVD incidence within a two-year follow-up period. However, the comprehensive proteomic screening identified several novel plasma proteins that demonstrated robust associations with CVD risk, offering promising targets for future biomarker validation and mechanistic exploration.

Discussion & Conclusion

This study highlights NBL1 as a novel pathogenic factor and potential biomarker in diabetic organ fibrosis. In vitro experiments demonstrated that exogenous NBL1 induces EndMT-like changes in endothelial cells, implicating it in vascular remodeling. In vivo, NBL1 expression was elevated in diabetic mouse models and associated with increased expression of fibrotic genes in both the kidney and heart, suggesting a systemic pro-fibrotic role. While plasma NBL1 was not significantly associated with incident cardiovascular events in hemodialysis patients, proteomic screening uncovered other candidate proteins with strong predictive value. These findings suggest that NBL1 may serve as a therapeutic target for fibrotic complications in diabetes, although its utility as a cardiovascular biomarker in advanced kidney disease remains limited. Further mechanistic studies and longitudinal validation in larger human cohorts are warranted to fully elucidate its clinical relevance and therapeutic potential.

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一般の皆様へ

本研究では、糖尿病によって腎臓や心臓に起こる「線維化（せんいか）」という病的な変化の原因を明らかにすることを目的としました。私たちは、NBL1 という血液中のたんぱく質が、細胞の性質を変化させ、腎臓や心臓の線維化を進行させる可能性があることを明らかにしました。特に、NBL1 が血管内皮細胞に影響を与え、組織のかたちや働きに悪影響を及ぼすことが分かりました。今後、このたんぱく質の働きを抑えることが、糖尿病による臓器障害の新たな治療法につながることが期待されます。

Opposite regulation by RNA splicesome factors for high and low temperature tolerance across species

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Summary Abstract

Adaptation and tolerance to temperature are essential for the survival of plants and animals. However, the common molecular mechanisms between animals and plants remain largely unknown. Here, we found that heat and cold tolerance in nematode *C. elegans* is oppositely regulated by RNA-binding protein EMB-4, whose plant homolog exhibits polymorphisms associated with heat tolerance diversity. *C. elegans* adjusts its heat and cold tolerance based on past temperatures, with EMB-4 acting as a positive and negative regulator of heat and cold tolerance, respectively. Among the genes regulated by EMB-4, phospholipid scramblase and acid sphingomyelinase were key factors in the negative regulation of heat tolerance.
Key Words : heat tolerance, cold tolerance, *C. elegans*, plant

Introduction

Temperature is a constant presence on Earth, and fluctuations in environmental temperature have a significant impact on the survival, adaptation, and reproduction of all living organisms. It has long been known that in insects and plants, temperature changes influence the composition of membrane lipids in cells and the accumulation of sugars that prevent freezing, thereby contributing to tolerance to both low and high temperatures. However, as molecular-level research has advanced, the study of temperature tolerance in animals and plants has tended to be conducted as separate systems, and the identification of shared molecular pathways between the two has remained limited.

Results

The heat tolerance of wild-type *Caenorhabditis elegans* strain N2 varies depending on past cultivation temperatures. When exposed to 31°C, individuals cultured at 15°C mostly died, whereas those cultured at 25°C survived. Worms cultured at 20°C displayed an intermediate phenotype, with approximately half surviving. These findings suggest that exposure to higher temperatures enhances heat tolerance, indicating that the acquisition or loss of heat tolerance depends on past temperature conditions.

The *LHT1* gene, identified in *Arabidopsis thaliana* as contributing to heat tolerance variability, was investigated for its role in animal temperature tolerance using *C. elegans*. *LHT1* encodes a protein with RNA and DNA helicase domains and functions as a spliceosomal factor. Its homolog in *C. elegans* is *emb-4*, which encodes the EMB-4 protein.

Survival analysis of *emb-4* mutants under heat stress (32°C for 24 hours) revealed a lower survival rate compared to wild-type worms, suggesting that EMB-4 positively regulates heat tolerance. Conversely, when exposed to cold stress (2°C for 48 hours), *emb-4* mutants showed a higher survival rate, indicating that EMB-4 negatively regulates cold tolerance. These results imply that EMB-4 promotes heat tolerance while suppressing cold tolerance in wild-type *C. elegans*.

Transcriptome analysis was performed to determine genes influenced by EMB-4 during heat and cold stress. Differential gene expression was analyzed under four conditions: (i) *emb-4* mutants cultured at 20°C without temperature changes (Up: 1,103 genes; Down: 173 genes); (ii) *emb-4* mutants shifted from 20°C to 2°C for 9 hours (Up: 799 genes; Down: 662 genes); (iii) *emb-4* mutants shifted from 20°C to 32°C for 1 hour (Up: 1,321 genes; Down: 133 genes); (iv) *emb-4* mutants shifted from 20°C to 32°C for 13 hours (Up: 480 genes; Down: 161 genes). A total of 55 genes showed consistent expression changes across all conditions (46 upregulated, 9 downregulated), highlighting their potential role in temperature tolerance.

Among the 55 differentially expressed genes, 12 mutants were analyzed for their involvement in heat and cold tolerance. Mutations in *asm-3* (fatty acid metabolism) and *scrm-4* (membrane phospholipid regulation) enhanced heat tolerance. Additionally, mutations in *gcy-19* (guanylyl cyclase) and *dyf-3* (clathrin-like protein) also improved heat tolerance. Furthermore, a mutation in an uncharacterized gene, *C38D9.2*, was found to enhance heat tolerance. Among these, *dyf-3* and *C38D9.2* mutants also exhibited increased cold tolerance.

Acid sphingomyelinase (ASM) is located in lipid bilayers, hydrolyzing sphingomyelin into ceramide and phosphorylcholine. Ceramide-containing sphingolipids contribute to lipid raft formation, essential for cellular signaling. In *C. elegans*, *asm-3* mutants exhibit excessive accumulation of omega-3 long-chain fatty acids, suggesting that ASM-3-mediated membrane signaling and fatty acid metabolism play a role in heat tolerance.

Phospholipid scramblase (SCRM), also present in lipid bilayers, facilitates phospholipid translocation and is primarily activated by Ca^{2+}. This process is linked to blood coagulation, apoptosis, and mitophagy signaling. Recent studies indicate that the *C. elegans* SCRM protein ATG-9 is involved in lipid mobilization from lipid droplets. These findings suggest that membrane phospholipid composition, regulated by SCRM-4, contributes to heat tolerance.

Previously, EMB-4 was primarily associated with embryonic development. However, this study reveals a novel role for EMB-4 in regulating the expression of neuronal genes, expanding its known functions in *C. elegans*.

Discussion & Conclusion

This study revealed that a spliceosomal factor previously reported to be involved in plant heat tolerance also plays a role in both heat and cold tolerance in the nematode *Caenorhabditis elegans*. Furthermore, we found that the corresponding gene in *C. elegans* (*emb-4*) regulates heat tolerance by influencing the expression of genes such as acid sphingomyelinase, which is involved in fatty acid metabolism, and phospholipid scramblase, which affects the local structure of membrane phospholipids.

Our findings suggest the existence of a common gene that regulates heat tolerance in both animals and plants. Since homologs of the *emb-4* gene are widely conserved across species, from animals, including humans, to plants, further analysis could contribute to the development of heat-resistant livestock and crops.

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一般の皆様へ

温度は地球上に常に存在し、環境の温度変化は、あらゆる生物の生存・適応・繁殖に大きな影響を及ぼす。従来より、昆虫や植物において、温度変化が細胞内の膜構造を構成する脂質の割合や、凍結を防ぐ糖の蓄積に影響を与え、結果として低温や高温に対する耐性に関与することが知られていた。一方で、分子レベルの研究が進むにつれ、動物と植物の温度耐性はそれぞれ独立したシステムとして解析される傾向があり、両者に共通する分子経路の解明は十分に進んでいなかった。こうした背景のもと、本研究では、植物の高温耐性の多様性に関与するRNAスプライシング因子が、動物（線虫C. elegans）の高温耐性にも関与することを明らかにした。

Understanding and Control of the Nuclear Transport Timer

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Summary Abstract

The cell nucleus is a crucial organelle that maintains cellular adaptability by regulating nucleocytoplasmic transport in response to various changes. In aging cells, transformation of this transport system—termed nuclear aging—disrupts intracellular information networks, leading to impaired adaptability. Conversely, cancer cells can evade nuclear aging, enabling prolonged proliferation, though the mechanisms remain unclear. This project aims to develop a system to identify long-lived nuclear proteins and uncover how the functional "nuclear lifespan timer" is regulated. The findings will provide foundational insights into cellular aging and pave the way for novel cancer therapeutic strategies.
Key Words : Nucleus, Nuclear pore complex, aging

Introduction

Aging is accompanied by functional decline in cells, often driven by cellular senescence involving DNA damage responses, telomere shortening, and metabolic changes. Recent studies reveal that the nuclear pore complex (NPC), essential for nucleocytoplasmic transport[1)], becomes structurally unstable in senescent cells[2)], disrupting intracellular signaling. Specific nucleoporins get degraded or mis-localized in aging cells[2)], suggesting the nucleus may act as a “lifespan timer.” While cancer cells evade such nuclear aging, the underlying mechanisms remain unclear. Understanding the dynamics and turnover of NPC components, and how their homeostasis is maintained through autophagy and proteasomal pathways, may reveal new strategies for aging control and cancer therapy.

Results

① Development of a Tracking System for Long-lived Nuclear Pores

This study aimed to (1) develop a RITE (Recombination-Induced Tag Exchange) system capable of distinguishing nuclear pores based on their lifespans, and (2) utilize this system to uncover the mechanisms regulating the structural dynamics of long-lived nuclear pores, thereby shedding light on nuclear aging. ELYS, a key nuclear pore component, is known to accumulate on segregated chromatin during mitosis and is essential for reassembling both the nuclear envelope and nuclear pores[3)]. To visualize long-lived ELYS proteins, we designed a RITE system where the tag fused to ELYS can be switched via Cre recombinase (Fig. 1).

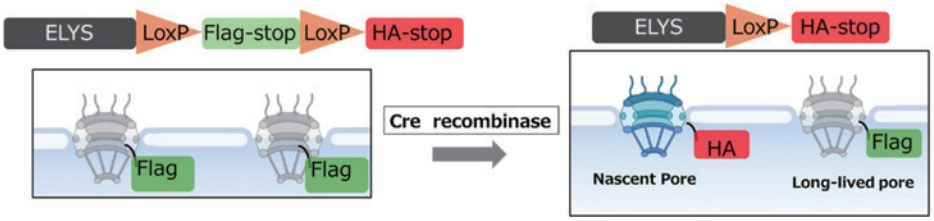

Figure 1. Overview of the RITE System for Tracking Long-lived NPCs

Using gene synthesis technology from GenScript, we generated a synthetic construct encoding the ELYS gene fused at the C-terminus with a RITE cassette (LoxP-3xFlag-Stop-LoxP-3xHA-Stop). This cassette was cloned into the lentiviral expression vector pLEX using restriction sites at both ends. Unexpectedly, the repeated tag sequences (Flag or HA) were found to undergo recombination in E. coli. Specifically, recombination occurred between the first and third Flag repeats, although no frameshift was detected. Thus, we proceeded with gene transduction experiments in HEK293T cells. qRT-PCR analysis showed a 40-fold increase in ELYS mRNA expression in cells transduced with pLEX-ELYS-RITE compared to controls. However, Western blotting using ELYS and Flag antibodies failed to detect any change in protein levels. Sequencing of the construct revealed no errors apart from the known partial deletion of the Flag repeat. We are currently investigating the cause of undetectable ELYS-Flag protein and optimizing codon usage to prevent recombination. Since a NheI site is present at the 3' end of ELYS, we plan to synthesize a new gene fragment for replacement.

② Protein Stability Analysis of Nuclear Pore Components

The nuclear pore complex (NPC), composed of ~30 proteins including ELYS, serves as the sole channel for nucleocytoplasmic transport and plays a vital role in coordinating gene expression. Despite this, little is known about the stability and

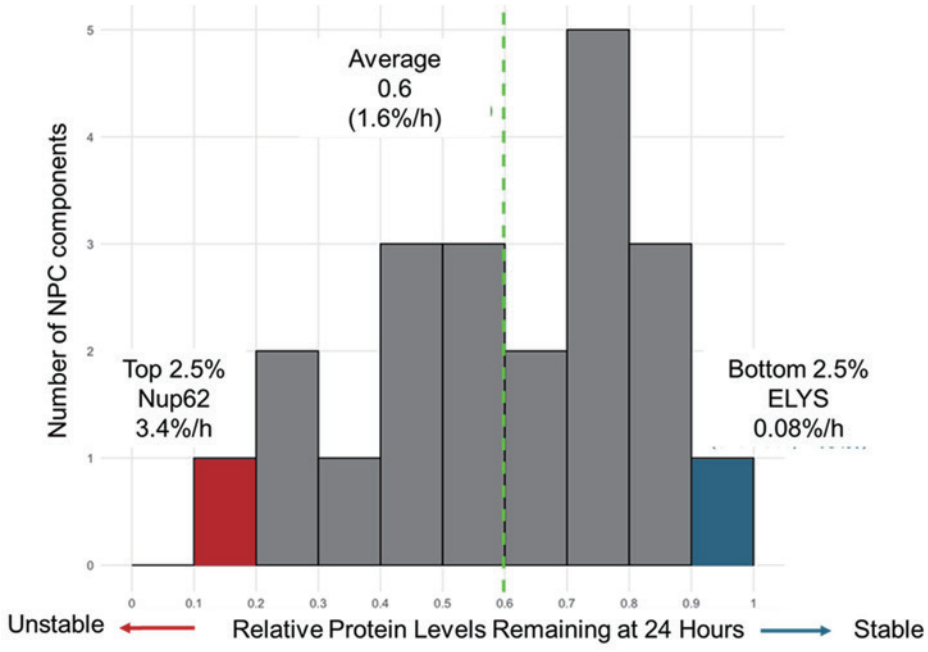

Figure 2. Stability of NPC Proteins

turnover of NPC components in cancer cells. To address this, we analyzed the half-lives of NPC proteins in HCT116 colorectal cancer cells using a cycloheximide (CHX) chase assay. Cells were treated with 50 μg/mL CHX and harvested at 0, 4, 8, and 24 hours. Lysates were prepared using a buffer containing protease and phosphatase inhibitors, heat-denatured, and ultrasonically lysed. Protein concentrations were measured via Bradford assay, and 10 μg of protein per lane was loaded for SDS-PAGE and Western blotting. KLF5, a short-lived protein, was used as a positive control and showed a half-life of approximately 1.05 hours, consistent with previous reports, confirming the assay's reliability.

We then assessed the stability of 21 NPC components, including representatives from the cytoplasmic filaments (e.g., Nup214, RanBP2), inner ring (e.g., Nup62, Nup93), Y-complex (e.g., Nup107, ELYS), linker Nups, membrane ring, and nuclear basket. Most proteins showed a time-dependent decrease in expression. Some Nups displayed transient increases at 4 hours, possibly due to rapid degradation of associated regulatory proteins, though this requires further validation.

Compared to KLF5, most NPC components had significantly longer half-lives. Their degradation rates were not uniform, averaging 1.6% per hour. NUP62 was the most unstable (3.4%/h), whereas ELYS was the most stable (0.083%/h), reinforcing its role as a long-lived structural protein (Fig.2).

③ Correlation Between Protein Stability and RNA Expression

To investigate whether RNA expression levels influence protein turnover, we hypothesized that proteins with high RNA expression may undergo faster degradation to maintain steady protein levels (Fig. 6). RNA expression (TPM) was retrieved from the DepMap Portal (Expression_Public_23Q4.csv), and the relationship between RNA levels and residual protein at 24 hours post-CHX treatment was analyzed (Fig. 3).

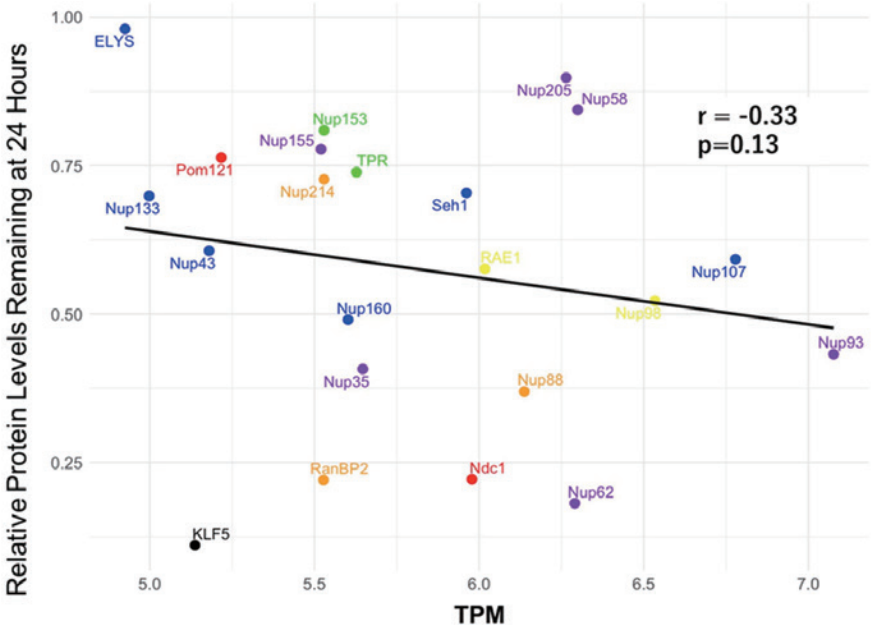

Fig.3 Correlation between protein stability and RNA expression levels

Overall, a weak inverse trend was observed (r = -0.33, p = 0.13), suggesting that higher RNA expression may be associated with faster protein degradation, although this correlation was not statistically significant. Notably, ELYS, the most stable protein, showed lower RNA expression than most Nups, whereas NUP62, the least stable, had relatively high RNA levels (TPM = 6.29 vs. mean = 5.83). These findings suggest that protein stability may not solely depend on transcription levels, but also on post-translational regulation, highlighting the need to integrate both datasets when evaluating protein lifespans.

Discussion & Conclusion

Abnormalities in the nuclear pore complex (NPC) in senescent cells may not merely reflect a decline in cellular function but could play a pivotal role in driving the progression of cellular aging. Disruption of NPC function affects intracellular signaling by disturbing the proper distribution of nuclear and cytoplasmic proteins, which is essential for maintaining cellular homeostasis. Notably, mislocalization of key transcription factors has been observed in senescent cells, potentially contributing to loss of function and enhanced inflammation.

In our study, we found that the degradation pathways of individual NPC components are not uniform. Analysis of protein stability revealed significant variability among nucleoporins: ELYS was remarkably stable, while NUP62 exhibited rapid turnover. Although there was no strong correlation between mRNA expression levels and protein stability, proteins with high mRNA levels tended to degrade more quickly. This suggests that protein abundance is influenced not only by transcription but also by degradation control mechanisms. ELYS, known to serve as a seed protein for NPC assembly during post-mitotic nuclear envelope reformation, was identified as a long-lived protein. Therefore, establishing a system such as RITE (Recombination-Induced Tag Exchange) to monitor the quality and dynamics of ELYS is essential to understanding how NPC integrity and nuclear homeostasis are maintained during aging

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一般の皆様へ

本研究は、細胞の中で情報のやりとりを担う「核膜孔」という構造の寿命や変化を追跡するための新しい仕組み（RITE システム）を開発するものです。年をとった細胞ではこの核膜孔の機能が低下し、細胞の働きが乱れることが知られています。がん細胞はこの仕組みをうまく回避して生き延びている可能性があり、その違いを明らかにすることで、老化やがんの理解が進むと期待されます。今後はこの技術を使って、細胞の「寿命のしくみ」に迫り、新たな治療法の手がかりを見つけたいと考えています。

Elucidation of the mechanism by which BST1 regulates the onset of NASH through adenosine synthesis.

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Summary Abstract

To investigate the role of BST1 in NASH development, we used a choline-deficient, high-fat diet-induced NASH model and metabolic tracing in mice. BST1 knockout did not affect liver pathology under diet-induced stress. Additionally, we analyzed the metabolic fate of nicotinamide riboside (NR), a substrate of BST1. Metabolomic analyses revealed that orally and intravenously administered NR is not directly used for NAD^+ synthesis but is processed via gut microbiota into nicotinic acid, supporting hepatic NAD^+ production. These findings highlight the essential role of microbiota in NR utilization and suggest that gut microbial function is a key determinant in the efficacy of NAD^+-boosting therapies.
Key Words : BST1, nicotinamide riboside, NASH

Introduction

We previously demonstrated that bone marrow derived stromal cell antigen 1 (BST1) catalyzes a base exchange reaction using nicotinamide riboside (NR) and nicotinic acid (NA) to produce nicotinic acid riboside[1]. Interestingly, in preliminary experiments, we found that: (1) BST1 can use adenine to exchange the base moiety in NR and produce adenosine, and (2) deletion of BST1 exacerbates fat accumulation in mice fed a high-fat diet. Based on these findings, we investigated whether this reaction is involved in the onset of non-alcoholic steatohepatitis (NASH).

Results

The methionine- and choline-deficient (MCD) diet is a typical model for inducing non-alcoholic steatohepatitis (NASH); however, it leads to substantial weight loss in mice, complicating the interpretation of disease-specific effects. Therefore, in this study, we used the choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD), which prevents weight loss in mice and induces liver fibrosis in a relatively short period. To investigate the role of bone marrow stromal antigen 1 (BST1) in NASH development, we fed a CDAHFD to wild-type and BST1 knockout (KO) mice. The food intake of BST1 KO mice was comparable to that of wild-type mice. The impact of CDAHFD on body weight of BST1 KO mice was also similar to that observed in wild-type mice. Additionally, the liver weight of wild-type and BST1 KO mice was comparably increased by CDAHFD, suggesting that BST1 deletion did not significantly affect the gross phenotype associated with this dietary model. Consistently, liver morphology assessed by histological analysis showed no marked differences between BST1 KO and wild-type mice, indicating that BST1 deficiency did not alter CDAHFD-induced hepatic changes at the tissue level.

Next, we investigated how the BST1 substrate, nicotinamide riboside (NR), is metabolized in the body. NR has been recognized as a promising precursor for nicotinamide adenine dinucleotide (NAD^+) synthesis, but its metabolic fate in vivo has remained poorly understood. To clarify this, we orally administered NR to mice and measured NAD^+ and its related metabolites. We found that NR increased hepatic NAD^+ levels; however, this effect was abolished in mice lacking gut microbiota, indicating that microbial activity is essential for NR utilization. To trace the metabolic route, we gavaged stable isotope-labeled NR and observed that NR was rapidly hydrolyzed to nicotinamide (NAM), which was then deamidated to nicotinic acid (NA) by gut microbiota. NA was subsequently converted to downstream metabolites that contributed to NAD^+ synthesis in the liver. These findings suggest that orally administered NR is not predominantly absorbed in its intact form but is instead metabolically processed via a gut microbiota-dependent route.

To examine whether this dependency on gut microbiota applies only to oral NR or extends to parenteral routes, we also evaluated the metabolic effects of intravenous NR administration. Unexpectedly, even with direct bloodstream delivery, NR was rapidly degraded to NAM, which was then secreted into bile. Analysis of bile and liver tissue revealed that NAM reached the gut, where it was again converted by microbiota into NA, contributing to hepatic NAD^+ synthesis. In germ-free mice, the increase in downstream metabolites of nicotinic acid (NA) was abolished, and hepatic NAD^+ synthesis was significantly reduced, even after intravenous NR injection. This highlights a previously unrecognized enterohepatic circulation of NAD^+ precursors, wherein gut microbiota remain essential for converting systemically delivered NAM into usable NA.

Collectively, these results demonstrate that gut microbiota play a central role in NAD^+ biosynthesis from NR, regardless of the route of administration. The microbial conversion of NAM to NA is not only critical for the efficacy of oral supplementation but also for intravenous strategies. Therefore, maintaining a healthy and functional gut microbiota is likely a key determinant for the success of NAD^+-boosting therapies utilizing NR. These findings have been published in *Science Advances*[2].

Discussion & Conclusion

In this study, we investigated the role of BST1 in a murine NASH model and examined the metabolic fate of nicotinamide riboside (NR), a known NAD^+ precursor. Although BST1 deletion did not markedly affect the NASH phenotype under CDAHFD feeding, our findings revealed key insights into NR metabolism. Orally administered NR did not significantly contribute to hepatic NAD^+ levels in the absence of gut microbiota, indicating that direct absorption is minimal. Stable isotope tracing showed that NR is hydrolyzed to nicotinamide (NAM), which is then deamidated to nicotinic acid (NA) by gut microbiota. This NA is further metabolized into NAD^+ in the liver. Surprisingly, even intravenously administered NR followed a similar microbiota-dependent route through enterohepatic circulation.

These findings challenge the conventional view that NR acts primarily through direct absorption and highlight the essential role of gut microbiota in NR utilization. Both oral and systemic NR supplementation require microbial processing for effective NAD^+ synthesis, suggesting that gut microbial health may significantly impact the efficacy of NAD^+-boosting therapies. Understanding this microbiota-mediated pathway provides a basis for optimizing nutritional or therapeutic interventions targeting NAD^+ metabolism, particularly in diseases associated with its decline.

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一般の皆様へ

非アルコール性脂肪性肝炎（NASH）は、アルコールをほとんど摂取しないにもかかわらず、肝硬変や肝がんへと進行する可能性のある病態です。国内の患者数は200万人以上と推定されていますが、その発症メカニズムには未解明な点が多く残されています。本研究では、以前に私たちが発見したアデノシン合成機構がNASHの発症に関与しているかどうかを検討しました。この合成機構には、bone marrow stromal cell antigen 1（BST1）という酵素が関わっています。アデノシンは、アデニンに糖が結合した分子であり、体内でエネルギー通貨として知られるATPの材料となるほか、細胞間のシグナル伝達にも関与する重要な物質です。

そこで、BST1を欠損したマウスを用いて、食事によってNASHを誘導し、通常のマウスとの違いを調べました。その結果、NASH誘導食に対する感受性には両群間で差が見られませんでした。一方、BST1が基質として利用するニコチンアミドリボシド（NR）の代謝について詳しく解析したところ、NRは経口投与でも経静脈投与でも腸内細菌によって代謝されることが明らかになりました。この結果は、BST1による代謝が腸内細菌と密接に関係していることを示唆しています。

NRから合成されるニコチンアミドアデニンジヌクレオチド（NAD）は、健康維持において重要な分子であることが知られており、本研究は、NADを介した健康増進戦略において腸内細菌の果たす役割の重要性を示すものとなりました。今後は、「この代謝に関わる腸内細菌は具体的に何か？」といったより詳細な研究へと発展していくと考えられます。

Establishment of a method for high-function neutralizing antibodies induction against SFTS viruses by strategic activation of germinal center

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Summary Abstract

Severe fever with thrombocytopenia syndrome (SFTS) is a viral infection with a high mortality rate. Antibody therapies for this infection have not yet been established due to the difficulty in efficiently inducing high-quality neutralizing antibodies. We hypothesize that this difficulty is caused by the characteristics of the “germinal center response”, which enhances antibody quality. In this study, we aim to establish a methodology to maximize the quality of low-quality neutralizing antibodies by artificially manipulating the germinal center response.
Key Words : SFTS, germinal center, antibody drug

Introduction

SFTS is a viral infectious disease with a high mortality rate. Although development research has been conducted, the efficient induction of high-quality neutralizing antibodies remains challenging, and as a result, antibody drugs have not yet been established. We hypothesize that the difficulty in inducing high-quality antibodies is due to the following two characteristics of the germinal center (GC) reaction: (I) the antigenic dominance of epitopes that could serve as targets for high-quality neutralizing antibodies is low (ref.1), and (II) insufficient affinity maturation of B cells in the GC due to antibody feedback against epitopes targeted by neutralizing antibodies (ref.2).

Results

In this study, we have established an experimental system based on the above hypothesis to achieve the following objectives: A) comprehensive evaluation of the repertoire of anti-SFTS virus antibodies and the epitopes recognized by these antibodies, B) antigen design for a neutralizing antibody target epitope vaccine utilizing the epitope information from item A based on Hypothesis I, and the establishment of a strategic method for inducing high-functioning neutralizing antibodies using a system that artificially excludes the influence of antibody feedback based on Hypothesis II.

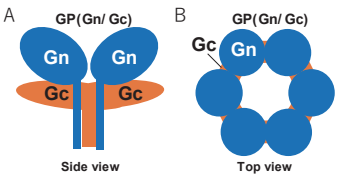

Fig1. SFTS virus envelope glycoprotein (GP)

We first identified the envelope GP proteins (Figure 1) on the virus surface, where the existence of neutralizing antibody target epitopes has been confirmed (ref.3,4), as the target for vaccine development and antibody drugs in this study. We successfully produced

four types of SFTS virus GP recognition B cell detection probes based on Hypothesis I: 1) Gn multimers, 2)Gn monomers, 3)Gn/Gc heterodimer, and 4)Gc monomer (Figure 2).

Next, we immunized wild-type C57BL/6 mice and humanized B cell receptor (BCR) mice (ref.5) with an SFTSV GP VLP vaccine via intraperitoneal injection. We then induced activation of SFTS virus-recognizing B cells and performed single-cell sorting of GP-recognizing B cells using the aforementioned four types of SFTS virus GP-recognizing B cell detection mix probes (Figure 2) on mouse spleen cells at 2 and 4 weeks post-immunization. The recovered single B cells were subsequently subjected to B cell receptor repertoire analysis, with a total of 432 cells analyzed: 160 cells from C57/BL6 mice (2 mice) and 272 cells from humanized BCR mice (3 mice). The analysis revealed a total of 90 BCR clones (35 from C57BL/6 mice and 55 from humanized BCR mice), and at least 51 BCR clones (22 from C57BL/6 mice and 29 from humanized BCR mice) were identified as having two or more identical BCR clones. Furthermore, to evaluate the characteristics of the identified BCR clones, we have successfully cloned a total of 35 B cell receptor-derived antibodies (15 from C57BL/6 mice and 20 from humanized BCR mice) from each BCR clone.

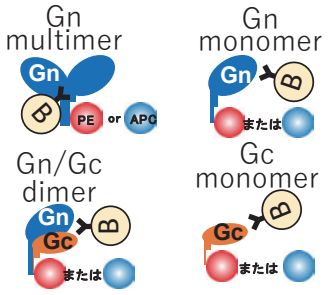

Fig.2 Preparation of four types of PE or APC-labeled GP probes and cell staining

For the BCR-derived antibodies successfully cloned, the following evaluations are being conducted on an ongoing basis: (1) antigen specificity evaluation to determine which of the four SFTS virus GP protein probes the antibodies are specific for, (2) neutralization activity assays using SFTS virus GP pseudoviruses, (3) antibody affinity-based evaluation using FACS beads and FACS detection probes (ref.1), and (4) competitive ELISA between clones as a preliminary screening step prior to cryo-electron microscopy evaluation for epitope evaluation.

To date, (1) has identified nine Gn monomer-recognizing antibodies and six Gc monomer-recognizing antibodies. (2) has identified five neutralizing antibody clones and six non-neutralizing antibody clones. Regarding (3), affinity evaluation has been completed for 16 clones. Regarding (4), In this study, we compared cloned antibodies with neutralizing antibodies reported in two previous studies (ref. 3,4) in terms of epitope recognition. As a result, only the Ab10-type clones(ref.4), which recognizes the Gn region, showed neutralizing activity.

For the remaining clones (antibodies) with incomplete evaluation, complete the evaluation process and classify the clones into groups. Subsequently, for each group classified by epitope classification using a competitive ELISA, identify the epitope region using cryo-electron microscopy and manufacture a neutralizing antibody target GP epitope vaccine.

Additionally, we have initiated experiments to introduce human BCRs recognizing neutralizing epitopes into B cells using the CRISPR-Cas9 system targeting BCRs with neutralizing activity, and plan to sequentially perform BCR-introduced adoptive B cell transplantation and immune induction tests. In particular, this study aims to thoroughly evaluate whether the immunological response of germinal center B cells induced after the administration of neutralizing BCR-introduced adoptive B cells leads to the reinduction of affinity maturation and an improvement in neutralizing activity.

Discussion & Conclusion

The objective of this research project is to establish a new methodology for the development of antibody drugs targeting SFTS virus infection, which has not yet been achieved.As an initial step, we have conducted a comprehensive evaluation of the characteristics of antibodies induced by SFTS virus immunity.To date, it has been clarified that among the GP proteins, only the previously reported Ab10-type clone recognizing the Gn subunit exhibits neutralizing activity against SFTS virus infection.Based on these results, we have concluded that focusing on Ab10-type antibodies is the optimal approach for future research.Going forward, we plan to continue our research with the theme of “how to improve the quality of the Ab10-type clones using the system currently under development.”

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一般の皆様へ

重要症熱性血小板減少症候群（SFTS）は、国内で発生している高い致死率を示すマダニ媒介性ウイルス感染症です。この感染症に対する有効な抗体医薬やワクチンは未だに確立されていません。本テーマは、抗体の質を高める免疫器官である「胚中心」の特徴に着目した、新規の抗体医薬開発法の確立を目指したテーマになります。この方法論は、既存の方法論とは視点が全く異なるため、将来的に抗体医薬開発技術における一つの選択肢として確立されていくことを強く期待しています。

Application of epigenome editing to advance the targeted therapy for sarcoma

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Summary Abstract

A fusion transcription factors are strong oncogenic drivers that are critical for growth and survival of bone and soft tissue sarcoma cells. Given the gross modification of transcriptome by fusion transcription factors, development of epigenetic drugs is expected for the cure of the disease. However, the chemicals targeting trans-acting factors lack specificity for oncogenic aberrations. We have developed CRISPR-based epigenome editing tool to target cancer-specific cis-elements such as oncogenic enhancers to inhibit fusion gene expression in sarcoma cells. Based on the histone modification data, a mini-library of gRNAs flanking the 5' region of EWSR1 was designed and introduced into Ewing sarcoma cells expressing dCas9-MeCP2-KRAB. Single gRNAs were identified by which significant suppression of EWSR1-FLI1 expression was achieved. These results underscore the power of epigenome editing for cancer therapy and provide deeper insights into the enhancer landscape of fusion transcription factor-associated sarcoma.
Key Words : epigenome editing, enhancer, fusion gene, sarcoma

Introduction

Tumor-specific fusion genes are strong oncogenic drivers that are critical for growth and survival of cancer cells. Thus, fusion genes, their products and downstream molecules are attractive targets for advanced cancer therapy. We have demonstrated that fusion transcription factors dynamically reprogram super-enhancers, which define tumor cell characteristics, thereby contributing to cell proliferation, maintenance of an undifferentiated state, and the formation of the tumor microenvironment [1, 2]. Super-enhancers are cis-regulatory elements that determine cell lineage and identity. We aim to establish an epigenome editing therapy targeting fusion gene-associated enhancers in bone and soft tissue sarcomas [3].

Results

This study aims to develop an epigenome-editing therapeutic technology that targets aberrant enhancer regions bound by fusion genes and fusion proteins in bone and soft tissue sarcomas caused by chromosomal translocations. By using guide RNAs (gRNAs) to recruit transcriptionally repressive dCas9 constructs to these enhancer regions, we seek to establish a novel therapeutic approach and validate its ability to suppress tumor growth in

vivo, thereby paving the way toward clinical application (Figure 1).

The methods are as follows: (1) to optimize functional gRNA and dCas9-KRAB combinations, (2) to evaluate the suppression of fusion gene expression or its downstream targets in vitro, and (3) to establish in vivo delivery systems using adeno-associated viruses (AAVs) or lipid nanoparticles (LNPs) and evaluate anti-tumor efficacy in mouse models.

Target diseases included fusion-positive sarcomas such as Ewing sarcoma, synovial sarcoma, alveolar soft part sarcoma, CIC-rearranged sarcoma, and myxoid liposarcoma. Using cell lines representing each sarcoma type, we performed ChIP-seq analysis for histone marks H3K27ac, H3K4me1, and H3K4me3. By comparing these data with enhancer profiles from other cancer types and normal cells, we identified candidate enhancers associated with fusion genes and sarcoma-specific enhancers. gRNAs were then designed to target these sarcoma-specific enhancer regions and introduced into a lentiviral vector to construct a gRNA mini-library.

To generate dCas9-KRAB sarcoma cell lines, we introduced three different types of dCas9 constructs, original KRAB, and improved versions fused with MeCP2 or ZIM3, into sarcoma cells and established stable clones. In preliminary experiments using alveolar soft part sarcoma cells, we found that among the three KRAB constructs, the dCas9-KRAB-MeCP2 showed the strongest gene repression upon lentiviral delivery of gRNAs. Subsequently, we introduced gRNA mini-library targeting the 5' region of EWSR1-FLI1 into Ewing sarcoma cells expressing dCas9-MeCP2-KRAB. As a result, we identified individual gRNAs that significantly suppressed EWS-FLI1 expression.

We plan to evaluate the effect of suppressing target gene expression in vitro in order to identify functional gRNAs from the constructed gRNA mini-library for other sarcomas. In parallel, we will initiate the development of a delivery system for the gRNA and dCas9 suitable for in vivo applications. This includes optimizing delivery vehicles such as AAVs, LNPs and ribonucleoproteins to achieve efficient and tissue-specific targeting in mouse sarcoma models, with the ultimate goal of assessing the therapeutic potential of epigenome editing in vivo.

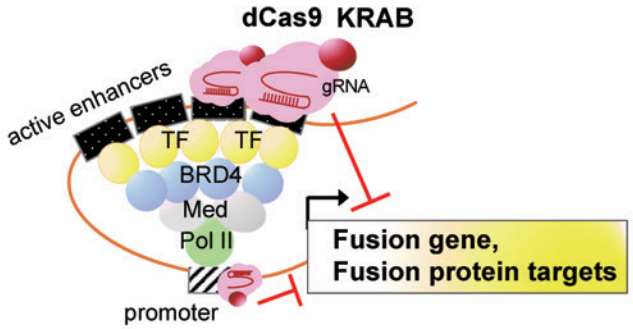

Figure 1. The primary goal of epigenetic engineering for cancer therapy.
Epigenetic therapy targeting cis-acting elements offers a distinct approach from conventional epigenetic drugs, which typically act on trans-acting factors. Advantages include tailor-made design for specific cis-elements and reduced toxicity.

Discussion & Conclusion

Our goal is to overcome disease by interfering with aberrant epigenomic functions through the recruitment of transcription-modifying dCas9 to tumor-specific enhancer regions in sarcomas using epigenome editing technology. Owing to the small number of patients, diagnostic tools and therapeutic drugs for sarcomas remain underdeveloped. Non-specific inhibitors of epigenetic abnormalities are effective drugs. These include inhibitors of histone deacetylases (HDACs), bromodomains (BRDs), histone acetyltransferase (HATs), and components of polycomb (PRCs) several studies have reported the potential efficacy of these inhibitors. Epigenome-editing-based cancer therapy modulates the expression of critical targets regardless of their mutational status. The tailor-made design of sgRNA for the epigenomic therapy will benefits patients with rare cancers, including sarcomas."

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一般の皆様へ

私たちはエピゲノム編集技術を応用して、がんの新しい治療法の基盤作りを進めています。いくつかの小児がんでは、融合遺伝子の形成ががんの発症に必要な唯一の要因であることが知られています。これらの融合遺伝子はがん細胞特異的であり、その除去によりがん細胞の増殖停止や細胞死が誘導されることが実験的に明らかになっています。骨軟部肉腫で認められる融合遺伝子の大半は転写因子で構成されており、これまで転写因子型の融合遺伝子を狙った創薬は困難でした。私たちは、骨軟部肉腫の融合遺伝子をドライブする特異領域を狙って、ゲノムを切断することなく、融合遺伝子の機能を抑制することを目指しています。

Elucidation of mechanism for the risk of the development of imprinting disorders caused by epimutation in assisted reproductive technology using targeted long-read sequencing

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Summary Abstract

Assisted reproductive technology (ART) has been associated with an increased incidence of imprinting disorders (IDs) due to the manipulation of gametes or zygotes during the process of genomic imprinting. To clarify whether ART itself or the underlying parental infertility contributes to the onset of IDs, we investigated the effects of ART on Silver-Russell syndrome (SRS) using target long-read sequencing (T-LRS) analysis. In ART-pregnancy SRS, multiple DMRs with more outliers in CpGs methylation levels were identified compared to natural pregnancy SRS, suggesting the possibility of genome-wide methylation abnormalities. The outliers were observed in both parental alleles, suggesting that methylation abnormalities in ART-pregnancy SRS are likely to arise from post-fertilization ART procedures rather than pre-fertilization methylation abnormalities in sperm or oocytes.
Key Words : Imprinting genes, DNA methylation, assisted reproductive technology, epimutation, long-read sequencing

Introduction

Imprinting genes with parental origin expression patterns are regulated by differentially methylated regions (DMRs) having different methylation statuses in each mother and father. Because assisted reproductive technology (ART) manipulates gametes and fertilized eggs, causing abnormal CpG methylation in DMRs, thereby increasing the risk of imprinting disorders (IDs) have been suspected. Using our IDs biobank, we first demonstrated that ART is a risk factor for epimutation (DMR methylation defects leading to IDs) and that maternal advanced age is not involved. However, it remains unclear whether the increased risk of epi-IDs is due to infertility requiring ART or the ART procedure itself.

Results

In this study, to clarify whether the increased risk of epi-IDs is due to infertility requiring ART or the ART procedure itself, we aim to elucidate the cellular mechanisms by which ART influences the onset of epi-IDs using blood samples from epi-IDs patients. We employed targeted long-read sequencing (T-LRS) analysis system. Nanopore-based long-read sequencing (LRS) (Oxford Nanopore Technologies, ONT) (LRS) obtains reads with sequences ranging from 10 to 100 kb in length and single-molecule DNA modifications such as 5mC. In addition, haplotype phasing analysis based on single nucleotide polymorphism

(SNPs) and insertions/deletions (indels) determines the parental origin of each read, clarifies the parental origin of alleles with SVs and SNVs, and shows the methylation levels in the DMRs on each parental allele. T-LRS, using adaptive sampling developed by ONT, enriches 0.1%-10% of the whole genome region and obtains four to five times the read depth compared to whole-genome LRS. We calculated the normal range of methylation levels in each CpG from six controls. We performed on three cases with SRS born via ART (ART-SRS) and three cases with SRS not born via ART (nonART-SRS), and the methylation levels of 76 known DMRs (approximately 7,000 CpG sites) were measured. The sequencing read with the information of DNA methylation was classified into paternal and maternal alleles. In this study, firstly, we evaluated the disease-associated DMRs in SRS (H19/IGF2: IG-DMR, IGF2: Ex9-DMR) and 12 known DMRs and compared the methylation levels of ART-SRS and non-ART-SRS in the 14 DMRs with those of normal controls and calculated the number of reads exceeding the maximum and minimum values of normal controls (outliers). In *H19/IGF2:*IG-DMR, ART-SRS and nonART-SRS showed outliers with low methylation at almost all CpG sites. In *PEG13*:TSS-DMR, *ZNF597*:3':DMR, *MCT2P*:TSS-DMR, and *L3MBTL1*:alt-TSS-DMR showed a higher number of outlier CpGs in ART-SRS compared to nonART-SRS. Outliers were present in both parental alleles. In other DMRs, no significant difference in the number of outlier CpGs was observed between ART-SRS and nonART-SRS.

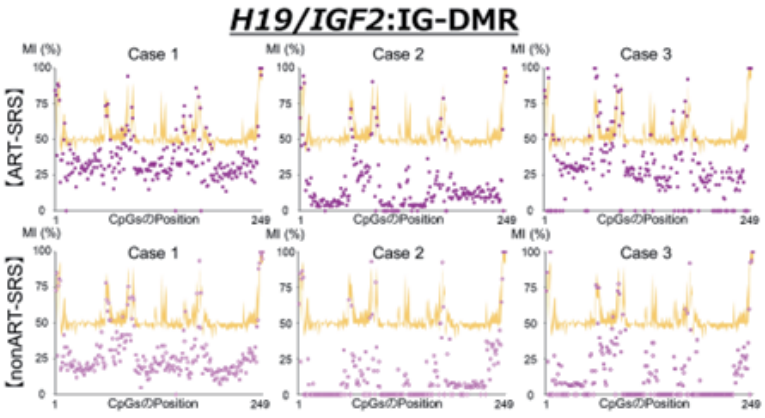

Figure 1. ART-SRS and nonART-SRS showed hypomethylation of the H19/IGF2:IG-DMR. The yellow region shows a normal range of methylation index of CpGs within the *H19-IGF2*-DMR.

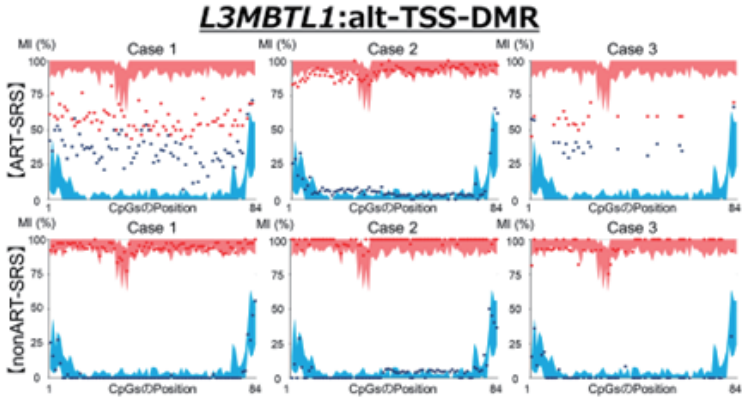

Figure 2. The results of methylation analysis using LRS in the L3MBTL1:alt-TSS-DMT. Cases 1 and 3 in ART-SRS had outliers, but no cases in nonART-SRS existed. The red region shows a normal range of methylation index of CpGs within the *H19-IGF2*-DMR on the maternal allele and blue on the paternal allele.

Discussion & Conclusion

In disease-responsible DMRs, both ART-SRS and nonART-SRS showed most CpGs as outliers with low methylation, similar to other analytical methods. In ART-SRS, multiple DMRs with a higher number of outlier CpGs compared to nonART-SRS were identified, suggesting the possibility that ART-SRS has genome-wide methylation abnormalities. Since the methylation abnormalities identified in this study were present in both parental alleles, it is considered that methylation abnormalities in ART-born infants are not caused by methylation abnormalities in sperm or eggs before fertilization but rather by ART procedures after fertilization. Currently, we are evaluating allele-specific methylation levels in the DMRs other than 14 DMRs. We are performing T-LRS in further ART-SRS and non-ART-SRS cases. Based on these results, we will be able to propose a robust mechanism for the risk of epimutation by ART.

References

This study is still in progress, so the paper has not been published.

一般の皆様へ

インプリンティング疾患（ID）は、遺伝子発現調節領域のエピジェネティック修飾の異常により生じる。生殖補助医療（ART）は、エピジェネティック機構が確立する卵子、精子、受精卵に操作を加えるため、ID 発症リスクが懸念され、我々は ART の ID 発症リスクを報告した。本研究ではロングリードシーケンスを用いて、ART 妊娠 ID（ART-ID）患者と自然妊娠 ID(nonART-ID）患者の DNA メチル化レベルを調査し、異常値を示す箇所の親由来アレルを調べ、ART-ID で nonART-ID に比較し異常メチル化を示す CpG を両アレルにわたりより多く認め、受精卵における ART の影響を示唆した。

Identification of molecular biomarkers representing alternations at the endoplasmic reticulum-mitochondria contacting sites.

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Summary Abstract

This study aimed to identify the molecules reflecting alternation at the mitochondria-associated membrane (MAM), where the endoplasmic reticulum (ER) is closely associated with the mitochondria, under various stress conditions. Using the MAMtracker, a MAM reporter we developed, we found that epidermal growth factor (EGF) is involved in maintaining the MAM. Moreover, we also found that a conditioned medium of the cells overexpressing TANK-binding kinase 1 (TBK1) also induces the MAM. We continue investigating further molecular details of these pathways.

Key Words : Mitochondria-associated membrane
Epidermal growth factor
TANK-binding kinase 1

Introduction

The mitochondria-associated membrane (MAM), where the endoplasmic reticulum (ER) is closely associated with the mitochondria, is a unique and dynamic structure essential for mitochondrial function. Our previous study reported that the MAM is disrupted in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). However, little was known about how the MAM alteration is induced and how we find the MAM alteration. Thus, we aimed to identify any biomarkers and/or cytokines reflecting the MAM alternation.

Results

First, we tried identifying the molecules associated with the MAM alteration from the conditioned medium. Unfortunately, however, we found that no molecules drastically changed in the media, possibly due to the amount of affected proteins or peptides in the conditioned medium being too small to detect.

Thus, instead of the experiment above, we performed inhibitor screening to identify the signaling pathways associated with the MAM dysfunction using a MAMtracker-Luc, a luciferase-based MAM reporter we previously developed (Ref 1) (Figure 1A). Briefly, we seeded the neuroblastoma cell line Neuro2a stably expressing MAMtracker-Luc into a 96-well plate. The cells were treated with various inhibitors for 24 hours, then the MAM amount was estimated using the Nano-Glo Live Cell Assay Kit (Promega) and Infinite 200 luminometer (Tecan). Among the inhibitors, we found that epidermal growth factor (EGF) receptor inhibitor Tryphostin-25 (Try25) and mitogen-activated protein kinase (MAPK) inhibitors significantly reduced the MAM. On the other hand, similar growth stimuli, such as phosphoinositol-3 kinase (PI3K) and mammalian target of rapamycin (mTOR), did not affect the MAM at all. Moreover, Try25 reduced the MAM within 30 min (Figure 1C), implying that

a direct target of this pathway is located in the MAM. To identify the downstream of the EGF receptor/MAPK pathway, we performed an MS analysis of phosphorylated proteins in the MAM of Try25-treated cells using Phos-tag. As a result, we found that about 30 phosphorylated proteins were affected in the MAM and mitochondria of the Try25-treated cells. Among the identified proteins, we focused on confidential candidate X, which interacts with the lipid bilayer and localizes to the mitochondria, to examine whether the EGF/MAPK pathway regulates X. Another inhibitor, NU-7441, targeting DNA-dependent protein kinase (DNAPK), an essential enzyme for DNA damage response represented by double strand break of DNA, increased the MAM. In contrast to Try25, NU-7441 induced the MAM after 24 h, suggesting that transcriptional changes induced by NU-7441 are responsible for the MAM induction. We are now also trying to identify candidate genes regulating the MAM level downstream of DNAPK.

In addition, we also examined whether the conditioned medium of the cells overexpressing TANK-binding kinase 1 (TBK1) induced the MAM in a manner similar to that of the TBK1-overexpressing cells. TBK1 is a well-known innate immune responder against virus infection, a causative of an inherited form of amyotrophic lateral sclerosis (ALS), and a MAM inducer. In our previous study (Ref 1), we found that TBK1 overexpression increased the MAM level. However, it was uncertain whether TBK1 directly phosphorylates MAM proteins or TBK1-induced cytokines are responsible for the MAM induction. As shown in Figure 2, we found that the conditioned medium of the TBK1 overexpressing cells (TBK1-conditioned medium) induced the MAM in a manner similar to that of the TBK1-overexpressing cells. We are planning which cytokine is crucial for the MAM induction.

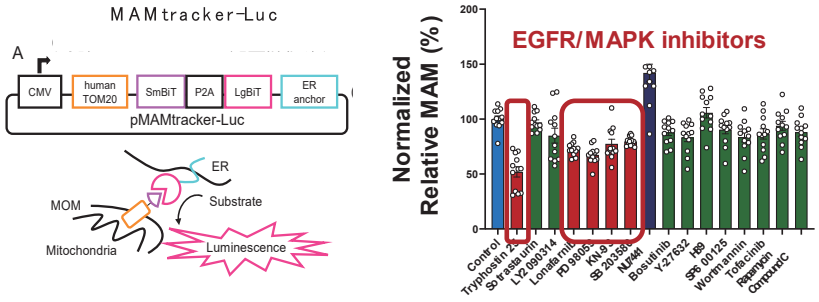

Figure 1 Identification of inhibitors affecting the MAM. Schematic illustration of the MAMtracker-Luc. (modified from Ref 1) (left panel). EGF receptor/MAPK pathway (red) predominantly reduced the MAM (right panel).

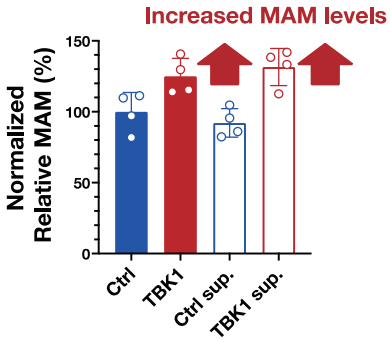

Figure 2 TBK1-conditioned medium is sufficient to induce the MAM formation. Amounts of the MAM were estimated using MAMtracker-Luc. Ctrl sup: conditioned medium of mock-transfected cells, TBK1 sup.: conditioned medium of TBK1-transfected cells.

Discussion & Conclusion

EGF is one of the most well-known growth stimuli. Interestingly, ERBB4, one of the ALS-causative gene products, is a member of the EGF receptor family. Thus, the deregulation of the EGF/MAPK pathway is potentially associated with disruption of the MAM in ALS pathology. We are now examining the candidates identified in the MS analysis, leading to a future therapeutic strategy targeting EGF to improve the MAM formation in neurons before their degeneration. Similarly, TBK1, another ALS-causative gene product associated with the MAM disruption, regulated the MAM possibly via cytokines. These results suggest that humoral factors can regulate the MAM, which may lead to a future strategy that prevents the MAM disruption in neurogenerative diseases such as ALS using growth factors or cytokines. We are going to reveal the detailed molecular mechanism for this purpose.

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一般の皆様へ

本研究では、神経変性疾患などで破綻することが知られている小胞体とミトコンドリアの間の接触領域（MAM）について、MAM の増加または減少を反映するバイオマーカーの探索に挑戦しました。当該のバイオマーカーは技術的な問題により同定することはできませんでしたが、代わりに MAM の増加や減少を引き起こすシグナル伝達経路を複数同定することに成功しました。今後、同定した経路に着目した詳細な解析により、神経変性疾患の治療薬開発につながると期待されます。

Dynamic structural analysis of a bacterial divisome using a small synthetic binding protein

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Key Words : Bacterial cell division, FtsZ, Monobody, Molecular mechanism

Introduction

Cell division is essential for bacterial survival and propagation. In *E. coli*, over 30 proteins participate in this process. FtsZ, conserved across most bacteria and many archaea, polymerizes into filaments in a GTP-dependent manner on the inner membrane. These filaments are bundled into a ring-like structure, known as a Z-ring, by proteins such as ZapA. Other cell division proteins then assemble on the Z-ring to drive membrane constriction. This study aims to elucidate the molecular mechanism by which cell division proteins function coordinately, analyzing FtsZ complexes using cryo-electron microscopy.

Results

This study aims to elucidate the regulation mechanism of bacterial cell division by analyzing the structure and function of the divisome, the bacterial cell division machinery. We previously reported atomic-resolution cryo-electron microscopy (cryo-EM) analysis of FtsZ, achieved through various strategies, including the use of synthetic binding proteins (Mbs) that specifically bind to FtsZ[1]. This work revealed the molecular mechanism by which FtsZ forms straight and curved filaments. However, the question still remains as to how FtsZ and other divisome proteins coordinate their function to drive membrane constriction. To address this question, we performed functional and cryo-EM structural analyses of FtsZ in complex with the other divisome proteins, aiming to clarify the molecular mechanisms underlying cell division control. Our first approach was to use Mbs. Mb is a protein engineered with diversified amino acid sequences in its loop regions. Like antibodies, these diversified loops allow Mbs to bind specifically to target proteins. Due to this property, Mbs have been widely used in medical and pharmaceutical research. In our previous study, we demonstrated that when Mbs bind to FtsZ, they are inserted between FtsZ filaments, resulting in the formation of double-helical structures. This highly regular and stable architecture enabled atomic-resolution cryo-EM analysis. In this structure, the regions of FtsZ predicted to interact with divisome proteins are exposed on the outer surface. Based on this observation, we hypothesized that it may be possible to visualize divisome proteins bound to this FtsZ-Mb complex using cryo-EM, forming the basis of the current research proposal. We first obtained Mbs that bind to FtsZ. While our previously obtained Mbs could

bind both GDP- and GTP-bound forms of FtsZ, we have isolated a Mb that specifically binds only to the GTP-bound form of FtsZ. However, when we attempted structural analysis using this Mb, we found that many complexes dissociated during the experiment, preventing successful structural determination; presumably due to its low binding affinity. We plan to improve the affinity of the Mb through amino acid mutations, followed by further structural and functional analyses. At the same time, we analyzed the FtsZ-ZapA complex, using ZapA, a divisome protein that binds FtsZ, as an alternative to Mb. As a result, we successfully determined the three-dimensional structure of the ZapA-FtsZ complex using cryo-electron microscopy (cryo-EM). This analysis revealed, for the first time, that the FtsZ filaments are bundled asymmetrically in a ladder-like configuration, consisting of one single filament aligned with two others. Focusing on the interaction between FtsZ and ZapA, we found that the head domains of the ZapA dimer interact with two pairs of FtsZ dimers arranged in antiparallel filaments. The N-terminal region of FtsZ is sandwiched between the two ZapA molecules. When we visualized the hydrophobicity of the ZapA dimer surface, we observed that the N-terminal seven residues of FtsZ bind within a hydrophobic pocket formed by the ZapA dimer. Among these seven residues, Phe2 and Pro4 of FtsZ showed prominent hydrophobic interactions, suggesting that the N-terminal region of FtsZ plays a crucial role in the formation of the ZapA–FtsZ complex. This analysis uncovered a unique binding mode in which the phenylalanine side chain acts like a "hook," anchoring itself into the hydrophobic pocket of ZapA.

Discussion & Conclusion

The elucidation of the 3D structure of the ZapA–FtsZ complex allowed us to examine the function of ZapA in more detail. We found that ZapA crosslinks FtsZ protofilaments, aligning them more uniformly and promoting the formation of a coherent Z-ring. This coherent Z-ring is thought to be essential for the initiation of cell division. FtsZ in its monomeric form possesses a flexible N-terminal tail. Previous cryo-EM analysis of GDP-bound FtsZ in complex with a monobody revealed that this N-terminal tail adopts a short helical structure, which stabilizes the curved conformation of FtsZ. Now, we have identified a direct interaction between ZapA and the N-terminal tail of FtsZ. Based on this observation, we propose a novel mechanism: ZapA captures the N-terminal region of FtsZ, enabling it to crosslink FtsZ protofilaments, align them more precisely, and promote the formation of a compact Z-ring.

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一般の皆様へ

細菌にとって、自分のコピーを作る細胞分裂は、種を守るために必要不可欠な仕組みです。彼らは、様々なストレスさらされながら生き抜いていますが、どのように細胞分裂を正確に進めているのでしょうか。私はバクテリアの細胞分裂に関わるタンパク質 FtsZ に注目し、その仕組みを解き明かすことを目的に研究を行いました。今回は、FtsZ と FtsZ に結合するタンパク質がどのようにして協調しながら機能しているかを明らかにしようとしました。初期の目的であった人工抗体を用いた実験はうまくいきませんでしたが、ZapA というタンパク質と FtsZ との複合体の構造解析に成功して、FtsZ と ZapA の協調現象を調べることができました。

The pathogenesis of Sjogren syndrome focused on T-B cell interactions on inflamed tissues

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Summary Abstract

We performed single-cell RNA sequencing on lip minor salivary glands from four untreated patients with primary Sjogren syndrome (SS) to characterize local immune responses. Among 7,232 cells, seromucous acinar cells (SMACs) were the dominant population and were divided into four subsets based on gene expression. SMAC_1 and SMAC_4 expressed pro-fibrotic genes (NRG2, RUNX2, EGFR) and interacted with T cells, suggesting roles of the potential contributions to inflammation and fibrosis. These findings highlight novel cellular interactions in SS pathogenesis.
Key Words : Sjogren syndrome, salivary glands, single cell RNA-seq, cell-cell interaction

Introduction

Sjogren syndrome (SS) is an autoimmune disease characterized by lymphocytic inflammation of exocrine glands. Its prevalence is relatively high, with an estimated 68,000 patients in Japan, but treatment remains largely symptomatic, underscoring the urgent need for curative therapies. So far, how cellular interactions within inflamed tissues contribute to the pathogenesis remains unclear. This study aims to elucidate local immune interactions in SS using single cell RNA sequencing of labial salivary glands.

Results

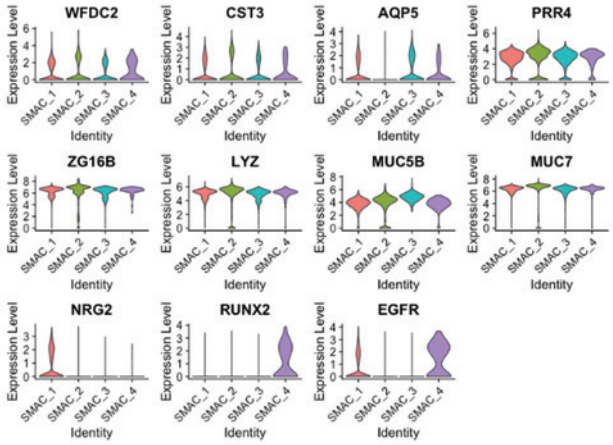
Single-cell RNA sequencing (scRNA-seq) analysis of labial salivary glands from four untreated primary Sjogren syndrome (SS) patients were performed. After quality control, a total of 7232 cells were obtained. Based on gene expressions, these cells were classified into 10 distinct clusters, including T cells, B cells, pericytes, keratinocytes, fibroblasts, endothelial cells, and seromucous acinar cells (SMACs). SMACs represented nearly half of the total cells and were further subdivided into four distinct subsets (SMAC_1 to SMAC_4) based on their transcriptional signatures. The distribution of these cell types was largely consistent across all four patients.

Detailed analyses revealed functional heterogeneity among SMAC subsets. SMAC_2 exhibited low expression of *AQP5*, a gene essential for fluid secretion, but showed high levels of *ZG16B*, suggesting a distinct molecular identity. In contrast, SMAC_3 was characterized by high *MUC5B* expression, indicating a more differentiated phenotype associated with mucin production. These transcriptional features imply that SMAC_2 and SMAC_3 may represent functionally specialized subpopulations within the acinar cell compartment (1). SMAC_1 and SMAC_4 demonstrated elevated expression of *RUNX2* and

EGFR, which previously implicated in promoting tissue fibrosis and remodeling (2,3). These finding suggest that SMAC_1 and SMAC_4 might not only participate in saliva secretion but also contribute to pathological processes such as inflammation and fibrosis in SS. This contrasts with the traditional view of acinar cells as passive structural or secretory units and highlights their potential involvement in disease pathogenesis.

To further explore the functional relevance of these cell subsets, we performed cell-cell communication analysis using CellChat (4). This approach enabled the inference of ligand-receptor interactions between distinct cellular populations within the inflamed salivary gland microenvironment. As expected, robust signaling networks were detected between T cells and B cells, supporting their central role in SS immunopathology. Moreover, we identified interactions between immune cells and non-immune epithelial subsets, particularly between T cells and SMAC_1 or SMAC_4. These interactions suggest that acinar cells, especially those with pro-fibrotic transcriptional profiles, may engage in direct crosstalk with immune cells at the site of inflammation.

The observed connections between epithelial and immune cell subsets imply a more active role for SMACs in SS pathophysiology than previously appreciated. Rather than serving solely as targets of immune-mediated destruction, certain acinar cell subsets may influence the local inflammatory milieu through cytokine signaling, antigen presentation, or modulation of fibrosis-related pathways. This hypothesis aligns with recent studies in other autoimmune and fibrotic diseases, which have identified epithelial–immune crosstalk as a key driver of chronic inflammation and tissue remodeling.



(Figure) Gene Expression Patterns in Seromucous Acinar Cells (SMACs).
SMAC_2 exhibited low expression of AQP5, while SMAC_3 showed high expression of MUC5B, indicating the presence of acinar cell subsets consistent with previous reports. In contrast, SMAC_1 and SMAC_4 displayed expression of genes related to fibrosis, including NRG2, RUNX2, and EGFR.

Discussion & Conclusion

Our study provides a comprehensive single-cell map of the SS labial salivary gland and reveals significant heterogeneity within the acinar cell population. The identification of SMAC subsets with distinct transcriptional and functional profiles, including those associated with fibrosis-related genes, offers new insight into the complex interplay between epithelial and immune cells in SS. These findings support the notion that epithelial cells are not merely passive bystanders but may actively participate in immune regulation and contribute to the characteristic dryness and tissue damage observed in SS. Further studies,

including T cell receptor (TCR) repertoire and clonotype analysis currently underway, will help elucidate the clonal dynamics of T cells and their interactions with both immune and epithelial cells in the inflamed regions of SS.

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一般の皆様へ

シェーグレン症候群（SS）は外分泌腺のリンパ球性炎症を特徴とする自己免疫疾患である。本邦の有病率は約 68000 人と比較的多い疾患であるが対症療法が主であり、根治的治療法の確立が最重要課題である。どのような病変局所での細胞間相互作用が病態に寄与するか不明であり single cell RNA-seq という手法を用いて解析を行った。これまで腺房細胞は唾液を分泌する細胞としてのみ考えられていたが、今回の結果からそれらの一部は「炎症・線維化」を誘導しうる可能性が示唆された。これらの細胞は T 細胞や B 細胞など免疫細胞とも関連することが示唆され、腺房細胞は単なる唾液腺の構成細胞でなく病態のドライネスに寄与する可能性が考えられた。

Understanding the role of androgen in sexual dimorphism of pain

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Summary Abstract

Using a mouse model of neuropathic pain, we identified androgen-responsive genes potentially involved in pain modulation. However, no direct AR-DNA binding was found, and androgens did not alter cytokine expression in BV2 microglia, suggesting indirect regulatory mechanisms and highlight the need for further validation in primary microglia or in vivo models.

Key Words : androgen, sexual dimorphism, pain

Introduction

Chronic pain conditions such as neuropathic pain, fibromyalgia, migraine, and rheumatoid arthritis significantly impair quality of life and show striking sex differences, with higher prevalence in women[1~3]. Despite this, most pain research has historically focused on males, neglecting sex as a biological variable. Consequently, the molecular and cellular basis of chronic pain in females remains poorly understood. What causes sex differences? How do mechanisms differ between sexes? These remain open questions. Recently, dysregulation of microglial suppression in the spinal cord has emerged as a key driver of chronic pain, highlighting the urgent need to elucidate its underlying regulatory mechanisms.

Results

To investigate androgen-dependent pathways involved in chronic pain regulation, we utilized a well-established mouse model of neuropathic pain induced by sciatic nerve ligation. RNA-seq and ATAC-seq analyses were performed on tissues collected from the model mice. From these datasets, several candidate genes were identified as potentially involved in pain modulation under androgen signaling. These included secreted factors previously known to be involved in nociceptive modulation, as well as genes with no prior association with pain signaling.

Subsequent RT-qPCR analysis was conducted to validate gene expression profiles of selected candidates. Among them, candidate genes were found to be specifically expressed in a cell population critical for pain regulation. Furthermore, these genes exhibited a possible sex-biased expression pattern, suggesting the involvement of androgen signaling. Given that androgens exert their effects via binding to androgen receptors (AR), which subsequently act as transcription factors within the nucleus, we explored whether the identified genes might be direct AR targets. To assess this, in silico analyses were carried

out using existing AR-ChIP-seq datasets and our ATAC-seq results. We broadly examined the regulatory regions associated with the candidate genes to identify potential AR binding motifs or enrichment patterns, without restricting the analysis to a specific genomic window. However, we were unable to identify any significant AR binding regions in the regulatory loci of these genes, suggesting that their androgen responsiveness may be mediated indirectly, rather than through direct AR-DNA interactions.

To further explore the relationship between androgens and pain regulation via immune cell mechanisms, we focused on microglial activation states. It is known that M1-type microglia become activated during the development of chronic pain and produce pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. In contrast, M2-type microglia secrete anti-inflammatory cytokines including CD206, ARG-1, and IL-10, contributing to the resolution of inflammation and potential pain suppression. Although previous studies have shown that androgens can induce IL-10 expression in T cells, it remains unclear whether a similar response occurs in microglia. To test whether androgens affect microglial phenotypes, particularly through suppression of M1-type activation or promotion of M2-type differentiation, we used the BV2 murine microglial cell line, which is commonly employed in studies of microglial signaling. Cells were treated with lipopolysaccharide (LPS) for 24 hours in the presence or absence of testosterone. Following treatment, expression levels of M1-associated cytokines (IL-1β, IL-6, TNF-α) and M2-associated cytokines (CD206, IL-10) were analyzed via quantitative real-time PCR. LPS stimulation successfully induced expression of M1-type cytokines, confirming microglial activation. However, no significant differences in cytokine expression were observed between androgen-treated and untreated groups. Specifically, androgen exposure did not upregulate IL-10 and Mrc1 expression, nor did it suppress pro-inflammatory cytokine levels. These results suggest that androgens do not exert a strong effect on cytokine gene expression under acute inflammatory stimulation in BV2 cells.

At present, we are conducting additional experiments using alternative microglial cell lines and primary cells to further validate these findings and determine whether cell-type-specific factors may influence androgen responsiveness in the context of microglial-mediated pain modulation.

Discussion & Conclusion

The present study aimed to identify androgen-dependent pain-regulated mechanisms using neuropathic pain models and microglial assays. While RNA-seq and ATAC-seq analyses revealed several candidate genes potentially regulated by androgens, no direct AR binding regions were identified, suggesting that the observed transcriptional changes may occur through indirect signaling pathways. Additionally, androgen treatment did not significantly alter the expression of M1- or M2-type cytokines in BV2 microglial cells under LPS stimulation. These results imply that androgen-mediated modulation of pain may not operate through classical genomic AR binding at these loci or via direct regulation of microglial cytokine profiles in vitro. The lack of significant response in BV2 cells may reflect limitations of this immortalized cell line, which does not fully recapitulate the behavior of primary microglia or in vivo microglial environments. Ongoing experiments using alternative

microglial models and in vivo validation will be essential to clarify the precise mechanisms by which androgens modulate inflammatory signaling and contribute to sex differences in chronic pain. Ultimately, a deeper understanding of these pathways could aid in the development of novel analgesic strategies that account for hormonal regulation and sex-specific responses.

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一般の皆様へ

本研究は、アンドロゲン依存的な慢性疼痛制御機構の解明を目的とし、坐骨神経結紮による神経障害性疼痛モデルマウスを用いて解析を行った。RNA-seq および ATAC-seq 解析から、発現に性差を示す疼痛関連候補遺伝子を同定したが、AR（アンドロゲン受容体）の直接的な結合は確認されず、間接的経路による制御の可能性が示唆された。BV2 ミクログリア細胞を用いた解析では、アンドロゲン投与によるサイトカイン発現変化は認められなかった。今後、他の細胞系や in vivo モデルでの検証を進め、性差疼痛の分子基盤の理解と新規治療戦略の構築を目指す。

Hippo-activated cells induce non-cell autonomous tumorigenesis in Drosophila

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Summary Abstract

The Hippo pathway is known as a tumor-suppressor pathway, and most related studies have indicated that the inhibition of the Hippo pathway leads to tumorigenesis. However, recent studies have suggested that the activated Hippo pathway can potentially promote tumorigenesis in some contexts. Here, we show that the activated Hippo pathway induces non-cell-autonomous tumorigenesis characterized by tumor markers in the *Drosophila* wing epithelium. This suggests that the Hippo-activated cells behave similarly to “oncogenic niche cells.” We found that the Hippo-activated cells distantly induced tumor-like cells exhibiting aberrant cell proliferation. Moreover, we identified the evolutionarily conserved amino acid transporters, Sat1/2, that redundantly work with the growth factors, Wingless and Spitz, for non-cell autonomous tumorigenesis. Our findings provide novel insights into the Hippo pathway and may be useful in developing cancer treatments targeting the Hippo pathway.

Key Words : Tumor suppressor, Hippo, Drosophila

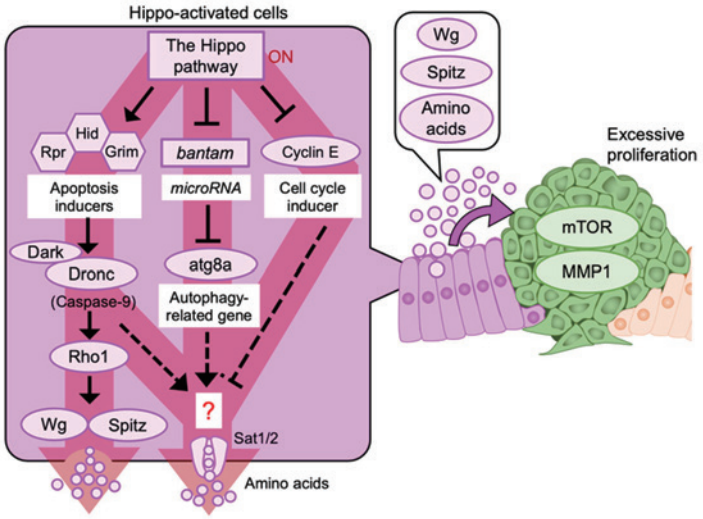
Introduction

The Hippo pathway, a highly conserved tumor-suppressor pathway, negatively regulates organ growth and tumorigenesis. The *Drosophila* Hippo pathway consists of the main components, Hippo (Hpo) and Warts (Wts), which target the transcriptional coactivator Yorkie (Yki). Given that the activated Hippo pathway suppresses Yki by sequestrating and degrading Yki in the cytosol, the inactivation of the Hippo pathway allows Yki to translocate into the nucleus and initiate tumorigenesis by promoting cell proliferation, cell competition, and migration. In contrast to this classic model in which the Hippo pathway acts as a tumor suppressor, recent reports suggest that the Hippo pathway acts as a tumor promoter. However, the mechanism underlying the tumorigenesis caused by Hippo activation remains poorly understood.

Results

We investigated the tumor-promoting role of the Hippo pathway using the *Drosophila* wing disc, where tumorigenesis can be analyzed by genetic manipulations. We found that Hippo activation can induce tumors characterized by phospho-S6 staining (tissue growth), matrix metalloproteinase 1 (MMP1) expression (invasive ability), and 5-ethynyl-2′-deoxyuridine (EdU) staining (excessive cell proliferation) in a non-cell-autonomous manner. This tumorigenesis occurs through the Hippo target genes, including *rpr*, *hid, grim* (an

apoptotic inducer), *atg8a* (an autophagy-related gene), *cyclin E* (a proliferative activator), and microRNA *bantam*. We found that the signaling pathway of the compensatory proliferation, Dronc-Wg/Spitz signaling, acting downstream of Rpr, Hid, and Grim is required for Hippo activation-mediated tumorigenesis. Moreover, we identified that the evolutionarily conserved amino acid transporters, Strip-interacting amino acid transporters 1/2 (Sat1/2), were required and redundantly worked with growth factors for Hippo activation-mediated tumorigenesis. Therefore, we suggest that the Hippo-activated cells cause non-cell-autonomous tumorigenesis through the growth factors Wg/Spitz and Sat1/2.



Current Model:
Hippo-activated cells induce non-cell-autonomous tumorigenesis through Spitz, Wg, and Sat1/2

The schematic depiction is the mechanistic hypothesis underlying the non-cell-autonomous tumorigenesis caused by the Hippo-activated cells. Hippo activation changes several gene expressions/activities, including *hid*, *bantam*, *atg8a*, and *cyclin E*. These alterations promote non-cell-autonomous tumorigenesis through Wg/Spitz secretion and Sat1/2.

Discussion & Conclusion

Many studies have asserted that the Hippo pathway acts as a tumor suppressor in *Drosophila* and mammals because the mutations in the Hippo pathway promote tumorigenesis. However, recent studies have suggested that the Hippo pathway acts as a tumor promoter. For example, the Hippo pathway promotes tumor growth and invasive behavior in a cell-autonomous manner. Here, we provide genetic evidence indicating that the Hippo-activated cells act as oncogenic niche cells, which promote tumorigenesis in a non-cell-autonomous manner.

Previous studies have suggested that tumor heterogeneity is crucial for tumor progression: cell-cell communication among oncogenic cells, including Src-activated and Ras-mutated cells, promotes invasive behaviors. Given that Hippo-activated cells are

crucial for tumorigenesis through cell-cell communication, Hippo-activated cells may also heterogeneously exist in human tumors.

The present study revealed the tumor-promoting role of the Hippo pathway, which is an unanticipated aspect of this pathway. This understanding will be useful in developing cancer treatments targeting the Hippo pathway.

一般の皆様へ

これまでのがん科学研究により、“Hippo 経路”は、がんを抑制する因子として知られている。すなわち、“Hippo 経路”が活性化すると、がんは消失すると考えられてきた。しかし、近年のがんゲノム科学の進展により、がん細胞内で“Hippo 経路”が活性化していることがわかってきた。この矛盾を解くべく、ショウジョウバエの遺伝学的解析法を駆使して“Hippo 経路”の機能理解を目指した。その結果、“Hippo 経路”が活性化すると確かにその細胞自体は消失する（細胞死する）ことを確認した。加えて興味深いことに、“Hippo 経路”を活性化している細胞の“周囲”にがん細胞が誘導されることが明らかになった。すなわち、“Hippo 経路”は単なるがん抑制因子ではなく、細胞間の相互作用を通じて、がんを誘導する活性も持ち合わせることが明らかになった。

Publication - FY2023 Grant Recipients

Title of the research project	Development of synthetic transformations based on installing group 13, 14 functional moieties
Recipient (Institution)	Chao Wang (Kanazawa University, Synthetic and Medicinal Element Chemistry Laboratory)
Journal article / other material	Chem. Eur. J. 2024, e202401546, doi.org/10.1002/chem.202401546
Title of the paper	Visible-Light-Driven Germyl Radical Generation via EDACatalyzed ET.HAT Process
Journal article / other material	JACS Au 2024, 4, 12, 4927–4933, doi.org/10.1021/jacsau.4c00907
Title of the paper	Transition-Metal-Free Thioboration of Terminal Alkynes

Title of the research project	Identification of transcriptional pause release regulators in leukemia progression
Recipient (Institution)	Takayuki Hoshii (Chiba University, Department of Molecular Oncology, Graduate School of Medicine)
Journal article / other material	Nucleic Acids Research , 2024, 52 , 9463.9480, doi.org/10.1093/nar/gkae605
Title of the paper	BOD1L mediates chromatin binding and non-canonical function of H3K4 meth yltransf erase SETD1A

Title of the research project	s of miR125-b involved in common molecular pathways of neurodegenerative diseases
Recipient (Institution)	Masato Yano (Niigata University, Neurobiology and Anatomy)
Journal article / other material	PNAS 2024 Vol. 121 No. 37 e2401531121, doi.org/10.1073/pnas.2401531121
Title of the paper	Qki5 safeguards spinal motor neuron function by defining the motor neuron-specific transcriptome via pre-mRNA processing

Title of the research project	Elucidation of Effects of Messenger RNA 5′ -Cap Structure Diversity on Protein Translation Activity
Recipient (Institution)	Masahito Inagaki (Nagoya University, Bioorganic Chemistry Laboratory)
Journal article / other material	Bulletin of the Chemical Society of Japan, 2025, 98, uoaf006, doi.org/10.1093/bulcsj/uoaf006
Title of the paper	Synthesis of hydrophobic-tagged 2' -deoxy-modified cap analogs and its effect on mRNA translation

Title of the research project	Opposite regulation by RNA splicesome factors for high and low temperature tolerance across species
Recipient (Institution)	Atsushi Kuhara (Konan University, Graduate school of Natural Science, Molecular and cellular regulation)
Journal article / other material	PNAS Nexus, 2024, 3, pgae293 doi.org/10.1093/pnasnexus/pgae293
Title of the paper	The intron binding protein EMB-4 is an opposite regulator of cold and high temperature tolerance in Caenorhabditis elegans
Journal article / other material	Neuroscience Research 13 November 2024 doi.org/10.1016/j.neures.2024.11.001
Title of the paper	Molecular, neural, and tissue circuits underlying physiological temperature responses in Caenorhabditis elegans

Title of the research project	Elucidation of the pathogenesis of NASH via adenosine synthesis by BST1
Recipient (Institution)	Keisuke Yaku (University of Toyama, Department of Molecular and Medical Pharmacology)
Journal article / other material	Yaku et al., Sci. Adv. 11, eadr1538 (2025) 21 March 2025, doi: 10.1126/sciadv.adr1538
Title of the paper	Nicotinamide riboside and nicotinamide mononucleotide facilitate NAD+ synthesis via enterohepatic circulation

Title of the research project	Identification of molecular biomarkers representing alternations at the endoplasmic reticulum-mitochondria contacting sites
Recipient (Institution)	Seiji Watanabe (Nagoya University, Department of Neuroscience and Pathobiology, Research Institute of Environmental Medicine)
Journal article / other material	BioEssays, 2025; 0:e70016, doi.org/10.1002/bies.70016
Title of the paper	Mitochondria and Endoplasmic Reticulum Contact Site as a Regulator of Proteostatic Stress Responses in Neurodegenerative Diseases

Title of the research project	Structural and functional analysis of a bacterial divisome using a small synthetic binding protein
Recipient (Institution)	Hiroyoshi Matsumura (Ritsumeikan University, College of Life Sciences)
Journal article / other material	bioRxiv July 18, 2024, doi.org/10.1101/2024.07.18.604045
Title of the paper	Structural basis for the interaction between the bacterial cell division proteins FtsZ and ZapA

＜論文掲載＞ 2023 年度受賞者

助成タイトル	13、14 族官能基の導入に基づく分子変換反応の開発
受賞者	王 超（金沢大学 医薬保健研究域 薬学系 元素創薬合成化学研究室）
論文掲載誌・書誌事項	Chem. Eur. J. 2024, e202401546, doi.org/10.1002/chem.202401546
論文タイトル	Visible-Light-Driven Germyl Radical Generation via EDACatalyzed ET.HAT Process
論文掲載誌・書誌事項	JACS Au 2024, 4, 12, 4927-4933, doi.org/10.1021/jacsau.4c00907
論文タイトル	Transition-Metal-Free Thioboration of Terminal Alkynes

助成タイトル	白血病進展における転写休止解除制御因子群の同定
受賞者	星居 孝之（千葉大学 大学院医学研究院 分子腫瘍学）
論文掲載誌・書誌事項	Nucleic Acids Research , 2024, 52 , 9463.9480, doi.org/10.1093/nar/gkae605
論文タイトル	BOD1L mediates chromatin binding and non-canonical function of H3K4 meth yltransf erase SETD1A

助成タイトル	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
受賞者	矢野 真人（新潟大学 医学部神経解剖学）
論文掲載誌・書誌事項	PNAS 2024 Vol. 121 No. 37 e2401531121, doi.org/10.1073/pnas.2401531121
論文タイトル	Qki5 safeguards spinal motor neuron function by defining the motor neuron-specific transcriptome via pre-mRNA processing

助成タイトル	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
受賞者	稲垣 雅仁（名古屋大学 生物有機化学研究室）
論文掲載誌・書誌事項	Bulletin of the Chemical Society of Japan, 2025, 98, uoaf006, doi.org/10.1093/bulcsj/uoaf006
論文タイトル	Synthesis of hydrophobic-tagged 2′ -deoxy-modified cap analogs and its effect on mRNA translation

助成タイトル	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
受賞者	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）
論文掲載誌・書誌事項	PNAS Nexus, 2024, 3, pgae293 doi.org/10.1093/pnasnexus/pgae293
論文タイトル	The intron binding protein EMB-4 is an opposite regulator of cold and high temperature tolerance in Caenorhabditis elegans
論文掲載誌・書誌事項	Neuroscience Research 13 November 2024 doi.org/10.1016/j.neures.2024.11.001
論文タイトル	Molecular, neural, and tissue circuits underlying physiological temperature responses in Caenorhabditis elegans

助成タイトル	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
受賞者	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）
論文掲載誌・書誌事項	Yaku et al., Sci. Adv. 11, eadr1538 (2025) 21 March 2025, doi: 10.1126/sciadv.adr1538
論文タイトル	Nicotinamide riboside and nicotinamide mononucleotide facilitate NAD+ synthesis via enterohepatic circulation

助成タイトル	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
受賞者	渡邊 征爾（名古屋大学 環境医学研究所　病態神経科学分野）
論文掲載誌・書誌事項	BioEssays, 2025; 0:e70016, doi.org/10.1002/bies.70016
論文タイトル	Mitochondria and Endoplasmic Reticulum Contact Site as a Regulator of Proteostatic Stress Responses in Neurodegenerative Diseases

助成タイトル	低分子人工抗体を用いた細胞分裂装置の構造機能解析
受賞者	松村 浩由（立命館大学 生命科学部）
論文掲載誌・書誌事項	bioRxiv July 18, 2024, doi.org/10.1101/2024.07.18.604045
論文タイトル	Structural basis for the interaction between the bacterial cell division proteins FtsZ and ZapA

Ⅲ.

Reports from the Recipients of Grants for International Meetings

Report on Research Meeting

Date: 2024/10/8

1. Name of Research Meeting / Conference
   18th International Zebrafish Conference (IZFC2024)

2. Representative
   Chair: Corinne Houart (King's College London, UK)
   Co-chair: Hitoshi Okamoto (RIKEN Center for Brain Science, Japan)

3. Opening period and Place
   August 17 ~ 21, 2024 at Miyako-Messe Kyoto (Kyoto, Japan)

4. Number of participants
   641 (+ Virtual 83)

5. Number of participating countries and areas
   38 countries (Australia 12, Austria 4, Belgium 1, Canada 34, Chile 1, China 43, Croatia 2, Denmark 4, Finland 2, France 12, Germany 27, Hong Kong 9, Hungary 2, India 4, Indonesia 1, Israel 14, Italy 6, Japan 187, Korea 33, Kuwait 1, Lithuania 1, Macao 1, Malaysia 3, Netherlands 4, New Zealand 1, Norway 3, Poland 1, Portugal 2, Qatar 1, Russia 2, Singapore 9, Spain 1, Sweden 7, Switzerland 2, Taiwan 30, Turkey 1, United Kingdom 32, United States 126, Unknown 15)

6. Total cost
   10,390,417 JPY

7. Main use of subsidy
   Student workers (part-time job)

8. Result and Impression
   The 18th International Zebrafish Conference (IZFC2024) took place from August 17 to 21, 2024, at Miyako Messe in Kyoto, Japan. This conference attracted 641 researchers from around the world along with 83 virtual participants, making it the largest gathering since the COVID-19 pandemic. The event featured two keynote lectures, three award lectures, 36 plenary talks, 90 oral presentations, and 374 poster presentations. Participants also engaged in luncheons, workshops, and community discussions, all aimed at exploring innovative technologies, enhancing scientific environments and strengthening connections within the research community. Additionally, the conference included cultural elements that allowed participants to immerse themselves in Japanese traditions, enriching their overall experience. This commitment to blending scientific inquiry with cultural appreciation ensured that participants gained valuable insights into the latest research while enjoying a taste of Japan's rich heritage. Overall, IZFC2024 successfully promoted collaboration and innovation in zebrafish research while celebrating the unique cultural aspects of Japan, making this conference an unforgettable event for everyone involved.

Keynote Lectures

The first keynote lecture took place after the opening remarks on the first day. Prof. Jie Qiao (Peking University Third Hospital, China) delivered a lecture titled "Mechanism research on epigenetic regulation of reproductive development from model animals to human beings". She has uncovered the molecular mechanisms behind human gametogenesis and embryonic development, providing epigenetic insights into fertility and early embryogenesis. Her talk was delivered with great enthusiasm, making it a great way start to the conference. The second keynote lecture was held on the third day. Prof. Emi Nishimura (The University of Tokyo, Japan) gave a presentation entitled “Searching for principles of tissue regeneration and aging in mammalian Skin.” She has demonstrated the role of stem cells in maintaining skin homeostasis. Her findings on the dynamics of skin stem cells brought significant hope for maintaining health and youthfulness in human aging.

Award Lectures

Three award lectures were featured at IZFC2024. The George Streisinger Award, recognizing a senior researcher who has made sustained and foundational contributions to the zebrafish research community, was awarded to Judith Eisen (University of Oregon, USA) this year. She attended IZFC2024 virtual and delivered her award lecture, titled "Making connections for a life in science" on the second day. Prof. Eisen is a pioneer in establishing zebrafish as a model organism for studying neural development and neural circuits. Her talk explored connections among neurons, glial cells, muscles and the microbiome in zebrafish as well as the relationships among scientists and the research community from its inception to the present. The Christiane Nüsslein-Volhard Award recognizes researchers who have made outstanding contributions to zebrafish research, such as establishing new techniques, infrastructure or funding resources. The European Zebrafish Society honored Stephen Wilson (University College London, UK) with this award this year. He delivered his award lecture titled "Left side story - a tale of genes, eyes and developing brains". Prof. Wilson discussed his pioneering and recent studies on brain development and neural connectivity, sharing humorous anecdotes and insights from his collaborations. He demonstrated that a researcher’s life can be deeply enriched through work with zebrafish. The Chi-Bin Chien Award honors exceptional graduate students, postdoctoral trainees or early-career principal investigators who have made significant contributions to zebrafish research. This award commemorates Prof. Chi-Bin Chien (1965-2011), who served the international zebrafish community in numerous ways. This year, the IZFS presented the Chi-Bin Chien Award to D’Juan Farmer (University of California, Los Angeles). He delivered his award-winning lecture titled "Mechanisms of growth in the vertebrate skull: lessons from zebrafish" on the fourth day. In his lecture, he discussed how genetic and cellular processes regulate craniofacial development, shedding light on human craniofacial disorders. He also conveyed a passion and love for zebrafish research that resonate with Chi-Bin Chien’s dedication and affection for science.

Sessions

Participants could submit an abstract for either oral or poster presentation. Following a review process, 36 abstracts were picked up for Plenary Sessions, which were held in a large hall where all participants could hear the talks. Ninety abstracts were selected for Concurrent Sessions, which took place simultaneously in three different halls. The remaining abstracts were assigned to Poster Sessions. Throughout all plenary sessions, the audience was captivated by the passionate presentations and the ensuing inspiring discussions. The Concurrent Sessions were held in three separate halls every

morning. The theme of the 15 concurrent sessions were as follows: Early development, morphogenesis and patterning I; Early development, morphogenesis and patterning II; Cardiovascular; Organ biology; Regeneration; Neurobiology I; Neurobiology II; Circuits and behavior; Cell biology; Reproduction (germline, sex determination and reproductive health); Physiology and metabolism; Lifespan and aging; Disease models; Evolution and comparative biology; Emerging technologies. All 90 presentations across these various themes were highly engaging, and the Q&A sessions featured lively debates, incorporating questions from both in person and virtual participants. The poster presentations were held over three days from the second to the fourth day, featuring a total of 374 posters. Each poster area was bustling with people, clutching snacks and coffee while engaging in lively discussions. At the closing remarks on the fifth day, the eight poster presentation prize winners were announced.

Banquet

The banquet was held at Hotel Okura Kyoto on the evening of the fourth day. While participants were mingling with glasses of wine, the banquet was suddenly kicked off by a dynamic performance of taiko, traditional Japanese drums. After the powerful drumming, Corinne Houart gave a brief speech to officially open the banquet. She conducted a traditional kagami-biraki ceremony, where they used wooden mallets to break open a sake barrel. They then served sake to everyone and led the toast. After some socializing, the taiko performers returned to the stage with players of shamisen and yokobue. They delivered a stunning performance of classical Japanese music. Participants were then invited to try playing the taiko drums themselves, creating a shared experience of traditional Japanese culture. The banquet concluded with great success.

9. Additional description

The 18th International Zebrafish Conference was made possible by financial support from the NOVARTIS Foundation (Japan). We deeply appreciate for this support.

Figure 1. Chairs, keynote lecturers and award winners in the IZFC2024.
(a) Chair: Corinne Houart. (b) Co-chair: Hitoshi Okamoto. (c) Keynote Lecturer: Jie Qiao (d) Keynote Lecturer: Emi Nishimura. (e) George Streisinger Award Winner: Judith Eisen. (f) Christiane Nüsslein-Volhard Award Winner: Stephen Wilson. (g) Chi-Bin Chien Award Winner: D'Juan Farmer. (h) Christine Beattie Award Winner: Kuo-Chang Tseng.

Figure 2. Snapshots in the IZFC2024.
(a) Registration desk with a sponsor board. (b) Education and outreach session. (c) Sustainability session. (d) Community session. (e) Kagami-biraki in the banquet. (f) Quartet of large drum, shamisen, small drum and flute.

Report on Research Meeting

Date: 2024/12/15

1. Name of Research Meeting / Conference
   The 12th 3R+3C International Symposium

2. Representative
   Tsutomu Katayama

3. Opening period and Place
   November 18-22, 2024
   ACROS Fukuoka Building, Fukuoka, Japan

4. Number of participants
   255

5. Number of participating countries and areas
   Japan 157, USA 24, UK 22, France 13, South Korea 10, China 6, Germany 4, Italy 4, Taiwan 3, Netherlands 3, Sweden 2, Switzerland 2, Denmark 1, Austria 1, Canada 1, Norway 1, Spain 1

6. Total cost
   16,039,660 JPY

7. Main use of subsidy
   Accommodation fee for oversea invited researchers

8. Result and Impression

This international symposium focused on cutting-edge research in molecular mechanisms and regulating systems involved in DNA replication, repair, and recombination (3R), and chromatin and chromosome dynamics and the cell cycle (3C). This event was held from November 18-22, 2024 and the venue was the International Conference Hall (oral presentations) and Exchange Gallery (poster presentations) in the ACROS Fukuoka Building located in the center of Fukuoka City. In order to promote mutual understanding and exchange between different fields, the oral presentations were intentionally given in one place, without any subcommittees. There were more people than expected who wanted to give general oral presentations (10 minutes, 5 minutes, or 3 minutes), and the high enthusiasm of young researchers, postdocs, and graduate students was evident. For this reason, the plan was changed from the past to extend the presentation time by starting at 8:30 in the morning, and as many topics as possible were accepted. Despite the large number of topics, all presentations including the Q&A sessions and poster sessions were extremely lively from start to finish.

Originally, preparations were underway for the 12th international symposium to be held in Chiba Prefecture in 2020. However, the event was canceled due to the COVID-19 pandemic and postponed to 2022. As the COVID-19 pandemic continued, the 2022 event was also canceled, and I took over as organizer, aiming to hold the event in Fukuoka in 2024. At that time, the impact of the COVID-19 pandemic was still significant, and we were fighting various anxieties in the preparations. As such, this was the first time in six years since the 11th symposium was held in 2018, but the fact that such a large number of participants (255) from Japan and abroad gathered confirms the importance of this symposium.

A plenary lecture was performed by Dr. John Diffley (Francis Crick Institute, UK) with a 30-minute presentation including Q&A session. Also, 17 oversea invited researchers (7 of whom were women) conducting cutting-edge research in the field of 3R+3C gave special lectures (20 minutes each including Q&A session). Meanwhile, 25 world-leading researchers from Japan also gave special lectures similarly. In addition to these, there were general presentations (10 minutes including Q&A: 33 presentations in total) mainly by young researchers, and poster presentations (167 presentations in total) mainly by graduate students and postdocs. Selected poster presentations were also given as short talks (5-minute or 3-minute oral presentations: 49 presentations in total). Each member of the Organizing Committee of the 12th 3R+3C International Symposium was responsible for his/her own field of expertise and supported the holding of this symposium in all aspects, including the research presentations, the selection of overseas invited researchers in advance, the selection of general presentations and short talks, and the selection of poster award winners.

As intended, the research presentations also featured lively presentations and discussions of the world's most cutting-edge research in 3R+3C. Important results were announced not only in basic researches of DNA replication, repair, recombination, epigenetics and chromosome dynamics such as DNA condensation and partition, in addition to advanced researches of the molecular basis of carcinogenesis, cancer cell proliferation, aging and diseases such as chromosomal abnormalities, developmental abnormalities, and hematopoietic abnormalities. In terms of methodology, the presentations also included new trends such as single-molecule/single-cell analysis, cryo-electron microscopy analysis, dynamic analysis of protein higher-order complexes, informatics analysis of genome dynamics, and new mathematical theoretical research. In this way, mutual understanding and personal exchanges were deepened at the world's most cutting-edge level, including among young researchers, graduate students, and postdoctoral researchers, and this has made a significant contribution to the development of molecular biology in Japan.

9. Additional description

In addition to the welcome reception and banquet, exchange promotion events included an excursion to experience making Kumiko Zaiku, a local historical wooden craft (1), and a visit to Dazaifu Tenmangu Shrine (2) (these were implemented by Nippon Travel Agency Co., at a separate fee from the participation fee). This was attended by 101 people, and the delicate Kumiko Zaiku was particularly well received. The overseas participants had a strong interest in Japanese culture, and it seems that this led to a living intellectual and cultural exchange that went beyond specialized research.

(1) Kumiko craft (Traditional artistic woodwork) experience with English/Japanese-speaking instructors

There are over 200 types of designs including hemp leaves with a combination of geometric patterns made by assembling designed wooden pieces without using nails. The thickness is adjusted in 0.01 mm increments using the craftsmen' skill with a history of approximately 300 years. 'Ohkawa Kumiko' is a specialty craft designated by the Fukuoka Prefecture in 1986.

(2) "Dazaifu Tenmangu Shrine"

"Dazaifu Tenmangu Shrine" is the head shrine of about 12,000 shrines that enshrine Sugawara no Michizane, also known as "Tenjin-sama" and widely revered as a god of education, culture, and the arts, as well as the symbol of warding off evil.

Report on Research Meeting

Date:2024/09/30

1. Name of Research Meeting / Conference
   The 4th International CADASIL & VCI Symposium

2. Representative
   Masafumi Ihara

3. Opening period and Place
   12/July/2024, SHIMADZU CORPORATION, Nakagyo-ku, Kyoto, Japan

4. Number of participants
   67

5. Number of participating countries and areas
   South Korea, Taiwan, Australia, USA and Japan

6. Total cost
   400,000 JPY

7. Main use of subsidy
   Venue setup costs, Printing costs, Office supplies

8. Result and Impression

Recent advancements in genomic research have revealed that approximately 9 individuals per 1,000 in East Asia carry cysteine-altering NOTCH3 variants within the epidermal growth factor-like repeat domains. These variants are the underlying cause of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Compared to CADASIL patients in Western countries, those in Asia show several notable differences. Specifically, there are variations in the predominant pathogenic NOTCH3 variants, a higher prevalence of hemorrhagic stroke, and a lower prevalence of white matter hyperintensities in the anterior temporal pole. These regional differences underscore the need for large-scale clinical studies in East Asia to establish more relevant clinical evidence for CADASIL patients in this region.

In response to this need, we have launched the CADREA (CADASIL Registry in East Asia) study. This study aims to investigate the clinical, genetic, and neuroradiological characteristics of CADASIL patients in Japan, Korea, and Taiwan. Since the inception of the CADREA study, several collaborative research projects have been initiated, leading to numerous important findings that have advanced our understanding of CADASIL.

At the 4th KYOTO INTERNATIONAL CADASIL & VCI SYMPOSIUM, there was a vigorous exchange of ideas regarding CADASIL. Researchers from Korea, Taiwan, and Japan came together to discuss the future direction of CADASIL treatment and medical care. Additionally, patients from Korea and Japan participated in the discussions, providing valuable perspectives from patient-public involvement. These discussions have highlighted the importance of integrating patient experiences into research and treatment planning. The next conference is scheduled to take place in Taiwan next year. The most significant achievement of this conference was the strengthening of the network of CADASIL researchers from Korea, Taiwan, and Japan. This enhanced collaboration underscores Japan’s active role within the regional network of CADASIL research.

9. Additional description

Acknowledgment

National Cerebral and Cardiovascular Center
Department of Neurology
Satoshi Saito (Secretary General)
Tomotaka Tanaka (Imaging study)
Yumi Yamamoto (Pre-clinical study)
Sonu Bhaskar (Registry)
Soichiro Abe (Registry)
Hiroyuki Ishiyama (Genome study)
Eriko Yamaguchi (Intl collaboration)
Takehito Kuroda (SPECT)
Shinsaku Nakazawa (ASL)
Yuriko Nakaoku (ASL)
Sakurako Abe (Symposium relations)
Testuya Chiba (Symposium relations)
Kotomi Nakazawa (National database)
Chikage Kakuta (Psychology)
Miho Yamauchi (Psychology)
Akino Ohta (CRC)
Takami Imazato (Secretary)
Yumi Ito (Secretary)
Yumiko Ito (Secretary)
Yoko Ohashi (Secretary)
Noriyuki Suzuki (Secretary)

Mie University Graduate school of Medicine
Hidekazu Tomimoto, Akihiro Shindo
University of Miyazaki
Kazuo Kitamura, Toshihiro Kita
Kyoto University Graduate School of Medicine
Ryosuke Takahashi, Takakuni Maki
RIKEN Ctr for Biosystems Dynamics Research
Toshihiko Aso
Kyoto Prefectural University
Ikuko Mizuno, Toshiki Mizuno
Niigata University
Osamu Onodera
Seoul National University College of Med
Hee-Joon Bae
Jeju National University Hospital
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Taiwan Veterans General Hospital
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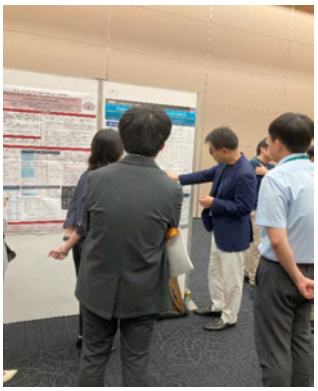
National Taiwan University Hospital
Sung-Chun Tang
Asan Medical Center
Bum Joon Kim, Jae-Sung Lim, Hyunjin Kim

We extend our sincere gratitude to all the CADASIL patients and their families involved in our research.



Report on Research Meeting

Date: 2024/10/10

1. Name of Research Meeting / Conference
   16th Congress of the Asia Continental Branch of the International Society of Paediatric Oncology (SIOP Asia 2024)

2. Representative
   Chair: Koichi Matsumoto
   Director, Children's Cancer Center, National Center for Child Health and Development.

3. Opening period and Place
   June 22 (Saturday) to June 25 (Tuesday), 2024, for 4 days.
   Pacifico Yokohama North
   1-1-1 Minatomirai, Nishi-ku, Yokohama, Kanagawa 220-0012, Japan.

4. Number of participants
   Japan : 294 pax.
   overseas : 443 pax.
   Total : 737 pax.

5. Number of participating countries and areas
   45 countries (including Japan)
   United States, Singapore, Vietnam, United Arab Emirates, Switzerland, Belgium, Armenia, Sweden, Malaysia, United Kingdom, Spain, Myanmar, Israel, Sri Lanka, Mongolia, Iran, Thailand, Jordan, India, Timor-Leste, Laos, Indonesia, Germany, Lebanon, Uzbekistan, Nepal, Russia, Australia, Bangladesh, China, Qatar, Pakistan, South Africa, Cameroon, Palestine, Taiwan, Cambodia, Philippines, Japan, Kyrgyzstan, Bulgaria, South Korea, Saudi Arabia, Brunei, Hong Kong.

6. Total cost
   ￥66,500,158

7. Main use of subsidy
   1. Equipment Rental Costs ￥150,000.
   2. Scholarship ￥150,000 1 pax.
   3. Awarded Travel Grants ￥50,000 2 pax.

8. Result and Impression
   Conference Overview
   SIOP Asia 2024, the largest international conference on pediatric oncology in the region, was held with the mission to eliminate childhood cancer fatalities. The conference aimed to enhance pediatric cancer care in Asia by presenting the latest research and clinical advancements to a global audience.

   Conference Highlights
   The conference featured a diverse array of sessions:
   - Educational Seminars: Opportunities to learn about fundamental knowledge and latest treatment methods

- Keynote Lectures: Insights on pediatric oncology genomics, immunotherapy, and long-term follow-up care
- Special Lectures: Discussions on CAR-T therapy, hematopoietic stem cell transplantation, and targeted molecular therapies
- Lunch Seminars: Seven seminars sponsored by various companies showcased cutting-edge research and treatment innovations
- Oral Sessions: Presentations on leukemia, brain tumors, neuroblastomas, and more
- Poster Sessions: Display of research findings and clinical achievements.

Major Speakers
Prominent speakers included:

- Godfrey Chan (University of Hong Kong)
- Matthew Shing (Hong Kong Children's Hospital)
- Ruzanna Papyan (Armenian Pediatric Oncology and Hematology Center)
- Alice Yu (Academia Sinica, Taiwan)
- Keon Hee Yoo (Samsung Medical Center)
- Hiroki Hori (Mie University)
- Atsushi Manabe (Hokkaido University)
- Eiso Hiyama (Hiroshima University)
- Motohiro Kato (University of Tokyo)

Sponsors and Exhibits
The event received support from numerous organizations:

- Lunch Seminars: 7 sponsors, including Alexion Pharma G.K., HIROTSU BIO SCIENCE INC., and Benesse Foundation for Children
- Exhibitions: Over 18 companies, such as Alfresa Pharma Corporation and Japan CliniClowns Association
- Program Book Advertisements: 17 companies

Summary
SIOP Asia 2024 provided a significant platform for discussing advancements in pediatric oncology. With over 700 participants and representatives from 43 countries, the conference facilitated meaningful discussions and knowledge exchange. Supported by organizations such as the Asian Pediatric Hematology and Oncology Group (APHOG), St. Jude Global, and Childhood Cancer International (CCI), the event successfully advanced the field of pediatric oncology. We extend our heartfelt gratitude to all supporters and collaborators, and we remain committed to contributing to the advancement of childhood cancer care.

9. Additional description
Conference Program
Please refer to the program book.


SIOP Asia
2024


You Raise It Up!
SIOP Asia
2024

Report on Research Meeting

Date: 2024/09/02

1. Name of Research Meeting / Conference
   The 76th Annual Meeting of the Japanese Society for Cell Biology

2. Representative
   Yasushi Okada

3. Opening period and Place
   July 17th ～ July 19th , 2024

4. Number of participants /
   725 people

5. Number of participating countries and areas
   6 countries and areas other than Japan

6. Total cost
   24,042,000 yen

7. Main use of subsidy
   Venue and equipment costs for the large hall hosting the plenary lecture
   Speaker fees for invited presenters of the plenary lecture

8. Result and Impression
   1) The conference, which featured academic programs consisting of approximately 20 symposia and workshops, and about 230 oral/poster presentations over the entire schedule (3 days), concluded successfully with 725 participants (including 210 students), far exceeding the expected number at the time of application. As evidenced by the fact that 320 people (about 44%) were participants from outside the Japanese Society for Cell Biology, the conference became an open event that involved many researchers interested in cell biology, not limited to society members. Throughout the entire period, it successfully deepened enlightenment and exchange among numerous researchers to a great extent.

   2) The conference also succeeded as a venue for international discussion and exchange in cell biology, welcoming numerous participants from overseas, including international students studying in Japanese graduate schools. In particular, the plenary lecture given by Dr. Fiona Watt, a world-renowned cell biologist who is the Director of the European Molecular Biology Organization (EMBO) in Heidelberg, Germany, and currently leads two active research groups at King's College London, UK, and in Heidelberg, Germany, was a great success. Many participants reported attending specifically to hear Dr. Watt's lecture, and her presentation on the first day filled the main hall to capacity. Dr. Watt's presentation of her findings, which spanned multiple levels from molecular to single-cell to tissue and organism, was excellently aligned with the conference's theme and truly befitting of a plenary lecture, leaving a strong impression on us. Despite her busy schedule, Dr. Watt stayed until the final day of the conference after her lecture, engaging in discussions and interactions with conference participants. Through these exchanges, she greatly influenced us not only in academic aspects but also in diverse areas such as the way of being a researcher, approaches to education, and management of scientific research.

3) The other plenary lecturer, Dr. Nozomu Yachie, is a rising star among young researchers who is vigorously conducting international research across the globe, primarily based at the University of British Columbia in Canada while also maintaining a laboratory at Osaka University in Japan. His approach to promoting a new style of cell biology research, backed by unique ideas and proprietary technologies, stood in contrast to Dr. Fiona Watt and left a strong impression on the participants, especially the younger ones.

9. Additional description

4) Another characteristic of this conference was the high participation of young researchers, particularly students and graduate students. Passionate discussions led by these young participants unfolded daily, especially in the poster session areas. Dr. Watt was impressed by this scene, noting that it was almost indistinguishable from conferences in Europe and North America. The Young Investigator Awards, Poster Awards, and Microscopy Image Contest, which were organized to foster the next generation of talent, were very well-received and proved to be valuable experiences that should be continued in future conferences.

5) The conference also featured exhibitions by nearly 30 companies and held luncheon seminars, providing a platform for participants to share information about various cutting-edge equipment, reagents, and resources that could be utilized in future research.

38th Grant Report (FY2024)

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 2024 are as follows.

38th Novartis Research Grant:	36 Researchers (JPY 1.0 mil.),	Subtotal JPY 36.0mil.
Research Meeting Grant:	5 Meetings (JPY 0.4 mil.),	Subtotal JPY 2.0mil.
		Total JPY 38.0mil.

38th Novartis Research Grant (FY2024)

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	Hideaki Morishita	Department of Molecular Cell Biology, Graduate School of Medical Sciences, Kyushu University	Professor	Elucidation of the molecular basis of intracellular breakdown phenomena
2	Mikiko Inaki	Grad. Sch. Sci., University of Hyogo	Professor	Manipulation of tissue morphology by regulating the expression of extracellular matrix
3	Shogo Kumagai	Research Institute Division of Cancer Immunology, National Cancer Center	Staff scientist	Development of new immunotherapy combined with cancer cell-selective radiotherapy
4	Eisaku Ohashi	Graduate School of Pharmaceutical Sciences, Synthetic Organic Chemistry, Nagoya City University	Assistant Professor	Medicinal Research on Next-Generation Immunosuppressant Candidates Based on the Development of Novel Synthesis Platform
5	Koichi Nishiyama	Laboratory for Vascular and Cellular Dynamics, Faculty of Medicineular, University of Miyazaki	Professor	Self-orgnization mechanism mediated by control of perivascular mechanical field in angiogenesis
6	Tsukasa Nabekura	Division of Immune Response, Aichi Cancer Center Research Institute	Chief	Artificial intelligence-based screening and experimental validation of an activator off anti-tumor activity of natural killer cells
7	Takahiro Matsui	Graduate School of Medicine, Department of Pathology, Osaka University	Associate Professor	The research for early cancer detection focusing on tissue-resident macrophages
8	Keiji Hirota	Lab. Integrative Biological Science, Kyoto University	Associate Professor	Novel function and regulation of neutrophil-producing IL-23a in the modulation of autoimmune diseases
9	Masahito Yoshida	Department of Chemistry, Institute of Pure and Applied Sciences, University of Tsukuba	Professor	Synthesis and application of a peptide probe toward the clarification of the mechanism of reversible V-ATPase inhibition
10	Kazutaka Shibatomi	Laboratory of Synthetic Organic Chemistry, Toyohashi University of Technology	Professor	Synthesis of Heterocyclic Compounds with a Spirocyclic Backbone for Expansion of Drug Library

#	Name	Institution	Title	Research Project
11	Motonao Nakamura	Department of Life Science, Graduate School of Science, Okayama University of Science	Professor	Blockage of multi-drug resistance of cancer cells by inhibition of bitter taste receptors
12	Tomonori Matsumoto	Laboratory of Ploidy Pathology, Graduate School of Frontier Biosciences, Osaka University	Associate Professor	Modeling and elucidation of polyploidization-driven carcinogenesis with a focus on Barrett's adenocarcinoma
13	Keiichiro Suzuki	Suzuki laboratory, Osaka University	Professor	Development of In Vivo Antibody-based Drug Production Technology for Dementia Prevention
14	Takayuki Fujiwara	Department of Computational Diagnostic Radiology and Preventive Medicine, The University of Tokyo	Project Assistant Professor	Investigation of intractable cardiovascular disease using three dimensional pathological analysis
15	Minoru Ueda	Graduate School of Science, Tohoku University	Professor	Development of OPDA-ID, a ligand-inducible animal cell-targeted proteolysis method using primordial plant hormones
16	Yoshihiro Omori	Laboratory of Functional Genomics, Graduate School of Integrated Sciences for Life, Hiroshima University	Professor	Understanding of bone formation mechanisms by genome-wide association study (GWAS) using the hybrids of ornamental medaka
17	Yuya Sanaki	Life Science Center for Survival Dynamics, University of Tsukuba	Assistant Professor	Identify a Functional Inhibitors for Metabolic Alteration in Cancer Patients
18	Masaaki Umeda	Graduate School of Science and Technology, Division of Biological Science, Laboratory of Plant Growth Regulation, Nara Institute of Science and Technology	Professor	Regulatory mechanisms of cell division underlying tissue regeneration in plants
19	Taku Kureha	Department of Immunology, The University of Tokyo	Lecturer	Inflammation Control in Obesity by innate-like memory CD8+ T Cells
20	Takaaki Sato	Department of Applied Chemistry, Faculty of Science and Technology, Keio University	Associate Professor	Development of Dynamic Asymmetric Crystallization for Synthesis of Medicinal Drugs
21	Takafumi Yokota	Department of Hematology, Osaka International Cancer Institute	Chief Director	Molecular mechanisms by which RNA splicing abnormalities induce acute myeloid leukemia
22	Haruka Sasaki	Department of Dento-oral Anesthesiology, Graduate School of Dentistry, Tohoku University	Assistant Professor	Development of novel asthma therapy based on the pathophysiology of airway hyperresponsiveness
23	Michihiro Mieda	Department of Integrative Neurophysiology, Kanazawa University	Professor	Mutually promoting effects of circadian rhythm center dysfunction and Alzheimer's disease onset/ progression
24	Saishu Yoshida	Laboratory of Animal Cell & Developmental Biology, Department of Biomolecular Science, Toho University	Senior Lecturer	Development of Next-Generation Hedgehog Inhibitors Targeting Drug-Resistant Cancer Cells
25	Akie Shimotohno	Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University	Lecturer	Exploration of functional molecules that transmit environmental changes
26	Naoto Fujiwara	Department of Gastroenterology and Hepatology, Mie University Hospital	Assistant Professor	Omics-driven insights into chemotherapy-induced modulation of tumor immune microenvironment in human biliary tract cancer

#	Name	Institution	Title	Research Project
27	Hideaki Fujiwara	Department of Hematology and Oncology, Okayama University Hospital	Research Associate Professor	Development of novel therapeutic strategies for chronic GVHD post allogeneic hematopoietic cell transplantation targeting oral microbiota
28	Yasutomi Kamei	Graduate School of Life and Environmental Sciences, Kyoto Prefectural University	Professor	Does DNA methylation cause skeletal muscle aging?
29	Takayuki Shimizu	Faculty Division of Natural Sciences, Nara Women's University	Associate Professor	Elucidation of the signaling mechanism for chloroplast biogenesis regulated by polysulfide
30	Norika Liu	Stem Cell Stress Laboratory (Liu Lab), International Research Center for Medical Sciences, Kumamoto University	Junior Associate Professor	Unraveling the Mechanism of Myocardial Fibrosis through Lineage-specific Macrophages
31	Chihiro Furumizu	Program of Biotechnology, School of Engineering, Hiroshima University	Assistant Professor	Novel inhibitory signaling mechanism of meristematic activities in plants
32	Sugiyama Shigeru	Research and Education Faculty, Natural Sciences Cluster, Sciences Unit, Kochi University	Professor	Elucidation of the molecular recognition mechanism of PFAS by human heart-type FABP
33	Yoshinori Katsumata	Institute for Integrated Sports Medicine, Keio University School of Medicine	Assistant Professor	Breath biopsy for nonalcoholic steatohepatitis
34	Daisuke Kurita	Molecular Biology Lab, Hirosaki University	Associate Professor	Effect of rRNA modification on ribosome rescue system
35	Satoru Torii	TMDU Advanced Research Institute, Pathological Cell Biology laboratory, Tokyo Medical and Dental University	Project Associate Professor	Elucidation of the pathogenesis of Parkinson's disease and development of curative compounds
36	Tomoki Nakashima	Faculty of Dentistry, Tokyo Medical and Dental University	Professor	Establishment of the molecular basis for systemic health care based on the identification of exerkines

FY2024 Research Meeting Grant

(JPY 400 thousand x5 = 2.0 million)

No.	Name	Institution	Title	Place/Date	Meeting
1	Masami Kanai	Department of disease model for human diseases, Tokyo Medical and Detal University	Professor	Nagano/ 2025.9.8-11	The VIIth International Workshop on SOX Transcription Factors
2	Koh Aoki	Graduate School of Agriculture, Department of Agricultural Biology Osaka Metropolitan University	Professor	Osaka/ 20255.7.7-11	The 7th International Conference on Plant Vascular Biology
3	Yoichi Morofuji	Department of Neurosurgery Nagasaki University	Associate Professor	Tokyo/ 2026.03.6-7	Minisymposium on the blood-brain barrier: from basic to clinical research
4	Yasuhito Kannya	Division of Hematopoietic Disease Control The University of Tokyo	Professor	Sapporo/ 2025.7.4-6	The 43rd Sapporo International Cancer Symposium
5	Yuki Tsuchikane	Plant Evolutionary Biology, The University of Tokyo	Project Assistant Professor	Hyogo/ 2025.5.19-22	Plant Reproductive Development and Genomics

第38期（2024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。2024年度は、下記の総額3,800万円の助成事業を実施しました。

第38回ノバルティス研究奨励金	36件（1件100万円）	3,600万円
研究集会助成	5件（1件 40万円）	200万円
		総額 3,800万円

第38回ノバルティス研究奨励金（2024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	森下　英晃	九州大学 大学院医学研究院 生体機能学分野	教授	細胞内破壊現象の分子基盤の解明
2	稲木美紀子	兵庫県立大学 大学院理学研究科	教授	細胞外マトリックスの発現制御による 組織形態の操作
3	熊谷　尚悟	国立がん研究センター 研究所 腫瘍免疫研究分野	研究員	がん細胞選択的放射線治療を併用した 新たな免疫治療開発
4	大橋　栄作	名古屋市立大学 大学院薬学研究科 薬品合成化学分野	助教	新規合成プラットフォーム開発に基づく 次世代免疫抑制剤候補化合物の探索研究
5	西山　功一	宮崎大学 医学部 機能制御学講座 血管動態生化学分野	教授	血管新生における血管周囲の力学場制御を 介した自己組織化機構
6	鍋倉　　宰	愛知県がんセンター研究所 腫瘍免疫応答研究分野	分野長	人工知能を用いたナチュラルキラー細胞 抗がん活性賦活化剤の探索と実験的検証
7	松井　崇浩	大阪大学 大学院医学系研究科 病態病理学	准教授	組織常在性マクロファージに着目した 早期がん検出法の研究
8	廣田　圭司	京都大学 医生物学研究所 統合生体プロセス分野	准教授	自己免疫疾患の病態抑制に関わる好中球が 産生するIL-23aの新規機能と制御機構
9	吉田　将人	国立大学法人筑波大学 数理物質系化学域	教授	可逆的V-ATPase阻害機構の解明を目指した ペプチドプローブの合成と応用
10	柴富　一孝	豊橋技術科学大学 有機反応化学研究室	教授	薬理活性化合物ライブラリーの拡充を志向した スピロ型複素環式化合物の合成
11	中村　元直	岡山理科大学大学院 理学研究科 臨床生命科学専攻	教授	苦味受容体の拮抗薬によってがん細胞の 抗がん剤耐性化を抑止する
12	松本　知訓	大阪大学 大学院生命機能研究科 倍数性病態学研究室	准教授	バレット腺癌に着目した多倍体発癌機構の モデル化と解明
13	鈴木啓一郎	大阪大学 鈴木研究室	教授	認知症予防に向けた 抗体薬生体内産生技術の開発
14	藤原　隆行	東京大学 医学部附属病院 コンピュータ画像診断学 予防医学講座	特任 助教	三次元病態解析による難治性心血管疾患の 病態解明

No.	氏　名	所　属	職位	研究課題
15	上田　実	東北大学 大学院理学研究科化学専攻	教授	始原植物ホルモンを用いるリガンド誘導性動物細胞標的タンパク質分解法 OPDA-ID の開発
16	大森　義裕	広島大学 大学院統合生命科学研究科 ゲノム機能科学研究室	教授	観賞メダカ品種群の雑種交配家系を用いたゲノムワイド関連解析（GWAS）による骨形成機構の解明
17	佐奈喜祐哉	筑波大学 生存ダイナミクス研究センター	助教	がん患者に見られる全身性代謝変容を抑制する機能的阻害剤の探索
18	梅田　正明	奈良先端科学技術大学院大学 先端科学技術研究科 バイオサイエンス領域、 植物成長制御研究室	教授	植物の組織再生を支える細胞分裂の制御機構
19	呉羽　拓	東京大学 大学院医学系研究科 免疫学	講師	自然免疫系 CD8+T 細胞による肥満における炎症制御のメカニズム
20	佐藤　隆章	慶應義塾大学 理工学部応用化学科	准教授	医薬品合成を指向した動的不斉結晶化法の開発
21	横田　貴史	大阪国際がんセンター 血液内科	主任部長	RNA スプライシング異常が急性骨髄性白血病を誘発する分子機構とその制御
22	佐々木晴香	東北大学 大学院歯学研究科 歯科口腔麻酔学分野	助教	気道過敏性のメカニズムをターゲットにした新規喘息治療薬の開発
23	三枝　理博	金沢大学 医学系・統合神経生理学	教授	概日リズム中枢の機能低下とアルツハイマー病発症・進行との相互促進作用
24	吉田　彩舟	東邦大学 理学部 生物分子科学科 動物発生制御学研究室	講師	薬物耐性がん細胞の克服に立脚した次世代 Hedgehog 阻害薬の開発
25	下遠野明恵	名古屋大学 トランスフォーマティブ 生命分子研究所（ITbM）	特任講師	環境認知を司る機能分子の探索とメカニズムの解明
26	藤原　直人	三重大学医学部附属病院 消化器病センター	助教	オミクス解析に基づく標準化学療法がヒト胆道癌微小環境へ与える影響の解明
27	藤原　英晃	岡山大学病院 血液・腫瘍内科	研究准教授	口腔内細菌叢を標的とした同種造血細胞移植後慢性 GVHD の新規予防・治療法の開発
28	亀井　康富	京都府立大学 生命環境科学研究科	教授	DNA のメチル化は骨格筋の老化を引き起こすか？
29	清水　隆之	奈良女子大学 研究院自然科学系	准教授	植物の葉緑体形成を制御する超硫黄分子シグナル伝達機構の解明
30	劉　孟佳	熊本大学 国際先端医学研究機構 幹細胞ストレス研究室（劉研究室）	特任講師	マクロファージの細胞系譜で紐解く心筋線維化機構の解明
31	古水　千尋	広島大学 工学部第三類生物工学プログラム	助教	植物ペプチドホルモンによる新たな成長制御機構の解明
32	杉山　成	高知大学 教育研究部 自然科学系理工学部門	教授	ヒト心臓型 FABP による有機フッ素化合物 PFAS の分子認識機構の解明
33	勝俣　良紀	慶應義塾大学 ス ポーツ医学総合センター	講師	非アルコール性脂肪肝炎の呼気バイオプシーの活用した診断技術の開発
34	栗田　大輔	弘前大学 分子生物学研究室	准教授	rRNA の修飾がリボソームレスキュー機構に与える影響
35	鳥居　暁	東京医科歯科大学 高等研究院 病態細胞生物学研究室	プロジェクト准教授	パーキンソン病の発症機構の解明と治癒化合物の開発
36	中島　友紀	東京医科歯科大学 歯学部	教授	運動器産生エグザカインの同定を基盤にした全身性ヘルスケアの分子基盤の確立

2024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。2024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	金井　正美	東京医科歯科大学 疾患モデル動物解析学	教授	長野／ 2025.9.8-11	第7回 国際 SOX カンファレンス
2	青木　考	大阪公立大学 大学院農学研究科応用 生物科学専攻	教授	大阪／ 2025.7.7-11	第7回 国際植物維管束生物学会議
3	諸藤　陽一	長崎大学 脳神経外科	准教授	東京／ 2026.03.6-7	血液脳関門シンポジウム ―基礎から臨床まで―
4	南谷　泰仁	東京大学 医科学研究所 造血病態制御学分野	教授	札幌／ 2025.7.4-6	第 43 回 札幌国際がんシンポジウム
5	土金　勇樹	東京大学 大学院理学系研究科 植物進化学研究室	特任 助教	兵庫／ 2025.5.19-22	国際会議 「植物の生殖発生とゲノミクス」

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
2024	36	3,600	5	200	0	0	0	0
Total	1,579	186,260	270	12,320	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
2024	0	0	0	0	41	3,800
Total	13	2,600	14	582	2,030	230,013

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
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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
2024	36	3,600	5	200	0	0	0	0
Total	1,579	186,260	270	12,320	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
2024	0	0	0	0	41	3,800
Total	13	2,600	14	582	2,030	230,013

38th Financial Report

Balance Sheet

As of March 31, 2025

(Unit：JP Yen)

Account	Amount
Ⅰ Assets	
1. Current Assets	
Current Assets Total	22,244,730
2. Fixed Assets	
(1) Basic Fund	
Basic Fund Total	1,100,000,000
(2) Other Long - term Assets	
Other Long - term Assets Total	85,039,036
Fixed Assets Total	1,185,039,036
Assets Total	1,207,283,766
Ⅱ Liabilities	
1. Current Liabilities	
Current Liabilities Total	39,654,538
Liabilities Total	39,654,538
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	167,629,228
(Amount Appropriating to)	(100,000,000)
Equity Total (Net Assets)	1,167,629,228
Liabilities & Equity Total	1,207,283,766

Statement of Net Assets

From April 1 st, 2024 to March 31, 2025

(Unit：JP Yen)

Account	Amount
Ⅰ General Net Assets Changes	
1. Ordinary income & Expenditure	
(1) Ordinary income	
Interest from basic fund	13,210,805
Donation	40,000,000
Other Income	693,844
Ordinary Income Total	53,904,649
(2) Ordinaty Expenditure	
Project Expense	11,914,728
Grant Expense	38,000,000
Novartis Research Grant	36,000,000
Research Meeting Grant	2,000,000
Administrative Expense	5,398,831
Ordinary Expenditure Total	55,313,559
Ordinary Balance without Appraisal Profit or Loss	△ 1,408,910
2. Nonrecurring Profit & Loss	
Nonrecurring Balance of Current Period	0
General Net Assets Ending Balance	167,629,228
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	1,167,629,228

第38期（2024年度）財務報告

貸借対照表

2025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	22,244,730
2．固定資産	
⑴ 基本財産	
基本財産合計	1,100,000,000
⑵ その他固定資産	
その他固定資産合計	85,039,036
固定資産合計	1,185,039,036
資産合計	1,207,283,766
Ⅱ負債の部	
1．流動負債	
流動負債合計	39,654,538
負債合計	39,654,538
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	167,629,228
（うち基本財産への充当額）	(100,000,000)
正味財産合計	1,167,629,228
負債及び正味財産合計	1,207,283,766

正味財産増減計算書

2024年4月1日から2025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	13,210,805
受取寄付金	40,000,000
雑収益	693,844
経常収益　計	53,904,649
⑵ 経常費用	
事業費	11,914,728
支払助成金	38,000,000
ノバルティス研究奨励金	36,000,000
研究集会助成金	2,000,000
管理費	5,398,831
経常費用　計	55,313,559
当期経常増減額	△ 1,408,910
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	167,629,228
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	1,167,629,228

[Board of Trustees] 5 trustees, 2 auditors As of July 1, 2025

Post	Name	Title
Chairman	Kuniaki Takata, Ph.D.	President, Gunma Prefectural Public University Corporation Professor Emeritus, Gunma University
Trustee	Sadayoshi Ito, M.D., Ph.D.	Special Administrator, Katta General Hospital Professor Emeritus, Tohoku University
	Mariko Hasegawa, Ph.D.	Chairman, Japan Arts Council Prof. Emeritus, Graduate University for Advanced Studies, SOKENDAI
	Fujio Murakami, Ph.D.	Professor Emeritus, Osaka University
	John Paul Pullicino	President and Representative Director, 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, 2025

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

[Grantee Selection Committee] 18 members As of July 1, 2025

Post	Name	Title
Chairman	Yoshihiro Sato, Ph.D	Professor, Hokkaido University Graduate School of Pharmaceutical Sciences
Member	Yutaka Takahashi M.D., Ph.D.	Professor, Nara Medical University
	Yasushi Sakata, M.D., Ph.D.	Professor, University of Osaka 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 Director, 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
	Hisashi Arase, M.D., Ph.D.	Professor, University of Osaka WPI Immunology Frontier Research Center/ Research Institute for Microbial Diseases
	Noriko Saitoh, Ph.D.	Director, Division of Cancer Biology, Cancer Institute of JFCR
	Takeshi Sakaba, Ph.D.	Professor, Doshisha University Graduate School of Brain Science
	Yukari Fujimoto, Ph.D.	Professor, Faculty of Science and Technology Department of Chemistry, Keio University
	Sachihiro Matsunaga, Ph.D.	Professor, University of Tokyo Graduate School of Frontier Science,
	Sayuri Yamazaki, M.D., Ph.D.	Professor, Nagoya City University Graduate School of Medical Science

公益財団法人ノバルティス科学振興財団

［理事・監事］

任期2024年6月19日～ 2026年6月　　2025年7月1日現在（敬称略）

職　名	氏　名	現　　職
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理　事	伊藤　貞嘉	公立刈田綜合病院 特別管理者 東北大学 名誉教授
	長谷川眞理子	独立行政法人日本芸術文化振興会 理事長 総合研究大学院大学 名誉教授
	村上富士夫	大阪大学 名誉教授
	ジョンポール・プリシーノ	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
監　事	春山 直輝	春山公認会計士事務所 所長
	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

［評議員］

任期2024年6月19日～ 2028年6月　　2025年7月1日現在（敬称略）

職　名	氏　名	現　　職
評議員長	清水　孝雄	公益財団法人 微生物化学研究会 常務理事 微生物化学研究所長 国立健康危機管理研究機構 国立国際医療研究所 シニアフェロー 東京大学 名誉教授
評 議 員	飯野　正光	東京大学ニューロインテリジェンス国際研究機構 機構長特別補佐 東京大学 名誉教授
	柴﨑　正勝	公益財団法人 微生物化学研究会 理事長 東京大学 名誉教授
	袖岡　幹子	理化学研究所　環境資源科学研究センター　センター長
	武田　洋幸	京都産業大学 生命科学部 教授 東京大学 名誉教授
	中野　明彦	東京科学大学 客員教授 理化学研究所 客員主管研究員・名誉研究員、東京大学 名誉教授
	鍋島　陽一	京都大学 大学院医学研究科　特任教授 京都大学 名誉教授
	藤本　豊士	順天堂大学 大学院医学研究科 特任教授 名古屋大学 名誉教授
	望月　眞弓	慶應義塾大学 名誉教授
	廣瀬　　徹	ノバルティス ファーマ株式会社 常務取締役 開発本部長

［選考委員］

2025年7月1日現在（敬称略）

職 名	氏 名	現　　職
選考委員長	佐藤　美洋	北海道大学　大学院薬学研究院　教授
選考委員	髙橋　　裕	奈良県立医科大学　教授
	坂田　泰史	大阪大学 大学院医学系研究科 教授
	竹内　　理	京都大学　大学院医学研究科　教授
	濡木　　理	東京大学　大学院理学系研究科　教授
	林（高木）朗子	理化学研究所　脳神経科学研究センター　チームディレクター
	林　　悠	東京大学 大学院理学系研究科　教授
	藤田　恭之	京都大学　大学院医学研究科　教授
	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
	荒瀬　　尚	大阪大学 免疫学フロンティア研究センター・微生物病研究所　教授
	斉藤　典子	がん研究会 がん研究所 がん生物部長
	坂場　武史	同志社大学 大学院脳科学研究科　教授
	藤本ゆかり	慶應義塾大学 理工学部化学科　教授
	松永　幸大	東京大学 大学院新領域創成科学研究科　教授
	山崎小百合	名古屋市立大学 大学院医学研究科　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。

当財団の事業は、基本財産の運用益並びに寄付金によって賄われており、財団では趣旨にご賛同いただける皆様からのご寄付を募っております。

当財団へのご寄付には、下記の税法上の優遇措置が適用されます。

優遇措置の概略

個人：年間寄付金の合計額もしくは年間所得の40％相当額のいずれか低い方から2千円を引いた金額が、所得税の寄付金控除額となります。

法人：「特定公益増進法人に対する寄附金の特例」により、一般の寄附金の損金算入限度額と別枠で損金算入できます。

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下さい。
（E-メール：foundation.japan@novartis.com）

事務局より

2025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。

新型コロナウイルス感染症という言葉もほとんど聞かれなくなり、社会全体が新たな日常に戻ってきましたが、科学技術の進歩と基礎研究の重要性を改めて実感する一年となりました。つい先日、大阪大学の坂口志文先生が2025年のノーベル賞を受賞されましたが、地道な基礎研究が継続されて進むことによって、思わぬ形で具体的な応用につながるという基礎研究の重要性を語っておられました。

近年、国も生命科学の基礎研究を社会実装の出発点ととらえその重要性を強調しておりますが、当財団の助成事業も試行錯誤しながら新しい発見を生み出す原動力の一部になりたいと考えているところです。

さて、本年度も、当財団の研究奨励金助成および研究集会助成事業には多くのご応募をいただき、研究者の方々の熱意と挑戦心に深く感銘を受けております。ありがとうございました。

当財団は1987年9月の設立以来、助成件数は総数で2030件、総額で約23.0億円となりました。自然科学の創造的研究への助成を通じて、日本の学術発展に寄与することを目指し、今後も新たな分野の創出につながることを願いながら事業を継続してまいります。

これまで助成事業が継続できているのも、ひとえにご理解・ご支援をいただいた皆様、助成を受けて研究を続けてこられた研究者の皆様、そして財団関係者のご尽力の賜物です。心より感謝申し上げます。

今後とも変わらぬご指導、ご支援を賜りますようお願い申し上げます。

事務局長　原　健記
2025年10月10日

公益財団法人 ノバルティス科学振興財団
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