

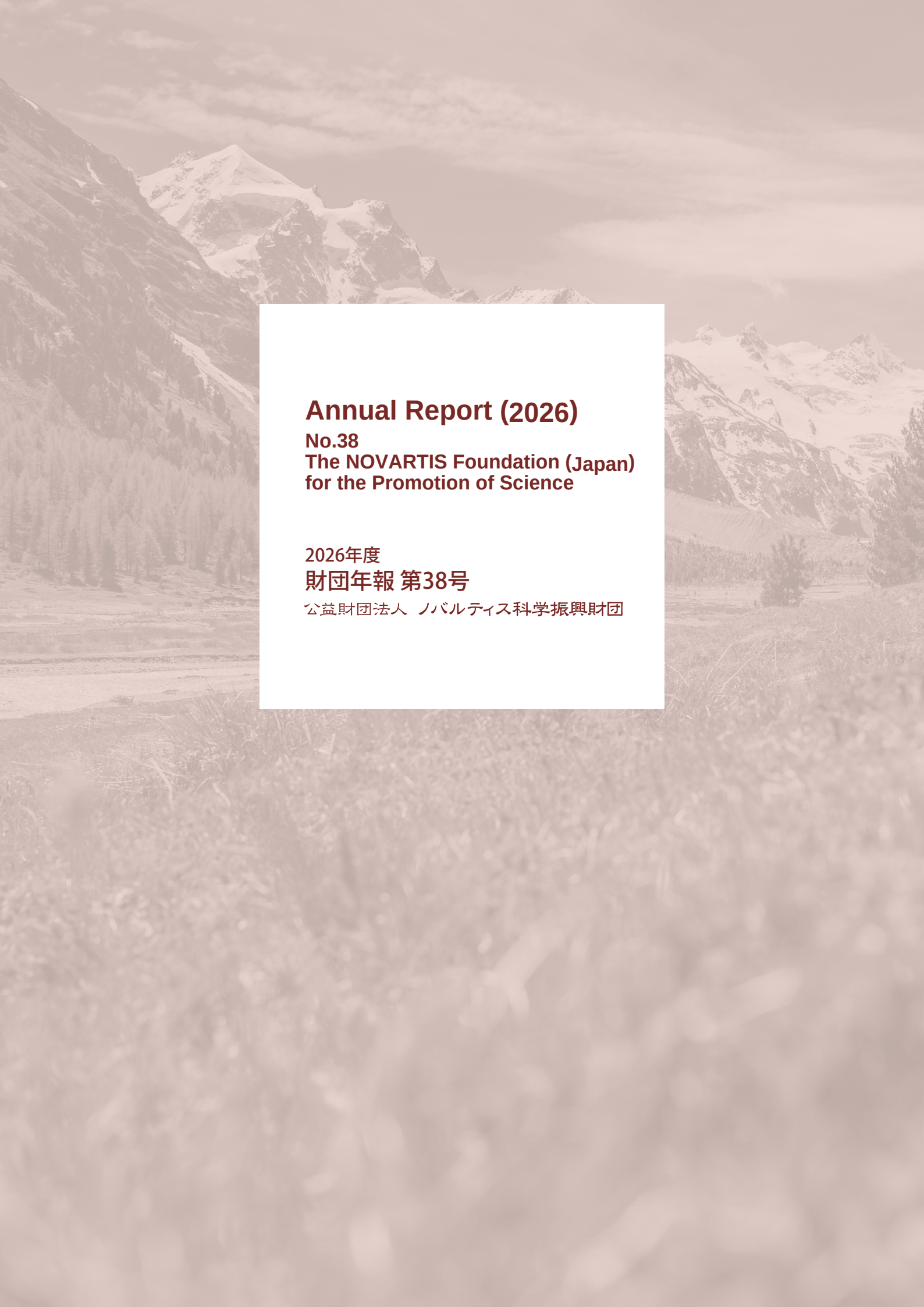
Annual Report (2026)

No.38
The NOVARTIS Foundation (Japan) for the Promotion of Science

2026年度

財団年報 第38号

公益財団法人 ノバルティス科学振興財団



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Ⅰ. Introduction　はじめに …… 9
Kuniaki Takata, Ph.D.
Chairman of the Board of Trustees

Ⅱ. Reports from the Recipients of the 38th Novartis Research Grants
第38回研究奨励報告 …… 11

1. Elucidation of the molecular basis of intracellular breakdown phenomena …… 12
Hideaki Morishita
Department of Molecular Cell Biology, Graduate School of Medical Sciences,Kyushu University

2. Manipulation of tissue morphology by regulating the expression of extracellular matrix …… 15
Mikiko Inaki
Graduate School of Science, University of Hyogo

3. Development of new immunotherapy combined with cancer cell-selective radiotherapy …… 18
Shogo Kumagai
Cancer Precision Medicine Center, Japanese Foundation for Cancer Research

4. Medicinal Research on Next-Generation Immunosuppressant Candidates Based on the Development of Novel Synthesis Platform …… 21
Eisaku Ohashi
Graduate School of Pharmaceutical Sciences, Synthetic Organic Chemistry,Nagoya City University

5. Self-organization mechanism mediated by control of perivascular mechanical field in angiogenesis …… 24
Koichi Nishiyama
Laboratory for Vascular and Cellular Dynamics, Faculty of Medicineular, University of Miyazaki

6. Artificial intelligence-based screening and experimental validation of an activator off anti-tumor activity of natural killer cells …… 28
Tsukasa Nabekura
Division of Immune Response,Aichi Cancer Center Research Institute

7. The research for early cancer detection focusing on tissue-resident macrophages …… 31
Takahiro Matsui
Graduate School of Medicine, Department of Pathology,Osaka University

8. Novel function and regulation of neutrophil-producing IL-23a in the modulation of autoimmune diseases ……33
Keiji Hirota
Lab. Integrative Biological Science,Kyoto University

9. Synthesis and application of a peptide probe toward the clarification of the mechanism of reversible V-ATPase inhibition ……36
Masahito Yoshida
Department of Chemistry, Institute of Pure and Applied Sciences,University of Tsukuba

10. Synthesis of Heterocyclic Compounds with a Spirocyclic Backbone for Expansion of Drug Library ……39
Kazutaka Shibatomi
Laboratory of Synthetic Organic Chemistry,Toyohashi University of Technology

11. Blockage of multi-drug resistance of cancer cells by inhibition of bitter taste receptors ……43
Motonao Nakamura
Department of Life Science, Graduate School of Science,Okayama University of Science

12. Modeling and elucidation of polyploidization-driven carcinogenesis with a focus on Barrett's adenocarcinoma……46
Tomonori Matsumoto
Laboratory of Ploidy Pathology, Graduate School of Frontier Biosciences,Osaka University

13. Development of In Vivo Antibody-based Drug Production Technology for Dementia Prevention ……49
Keiichiro Suzuki
Suzuki laboratory,Osaka University

14. Investigation of intractable cardiovascular disease using three dimensional pathological analysis ……52
Takayuki Fujiwara
Department of Computational Diagnostic Radiology and Preventive Medicine,The University of Tokyo

15. Development of OPDA-ID, a ligand-inducible animal cell-targeted proteolysis method using primordial plant hormones ……55
Minoru Ueda
Graduate School of Science,Tohoku University

16. Understanding of bone formation mechanisms by genome-wide association study (GWAS) using the hybrids of ornamental medaka 58
Yoshihiro Omori
Laboratory of Functional Genomics, Graduate School of Integrated Sciences for Life,Hiroshima University

17. Identify a Functional Inhibitors for Metabolic Alteration in Cancer Patients 61
Yuya Sanaki
Life Science Center for Survival Dynamics,University of Tsukuba

18. Regulatory mechanisms of cell division underlying tissue regeneration 64
Masaaki Umeda
Graduate School of Science and Technology, Division of Biological Science, Laboratory of Plant Growth Regulation,Nara Institute of Science and Technology

19. Inflammation Control in Obesity by innate-like memory CD8+ T Cells 67
Taku Kureha
Department of Immunology,The University of Tokyo

20. Development of Dynamic Asymmetric Crystallization for Synthesis of Medicinal Drugs 69
Takaaki Sato
Department of Applied Chemistry, Faculty of Science and Technology,Keio University

21. Molecular mechanisms by which RNA splicing abnormalities induce acute myeloid leukemia 72
Takafumi Yokota
Department of Hematology,Osaka International Cancer Institute

22. Development of novel asthma therapy based on the pathophysiology of airway hyperresponsiveness 75
Haruka Sasaki
Department of Dento-oral Anesthesiology, Graduate School of Dentistry,Tohoku University

23. Mutually promoting effects of circadian rhythm center dysfunction and Alzheimer's disease onset/progression 78
Michihiro Mieda
Department of Integrative Neurophysiology,Kanazawa University

24. Development of Next-Generation Hedgehog Inhibitors Targeting Drug-Resistant Cancer Cells 81
Saishu Yoshida
Laboratory of Animal Cell & Developmental Biology, Department of Biomolecular Science,Toho University

25. Exploration of functional molecules that transmit environmental changes 84
Akie Shimotohno
Institute of Transformative Bio-Molecules (WPI-ITbM),Nagoya University

26. Omics-driven insights into chemotherapy-induced modulation of tumor immune microenvironment in human biliary tract cancer ... 87
Naoto Fujiwara
Department of Gastroenterology and Hepatology,Mie University Hospital

27. Development of novel therapeutic strategies for chronic GVHD post allogeneic hematopoietic cell transplantation targeting oral microbiota 91
Hideaki Fujiwara
Department of Hematology and Oncology,Okayama University Hospital

28. Does DNA methylation cause skeletal muscle aging? 94
Yasutomi Kamei
Graduate School of Life and Environmental Sciences,Kyoto Prefectural University

29. Elucidation of the signaling mechanism for chloroplast biogenesis regulated by polysulfide 97
Takayuki Shimizu
Faculty Division of Natural Sciences,Nara Women's University

30. Unraveling the Mechanism of Myocardial Fibrosis through Lineage-specific Macrophages 100
Norika Liu
Stem Cell Stress Laboratory (Liu Lab),International Research Center for Medical Sciences, Kumamoto University

31. Novel inhibitory signaling mechanism of meristematic activities in plants 103
Chihiro Furumizu
Department of Life Sciences,Faculty of Life and Environmental Science,Shimane University

32. Elucidation of the molecular recognition mechanism of PFAS by human heart-type FABP 106
Shigeru Sugiyama
Research and Education Faculty, Natural Sciences Cluster, Sciences Unit,Kochi University

33. Breath biopsy for nonalcoholic steatohepatitis …… 111
Yoshinori Katsumata
Institute for Integrated Sports Medicine,Keio University School of Medicine

34. Effect of rRNA modification on ribosome rescue system …… 115
Daisuke Kurita
Molecular Biology Lab,Hirosaki University

35. Elucidation of the pathogenesis of Parkinson's disease and development of curative compounds …… 118
Satoru Torii
Pathological Cell Biology Laboratory, Advanced Research Initiative, Institute of Integrated Research, Institute of Science Tokyo

36. Establishment of the molecular basis for systemic health care based on the identification of exerkines …… 121
Tomoki Nakashima
Faculty of Dentistry,Institute of Science Tokyo

Publication - FY2024 Grant Recipients …… 124

Ⅲ. Reports from the Recipients of Grants for International Meetings
研究集会報告 …… 129

1. The VIIth International Workshop on SOX Transcription Factors 130
Masami Kanai
Department of disease model for human diseases,Tokyo Medical and Detal University

2. The 7th International Conference on Plant Vascular Biology …… 132
Koh Aoki
Graduate School of Agriculture, Department of Agricultural Biology,Osaka Metropolitan University

3. The 43rd Sapporo International Cancer Symposium …… 135
Yasuhito Nannya
Division of Hematopoietic Disease Control,The University of Tokyo

4. Plant Reproductive Development and Genomics…… 137
Yuki Tsuchikane
Plant Evolutionary Biology,The University of Tokyo

Ⅳ. The 39th (fiscal year 2025) Promotion Report
第39期（2025年度）助成事業報告 …… 143

Ⅴ. The 39th (fiscal year 2025) Financial Report
第39期（2025年度）財務報告 …… 150

Ⅵ. List of the Trustees, Auditors, Councilors and Grant Selection Committee Members
理事・監事・評議員・選考委員名簿 …… 152

Ⅶ. Information from the Secretariat
事務局便り …… 156

Introduction



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

This annual report contains research summaries submitted by recipients of the 38th Novartis Foundation (Japan) for the Promotion of Science Research Grants, covering research conducted between April 2025 and March 2026. The report includes 36 Research Grants and 5 Scientific Meeting Grants.

Recent advances in science and technology have been truly remarkable, spanning fields such as neuroscience, immunology, molecular and cellular biology, and computer science represented by artificial intelligence. When we trace these achievements back to their origins, we invariably find stories of discovery and invention driven by the curiosity of scientists. Small ideas and findings, much like seeds that germinate and grow, develop in diverse ways and ultimately bear fruits that support modern society. Research funding serves as the water and nourishment that sustain this growth.

In recent years, the distribution of research funding in Japan has shifted from a model of broad allocation toward one of greater selectivity and concentration. While this approach has certain advantages, it has also created new challenges. Large-scale research projects supported by substantial public funding are often expected to avoid failure, leading to an emphasis on predictable outcomes. Under such circumstances, truly groundbreaking discoveries are less likely to emerge. Diverse forms of research support for diverse scientific approaches are essential to opening the path toward transformative breakthroughs. In this respect, the activities of private foundations, including our own, are significant not only because of the amount of funding they provide but also because of the unique perspectives they bring to the promotion of scientific research.

The Foundation was established in 1987 through a donation of one billion yen from Ciba-Geigy Ltd. of Switzerland, now Novartis AG. The Foundation's Articles of Incorporation state that its purpose is "to promote academic advancement through the encouragement of creative research in the natural sciences, thereby contributing to the improvement of public health and welfare." In addition, a document prepared at the time of the Foundation's establishment, entitled *Purpose of the Foundation*, expressed the aspiration that the Foundation would "promote and support creative research in the natural sciences, which will form the foundation of science in the twenty-first century, thereby contributing to the welfare of humankind." It further emphasized both "financial support for research" and "the provision of opportunities for international exchange beyond national borders."

Guided by these principles, the Foundation has provided a total of 2,070 grants over the past 39 years, amounting to more than JPY 2.3 billion in funding. In today's world of rapid and constant change, we believe it is more important than ever to return to these founding ideals and support outstanding researchers who are developing original ideas and embarking on new scientific endeavors that will help shape the future.

This annual report presents the achievements of the excellent research projects supported by the Foundation. The accomplishments described herein are particularly impressive given that they were achieved within the span of a single year. Among the past recipients of our grants are many researchers who have gone on to become leaders in their respective fields, including Nobel Prize laureates such as Dr. Hideki Shirakawa and Dr. Susumu Kitagawa in Chemistry, and Dr. Tasuku Honjo in Physiology or Medicine.

It is our sincere hope that the researchers supported in this funding cycle will use these achievements as a springboard to even greater accomplishments in the future. Finally, I would like to express my deepest gratitude to the members of our Selection Committee for their careful evaluation of the applications, and to all those who support the activities of the Foundation, including Novartis Pharma K.K., whose generous endowment made the Foundation possible.

はじめに

代表理事　高田　邦昭

本年報には、第 38 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（2025 年 4 月～ 2026 年 3 月の研究：研究奨励金 36 件、研究集会 5 件）を収録しました。

神経科学、免疫学、分子細胞生物学などの医生物学や、AI に代表されるコンピュータサイエンスなど、近年の科学技術の発展には目を見張るものがあります。これらの源を辿っていくと、科学者のキュリオシティに基づく発見、発明のドラマがあります。小さなアイデアや発見が、ちょうど種が発芽して育っていくように、様々な形で発展した果実が現代社会を支えています。科学技術の発展を促す研究費は、この成長を支える水や栄養にあたります。近年、日本での研究費の配分は、「均等な分配」から「選択と集中」へと舵を切ってきました。このような、集中化は、また新たな問題も生んでいます。巨額の公的資金を投入する研究プロジェクトは失敗が許されない、すなわち予測可能な結果が出るものとなりがちです。これでは、全く新しい成果は期待できません。多様なアプローチへの多様な研究助成が、画期的なブレークスルーへとつながる道を拓くこととなるでしょう。当財団をはじめとする様々な民間財団が、それぞれ独自の視点から研究助成を行っていることは、その助成額以上に大きな意味があると考えます。

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、39 年間で総計 2070 件、金額にして約 23 億円を超える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代においてこそ、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北川進博士、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

Elucidation of the molecular basis of intracellular breakdown phenomena

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Summary Abstract

During the terminal differentiation of skin keratinocytes, all intracellular organelles are rapidly degraded to form the cornified layer. While essential for the skin barrier, its molecular mechanism has remained unknown for over a century. In this study, we established a 4D-live imaging system and a 3D-human keratinocyte culture model. Through spatial transcriptomics and siRNA screening, we identified several candidate hydrolases responsible for organelle degradation, providing the first molecular insights into skin barrier formation.
Key Words : Keratinocyte, Organelle degradation, Skin barrier, Spatial transcriptomics

Introduction

All organelles are cleared to optimize specialized cellular functions in lens cells and skin keratinocytes. We previously identified PLAAT family phospholipases as the executioners in the lens (Morishita et al., Nature 2021); however, organelle degradation in the skin occurs independently of PLAAT and autophagy. This research aimed to identify the unknown factors governing skin organelle degradation. By combining advanced morphology with a newly established 3D-culture screening system, we sought to uncover the molecular basis of this fundamental biological process.

Results

The research was executed according to the three planned phases: morphological analysis, establishment of a screening platform, and functional identification of the executioner factors.

1. 4D Morphological Analysis of Organelle Degradation

Using newborn mouse tail skin, we established a high-resolution 4D-live imaging system. By employing super-resolution confocal microscopy (AX R with NSPARC), we successfully visualized the intracellular breakdown in the transition zone between the granular and cornified layers. Our observations confirmed that mitochondria and nuclear DNA disappear almost simultaneously within a very narrow time window (approximately 10–20 minutes). Furthermore, FIB-SEM analysis revealed that organelle membranes undergo fragmentation and collapse prior to complete enzymatic digestion. This suggests that the process is initiated by specific membrane-disrupting factors rather than general cellular decay.

2. Establishment of a 3D-Human Keratinocyte Screening System

To identify the molecules responsible for this phenomenon, we optimized a 3D-human primary keratinocyte culture system (using J-TEC culture models). We confirmed that this 3D model faithfully recapitulates the terminal differentiation and organelle clearance observed in vivo. By integrating high-NA objectives, we established a protocol for deep-tissue 4D visualization (up to 200 μm depth), enabling us to monitor organelle dynamics in real-time during gene knockdown experiments.

3. Identification of Candidate Factors via Spatial Transcriptomics

To narrow down the potential executioner genes, we performed spatial transcriptomic analysis (Visium HD) on multiple mouse keratinized tissues (tail, ear, esophagus, and tongue) and the 3D-human culture model. By comparing gene expression profiles immediately before and after organelle disappearance, we selected approximately 200 candidate genes. We focused specifically on hydrolases, lipid metabolic enzymes, and transcription factors that were highly and specifically upregulated in the granular layer.

4. Functional Screening and Identification of Key Factors

We conducted a targeted siRNA knockdown screen of the top candidates using our 3D-culture system. This screening successfully identified a group of specific proteins whose depletion led to the abnormal retention of organelles (mitochondria and nuclear fragments) in the cornified layer. Further analysis revealed that these "organelle-retaining" cells failed to form a robust skin barrier. These results indicate that these proteins are not only essential for organelle degradation but also for the maturation of the skin’s barrier function.

Discussion & Conclusion

This study has identified the first potential executioner factors for intracellular organelle breakdown in skin keratinocytes, a mystery that has persisted for over 100 years in cell biology. Our findings demonstrate that while the phenomenon of organelle degradation is shared between the lens and the skin, the molecular machinery involved is distinct, reflecting the specialized requirements of each tissue.

The identification of these factors provides a new perspective on skin barrier diseases. For instance, incomplete organelle degradation may contribute to the pathogenesis of parakeratosis or atopic dermatitis. Furthermore, our established 3D-screening platform serves as a powerful tool for future drug discovery aimed at enhancing skin barrier functions. In conclusion, this research provides a molecular foundation for understanding how cells "break themselves" to build a protective shield for the body, offering broad implications for dermatology, evolutionary biology, and regenerative medicine.

References

Morishita H, et al. Organelle degradation in the lens by PLAAT phospholipases. Nature 592, 634-638 (2021).

一般の皆様へ

私たちの皮膚の表面には、厚い「バリア」が存在し、外部の刺激や乾燥から体を守っています。このバリアができる過程で、細胞の中にある核やミトコンドリアといった全ての「細胞小器官（オルガネラ）」が、わずか数分という短時間で跡形もなく分解されることが100年以上前から知られていましたが、その犯人（実行因子）は不明でした。本研究では、最新の顕微鏡技術とヒトの皮膚を再現した3次元培養モデルを駆使することで、この分解を担う新たなたんぱく質の候補を特定することに成功しました。この成果は、アトピー性皮膚炎などのバリア機能が低下する疾患の原因解明や、新たなスキンケア製品の開発につながる画期的な一歩となります。

Manipulation of tissue morphology by regulating the expression of extracellular matrix

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Summary Abstract

Organ morphology is genetically encoded and executed with high precision. Although it is widely accepted that morphogenesis results from the migration and deformation of constituent epithelial cells, the role of the surrounding extracellular matrix remains poorly understood. This project seeks to elucidate the mechanisms of organogenesis by focusing on the transcriptional regulation of the extracellular matrix, ultimately aiming to manipulate organ shape through the modification of transcriptional regulatory regions.
Key Words : Drosophila, Organogenesis, Extracellular matrix

Introduction

Organ morphology is genetically determined and optimized for its physiological function. The *Drosophila* embryonic hindgut exhibits left-right (LR) asymmetry. Initially forming as a bilaterally symmetrical structure, the hindgut subsequently rotates counterclockwise to achieve its final LR asymmetrical shape. I discovered that basement membrane components are spatiotemporally localized and regulated at the basal surface of the hindgut during this rotation. Specifically, while collagen is secreted by macrophages, laminin is expressed in the epithelium, leading to the hypothesis that laminin-mediated regulation of collagen localization is essential for driving hindgut rotation.

Results

To clarify the relationship between extracellular matrix accumulation and organogenesis mediated by laminin transcriptional regulation, the following experiments were conducted:
(1) Identification of the transcriptional regulatory region of the extracellular matrix

Previous studies suggested that the spatiotemporal regulation of collagen IV localization in hindgut epithelial cells might be due to the regulation of laminin expression by the hindgut epithelial cells themselves. Since laminin forcibly expressed in the hindgut epithelium was secreted from the pre-rotation stage, it was thought that laminin expression regulation was transcriptional rather than post-translational. Therefore, the transcriptional regulatory region of laminin was identified. The transcriptional regulatory region was predicted using the chromatin immunoprecipitation sequencing (ChIP-seq) database, and this region was incorporated into the pGreenRabbit plasmid, which is specialized for reporter assays, to identify the enhancer region. Specifically, an upstream region of approximately 5 kilobase pairs including the intron was incorporated into the vector, and the expression region was analyzed using embryos before hindgut rotation, with the expression of the fluorescent

protein GFP as a reporter. As a result of investigating three genes encoding laminin, the enhancer regions were successfully identified. It was found that two of the three genes were expressed in the epithelium. In addition, expression was observed in blood cells and visceral muscle. Subsequently, the enhancer region was divided into four parts, and a series of partial deletions were created, each lacking a specific part. As a result, no region involved in expression suppression in the hindgut could be identified. Enhancer regions specifically required for expression in epithelial tissue on both sides were identified.

(2) Morphogenesis control by genome editing of transcriptional regulatory regions

We attempted to clarify the relationship between collagen localization and epithelial morphological changes by deleting the expression regulatory region in epithelium identified in (1) using genome editing to eliminate laminin expression. If it is shown that collagen does not accumulate and epithelial tissue does not extend or migrate forward, it can be shown that the regulation of laminin expression by epithelial cells themselves promotes collagen accumulation by macrophages and leads to normal morphogenesis. Since a deletion individual could not be obtained in an experiment in which two guide RNAs and the Cas9 enzyme were injected for genome editing, the experiment was modified to use four guide RNAs, and the fly with a deletion was obtained. It is thought that this mechanism can be clarified by further detailed examination of visceral organ formation in this fly. Furthermore, while collagen is encoded by two genes, we have already created a strain in which the common enhancer for expression in macrophages has been deleted through genome editing. In the future, we will investigate whether it is possible to change the shape of the digestive tract by expressing this genetically modified collagen.

Discussion & Conclusion

At least two of the three laminins were found to be expressed in epithelial tissue. Regarding the remaining one, a new possibility was suggested, contrary to previous reports, that its expression source is other than epithelium or blood cells. For the two laminins expressed in epithelium, it was predicted that their expression was specifically repressed in the hindgut, but the repressive element could not be identified in this study. It is possible that there is no repressive element, or that it cannot be identified in this experimental system. Since the regions necessary for expression in the epithelial tissues on both sides of the hindgut were identified, we successfully obtained individuals with the region deleted using genome editing. In the future, it is expected that these individuals can be used to clarify the relationship between extracellular matrix accumulation and morphogenesis.

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一般の皆様へ

私たちの体にある心臓や消化管などの臓器は、どれも遺伝子によって正確な形に作られています。これまでは「細胞自身の動き」だけでその形が作られると考えられてきましたが、本研究では細胞の周りを埋めている「細胞外マトリックス」という物質の重要な役割に注目しました。遺伝子がどのようにこの物質に働きかけて臓器の形をコントロールしているのか、その仕組みを解明しました。さらに研究では、この遺伝子を改変することで、臓器の形を人工的に変化させることにも挑戦しました。この成果は、生まれつきの変形（先天性疾患）の原因究明や、傷ついた臓器を再生させる未来の医療（再生医療）に大きく貢献する可能性を秘めています。

Development of new immunotherapy combined with cancer cell-selective radiotherapy

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Summary Abstract

This study aimed to develop a novel combinatorial immunotherapy integrating Boron Neutron Capture Therapy (BNCT) with Chimeric Antigen Receptor T (CAR-T) cell therapy for glioblastoma (GBM). BNCT-mediated tumor destruction and immune modulation enhanced CAR-T cell function under optimized irradiation conditions. Multi-omics analyses of clinical samples revealed that pre-existing immune context and metabolic pathways critically regulate therapeutic response and resistance, providing a framework for patient stratification and next-generation personalized immunotherapy design.
Key Words : Glioblastoma; BNCT; CAR-T; Tumor microenvironment; Immunotherapy

Introduction

Immune checkpoint inhibitors have transformed cancer therapy but benefit only a subset of patients due to immunosuppressive tumor microenvironments. Glioblastoma (GBM) represents an extreme example, characterized by low immunogenicity, limited immune infiltration, and strong physical barriers, resulting in poor prognosis despite standard treatments. While CAR-T therapy has shown remarkable efficacy in hematological malignancies, its success in solid tumors remains limited. BNCT offers selective tumor cell killing with potential immunostimulatory effects. This study investigates whether BNCT-induced microenvironmental remodeling can enhance CAR-T cell efficacy and overcome resistance mechanisms in GBM.

Results

This study established a mechanistic framework for combining BNCT with CAR-T cell therapy in glioblastoma. We generated antigen B–specific CAR-T cells engineered with gene A, enhancing responsiveness to neuroendocrine signals and supporting function within the central nervous system microenvironment. This design enabled sustained activity under metabolically restrictive and immunosuppressive conditions typical of GBM. These CAR-T cells showed stable expression and were evaluated in an orthotopic GBM mouse model under BNCT combination conditions.

In vivo experiments showed that sequential CAR-T administration followed by BNCT significantly suppressed tumor growth compared to either monotherapy. Tumor kinetics demonstrated delayed progression, indicating an early synergistic effect. Survival extension was observed within a defined irradiation range, suggesting an optimal therapeutic window.

Histological analysis revealed increased tumor necrosis and reduced tumor cell density in the combination group.

The relationship between BNCT dose and CAR-T function was evaluated in vivo and ex vivo. At low-to-moderate doses, CAR-T cells retained or enhanced cytotoxicity, cytokine production (e.g., IFN-γ, TNF-α), and persistence, with increased activation markers and reduced exhaustion phenotypes. In contrast, high-dose BNCT impaired proliferation, cytokine secretion, and effector function, highlighting the need for precise dose optimization to balance tumor killing with immune preservation.

To investigate resistance mechanisms, a CRISPR/Cas9-based screening was performed using CAR-expressing T cells with a genome-wide sgRNA library. After BNCT exposure, surviving functional T cells were analyzed, identifying candidates involved in DNA damage response, cell cycle regulation, oxidative stress resistance, and metabolic adaptation. Enrichment of genes related to redox homeostasis and mitochondrial function suggested metabolic resilience as a key determinant of survival under BNCT stress. These results provide targets for engineering radiation-tolerant CAR-T cells.

Multi-omics analyses of tumor and blood samples from BNCT-treated patients—including exome sequencing, bulk and single-cell RNA-seq, and spatial transcriptomics—demonstrated that therapeutic response depends strongly on the pre-treatment immune landscape. Tumors enriched with effector CD8 T cells and dendritic cells showed higher response rates and systemic immune activation, including abscopal-like effects, along with coordinated antigen presentation and interferon signaling.

Non-responsive cases were characterized by immunosuppressive myeloid cells and regulatory T cells expressing molecules C and D, with transcriptional signatures of immune inhibition and metabolic suppression. Spatial analyses indicated these populations localized near tumor niches, potentially limiting CAR-T infiltration and function.

Further spatial analyses showed that effector T cells expressing molecule E correlated with improved outcomes and were enriched at tumor–stroma interfaces undergoing remodeling. Multiplex imaging confirmed their proximity to BNCT-damaged regions, suggesting coupling between tumor disruption and immune activation. On the tumor side, LAT1 and proteasome-related gene expression correlated with BNCT efficacy, potentially influencing boron retention and radiation sensitivity. Tumors with higher expression exhibited increased susceptibility, linking tumor metabolism to radiotherapeutic response. Deep learning–based integration of cellular composition, gene expression, and spatial organization enabled predictive modeling of treatment response. These models identified key determinants such as immune cell density, spatial patterns, and metabolic signatures, with spatial data significantly improving predictive accuracy. This framework supports patient stratification and precision immunotherapy development. Collectively, these findings demonstrate that BNCT reshapes the tumor microenvironment to modulate CAR-T activity. The interplay between structural remodeling, immune activation, and stress responses defines therapeutic efficacy, providing a rationale for optimizing BNCT–CAR-T strategies in GBM.

Discussion & Conclusion

This study demonstrates that integrating BNCT with CAR-T therapy represents a promising strategy to overcome key barriers in solid tumor immunotherapy. BNCT functions not only as a local cytotoxic modality but also as an inducer of tumor microenvironment remodeling, enhancing immune accessibility and activation. Importantly, we identified a dose-dependent balance between tumor control and CAR-T cell preservation, emphasizing the need for precise treatment optimization.

Clinical sample analyses revealed that pre-existing immune context critically determines therapeutic outcome, underscoring the importance of patient selection and stratification. The identification of resistance-associated pathways and candidate genes provides a foundation for engineering next-generation CAR-T cells with improved resilience to radiation stress. Additionally, the integration of spatial and multi-omic data with deep learning highlights the potential for predictive modeling in personalized therapy.

Overall, this work establishes a multidisciplinary framework combining immunology, radiation biology, and computational science, offering a scalable platform for developing effective combination therapies and advancing precision oncology in GBM and other solid tumors.

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一般の皆様へ

本研究は、脳の難治がんである膠芽腫に対し、放射線治療の一種であるBNCTと、免疫細胞を利用するCAR-T療法を組み合わせた新しい治療法の可能性を探るものです。BNCTはがん細胞を選択的に壊すだけでなく、免疫の働きを高める効果も期待されます。本研究では、この作用によってCAR-T細胞がより働きやすくなることを明らかにしました。また、患者ごとの免疫状態が治療効果を左右する重要な要因であることも示されました。将来的には、個々の患者に最適化された精密ながん免疫療法の実現につながると期待されます。

Medicinal Research on Next-Generation Immunosuppressant Candidates Based on the Development of Novel Synthesis Platform

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Summary Abstract

The author developed an efficient strategy for constructing *trans*-fused bicyclic skeletons by combining three-component coupling reactions with cyclic conjugated enones and gold-catalyzed 6-*endo* cyclization of 1,6-enynes. Improvements in tin reagent synthesis, triflate electrophile applicability, and 6-*endo* selectivity enabled application to estrone synthesis and the construction of the diterpene framework of brasilicardin A. The remaining ring construction is currently being investigated based on mechanistic insights from DFT calculations.

Key Words : three-component coupling, 6-*endo* cyclization, gold-catalyzed reaction, brasilicardin A, next-generation immunosuppressive agents

Introduction

Unmet clinical needs in organ transplantation remain to be addressed by new immunosuppressive agents, and their economic impact is substantial.[1,2] Although brasilicardin A is regarded as a promising natural-product candidate for next-generation immunosuppressive agents, the development of lead compounds based on this molecule remains challenging owing to its unusual *trans*/*syn*/*trans*-hydrophenanthrene skeleton.[3,4] In this study, the author aimed to develop an efficient synthetic method for constructing the *trans*/*syn*/*trans*-hydrophenanthrene skeleton based on a gold-catalyzed 6-*endo* cyclization of 1,6-enynes developed in our laboratory.

Results

Although the author had previously found that *trans*-fused bicyclic skeletons could be constructed by combining three-component coupling with cyclic conjugated enones and 6-*endo* cyclization of 1,6-enynes, several issues remained to be addressed before this strategy could be broadly applied. Specifically, the preparation of the tin reagent required for the three-component coupling was problematic in terms of the number of steps and overall yield; the triflate reagents used as electrophiles exhibited poor stability, and their substrate generality had not been sufficiently investigated; and the 6-*endo* cyclization suffered from competition with 5-*exo* cyclization. To overcome these limitations, the synthesis of the tin reagent, the applicability of triflate electrophiles, and the selectivity of the 6-*endo* cyclization were investigated.

For the tin reagent, the author established an improved synthetic method via

hydroboration of a propargylsilane followed by transmetallation to tin. This method provided the tin reagent in higher yield than the conventional procedure and was reproducible on the required scale. Subsequent optimization of the three-component coupling conditions also allowed the amounts of both the tin reagent and the triflate reagent to be reduced.

For the triflate electrophiles, the author evaluated their stability in a simple manner by reacting them with a readily prepared enolate. Aryl-conjugated alkynyl triflates with limited stability were found to participate in the three-component coupling when used immediately after preparation.

For the 6-*endo* cyclization, the author investigated the factors governing reactivity and selectivity through ligand screening. This study revealed that the use of a specific phosphine ligand suppressed 5-*exo* cyclization and enabled extremely high 6-*endo* selectivity.

Building on these achievements, the author initiated the total synthesis of natural products possessing *trans*-fused bicyclic skeletons to evaluate the utility of this method. Consequently, estrone was synthesized in four steps from 2-methylcyclopentenone.

Having established a method for constructing *trans*-fused bicyclic skeletons by combining three-component coupling reactions with cyclic conjugated enones and 6-endo cyclization of 1,6-enynes (the manuscript on this work is currently being prepared for submission), the author next focused on developing an efficient method for constructing the *trans*/*syn*/*trans*-hydrophenanthrene skeleton of brasilicardin A.

First, the author examined the three-component coupling reaction of 3-methyl-2-cyclohexenone. However, the 1,4-addition proceeded only sluggishly, and the desired product was obtained in low yield. Screening of various nucleophiles revealed that the use of an alanate reagent enabled the 1,4-addition to proceed almost quantitatively. After the resulting 1,4-adduct was trapped with TMSCl, the corresponding silyl enol ether was converted into the lithium enolate and treated with propargyl triflate in the presence of a specific chelating agent to afford the three-component coupling product in good yield. The subsequent gold-catalyzed reaction proceeded through highly selective 6-*endo* cyclization in high yield, enabling the construction of the *trans*-fused bicyclic skeleton of the tricyclic diterpene moiety. Toward the construction of the remaining ring, the author investigated a sequence involving formylation at the carbonyl α-position, Michael addition with methyl vinyl ketone, deformylation, and subsequent intramolecular aldol condensation. Although the sequence proceeded smoothly up to the deformylation step, the desired tricyclic enone was not obtained. DFT calculations indicated that the desired stereoisomer with the 2-oxobutyl group in an axial orientation is significantly more stable, suggesting that the intramolecular aldol condensation is energetically unfavorable.

Discussion & Conclusion

In this study, the author addressed key limitations associated with the construction of *trans*-fused bicyclic skeletons by combining three-component coupling reactions with cyclic conjugated enones and gold-catalyzed 6-*endo* cyclization of 1,6-enynes. An improved synthesis of the tin reagent was established, which enhanced both operational simplicity and reproducibility, and optimization of the coupling conditions reduced the required amounts of the coupling partners. The study of triflate electrophiles further revealed that even unstable alkynyl triflates could be effectively employed when used immediately after preparation, thereby expanding the synthetic utility of this strategy. In addition, ligand screening identified conditions that strongly favored 6-*endo* cyclization over competing 5-*exo* cyclization. The successful four-step synthesis of estrone from 2-methylcyclopentenone demonstrated the applicability of this method to natural product synthesis. Furthermore, extension of this strategy toward the diterpene framework of brasilicardin A enabled efficient construction of the *trans*-fused bicyclic core, although formation of the remaining ring proved challenging. DFT calculations suggested that the intramolecular aldol condensation is intrinsically unfavorable, providing important insights for redesigning the synthetic route. Based on these insights, the author is currently investigating an alternative strategy for the construction of the remaining ring.

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一般の皆様へ

本研究では、新しい免疫抑制剤の候補となる天然物「ブラシリカルジンA」に着目し、その複雑な骨格を効率よく作るための手法を開発しました。特に、複数の分子を一度に連結する反応と、金触媒を用いた環化反応を組み合わせることで、複雑な炭素骨格を短工程で構築できる可能性を示しました。一方で、ブラシリカルジンA骨格の残る環を構築する段階では課題も明らかになりました。現在は、計算化学から得られた知見をもとに、より有効な環構築法を検討しています。本研究は、将来的な次世代免疫抑制剤の創製に向けた基盤技術になると期待されます。

Self-organization mechanism mediated by control of perivascular mechanical field in angiogenesis

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Summary Abstract

Angiogenesis is regulated by mechanical interactions between blood flow and the vascular microenvironment. Using a microfluidic angiogenesis model, we found that intraluminal pressure–induced vessel expansion suppresses endothelial polarity and directional migration, thereby inhibiting vessel elongation. Pericyte-dependent type IV collagen deposition stiffens the perivascular environment and counteracts this effect. These findings identify a biomechanical mechanism that coordinates vascular morphogenesis through the balance between blood flow and vascular basement membrane mechanics.
Key Words : Angiogenesis, Mechanobiology, Type IV collagen, Pericyte, Endothelial polarity

Introduction

Angiogenesis is a self-organizing process of vascular endothelial cells that occurs under continuous blood-flow stimulation. Previous studies have shown that blood-flow–derived mechanical forces influence endothelial behavior, yet how vessel elongation and lumen formation are coordinated during angiogenesis remains unclear. We hypothesized that the mechanical balance between intraluminal pressure and the perivascular microenvironment regulates endothelial polarity and collective migration. The objective of this study was to determine how these mechanical cues govern angiogenic morphogenesis and vascular network formation.

Results

To investigate how blood flow and the perivascular microenvironment regulate angiogenic morphogenesis, we established a three-dimensional microfluidic angiogenesis model. Human endothelial cells were cultured alone or together with pericytes within a fibrin/collagen gel matrix, while human lung fibroblasts were maintained in a separate compartment to support angiogenic growth. Using hydrostatic pressure differences across the culture medium, we further reproduced blood flow–derived intraluminal pressure within newly formed vessels.

Time-lapse imaging combined with whole-mount immunostaining was used to analyze endothelial behavior during angiogenesis. In endothelial cell monocultures, actively elongating sprouts exhibited pronounced front–rear polarity, as determined by the relative positioning of the Golgi apparatus and nucleus, and endothelial cells migrated collectively in a directional manner. However, once lumens formed and expanded, endothelial polarity progressively disappeared. This loss of polarity was accompanied by the cessation of directional migration

and arrest of sprout elongation.

To determine whether lumen expansion directly caused these changes, intraluminal pressure was artificially increased to induce vessel dilation. Under these conditions, endothelial front–rear polarity and directional migration were markedly disrupted. Physiologically,lumen expansion induced actomyosin-dependent contractile responses characterized by phosphorylation of myosin light chain, which appeared to counteract excessive vessel expansion. When this contractile response was pharmacologically inhibited, excessive lumen dilation was further enhanced and endothelial polarity and directional migration were lost. These findings established a causal relationship between vessel expansion and the suppression of endothelial collective migration.

We next examined whether the surrounding mechanical environment influences this process. When the fibrin gel was stiffened by transglutaminase-mediated crosslinking, pressure-induced vessel expansion was significantly suppressed. Under these conditions, endothelial front–rear polarity and directional migration were maintained, allowing continued sprout elongation despite lumen formation. These observations strongly suggested that tensile forces generated by vessel-wall stretching play a critical role in regulating endothelial polarity.

Because angiogenesis proceeds efficiently in vivo despite blood flow–induced lumen expansion, we hypothesized that endogenous mechanisms exist to counteract excessive vessel dilation. We therefore focused on pericytes, which closely associate with endothelial cells during vascular development. Co-culture with pericytes significantly reduced vessel diameter, promoted endothelial directional migration, and enhanced vascular elongation under flow conditions. These results suggested that pericytes protect angiogenic vessels from excessive mechanical deformation.

To identify the underlying mechanism, we analyzed the mechanical properties of the perivascular environment using particle-tracking microrheology (PTM). As expected, the presence of pericytes significantly increased perivascular stiffness. We therefore investigated the role of type IV collagen, a major structural component of the vascular basement membrane and an important determinant of tissue stiffness. Pericyte recruitment markedly enhanced deposition of type IV collagen around newly formed vessels.

Conversely, knockdown of endothelial type IV collagen expression reduced basement membrane deposition, decreased perivascular stiffness, and resulted in abnormal vessel dilation accompanied by impaired endothelial migration and reduced vessel elongation. Importantly, these defects were rescued when the surrounding gel was artificially stiffened, demonstrating that the physical stiffening provided by type IV collagen deposition is essential for normal angiogenic morphogenesis.

Finally, we investigated how endothelial cells sense vessel-wall stretching and convert mechanical signals into changes in polarity and migration. We focused on the membrane-associated F-BAR proteins TOCA-1 and CIP4. During active sprout elongation, TOCA-

1 and CIP4 accumulated at the leading edge of migrating endothelial cells, where they promoted localized Arp2/3-dependent actin polymerization and the formation of filopodia and lamellipodia. In contrast, vessel expansion disrupted the polarized localization of TOCA-1 and CIP4, resulting in loss of localized actin assembly and directional migration.

Together, these findings demonstrate that angiogenic morphogenesis is regulated by a biomechanical balance between blood flow–derived intraluminal pressure and pericyte-dependent type IV collagen deposition in the vascular basement membrane. This mechanical balance controls endothelial polarity, collective migration, and vascular elongation through TOCA-1/CIP4-mediated mechanosensing mechanisms.

Discussion & Conclusion

Our findings reveal that angiogenic morphogenesis is governed by a dynamic mechanical balance between blood flow–derived intraluminal pressure and perivascular mechanical support. While blood flow is essential for vascular function, lumen expansion induced by intraluminal pressure suppresses endothelial polarity and directional migration, thereby limiting sprout elongation. This inhibitory effect is counteracted by pericyte-dependent deposition of type IV collagen in the vascular basement membrane, which stiffens the perivascular environment and prevents excessive vessel expansion.

Mechanistically, endothelial cells sense vessel-wall stretching through TOCA-1/CIP4-dependent regulation of localized actin remodeling, linking extracellular mechanical cues to collective cell migration. These findings establish a new conceptual framework in which angiogenesis is viewed as a mechanically regulated self-organizing process rather than a phenomenon controlled solely by biochemical signals. Furthermore, our study identifies the vascular basement membrane as a critical regulator of angiogenic morphogenesis and suggests that modulation of perivascular mechanics may provide a novel strategy for controlling pathological angiogenesis and promoting vascular regeneration.

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一般の皆様へ

私たちの体の中では、新しい血管がつくられる「血管新生」が、発育や傷の修復に重要な役割を果たしています。しかし、どのようにして血管が適切な太さや長さを保ちながら成長するのかは十分にわかっていませんでした。

本研究では、血管の中を流れる血液の力と、血管を支える周囲の組織の硬さとのバランスが、血管の成長を決める重要な要因であることを明らかにしました。特に、ペリサイトと呼ばれる細胞が血管の周りにコラーゲンを蓄積させることで血管を支え、正常な血管形成を助けていることを見いだしました。

これらの成果は、創傷治癒や再生医療における血管再生の促進、さらにはがんなどの病気でみられる異常な血管形成を制御する新しい治療法の開発につながることが期待されます。

Artificial intelligence-based screening and experimental validation of an activator of anti-tumor activity of natural killer cells

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Summary Abstract

Natural killer (NK) cells are cytotoxic lymphocytes that play a critical role in anti-tumor immunity. However, no drugs that enhance their anti-tumor activity have yet been developed. Here, we screened chemical compounds that bind to previously undruggable inhibitory signaling molecules in NK cells using artificial intelligence (AI). Among the candidate chemicals, a small compound X promoted the anti-tumor activity of primary human NK cells *in vitro*. These findings demonstrate that AI-based screening is a useful approach for identifying hit compounds to address unmet medical needs.
Key Words : Natural killer cells, Anti-tumor immunity, Artificial intelligence

Introduction

Angiogenesis is a self-organizing process of vascular endothelial cells that occurs under continuous blood-flow stimulation. Previous studies have shown that blood-flow–derived mechanical forces influence endothelial behavior, yet how vessel elongation and lumen formation are coordinated during angiogenesis remains unclear. We hypothesized that the mechanical balance between intraluminal pressure and the perivascular microenvironment regulates endothelial polarity and collective migration. The objective of this study was to determine how these mechanical cues govern angiogenic morphogenesis and vascular network formation.

Results

To identify hit chemical compounds for the development of NK cell activators, we utilized an AI-based chemical screening platform that predicts the interaction between proteins and chemicals. Through this AI prediction, we obtained multiple small compounds that potentially bind to previously undruggable inhibitory signaling molecules at the proximity of NK receptors.

Primary human peripheral or cord blood NK cells were cultured in the presence of candidate compounds for 24 hours and then co-cultured with human leukemia cell lines to evaluate their effector functions. Amond the candidates, compound X, which targets inhibitory molecule Y, enhanced the cytotoxicity and interferon (IFN)-γ production of NK cells. Furthermore, pretreatment of human NK cells with compound X augmented antibody-dependent cellular cytotoxicity (ADCC) against tumor cell lines derived from B cell lymphoma, multiple myeloma, head and neck squamous cell carcinoma, and lung carcinoma, some of which are resistant to NK cell-mediated killing.

To elucidate the molecular mechanisms underlying NK cell activation by compound X, we performed immunophenotyping by flow cytometry, as well as transcriptomic profiling by RNA-sequencing (RNA-seq) and proteomics analyses of compound X-treated NK cells. Although compound X did not affect expression of NK receptors and NK cell-associated effector molecules, such as granzyme B, perforin 1, granulysin, and IFN-γ, it induced transcriptional programs that promote differentiation into a hybrid state exhibiting functional features of both immature and mature NK cells: enhanced cytokine production including IFN-γ, augmented cytotoxicity, reduced sensitivity to apoptosis, and increased proliferative activity in response to cytokines and NK receptor simulation. In addition, compound X promoted acquisition of myeloid-like functional programs, including production of chemokines and inflammatory cytokines and also neutrophil-associated effector functions, possibly through upregulation of a transcription factor Z, which is involved in myeloid lineage commitment. These findings suggest that compound X reprograms NK cells toward a non-canonical activation state.

Biochemical analyses revealed that inhibitory molecule Y binds to phosphatases as a downstream event of NK receptor signaling, suggesting that compound X physically inhibits the interaction between inhibitory molecule Y and the phosphatases. Molecular dynamics calculations using predicted conformations *in silico* failed to simulate a stable interaction between compound X, inhibitory molecule Y, and the phosphatases. These results suggest that the binding of compound X might be dependent on a specific conformation of inhibitory molecule Y, potentially influenced by post-translational modification.

To evaluate *in vivo* efficacy of compound X, we established a cancer immunotherapy model using immunodeficient NSG-IL-15 transgenic mice, primary human blood NK cells, and human hematological malignant cell lines. Mice were transplanted with a B cell lymphoma cell line, followed by adoptive transfer of human NK cells expanded *in vitro* with cytokines and NK receptor stimulation in the presence of compound X. To mimic a therapeutic setting and evaluate anti-tumor effects through NK cell-mediated ADCC, a monoclonal antibody against CD19 rituximab was administered with NK cells in some experiments. Administration of compound X alone had no effects on tumor growth, consistent with no impact of compound X on proliferation, survival, and expression of ligands for NK receptors of the tumor cell line *in vitro*. Adoptive transfer of NK cells expanded in the absence of compound X (untreated NK cells) had little effect on tumor growth. By contrast, a combination therapy of untreated NK cells with rituximab improved overall survival and control of tumor growth. Ongoing studies are evaluating the efficacy of not only adoptive transfer of NK cells expanded in the presence of compound X, but also a combination therapy of compound X-pretreated NK cells with rituximab.

Discussion & Conclusion

We identified a small compound that enhances anti-tumor activity of NK cells through AI-based screening. Compound X enhanced the cytotoxicity and IFN-γ production of primary human NK cells against multiple tumor cell lines, including hematological malignancies and solid tumors. RNA-seq and proteomics analyses of compound X-treated NK cells revealed that compound X promotes differentiation into a hybrid state of immature and mature NK cells. Additionally, compound X promoted acquisition of myeloid-like functions in NK cells.

These findings suggest that compound X induces non-canonical activation of NK cells.

Collectively, these results demonstrate that the combination of AI-based chemical screening and functional cellular assays represents an effective approach for identifying hit compounds targeting previously undruggable molecules. Validation of the anti-tumor efficacy of compound X is underway in a mouse model transplanted with a human B cell lymphoma cell line. In parallel, the structural optimization through chemical library screening following *in silico* structure-activity relationship is planned to further enhance anti-tumor activity of NK cells.

一般の皆様へ

ナチュラルキラー（NK）細胞は、がんを傷害して排除できる免疫細胞です。しかし現在まで、NK 細胞の活性を増強する薬剤は開発されていません。我々は人工知能（AI）を用い、NK 細胞活性を抑制する因子の機能を阻害する化合物を探索しました。その結果、化学物質 X を見出しました。化合物 X でヒトの血液 NK 細胞を処理すると、試験管内でがん細胞を傷害する効率が上昇しました。以上から、これまで不可能だと思われていた、NK 細胞を活性化する新しい抗がん剤を開発する端緒が得られました。

The research for early cancer detection focusing on tissue-resident macrophages

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Summary Abstract

This study investigated how tissue-resident macrophages, especially Kupffer cells in the liver, change their phenotypes in response to hepatocellular carcinoma. Using single-cell RNA sequencing data and human tissue samples, I identified gene X as significantly altered in macrophages within cancer tissues. In normal liver tissue, Kupffer cells highly expressed gene X only a small portion of liver tissues, while this spatial pattern was disrupted in cancer tissues. These findings suggest that gene X expression in Kupffer cells may serve as a useful biomarker for early hepatocellular carcinoma detection and provide insights into cancer-related immune microenvironment changes.
Key Words : tissue-resident macrophages, early cancer, diagnosis

Introduction

Cancer tissues contain complex microenvironments with a wide variety of non-neoplastic cells in addition to tumor cells, and diverse cells such as immune cells are involved in regulating tumor progression and suppression. In this study, I focused on tissue-resident macrophages and analyzed the differences in phenotypes between the physiological environment in a non-neoplastic state and the microenvironment with tumor cells.

Results

I conducted the research in order to detect the biological changes of the tissue-resident macrophages associated with the presence or absence of cancer cells as well as their proliferation and progression. The subjects of analysis in this study were hepatocellular carcinoma. Hepatocellular carcinoma is one of the major malignant tumors, and its carcinogenesis is linked to chronic liver diseases such as viral hepatitis. In recent years, there has also been an increase in hepatocellular carcinomas arising from non-alcoholic fatty liver disease. Therefore, their disease control is one of the major social issues all over the world. Although hepatocellular carcinoma is a disease in which hepatocytes turn to become cancerous, normal liver tissue also contains a variety of cells other than hepatocytes. Kupffer cells are macrophages specific to the liver tissue. First, using a public dataset of single-cell RNA-sequence analysis, I compared the comprehensive gene expression levels in macrophages from normal liver tissue and hepatocellular carcinoma tissue samples. Among the genes whose gene expression showed significant changes compared to macrophages in hepatocellular carcinoma tissue, I focused on a gene, the gene X, and conducted the molecular biological investigation. Analysis of the public dataset (GSE149614) for single-cell RNA-sequence analysis revealed gene X, which showed a significant difference in gene

expression in macrophages in hepatocellular carcinoma tissues compared to macrophages in normal liver tissues. Next, using human pathological tissue samples, the protein expression level of the gene X in Kupffer cells, which are tissue-resident macrophages in liver tissue, was evaluated by multiplex immunohistochemical staining. This was analyzed along with the expression and localization in surrounding inflammatory cells other than macrophages, such as hepatocytes, bile duct epithelial cells, and lymphocytes. Multiplex immunofluorescence staining of human pathological tissue samples showed that Kupffer cells, which are tissue-resident macrophages in normal liver tissue, expressed the protein encoded by the gene X. In contrast, Kupffer cells within hepatocellular carcinoma tissue showed a significant difference in the expression of the same protein. Moreover, in order to conduct the analysis of the animal experimental model, tumor cell lines were transplanted into wild-type and genetically modified mice to create tumor-bearing model mice, and I attempted to detect molecular pathological insights that can contribute to tumor control. Finally, the fluorescence staining image data obtained by the method indicated above was analyzed with image analysis software to detect quantitative differences. I found that even within liver tissue, Kupffer cells clearly exhibit altered gene expression depending on their localization, which is determined by other cell fractions such as hepatocytes, bile duct epithelial cells, and lymphocytes. Specifically, in normal liver tissue, Kupffer cells highly expressed gene X near bile duct epithelial cells, while protein expression was reduced in regions far from the bile duct epithelium. On the other hand, in hepatocellular carcinoma tissue, these localization-based changes in Kupffer cells were less pronounced. The association between the presence or absence of tumor cells as well as the zonation of Kupffer cell gene expression was considered to reflect the biological changes in Kupffer cells in response to carcinogenesis.

Discussion & Conclusion

A series of my results listed above suggest that the measurement of the expression level of the gene X in Kupffer cells in hepatocellular carcinoma tissues could be one of the most effective tools for the cancer detection in early phase. Further research is indispensable to investigate the molecular biological significance of the gene through analyses using tumor-bearing mouse models, cultured cell lines, and human pathological tissue samples. Especially, I will focus on the analysis of early cancer lesions as well as the investigation of chronic liver diseases triggered to hepatocellular carcinoma. Ultimately, it is extremely important to translate these results into clinical applications.

一般の皆様へ

がん組織は腫瘍細胞以外にも多種多様な細胞が混在する複雑な微小環境を形成しており、免疫細胞などの多彩な細胞が腫瘍の進展や抑制などの制御に関わっています。本研究ではその中から組織常在性マクロファージに着目し、非腫瘍下での生理的な環境と担がん状態における微小環境での細胞の性状の差異を解析しました。今回の結果を基に、今後は早期がん病変での解析に注力するとともに、癌に進展する前の慢性炎症におけるマクロファージの性状評価についても検証を重ね、早期がんの検出につなげたいと考えています。

Novel function and regulation of neutrophil-producing IL-23a in the modulation of autoimmune diseases

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Summary Abstract

This study identified a previously unrecognized role of IL-23a (p19) in Th17-dependent autoimmune inflammation. Using a newly established IL-23a (p19)-Venus reporter mouse, we found that neutrophils, rather than dendritic cells, were a major source of IL-23a (p19) in inflamed tissues in experimental autoimmune encephalomyelitis and autoimmune arthritis. Functional analysis with neutrophil-specific conditional IL-23a (p19) knockout mice showed that loss of neutrophil-derived IL-23a (p19) exacerbated disease, indicating a protective role. Because these neutrophils lacked IL-12b (p40) expression, the suppressive effect is likely mediated by a noncanonical, p40-independent function of IL-23a (p19) during chronic autoimmune inflammation.

Key Words : IL-23a, Autoimmune disease, T helper 17

Introduction

IL-23 functions as a proinflammatory cytokine through formation of a heterodimer composed of IL-23α (p19) and IL-12β (p40). Accumulating evidence has established IL-23α (p19) as an essential component in the development of autoimmune disease, as demonstrated by disease suppression in knockout mice and by treatment with neutralizing anti-IL-23α antibodies[1)]. In humans, IL-23 receptor variants are strongly associated with susceptibility to multiple autoimmune and inflammatory disorders. To investigate IL-23α-producing cells and their regulatory mechanisms in vivo, we generated a unique IL-23α (p19)-Venus reporter mouse. Using this model, we aim to define novel functions of IL-23α and disease-suppressive mechanisms during chronic autoimmune inflammation.

Results

Following induction of the Th17 cell-dependent autoimmune disease models, experimental autoimmune encephalomyelitis (EAE) and autoimmune arthritis, we analyzed IL-23a (p19) reporter-expressing cells in inflamed tissues using our IL-23a (p19)-Venus reporter mice. In both models, reporter expression was not detected in dendritic cells (DCs) within inflammatory lesions. Instead, unexpectedly, neutrophils infiltrating the inflamed tissues exhibited strong IL-23a (p19) reporter expression. To validate these findings, reporter-positive and reporter-negative cell populations were isolated by cell sorting, and Il23a transcript levels were quantified by real-time PCR. The qPCR results were consistent with the reporter expression pattern, confirming that neutrophils, rather than DCs, represented the predominant IL-23a

(p19)-expressing population in these inflammatory settings. These results indicate that the IL-23a (p19)-Venus reporter mouse is a highly useful tool for visualizing disease-specific IL-23a (p19)-producing cells and analyzing their dynamics in vivo.

To determine whether neutrophil-derived IL-23a (p19) contributes functionally to disease pathogenesis, we generated neutrophil-specific conditional IL-23a (p19) knockout mice and induced EAE. Unexpectedly, neutrophil-specific deletion of IL-23a (p19) exacerbated disease severity, indicating that IL-23a (p19) expressed by neutrophils does not promote pathology in this context, but instead plays a protective role. This finding was notable because IL-23 has generally been regarded as a proinflammatory cytokine required for Th17-mediated autoimmune responses. Our data therefore suggest that the role of IL-23a (p19) is more complex than previously appreciated and may depend on its cellular source and molecular context.

We next investigated whether neutrophil-derived IL-23a (p19) in inflammatory lesions functions as part of the conventional IL-23 heterodimer. Canonical IL-23 is composed of IL-23a (p19) associated with IL-12b (p40). However, analysis of neutrophils isolated from inflamed tissues demonstrated that these cells lacked IL-12b (p40) expression, the binding partner required for IL-23 heterodimer formation. This result strongly suggests that neutrophil-derived IL-23a (p19) does not act as conventional IL-23 in these disease models. Rather, the data support the existence of a previously unrecognized function of IL-23a (p19) independent of p40.

Taken together, our findings reveal an unexpected cellular source and a potentially novel mode of action of IL-23a (p19) during Th17-dependent autoimmune inflammation. In both EAE and autoimmune arthritis, infiltrating neutrophils, but not DCs, were identified as prominent IL-23a (p19)-expressing cells in the target tissues. Functional analysis using neutrophil-specific conditional knockout mice further demonstrated that neutrophil-derived IL-23a (p19) suppresses, rather than enhances, autoimmune pathology. Because inflammatory neutrophils did not express IL-12b (p40), this suppressive effect is unlikely to be mediated by classical IL-23. Instead, our results strongly imply that IL-23a (p19) produced by neutrophils contributes to inhibition of Th17-dependent disease in a form not associated with p40.

Discussion & Conclusion

Our findings reveal a previously unappreciated role of IL-23a (p19) in Th17-dependent autoimmune inflammation. Although IL-23 has been widely regarded as a pathogenic cytokine that promotes autoimmune disease, our results indicate that IL-23a (p19) can also exert a suppressive function depending on its cellular source and molecular context. Using the IL-23a (p19)-Venus reporter mouse, we identified infiltrating neutrophils, rather than dendritic cells, as a major source of IL-23a (p19) in inflamed tissues. Moreover, neutrophil-specific deletion of IL-23a (p19) exacerbated EAE, demonstrating a protective role for neutrophil-derived IL-23a (p19). Because these neutrophils lacked IL-12b (p40) expression, this effect is unlikely to be mediated by canonical IL-23, suggesting a noncanonical, p40-independent function of IL-23a (p19). Collectively, these findings establish a new conceptual framework in which

neutrophils actively regulate chronic autoimmune inflammation and may provide a basis for novel therapeutic strategies targeting inflammatory control mechanisms.

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一般の皆様へ

この研究では、自己免疫疾患の炎症の仕組みを調べる中で、これまで主に「炎症を悪化させる」と考えられていた IL-23a(p19) に、逆に炎症を抑える働きがある可能性を見いだしました。特に、炎症部位に集まる好中球という免疫細胞が、病気の進行を抑える役割を担うことが示されました。これは、好中球が単に炎症を起こす細胞ではなく、炎症を調節する細胞でもあることを示す新しい発見です。今後、この仕組みの解明が、自己免疫疾患の新しい治療法の開発につながることが期待されます。

Synthesis and application of a peptide probe toward the clarification of the mechanism of reversible V-ATPase inhibition

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Summary Abstract

In this study, we investigated the structure-activity relationships of natural cyclic peptides, destruxins, which exhibit reversible V-ATPase inhibitory activity, with the aim of developing molecular probes for target identification. To synthesize molecular probes capable of interacting at two reactive sites, we prepared *N*-methyl group-modified derivatives and evaluated their biological activities. As a result, we successfully identified specific substitution sites that do not compromise biological activity, thereby obtaining crucial insights for the development of molecular probes for target identification.
Key Words : Natural product, Cyclic peptide, V-ATPase

Introduction

The reversible V-ATPase inhibitory activity exhibited by natural cyclic peptides, destruxins, remains incompletely understood in terms of not only the target protein but also whether the compounds act directly or indirectly. Reversible inhibition of V-ATPase has not been reported elsewhere and given its potential to lead to innovative drug discovery research based on a novel mechanism of action, understanding the mechanism of action underlying destruxin-mediated activity is an important research objective. Therefore, in this study, we aimed to develop molecular probes utilizing the cyclic peptide structure, which functions as a molecular recognition site, with the goal of elucidating the reversible inhibitory mechanism against V-ATPase.

Results

Toward the preparation of a molecular probe for target identification, introduction of functional groups for target molecule binding to the structure of destruxin E is required. To date, we have elucidated the structure–activity relationships (SARs) of destruxin E[1)], particularly focusing on the side chains of its amino acid residues. However, no SAR data regarding the substitution of the *N*-methyl moiety have been available. Therefore, we initially attempted the synthesis of destruxin E analogs possessing a long alkyl chain instead of the *N*-methyl group at either the MeAla or MeVal residue to determine the optimal position for installation of functional groups.

The synthesis of these destruxin analogs was performed based on our established total synthesis. Coupling H-β-Ala-OTMSE with a Boc-protected *N*-alkyl Ala derivative provided the desired dipeptide, which was then treated under acidic conditions to yield the *N*-alkyl

secondary amine. Acylation of the resulting *N*-alkyl secondary amine proved challenging due to steric hindrance of the *N*-alkyl group; thus, harsh conditions were required to couple the two segments, affording the desired tripeptide in an acceptable yield without epimerization. After removal of the protecting group at the *N*-terminus, the tetrapeptide was obtained by condensation with Fmoc-Ile-OH using standard conditions. The cyclization precursor was prepared via a hexapeptide by coupling the tetrapeptide with the acylproline segment prepared by condensation of α-hydroxycarboxylic acid derivative and H-Pro-OBn. Macrocyclization was subsequently achieved via macrolactonization using MNBA/DMAPO to afford the corresponding macrolactone. Finally, construction of the side-chain moiety containing the terminal epoxide furnished the desired destruxin E analog bearing the *N*-alkyl Ala residue. Additionally, the analog containing the *N*-alkyl Val residue was synthesized using the same procedure.

Having successfully synthesized these analogs, we next evaluated their biological activity using colon cancer cells (HCT-116). The cytotoxicity of the synthetic analogs against HCT-116 cells was evaluated using the MTT assay. Notably, the analog containing the *N*-alkyl Val residue exhibited potent cytotoxicity comparable to that of natural destruxin E. In contrast, the cytotoxicity of the analog possessing the *N*-alkyl Ala residue was significantly weaker than those of the natural product and the *N*-alkyl Val-containing analog. These results clearly indicated that the MeVal residue in the structure of destruxin E can be suitable position to install functional groups for the preparation of a molecular probe without losing a biological activity. Consequently, we further modified the *N*-alkyl moiety of the Val residue.

To develop the molecular probe, we planned to install an azide moiety as a functional group onto the *N*-alkyl chain, as the azide group is a versatile handle for 1,3-dipolar cycloaddition (click chemistry) under ambient conditions. For this purpose, the same synthetic route was applied to prepare a further modified destruxin E analog possessing the *N*-alkylazide moiety instead of the *N*-methyl moiety at the Val residue, successfully yielding the desired analog. The cytotoxicity of this new analog was also evaluated against HCT-116 cells, and it exhibited potent cytotoxicity comparable to natural destruxin E. These findings demonstrate that structural modification of the *N*-alkyl moiety at the Val residue does not impair the biological potency of the analog.

Discussion & Conclusion

In this study, we demonstrated the synthesis and biological evaluation of destruxin E analogs in which the *N*-methyl group was replaced with an *N*-alkyl moiety, aimed at clarifying the mechanism of action. Based on our previously established total synthesis, we successfully synthesized these analogs via efficient peptide elongation and macrolactonization, and the cytotoxicity of the synthetic analogs against HCT-116 was evaluated using the MTT assay. Notably, the analog bearing an *N*-alkyl moiety at the Val residue exhibited stronger cytotoxicity compared to the other diastereomer. As a result, we successfully identified specific substitution sites that do not compromise biological activity, thereby obtaining crucial insights for the development of molecular probes for target identification.

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一般の皆様へ

V-ATPase は細胞の生命活動における重要性から、創薬研究の標的として国内外で注目されているが、創薬展開に資する機能解明には至っていない。本研究で取り扱うデストラキシン類のようなペプチド系阻害剤はこれまでに報告例がなく、可逆的な阻害様式からも作用機序が新規である可能性が極めて高い。そこで、特徴的な生物活性を示すデストラキシン類について、有機合成と生物活性評価を基盤とした生物有機化学研究を進めることで、「なぜ可逆的に V-ATPase を阻害できるのか？」という謎の解明に挑戦する。本研究の推進により得られる知見は、V-ATPase が高発現している破骨細胞を生かしたまま骨吸収機能のみを制御するという、新しい骨吸収抑制剤の開発への利用が期待される。

Synthesis of Heterocyclic Compounds with a Spirocyclic Backbone for Expansion of Drug Library

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Summary Abstract

In this research, we developed a new synthetic method of nitrogen-containing spirocyclic compounds with high enantiopurity. The spiro compounds were synthesized from β-keto acids with a nitrogen substituent at the terminal of side chain by enantioselective decarboxylative chlorination and subsequent S_N2 reaction. Resulting spirocyclic compounds are attractive as building blocks of new drug candidates.

Key Words : Organic synthesis, Asymmetric synthesis, Spiro compounds, Heterocyclic compounds

Introduction

Spiro cyclic structure is often found in natural products and biologically active compounds. It is thought that high rigidity and three dimensionality of spiro compounds help the expression of biological activity[1]. It is also known that nitrogen-containing heterocycles are the most important building block in drug design. Therefore, their efficient synthetic methods are highly required in medicinal chemistry. Hence, we aimed to develop a new method for asymmetric synthesis of nitrogen-containing spiro cyclic compounds.

Results

We previously developed an enantioselective decarboxylative chlorination of β-keto carboxylic acids with chiral primary amine catalyst **C1** [2]. The reaction yielded the corresponding α-chloroketones with high enantioselectivity (Figure 1). We also found that S_N2 reaction of the resulting α-chloroketones proceeded smoothly even the fact that the reaction occurred at a tertiary carbon to form a tetrasubstituted chiral carbon center[2],[3]. In general, S_N2 reaction rarely occurs at a tertiary carbon due to the steric hinderance. However, in this case, carbonyl group next to reaction center assisted S_N2 reaction. Based on these findings, we envisaged that the intramolecular S_N2 reaction of α-chloroketones with a nitrogen substituent at a terminal of the side chain would yield nitrogen-containing spiro compounds (Figure 2).

Figure 1. Our previous research

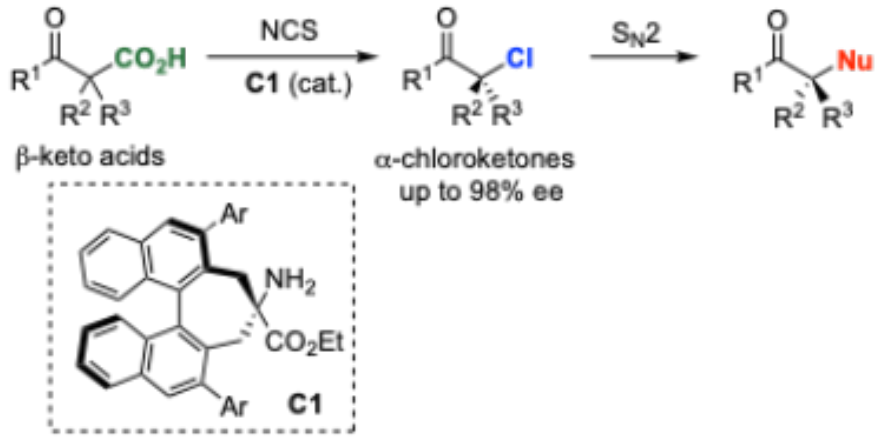
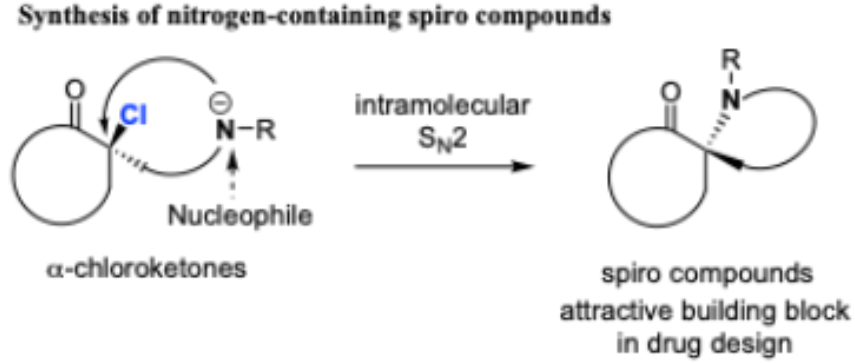
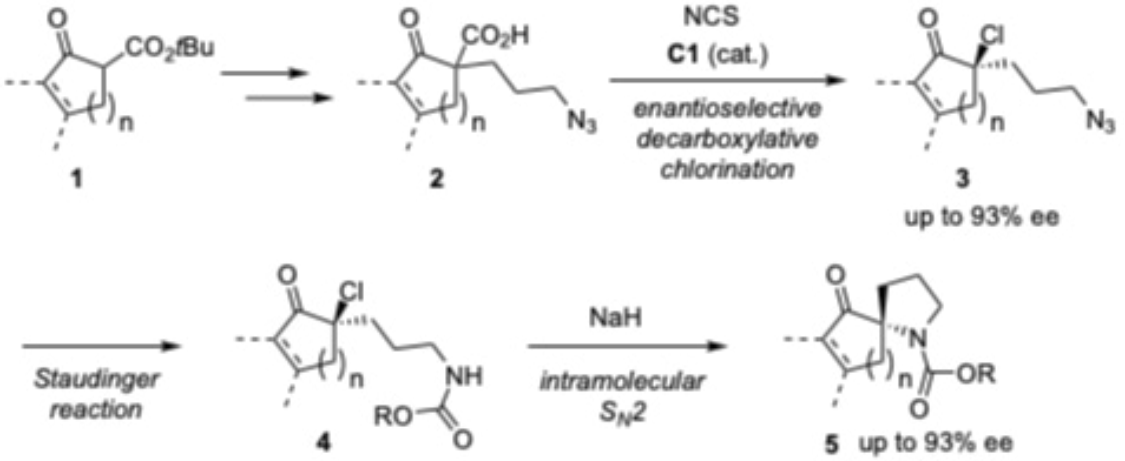


Figure 2. Aim of this research



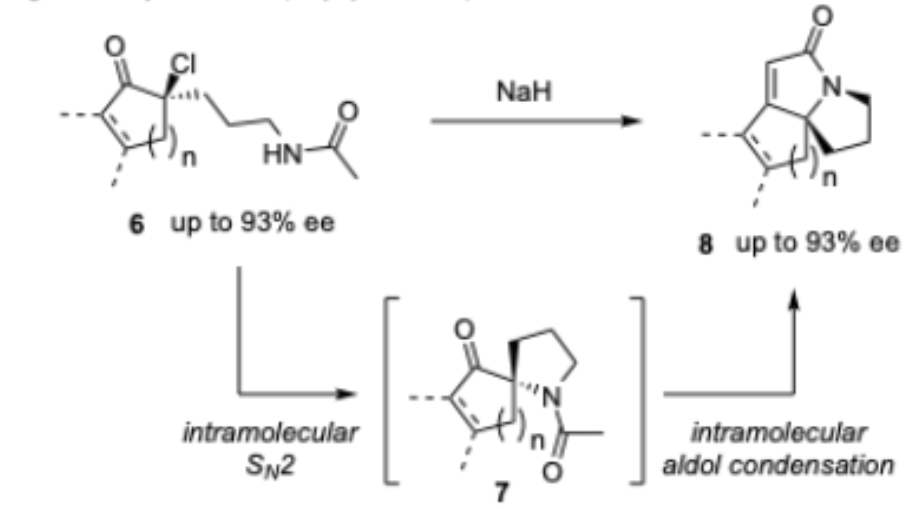
First, we synthesized α-chloroketones **4** with a carbamate moiety at the terminal of a side chain as the precursor of spiro compounds (Figure 3). β-Keto carboxylic acids **2** with an azido moiety were synthesized from *t*-butyl β-keto esters **1** by a three-step sequence. Then, enantioselective decarboxylative chlorination of **2** was performed with *N*-chlorosuccinimide (NCS) in the presence of chiral amine catalyst **C1**. The reaction successfully yielded the desired α-chloroketone **3** with high enantioselectivity, up to 93% ee. Azido moiety of **3** was converted into carbamates **4** by Staudinger reaction. Then, we tried intramolecular S_N2 reaction of the resulting α-chloroketones **4**. After the screening of reaction conditions, we found that the treatment of **4** with sodium hydride in acetonitrile yielded the desired spiro compounds **5** without loss of enantiopurity. This result indicates that the reaction proceeded in a S_N2 fashion involving Walden inversion at a chiral carbon.

Figure 3. Synthesis of spiro compounds



Next, we synthesized an α-chloroketone **6** with an acetamide moiety at the terminal of a side chain. S_N2 reaction of **6** with sodium hydride did not afford expected spiro compound **7** while a tetracyclic compound **8** was obtained (Figure 4). We consider that the reaction proceeded via a two-step process; an amide nitrogen attacked a chlorinated carbon in a S_N2 fashion to give spiro compound **7** and subsequently acetyl proton was deprotonated to undergo intramolecular aldol condensation yielding **8** as shown in Figure 4. It is noteworthy that simply changing the structure of the terminal of side chain from carbamate to amide drastically changed the structure of the resulting compounds. SN2 fashion to give spiro compound 7 and subsequently acetyl proton was deprotonated to undergo intramolecular aldol condensation yielding 8 as shown in Figure 4. It is noteworthy that simply changing the structure of the terminal of side chain from carbamate to amide drastically changed the structure of the resulting compounds.

Figure 4. Synthesis of polycyclic compounds



Discussion & Conclusion

In this research, we developed a new synthetic method of chiral nitrogen-containing spiro compounds with high enantiopurity. The reaction does not require the use of any transition metals. Most compounds synthesized in this research are difficult to synthesize by known methods even in a racemic form. Recently, spiro compounds are attracted much attention as building blocks of drug candidate. In addition, nitrogen-containing heterocycles are the most important building block in drug design. Thus, our method would help to expand the range of compound library for drug discovery.

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一般の皆様へ

医薬品のほとんどは有機化合物であり、有機化合物を精密に効率良く合成する技術は創薬研究において不可欠です。最近ではコンピューターの進歩も相まって、複雑な構造を持つ医薬品の設計が盛んに行われています。一方で、これら複雑な有機分子を自在に合成する技術は未だ十分とは言えません。本研究では医薬品の部分構造として注目度の高い、含窒素環状構造、およびスピロ構造を合わせ持つ化合物の精密合成法を開発しました。従来の手法では合成困難な化合物を提供できるため、創薬研究の一助となることを期待してます。

Blockage of multi-drug resistance of cancer cells by inhibition of bitter taste receptors

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Summary Abstract

Anticancer drug resistance is a significant obstacle in chemotherapy. Although ABCB1, an ABC transporter, confers chemoresistance owing to the extrusion of drugs, ABCB1 blockers have not been developed. Here, we clarified the chemosensory role of bitter taste receptors (TAS2Rs) in response to incorporated drugs. The underlying mechanisms by which ABCB1 is regulated in cancer cells involve TAS2Rs, the activation of which triggers drug excretion by ABCB1. In MCF-7 cells, intracellular TAS2Rs were activated by incorporated drugs, which elicited the Gα12/13/p38MAPK/NF-κB pathway, leading to ABCB1 up-regulation. Thus, intracellular TAS2Rs act as gatekeepers that drive drug excretion. Blockers for TAS2Rs could be used as efficacious agents to attenuate the excretion of anticancer drugs.

Key Words : GPCR, bitter taste receptors, drug excretion, ABCB1, cancer cells

Introduction

In this study, we demonstrated the chemosensory role of intracellular TAS2Rs against incorporated anticancer drugs, the activation of which triggers the excretion of drugs by ABCB1 in cancer cells. In cancer cells, TAS2Rs is located intracellularly and activation of TAS2Rs in these cells leads to increased expression of ABCB1. Furthermore, we demonstrated that long-term culture of these cells in the presence of anticancer drugs leads to increased function of ABCB1 due to activation of TAS2Rs, resulting in resistance to the anticancer drugs. The aims of this study were to demonstrate that intracellularly localized TAS2Rs in cancer cells function as gatekeepers against anticancer drugs, and to clarify the signaling pathway enhancing the function of ABCB1 via TAS2Rs.

Results

Intracellular localization of TAS2Rs in human cancer cells - Firstly, we investigated the expression of human TAS2Rs in 17 human cancer cell lines. In all cells, the expressions of 25 TAS2Rs were confirmed by RT-PCR. Although there have been several reports on the expression of TAS2Rs in individual tumor tissues, there have been no studies that have analyzed the expression of all 25 types of TAS2Rs broadly across different tumors. Using TAS2R14 and TAS2R38 antibodies, the localization of these TAS2Rs in MCF-7 cells was determined by co-staining with several organelle markers. Endogenous TAS2R14 and TAS2R38 proteins accumulated intracellularly in MCF-7 cells and presumably colocalized with calnexin. The colocalizations of TAS2Rs with calnexin were further confirmed in A549,

and HuH7 cells. Collectively, these data suggest that TAS2R14 and TAS2R38 are localized intracellularly in cancer cells, presumably in the ER, although it has not yet been possible to determine the subcellular localization of other 23 TAS2Rs.

Activation of intracellular TAS2Rs leading to the enhanced expressions of ABCB1 via Gα12/13/p38MAPK/NF-κB axis - We explored signaling pathways via TAS2Rs in MCF-7 cells using a NanoLuc reporter system. Stimulation with bitter compounds, such as Denatonium and phenylthiocarbamide (PTC), led to an increase in NanoLuc reporter activity in MCF-7 cells expressing serum response factor-response element-fused NanoLuc reporter, suggesting coupling with Gα12/13. Next, we examined the signaling pathway via the TAS2R/Gα12/13 axis, particularly the involvement of various MAP kinases elicited by intracellular receptors. p38MAP kinase (p38MAPK) was activated after addition of Denatonium or PTC to MCF-7 cells; however, ERK1/2 and JNK1/2/3 were not phosphorylated. According to several studies, potentiation of p38MAPK activity is required for the action of NF-κB. Thus, we examined the activation of NF-κB, namely translocation of phosphorylated NF-κB, via stimulation with Denatonium or PTC in MCF-7 cells. Enhanced phosphorylation of p65, a subunit of NF-κB, was detected in stimulated cells, demonstrating the activation of NF-κB via the TAS2R/Gα12/13 axis. As various harmful substances are excreted by several ABC transporters such as ABCB1 and ABCG2, we examined the effects of bitter compounds on the expression of these ABC transporters in MCF-7 cells. When MCF-7 cells were stimulated with Denatonium or PTC, the expressions of ABCB1 and ABCG2 increased. These data suggest that the activation of TAS2Rs by bitter compounds increases the production of ABC transporters via the TAS2Rs/Gα12/13/p38MAPK/NF-κB axis.

Excretion of drugs by ABCB1 in cancer cells treated with bitter substances - Several reports have demonstrated that long-term treatment with anticancer drugs results in multi-drug resistance, possibly due to excretion by ABC transporters. Because the activation of TAS2Rs by bitter substances enhanced the production of ABCB1, we speculated that long-term treatment with bitter substances or anticancer drugs caused drug resistance due to excretion by ABCB1. To further understand the significance of the enhanced expression of ABCB1 through TAS2R activation, MCF-7 cells were cultured long-term (> 30 passages) in the presence of Denatonium (MCF-7/Denatonium cells) or Doxorubicin, an anticancer drug, (MCF-7/DOX cells). From the viewpoint of obtaining resistant strains, Denatonium at 0.5 mM was used, which showed toxicity, and resistant strains were obtained. In these cells, we found a further increase in the expression of ABCB1 and ABCG2. In particular, we confirmed the enhanced production of the ABCB1 protein in these cells. In the presence of 40 nM DOX, parental MCF-7 cells showed a significant reduction in cell growth, whereas MCF-7/DOX and MCF-7/Denatonium cells exhibited a higher proliferation rate than parental cells. In parental MCF-7 cells, fluorescence microscopy images of DOX-treated cells showed high accumulation of this drug, which was concentrated in the nucleus. In contrast, DOX accumulation was considerably lower in MCF-7/DOX and MCF-7/Denatonium cells. The fluorescence of DOX in these cells was increased by treatment with verapamil, an inhibitor of ABCB1, indicating enhanced activation of ABCB1 in MCF-7/DOX and MCF-7/Denatonium cells. These results suggest that long-term exposure to ligands for TAS2Rs leads to increased ABCB1 production, resulting in the enhanced excretion of substances incorporated into these cells.

Discussion & Conclusion

In summary, by the treatment of cancer cells with activators of intracellular TAS2Rs, the TAS2Rs/Gα12/13/p38MAPK/NF-κB pathway was evoked, and the expression of several ABC transporters such as ABCB1 increased. An increase in ABCB1 leads to enhanced excretion of incorporated activators including anticancer drugs, resulting in conferring resistance to chemotherapy. These data propose that membrane-permeable blockers for TAS2Rs may be used as efficacious agents to attenuate the excretion of incorporated anticancer drugs, leading to an escape from resistance to chemotherapy. Although more extensive studies are required to better understand the functional significance of TAS2Rs in cancer cells, we suggest that intracellular TAS2Rs act as gatekeepers that drive the excretion of anticancer drugs in cancer cells. In this study, we demonstrated that harmless blockers for TAS2Rs may be used as efficacious agents to suppress the efflux of anticancer drugs, leading to increase in the sensitivity of the cells against anticancer drugs. Unlike GPCRs that are localized on the cell surface, drug discovery targeting TAS2R, which is localized intracellularly, is extremely difficult. In this case, it is necessary to overcome obstacles in the developmental stage of drugs, such as the cell membrane permeability and off-target effects. “Good medicine tastes bitter.” This ancient teaching will likely be verified in modern molecular biology in the near future.

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一般の皆様へ

私達は、がん化学療法時の多剤耐性化の成因の一端を明らかにし、この耐性の回避薬の創製コンセプトを社会に提案します。私達は、がん細胞の内部に苦味受容体が局在することを発見しました。苦味受容体は細胞内に浸潤した抗がん剤と結合して活性化され、これを細胞外へ排出する機構を作動することで抗がん剤蓄積が回避されます。この機構の恒常化が多剤耐性化を促進することから、抗がん剤 / 苦味受容体の結合を遮断する拮抗薬が抗がん剤耐性化を抑制すると考えます。“良薬口に苦し”この古い教えは、近い将来、現代の分子生物学によって検証されるでしょう。本研究の成果から免疫療法、外科治療などに加え、がん化学療法も新たなステージに進むことを期待します。

Modeling and elucidation of polyploidization-driven carcinogenesis with a focus on Barrett's adenocarcinoma

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Summary Abstract

In this study, we tried to elucidate how polyploid cells, despite their presumed proliferative disadvantage, gain a selective advantage over diploid cells and give rise to polyploid cancers. We found that continuous culture of CP-A cells induced spontaneous polyploidization, after which cells proliferated despite extensive genomic damage. Although multicolor lineage tracing was attempted to monitor clonal dynamics, high cell motility limited definitive evaluation of clonal dominance. Mechanistically, polyploid cells exhibited attenuated p53 activation without gene mutation, associated with upregulation of the p53 phosphatase PPM1D. Inhibition of PPM1D preferentially suppressed polyploid cell growth, indicating its critical role in post-polyploidization proliferation.

Key Words : Polyploid cancer, Whole genome doubling, Barrett esophageal adenocarcinoma, Carcinogenesis

Introduction

Organ-agnostic cancer therapies have rapidly expanded in recent years. However, cancers characterized by chromosomal instability and polyploidization remain highly refractory, and no selective therapeutic strategies have been established. Targeting polyploid cancer cells may provide an effective approach to treating aggressive malignancies. Yet it remains largely unclear how polyploid cells, despite their presumed proliferative disadvantage, gain a selective advantage over diploid cells and give rise to polyploid cancers. Focusing on Barrett esophageal adenocarcinoma, a prototypical polyploid malignancy, we will leverage our unique cell culture system to prospectively model clonal evolution during polyploid tumorigenesis and elucidate its underlying mechanisms.

Results

We found that continuous culture of CP-A cells, the Barrett's non-dysplastic esophageal epithelium cell line, resulted in spontaneous polyploidization. Notably, once polyploidization occurred, these cells continued to proliferate despite harboring substantial genomic damage, suggesting that they had acquired adaptive mechanisms that permitted survival and growth under conditions that would normally constrain polyploid cells. Based on this observation, we sought to elucidate in detail the clonal evolutionary processes by which CP-A cells undergo polyploidization and subsequently establish stable polyploid populations.

To investigate how polyploid clones emerge and expand, we first asked whether a small subset of polyploidized cells acquires a marked proliferative advantage and becomes dominant, or whether multiple clones coexist and expand while maintaining a degree of clonal diversity. To address this question, we introduced a multicolor lentiviral labeling system into CP-A cells to enable longitudinal tracking of clonal dynamics. The multicolor panel was designed to express eight spectrally distinct fluorescent proteins, each targeted to different subcellular compartments, such as the nucleus or plasma membrane, thereby increasing the combinatorial diversity of clonal labeling. Lentiviral infection was performed at a low multiplicity of infection below 0.3 to ensure that, on average, a single viral copy was integrated per cell, allowing each labeled cell to serve as a unique clonal unit. Following labeling, cells were monitored over time by fluorescence microscopy, and we attempted to evaluate differences in colony formation and clonal expansion among distinct color-coded populations. However, contrary to our expectations, CP-A cells displayed high migratory activity during serial passaging. As a result, instead of forming clearly demarcated single-color colonies, labeled clones became extensively intermixed. This unexpected cellular behavior precluded robust assessment of clonal dominance or competitive expansion based on spatially defined colony formation. Although this limitation prevented definitive conclusions from this experimental setup, the observations highlighted the dynamic and highly motile nature of CP-A cells during polyploidization.

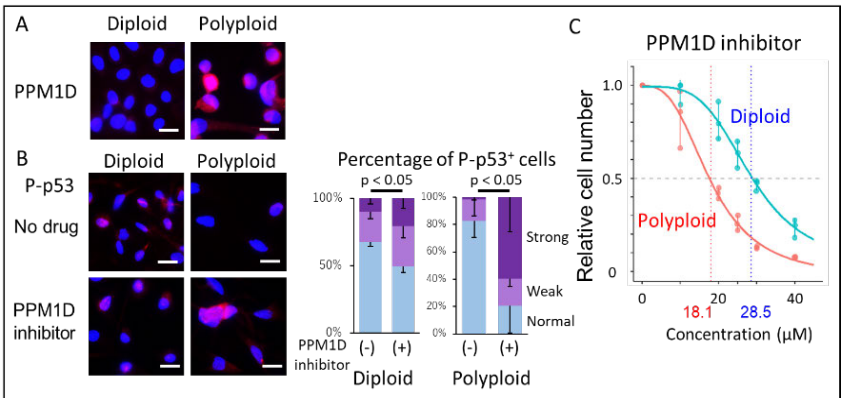


Figure1. Involvement of PPM1D in polyploid CP-A proliferation

In parallel with the clonal tracking experiments, we examined molecular alterations associated with polyploidization. We found that polyploid CP-A cells accumulated high levels of DNA damage and exhibited activation of the DNA damage response pathway. Given that activation of this pathway typically leads to p53-mediated cell cycle arrest or apoptosis, we focused on the p53 axis to clarify how polyploid cells circumvent growth suppression. Genomic analysis revealed no detectable mutations in the p53 gene in polyploid CP-A cells, indicating that canonical genetic inactivation of p53 had not occurred. However, immunostaining analyses demonstrated that phosphorylation-dependent activation of p53 was markedly attenuated in polyploid cells compared with their diploid counterparts. These findings suggested functional suppression of p53 signaling despite the absence of structural gene alterations. To identify the mechanism underlying this attenuation, we analyzed the expression of known negative regulators of p53. Among these, PPM1D, a phosphatase that dephosphorylates p53, was found to be substantially upregulated in polyploid CP-A cells relative to diploid cells (Fig. 1A). Functional inhibition of PPM1D led to a pronounced

suppression of cell proliferation specifically in the polyploid population, whereas the effect on diploid cells was comparatively modest (Fig. 1B, 1C). These results indicate that PPM1D-mediated downregulation of p53 activity is a key mechanism enabling continued proliferation after polyploidization and suggest that PPM1D represents a potential therapeutic vulnerability in polyploid cancer cells.Finally, to comprehensively identify additional molecular determinants required for clonal expansion after polyploidization, we introduced a genome-wide shRNA library into CP-A cells. The infected population is currently being serially passaged until stable polyploid clones are established. By comparing shRNA representation between diploid and polyploid populations using deep sequencing, we aim to identify genes that are selectively required for survival and expansion in the polyploid state. This unbiased screening approach is expected to further delineate the molecular framework that supports polyploid tumor evolution.

Discussion & Conclusion

Our findings demonstrate that spontaneous polyploidization in CP-A cells is not merely a passive consequence of genomic instability but is accompanied by adaptive mechanisms that enable sustained proliferation despite extensive DNA damage. Although multicolor lineage tracing was limited by unexpected cell motility and clonal intermixing, our molecular analyses revealed a clear mechanism supporting post-polyploidization growth. Specifically, polyploid cells exhibited attenuated phosphorylation-dependent activation of p53 without detectable genetic mutation, indicating functional suppression of the DNA damage checkpoint. The marked upregulation of the p53 phosphatase PPM1D and the preferential growth inhibition observed upon PPM1D blockade suggest that enhanced p53 dephosphorylation is a critical vulnerability of polyploid cells.

Collectively, these results support a model in which attenuation of p53 signaling through PPM1D overexpression facilitates clonal expansion after polyploidization. Targeting this pathway may therefore represent a rational therapeutic strategy for cancers characterized by whole genome doubling and chromosomal instability.

一般の皆様へ

通常よりも多くの染色体を持つ「多倍体化」はがんの発生や難治化の原因となるとされています。本研究では、そのような多倍体細胞が増え続ける仕組みを明らかにすることを目指しました。

多倍体化しながらクローン拡大する、発がん過程を模倣する細胞株を用いて、私たちは、特定の分子が本来がんの増殖を抑える働きを弱めることで多倍体細胞の増殖を支えている可能性を見出しました。

今後、この分子を標的とすることで、治療が難しいがんに対する新たな治療法の開発につながることが期待されます。

Development of *In Vivo* Antibody-based Drug Production Technology for Dementia Prevention

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Summary Abstract

We aim to establish a genome-editing–based platform that enables sustained *in vivo* production of biologics for disease prevention. During the funding period, we demonstrated the concept of preventive biologic production through *in vivo* genome editing, resulting in a publication in *Communications Medicine*. We further identified limitations in antibody secretion from genome-edited hepatocytes and subsequently performed systematic analyses of hepatic secretory proteins, leading to the identification of candidate signal peptides that enhance secretion of large therapeutic proteins.

Key Words : *In vivo* genome editing, Biologics production, Signal peptide, Antibody therapeutics, Disease prevention

Introduction

Biologics such as therapeutic antibodies have transformed modern medicine, yet their clinical use remains limited by repeated administration, high cost, and poor patient adherence. We previously demonstrated that *in vivo* genome editing can generate long-term secretion of therapeutic peptides from hepatocytes. Building on this concept, the present study aimed to establish a platform for sustained production of larger biologics, including therapeutic antibodies for Alzheimer's disease prevention. We therefore investigated key technological barriers and developed strategies to improve secretion efficiency from genome-edited liver cells.

Results

We first sought to validate the concept that genome editing can be utilized not only for genetic disorders but also for preventive treatment of complex diseases through sustained production of therapeutic proteins. Using our previously developed Homology-Independent Targeted Integration (HITI) platform (Suzuki et al., 2016), we introduced a secretion-enabled GLP-1 receptor agonist gene into hepatocytes and demonstrated long-term therapeutic protein production following a single administration. Continuous secretion of the therapeutic peptide improved obesity- and prediabetes-related phenotypes in mice without detectable adverse effects. These findings established a proof-of-concept that *in vivo* genome editing can function as a platform for preventive biologic production rather than solely for mutation correction. The work was subsequently published in *Communications Medicine* (Hirose et al., 2025).

Building on this foundation, we investigated whether the same strategy could be

extended to larger biologics such as therapeutic antibodies. As proposed in the original application, anti-amyloid-β antibodies were selected as model therapeutics for dementia prevention. Multiple antibody expression constructs were generated and evaluated for secretion and antigen-binding activity. Recombinant antibodies secreted from cultured cells retained amyloid-β binding activity comparable to commercially available antibodies. However, attempts to achieve efficient secretion after genome integration revealed a major technical limitation. While antibody heavy-chain and light-chain genes could be expressed individually, simultaneous production of properly assembled antibodies following targeted genomic integration was inefficient, resulting in limited secretion.

To overcome this limitation, we generated single-chain variable fragment (scFv) derivatives and evaluated various expression architectures. These experiments demonstrated that simplified antibody formats improved secretion while maintaining antigen recognition capability, thereby clarifying an important design principle for future *in vivo* antibody production systems. Nevertheless, secretion efficiency remained insufficient for practical therapeutic applications, suggesting that further optimization of protein export mechanisms would be required.

We therefore initiated a systematic investigation of protein secretion mechanisms in hepatocytes. Secreted proteins and intracellular proteins were comprehensively analyzed using proteomic approaches, and proteins exhibiting particularly high secretion efficiency were identified. Comparative sequence analysis revealed characteristic structural features within their signal peptides, including differences in length, hydrophobicity, and cleavage-site composition. Based on these findings, a series of engineered signal peptide candidates were designed and experimentally evaluated using reporter proteins. Several candidate sequences exhibited enhanced secretion compared with conventional signal peptides, indicating that secretion efficiency can be rationally improved through architecture-guided signal peptide engineering.

Collectively, these studies established a technological roadmap toward *in vivo* production of large biologics. The work progressed from conceptual validation of preventive biologic production, through identification of key bottlenecks in antibody secretion, to the discovery of candidate signal peptides capable of improving secretion efficiency. These advances provide an important foundation for future development of genome-editing–based antibody production systems for dementia prevention and other chronic diseases.

Discussion & Conclusion

This study advanced the development of *in vivo* biologic production technology at multiple levels. First, we demonstrated that genome editing can provide long-term preventive therapeutic effects through sustained production of biologics *in vivo*. Second, we identified secretion efficiency as a major bottleneck for extending this strategy to large therapeutic proteins such as antibodies. Finally, systematic analysis of hepatocyte secretory proteins enabled the identification of candidate signal peptides capable of enhancing secretion. These findings provide both conceptual and technical foundations for future

genome-editing–based antibody production systems. Continued optimization of secretion control and expression architectures is expected to broaden the applicability of this platform to Alzheimer's disease prevention and other chronic disorders requiring long-term biologic administration.

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一般の皆様へ

私たちは「薬を体の中でつくる」新しい医療技術の基礎研究に取り組んでいます。今回、ゲノム編集を使って、肝臓の細胞に治療用タンパク質を作る機能を持たせ、その薬を血液中に長期間分泌させ続けることに成功しました。その結果、マウスで肥満や糖代謝異常の改善が見られました。

さらに、認知症治療などに使われる抗体医薬を体内で効率よく作るため、肝臓からタンパク質を血液中へ分泌する仕組みを解析し、有望な分泌シグナル候補を見いだしました。将来的には、一度の治療で長期間効果が続く新しい予防・治療法の実現を目指しています。

Investigation of intractable cardiovascular disease using three dimensional pathological analysis

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Summary Abstract

We established novel mouse models of pulmonary arterial hypertension (PAH) and thoracic aortic aneurysm and dissection (TAAD) combined with a three-dimensional vascular imaging system to investigate spatial mechanisms of vascular disease. Comprehensive transcriptomic analyses revealed cellular senescence-associated pathways in both models. We identified endothelial *Cd74* as a potential therapeutic target in PAH and demonstrated therapeutic efficacy through its suppression. In addition, we found impaired DNA repair signaling in TAAD models, suggesting a critical role of cellular senescence and DNA damage responses in vascular remodeling and disease progression.

Key Words : Three-dimensional analysis, pulmonary arterial hypertension, thoracic aortic aneurysm and dissection

Introduction

Recent advances in high-throughput technologies, particularly single-cell RNA sequencing, have enabled identification of disease-specific cell populations and molecular signatures with high resolution. However, these approaches lack spatial and three-dimensional information, limiting analyses of structurally organized tissues such as vessels and nerves. To address this issue, I established a whole-lung 3D vascular imaging system and generated novel physiologically relevant mouse models of refractory vascular diseases, including PAH and AAD. By integrating 3D imaging with comprehensive transcriptomic analyses, this study aims to elucidate disease mechanisms and validate findings using human specimens.

Results

We previously generated a novel PAH mouse model by introducing a human PAH-associated mutation, BMPR2 p.Try247X[1], together with endothelial cell-specific deletion of PGC-1α, whose expression is known to be reduced in pulmonary endothelial cells from patients with PAH. This double-mutant mouse (Bmpr2-KI;Pgc1α^{eKO}) successfully recapitulated severe PAH pathology. In parallel, we established a novel thoracic aortic aneurysm and dissection (TAAD) model mouse by introducing a mutation corresponding to a newly identified human familial TAAD mutation, MYH11 p.Leu1264Pro[2], into mice (Myh11 p.Leu1261_Gln1262del; Myh11-KI). Comprehensive transcriptomic analyses of both models revealed marked upregulation of gene sets associated with cellular senescence.

To clarify the significance of cellular senescence in disease progression, we next attempted three-dimensional visualization of senescent cells. By combining p16-ert-Cre

mice, in which Cre recombinase is specifically expressed in p16-positive senescent cells, with the fluorescent reporter mouse R26-lsl-tdTomato, we successfully established a system enabling three-dimensional visualization of senescent cells in vivo. Using this approach, we demonstrated the accumulation of senescent cells in the aortic root and ascending aorta, the major sites of aneurysm formation, in a Marfan syndrome mouse model, one of the representative forms of TAAD. These findings suggested a spatial association between senescent cells and aneurysm-prone regions.

In the novel PAH mouse model, expression of Cd74, one of the senescence-associated secretory phenotype (SASP) factors, was markedly increased in endothelial cells. Importantly, elevated CD74 expression was also confirmed in lung specimens from patients with PAH. We therefore hypothesized that endothelial Cd74 contributes to disease progression. To test this hypothesis, we performed endothelial cell-specific knockdown of Cd74 using an adeno-associated virus (AAV) vector with endothelial tropism. Remarkably, Cd74 knockdown improved pulmonary arterial pressure and attenuated pulmonary vascular remodeling. Although previous studies have already implicated cellular senescence in PAH pathogenesis, the field remains controversial because elimination of senescent cells has paradoxically been reported to exacerbate PAH in certain settings. Our findings suggest that targeting downstream mediators of senescence, rather than indiscriminate elimination of senescent cells themselves, may represent a more effective therapeutic strategy. In particular, regulation of Cd74 signaling may provide a novel therapeutic approach for PAH.

In the novel TAAD model, transcriptomic analyses additionally demonstrated upregulation of genes related to DNA damage responses and downregulation of genes involved in DNA repair, both of which are closely linked to cellular senescence. Among these, we identified a marked reduction of DNA repair factor X, particularly in aortic smooth muscle cells. Given the critical role of vascular smooth muscle cells in maintaining aortic wall integrity, we hypothesized that loss of factor X may contribute to TAAD development through impaired DNA repair and promotion of cellular senescence. To investigate this possibility, we successfully generated floxed mice for factor X. We are currently planning smooth muscle cell-specific deletion of factor X to further elucidate its functional role in TAAD pathogenesis. Through these studies, we aim to clarify the molecular links among DNA damage, cellular senescence, and vascular remodeling, ultimately contributing to the development of novel therapeutic strategies for refractory vascular diseases.

Discussion & Conclusion

In the present study, we identified endothelial *Cd74* as a potential therapeutic target in our novel PAH mouse model. Therapeutic intervention was achieved by directly suppressing *Cd74* expression using an endothelial cell-targeted AAV vector, resulting in improvement of pulmonary hypertension and vascular remodeling. However, viral gene therapy is currently not considered a realistic therapeutic strategy for human PAH in clinical settings. Therefore, future studies will focus on developing alternative approaches to regulate *Cd74* expression or signaling pathways, including pharmacological or molecular-targeted strategies, and evaluating their therapeutic efficacy and safety.

In addition, we identified downregulation of DNA repair factor X in our novel TAAD

model mouse. We plan to investigate whether reduced expression of factor X is commonly observed across other TAAD models, which may indicate a shared pathogenic mechanism. Furthermore, by generating factor X-deficient models in multiple TAAD backgrounds, we aim to clarify the functional significance of factor X in disease progression and its potential contribution to vascular degeneration and aneurysm formation.

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一般の皆様へ

本研究では、肺高血圧症（PAH）や大動脈瘤・大動脈解離（TAAD）といった難治性の血管病に対して、病気の原因をより詳しく理解することを目指しました。特に、細胞の「老化」が病気の進行に深く関わっている可能性に注目し、血管を三次元で観察できる新しい解析技術と、患者さんの遺伝子変異を再現したマウスモデルを組み合わせて研究を行いました。その結果、PAH では Cd74 という分子が病気の悪化に関与する可能性を見出し、その働きを抑えることで病態が改善することを確認しました。また TAAD では、DNA 修復機能の低下が細胞老化や血管の脆弱化につながる可能性が示されました。今後は、これらの分子を標的とした新しい治療法の開発につなげることで、現在有効な治療法が限られている血管疾患の克服に貢献したいと考えています。

Development of OPDA-ID, a Ligand-Inducible Target Protein Degradation System for Animal Cells Using an Ancestral Plant Hormone

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Summary Abstract

We developed the initial biochemical basis for the OPDA-ID system, a ligand-inducible protein interaction platform using the bryophyte MpCOI1–MpJas module and dn-iso-OPDA. GFP-fused MpJas constructs were successfully expressed, and pulldown assays showed dn-iso-OPDA-dependent interaction with GST-MpCOI1, particularly for C-terminally GFP-fused MpJas. Membrane permeability analysis further suggested that dn-iso-OPDA has sufficient permeability for cellular applications. These results support OPDA-ID as a promising new tool for chemically controlled protein regulation.

Key Words : jasmonate, molecular glue, receptor

Introduction

Targeted protein degradation using molecular glues and PROTACs has emerged as a promising drug-discovery modality, particularly for previously “undruggable” proteins. We propose to develop OPDA-ID, a new ligand-dependent protein degradation system inspired by the hormone signaling machinery of the basal land plant Marchantia polymorpha. This strategy exploits a highly cell-permeable ancestral plant hormone and its receptor to reconstitute, in animal cells, the plant mechanism of target protein ubiquitination and 26S proteasome-mediated degradation. Because plant hormones act as highly specific molecular glues, transplantation of this degradation module into animal cells may enable selective, efficient, and low-toxicity degradation of desired proteins.

Results

During this reporting period, we made substantial progress toward establishing the OPDA-ID system, a ligand-induced protein interaction/degradation platform based on the bryophyte jasmonate receptor pair MpCOI1–MpJas and its endogenous ligand dn-iso-OPDA. The work focused on three key objectives: construction of GFP-tagged MpJas expression systems, evaluation of dn-iso-OPDA-dependent interaction between MpCOI1 and MpJas, and assessment of the membrane permeability of dn-iso-OPDA in comparison with ligands used in existing degron systems.

First, we constructed bacterial expression systems for GFP-fused MpJas peptides. Because the position of the GFP tag and the linker sequence could strongly affect the interaction between MpJas and MpCOI1, six constructs were prepared: MpJas-linker-GFP

and GFP-linker-MpJas, each with no linker, an AS linker, or an SG linker. These constructs were introduced into the pMAL-C2 vector and expressed in *E. coli* BL21(DE3). Western blot analysis using an anti-GFP antibody confirmed the expression of all GFP-tagged MpJas variants. This established the basic protein resources required for subsequent in vitro binding assays.

Second, we examined whether dn-iso-OPDA could induce interaction between GST-MpCOI1 and GFP-tagged MpJas. GST-MpCOI1 was prepared using an Sf9 insect-cell expression system, and pulldown assays were performed in the presence of dn-iso-OPDA and IP5. At 500 nM dn-iso-OPDA, all GFP-tagged MpJas constructs showed ligand-dependent interaction with GST-MpCOI1. Importantly, MpJas-linker-GFP, in which GFP was fused to the C-terminus of MpJas, showed stronger binding than GFP-linker-MpJas, in which GFP was fused to the N-terminus. This result suggests that the N-terminal region of the Jas motif is important for efficient receptor interaction. Since structural studies of the AtCOI1–JA-Ile–JAZ complex indicate that the N-terminal region of the Jas motif acts as a clamp over the ligand-binding pocket, N-terminal fusion of the bulky GFP protein may sterically interfere with proper complex formation. Therefore, C-terminal GFP fusion appears to be the more suitable design for OPDA-ID.

Third, we analyzed the ligand concentration dependence of the MpJas-linker-GFP interaction with GST-MpCOI1. Using dn-iso-OPDA at 1, 5, 50, and 500 nM, ligand-dependent binding was observed from at least 50 nM in all three MpJas-linker-GFP constructs. Among them, the AS-linker construct appeared to show the strongest interaction, although further quantitative validation will be necessary. These results indicate that the MpCOI1–MpJas system can respond to dn-iso-OPDA at relatively low nanomolar concentrations, supporting its potential utility as a chemically inducible interaction module.

Fourth, the concentrations of the proteins used in the pulldown assays were estimated by western blotting. The GST-MpCOI1 concentration in the pulldown system was estimated to be approximately 14 nM, whereas MpJas-linker-GFP was present at approximately 2.9 μM. Thus, the assays were performed under conditions in which MpJas-linker-GFP was present in large excess over GST-MpCOI1 and dn-iso-OPDA. Thisinformation is important for interpreting the current binding data and for optimizing future assay conditions.

Finally, we performed a PAMPA assay to compare the membrane permeability of dn-iso-OPDA with that of 5-Ph-IAA, the ligand used in the AID2 system, and JA-Ile. The apparent permeability values of 5-Ph-IAA, JA-Ile, and dn-iso-OPDA were estimated to be approximately 2.16×10^{-5}, 1.90×10^{-5}, and 1.05×10^{-5} cm/s, respectively, with no significant differences among the three compounds. Since compounds with Pe values above 1×10^{-5} cm/s are generally considered to show high permeability, dn-iso-OPDA appears to possess sufficient membrane permeability for cellular applications. However, the present PAMPA data require further validation because of technical limitations, including delayed LC-MS/MS analysis, imperfect initial concentration matching, limited acceptor-well volume for 5-Ph-IAA, and lack of appropriate controls.

Overall, these results demonstrate that the core biochemical components of OPDA-ID have been successfully established. The most promising current configuration is the C-terminally GFP-fused MpJas construct, particularly with the AS linker. Future work should

focus on quantitative binding analysis, optimization of protein stoichiometry, improved permeability assays, and validation of dn-iso-OPDA-induced target protein degradation in living cells.

Discussion & Conclusion

In this study, we established the initial biochemical basis for the OPDA-ID system using the bryophyte MpCOI1–MpJas receptor module and its ligand dn-iso-OPDA. GFP-tagged MpJas variants were successfully expressed in *E. coli*, and pulldown assays demonstrated dn-iso-OPDA-dependent interaction between GST-MpCOI1 and GFP-fused MpJas. Notably, C-terminally GFP-fused MpJas showed stronger binding than N-terminally fused MpJas, suggesting that the N-terminal region of the Jas motif is important for proper co-receptor formation and may be disrupted by bulky GFP fusion. Ligand-dependent binding was detected from at least 50 nM dn-iso-OPDA, indicating that this module can respond to low nanomolar ligand concentrations. PAMPA analysis further suggested that dn-iso-OPDA has membrane permeability comparable to 5-Ph-IAA and JA-Ile, although further validation is required. Overall, these results support the feasibility of OPDA-ID as a new ligand-inducible protein interaction system. Future work should focus on quantitative binding analysis and validation of target protein degradation in living cells.

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一般の皆様へ

本研究では、植物ホルモンのしくみを利用して、細胞内の特定のタンパク質を必要なタイミングで分解する新しい技術「OPDA-ID 法」の開発を目指しており、今回、ゼニゴケ由来のホルモン受容体とその結合相手が、特定の低分子化合物によって結合することを試験管内で確認しました。この低分子化合物が細胞膜を通過しやすい性質を持つ可能性も示されました。将来的には、病気に関わるタンパク質の機能解析や、新しい創薬技術の基盤として役立つことが期待されます。

Understanding of bone formation mechanisms by genome-wide association study (GWAS) using the hybrids of ornamental medaka

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Summary Abstract

Ornamental medaka strains in Japan have rapidly diversified, exhibiting various morphological and color traits. Unlike laboratory mutants, these strains carry non-lethal, heritable mutations, making them valuable for genetic studies. This study analyzed 86 strains and identified 34 phenotypes, many previously unreported. Whole-genome sequencing of 201 individuals revealed over 17 million variants. GWAS of the bone morphology phenotypes identified candidate genomic regions associated with traits. Fin morphology analysis highlighted key loci, including kcnq5a on chromosome 15 and and2 on chromosome 17. Potassium channel genes were also implicated. These findings demonstrate that ornamental medaka provide a rich resource for uncovering genetic mechanisms underlying phenotypic diversity.

Key Words : genome, GWAS, medaka, bone formation

Introduction

In recent years, the number of ornamental medaka varieties has increased dramatically, with over 600 strains developed and marketed in Japan. Some exhibit abnormalities in eye morphology or skeletal structure, while others show variations in body coloration; combinations of these traits have produced diverse strains. Since the late 1990s, small fish such as medaka and zebrafish have been used to screen mutants for studying human genetic diseases, though many mutations were lethal. In contrast, ornamental fish possess non-lethal, heritable, and potentially multiple mutations, offering high potential to identify novel phenotypes and causative genes.

Results

This study aimed to characterize genomic variation and its relationship to phenotypic diversity in ornamental medaka strains. A total of 86 ornamental strains displaying a wide range of body colors and morphologies were collected. From these strains, 34 distinct phenotypes were identified. While some phenotypes had been previously described in laboratory mutants, many were newly reported.

To investigate genetic variation, whole-genome sequencing was performed on 201 individuals, including 181 from ornamental strains and 20 from five laboratory strains, using Illumina short-read sequencing. This generated 2.7 Tb of data, with an average sequencing depth of 18× per individual. Sequence reads were aligned to the Hd-rR reference genome,

resulting in the identification of over 17 million genomic variants, including approximately 15.2 million single-nucleotide variants (SNVs) and 2.0 million small insertions and deletions (indels).

To explore genotype–phenotype relationships, genome-wide association studies (GWAS) were conducted using 181 ornamental individuals representing 34 phenotypes. Among these, 29 phenotypes observed in at least four individuals were analyzed. Candidate genomic regions were defined as chromosomal regions enriched with the top 0.01% of associated SNVs.

The study focused particularly on four fin morphology phenotypes: hirenaga, swallow, longfin, and real-longfin. Compared to wild-type fish, these strains exhibited varying degrees of fin elongation. GWAS identified significant association peaks for all four phenotypes. For the hirenaga phenotype, which shows elongation of all fin rays and partial membrane extension, a strong association signal was detected on chromosome 15. A nearby candidate gene, kcnq5a, encoding a potassium channel, was identified. Although no coding mutations specific to hirenaga were found, a large deletion in intron 1 of kcnq5a was observed, suggesting a regulatory effect on gene expression.

A similar GWAS peak on chromosome 15 was identified for the swallow phenotype. Given the similar fin morphology and proximity of associated loci, these two phenotypes are likely caused by different alleles of the same gene.

For the longfin and real-longfin phenotypes, candidate regions were identified on chromosomes 7 and 17, respectively. Although gene ontology analysis did not yield clear candidates, several potassium channel genes (kcna3, kcna10, kcnd3) were found in the longfin-associated region. In the real-longfin region, the gene and2, known to regulate fin development, was identified as a strong candidate.

Overall, this study demonstrates that ornamental medaka strains harbor extensive genetic variation and provide a valuable resource for identifying genes associated with morphological traits.

Discussion & Conclusion

If similar phenotypes are caused by mutations in independent multiple genes, and/or additive stimulus mutations in several genes, a strong association would not be observed in the GWAS analysis. In other cases, multiple different causative alleles decrease the association between the phenotype and the responsible loci in GWAS analysis. For example, the goldfish telescope-eye phenotype was associated with lrp2a having more than three allele types, showing a relatively weak GWAS peak in its Manhattan plot. In these cases, gene mapping using F2 hybrid individuals may help identify the responsible genes for such phenotypes. In such cases, our data will provide the genetic background of ornamental mutant strains and help find mutations by comparing our genomic data of these strains and those of F2 hybrid individuals.

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一般の皆様へ

観賞メダカ品種の大規模なゲノム DNA 解析を行いました。さまざまな体色や体型の変化に関連するゲノムワイド関連解析（GWAS）を実施し、変異の原因と考えられる遺伝子領域を特定しました。これらの中には、ヒレの長さを制御する遺伝子が含まれます。その結果、メダカ品種に見られる体型の変化を引き起こす遺伝子変異の候補が見つかり、体型が多様に変化する仕組みを明らかにする研究へとつながることが期待されます

Identify a Functional Inhibitors for Metabolic Alteration in Cancer Patients

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Summary Abstract

Cancer cachexia causes severe, multi-tissue wasting that remains difficult to treat. Driven by mechanistic insights from *Drosophila* models, we focused on key metabolic alterations shared across species. Through a targeted compound screening, we discovered that a drug approved for other diseases effectively suppresses muscle and adipose tissue atrophy in tumor-bearing mice. This study highlights a promising drug repositioning strategy to counteract systemic wasting syndromes and improve the quality of life for cancer patients.

Key Words : Cachexia Metabolic disorder Microbiota

Introduction

In cancer patients, catabolism becomes predominant in non-tumor tissues, such as skeletal muscle and adipose tissue, often leading to the development of cachexia—a severe tissue-wasting syndrome. Effective therapeutic options for this condition remain extremely limited. Based on insights gained from *Drosophila* research, we have discovered a novel agent that suppresses muscle and adipose tissue atrophy in tumor-bearing mice.

Results

Using a *Drosophila* tumor-bearing model generated by the local transplantation or genetic induction of malignant tumor cells, we initially sought to elucidate the systemic factors driving the pathogenesis of cancer cachexia. Through a series of survival and phenotypic analyses, we discovered that the host gut microbiota poses a critical risk factor for the onset and acceleration of cancer-induced systemic wasting. To rigorously validate the contribution of these microbes, we established germ-free tumor-bearing flies via the oral administration of a broad-spectrum antibiotic cocktail. Remarkably, in these microbiota-depleted tumor-bearing flies, the characteristic cachectic phenotypes—most notably the progressive degradation of skeletal muscle fibers and the severe atrophy of fatbody cells—were completely absent. Furthermore, this ablation of the gut microbiota significantly prolonged the lifespan of the host flies compared to their conventional, microbiota-harboring counterparts. These striking findings suggested a potent, microbiota-dependent mechanism that non-autonomously drives tissue degeneration during tumorigenesis.

Intrigued by these robust phenotypes in the invertebrate model, we next examined whether the impact of the gut microbiota on cachexia is evolutionarily conserved in a mammalian system. To test this hypothesis, we utilized a well-established mammalian preclinical model

consisting of tumor-bearing mice. We evaluated the effects of microbiota depletion by maintaining a cohort of mice on drinking water supplemented with a robust antibiotic regimen, subsequently comparing them with conventional tumor-bearing controls. Unexpectedly, longitudinal monitoring of body weight, lean mass, and fat mass revealed that severe tissue wasting and organ atrophy progressed similarly in both groups, regardless of the presence or absence of the gut microbiota. These results clearly demonstrated that the direct impact of the gut microbiota on the pathogenesis of cancer cachexia is remarkably minor in mammals, revealing a striking phenotypic divergence or species difference between Drosophila and mice.

To bridge this cross-species gap and uncover clinically relevant therapeutic targets, we hypothesized that the underlying downstream metabolic networks might still be conserved. We performed a comprehensive metabolic profiling and identified a specific metabolic pathway in Drosophila that altered drastically and systematically depending on the presence of the gut microbiota. Recognizing its potential role as a conserved metabolic link, we focused our investigation on its mechanistic importance in the context of mammalian cachexia.

We then conducted a targeted, small-scale compound screening in tumor-bearing mice by administering a panel of small-molecule compounds designed to selectively block this specific, microbiota-regulated pathway. Through this focused in vivo screen, we successfully identified a promising lead compound that markedly suppresses the progression of mouse cachexia, effectively decoupling tumor growth from systemic tissue wasting.

However, during the preclinical optimization phase, we encountered a significant pharmacological problem. While the initial oral administration of this lead compound successfully inhibited adipose tissue atrophy, its protective efficacy against skeletal muscle atrophy lacked reproducibility across independent experimental cohorts, potentially due to high absorption or rapid metabolism in the liver. To overcome this critical pharmacological limitation and maximize the therapeutic potential of the molecule, we optimized the drug delivery strategy. We established that intraperitoneal injection, when combined with a specific stabilizing and solubilizing additive, completely showed the compound's efficacy in the muscles. This optimized formulation robustly and reproducibly suppresses the severe atrophy of both adipose and skeletal muscle tissues in tumor-bearing mice, establishing a highly effective and reproducible therapeutic intervention against cancer-induced wasting.

Discussion & Conclusion

Given that this therapeutic agent is already clinically approved and widely administered to human patients for other indications, it holds significant promise for rapid drug repositioning to treat cancer cachexia. However, the precise molecular and cellular mechanisms through which this compound suppresses cachectic tissue wasting remain entirely elusive, highlighting a critical area for future investigation.

Elucidating the exact mode of action of this drug will not only validate its clinical utility but also likely uncover novel, highly conserved metabolic or signaling pathways involved in systemic wasting. Ultimately, mapping these mechanistic underpinnings possesses high potential to drive the design and creation of even more effective, targeted therapeutic intervention points, offering next-generation strategies to combat this devastating syndrome.

References

Patent application in preparation; manuscript submission to follow upon approval.

一般の皆様へ

疲れていても、ご飯を食べて眠り、朝起きれば元気になる。そんな生き物の仕組みは、細胞の働きと全身を駆け巡るホルモンが調和して機能する、身体のメンテナンス機構によるものです。

この身体のメンテナンスは、健康な時には意識されませんが、がんや慢性疾患にかかると疎かになり、極端に痩せてしまうことがあります。その結果、ベッドから起き上がることも、会話することもできなくなってしまいます。この病気はヒポクラテスの時代から知られていますが、現代でも治療することは極めて困難です。ショウジョウバエというとても小さな虫にも、私たちと同じ身体のメンテナンス機構があり、これを研究することで哺乳類の病気を治癒する薬を見つけるきっかけとなりました。

Regulatory Mechanisms of Cell Division Underlying Plant Tissue Regeneration

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Summary Abstract

Plants possess a remarkable ability to regenerate tissues through autonomous regulation of cell division. In this study, we demonstrated that the transcription factor ERF115 upregulates the expression of the brassinosteroid receptor BRL3, thereby activating cell division in cells adjacent to dead ones. This finding provides new insights into plant-specific mechanisms underlying tissue regeneration and maintenance.

Key Words : Regeneration, Cell division, *Arabidopsis*, Root

Introduction

Plants possess a remarkable capacity for tissue regeneration, enabling continuous growth under changing environmental conditions. For example, when DNA damage induces stem cell death in the root tip, cell division is activated in the quiescent center (QC), thereby maintaining the stem cell population. In this study, we aimed to elucidate the mechanisms that fine-tune regenerative cell division and preserve tissue architecture.

Results

We previously reported that, in the QC, expression of the brassinosteroid receptor *BRL3* is induced by DNA damage signaling, which subsequently upregulates the transcription factor *ERF115* and activates QC cell division (Takahashi *et al*., 2024). To investigate whether this mechanism generally contributes to regenerative cell division following DNA damage-induced cell death, we conducted genetic experiments to determine whether the BRL3–ERF115 pathway is also involved in endodermal cell division after treatment with a DNA-damaging agent. Our results showed that BRL3 is not required for *ERF115* induction; rather, ERF115 enhances *BRL3* expression.

This result prompted us to identify the cis-regulatory element(s) within the *BRL3* gene that interact with ERF115. Because ERF transcription factors are known to bind GCC motifs, we searched the *BRL3* genomic region for GCC sequences and substituted them with TAA sequences. The *BRL3* constructs containing these GCC-to-TAA substitutions were then fused to the luciferase gene and co-transfected with *ERF115* into *Arabidopsis* protoplasts. Our transient expression assays revealed that ERF115 binds to one of the GCC motifs and upregulates *BRL3* expression.

To investigate whether the ERF115–BRL3 pathway is required for regenerative cell division, we introduced *BRL3-GFP* containing the GCC-to-TAA substitution into the *brl3*

knockout mutant and quantified periclinal cell division adjacent to dead cells. The results showed that cell division in the endodermis was reduced to the level observed in the original *brl3* mutant, whereas *BRL3-GFP* without the GCC-to-TAA substitution fully complemented the mutant phenotype. These findings suggest that ERF115-mediated induction of *BRL3* is involved in regenerative cell division.

We then carefully monitored GFP expression over a time course following treatment with a DNA-damaging agent. Twenty-four hours after DNA damage induction, fluorescence levels were comparable between the wild type and the mutated version of *BRL3-GFP*. Our previous study showed that DNA damage signaling directly induces *BRL3* expression (Takahashi *et al*., 2024); therefore, this result suggests that the ERF115–BRL3 pathway does not play a major role during the DNA damage response itself. In contrast, when roots were transferred to a medium lacking the DNA-damaging agent, fluorescence levels in the endodermis were lower in the line carrying the GCC-to-TAA substitution than in the line without the substitution. These results suggest that the ERF115–BRL3 pathway plays an important role during the regeneration phase by enhancing *BRL3* expression, thereby promoting endodermal cell division to restore tissue structure.

The results described above indicate that, during the regeneration stage, the ERF115–BRL3 pathway plays a major role in enhancing cell division, whereas the BRL3–ERF115 pathway is no longer active. These findings contradict our original hypothesis that BRL3 and ERF115 form a positive feedback loop that regulates not only the activation but also the termination of cell division to maintain tissue structure. We therefore began to investigate how *ERF115* expression is activated during the regenerative stage and how cell division is automatically terminated to prevent overproliferation. To address these questions, we established an experimental system that enables cell type-specific induction of cell death in *Arabidopsis* roots. We have already confirmed that *ERF115* is induced in cells neighboring dead cells, indicating that this system is useful for observing the regenerative process following cell death induction at the cellular level.

Discussion & Conclusion

Our results show that the ERF115–BRL3 pathway is involved in regenerative cell division in neighboring cells following cell death induction. Considering our previous findings on DNA damage–induced QC cell division (Takahashi *et al*., 2024), regenerative cell division itself does not require brassinosteroid-dependent induction of *ERF115*; rather, ERF115 appears to regulate brassinosteroid signaling to promote regeneration. We will test this hypothesis using the cell death induction system described above in a more general context. However, it remains unknown how regenerative cell division ceases autonomously. Addressing this question may provide mechanistic insight into the plant-specific capacity to prevent cancer-like overgrowth. We are therefore currently establishing an experimental system to induce overproliferation in specific root cell layers. We believe that this system will allow us to investigate how plants autonomously suppress cell division during tissue regeneration, likely through signaling molecules such as phytohormones.

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一般の皆様へ

植物の組織が傷つくと細胞が死にますが、その際死細胞の周囲の細胞が分裂を活性化させ、死細胞と同じ性質を持つ細胞を作りだして組織構造を回復します。植物はこのような組織再生を自律的に行いますが、その仕組みの多くは未だ不明です。私達は、ブラシノステロイドという植物ホルモンの受容体を発現させる制御系が再生のための細胞分裂を活性化させるのに重要な役割をもつことを明らかにしました。この発見は、植物独自の組織再生機構を理解する上で重要な手がかりになるものと考えています。

Inflammation Control in Obesity by innate-like memory CD8+ T Cells

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Summary Abstract

Obesity is associated with chronic inflammation in visceral adipose tissue, leading to insulin resistance and metabolic dysfunction. This study aims to clarify the role of memory-like CD8+ T cells in obesity-associated inflammation and to provide new insights into the pathogenesis and treatment of obesity and type 2 diabetes.

Key Words : Inflammation, Innate-like CD8+ T cell, Obesity, NKT cell

Introduction

Virtual memory CD8+ T (vmCD8+: Innate-like CD8+ T cell) cells are memory-like T cells that develop independently of prior antigen exposure and are regulated in part by IL-4. While immune cells in visceral adipose tissue are known to regulate obesity-associated inflammation, the role of vmCD8+ T cells remains unclear. This study aims to elucidate the function of vmCD8+ T cells in adipose tissue inflammation and metabolic dysfunction, providing new insights into obesity and type 2 diabetes.

Results

(1) Identification and characterization of vmCD8+ T cells in visceral adipose tissue

To investigate the potential role of virtual memory CD8+ T (vmCD8+) cells in obesity, we first established a flow cytometry-based platform for the identification and isolation of vmCD8+ T cells from lymphoid and metabolic tissues. Using established markers, including CD122 and CD49d, we successfully detected vmCD8+ T cells in the spleen, lymph nodes and visceral adipose tissue. Optimization of tissue digestion, cell isolation and staining conditions enabled reliable detection of this population from adipose tissue, where immune cell recovery is often technically challenging. These results confirmed the presence of vmCD8+ T cells in visceral adipose tissue and established an experimental foundation for subsequent phenotypic and functional analyses.

(2) Analysis of vmCD8+ T cells in diet-induced obesity

To determine whether obesity influences the abundance or phenotype of vmCD8+ T cells, mice were fed a high-fat diet and compared with age-matched control animals maintained on a normal diet. Visceral adipose tissue was harvested, and vmCD8+ T cells were analyzed by flow cytometry. We examined the frequency of vmCD8+ T cells as well as the expression of characteristic markers, including CD122 and CD49d. No significant differences were

observed in the proportion or phenotype of vmCD8+ T cells between obese and control mice. These findings suggest that diet-induced obesity does not markedly alter the size of the vmCD8+ T-cell compartment in visceral adipose tissue. However, isolation of vmCD8+ T cells from adipose tissue proved technically challenging because of the relatively low cell numbers and limited recovery efficiency. Therefore, further optimization of tissue digestion, cell isolation, and enrichment procedures will be required to improve the sensitivity of downstream phenotypic and functional analyses. Future studies using optimized isolation protocols and larger sample sizes will be necessary to fully evaluate the impact of obesity on adipose tissue vmCD8+ T cells.

(3) Contribution to vaccine-related research

In addition to the original aims of this project, the experimental platform and immunological expertise established through this study contributed to collaborative research on vaccine-induced immune responses. In particular, the flow cytometric analysis and characterization of T-cell populations developed in this project were applied to the evaluation of immune responses following vaccination. These contributions supported the completion of a collaborative study, and the present grant was acknowledged in the resulting publication. These findings demonstrate the broader applicability of the methodologies established in this project beyond obesity research.

Discussion & Conclusion

(3) Future directions

Although obesity did not induce obvious quantitative changes in vmCD8+ T cells, the absence of changes in cell frequency does not exclude the possibility that their functional properties are altered during obesity. Indeed, many immune cell populations undergo substantial transcriptional and functional reprogramming without major changes in cell number. Therefore, future studies will focus on evaluating the activation status and effector functions of adipose tissue vmCD8+ T cells. In particular, cytokine production, cytotoxic molecule expression, and transcriptional profiles will be analyzed to determine whether obesity affects the functional state of these cells.

Collectively, this study established a robust methodology for the identification and isolation of vmCD8+ T cells from visceral adipose tissue and demonstrated that their abundance is largely maintained during obesity. These findings provide an important basis for future studies aimed at elucidating the functional contribution of vmCD8+ T cells to adipose tissue inflammation, metabolic homeostasis, and the pathogenesis of obesity-associated metabolic disorders.

一般の皆様へ

肥満は糖尿病などの生活習慣病の大きな原因であり、その発症には脂肪組織の慢性炎症が深く関わっています。本研究では、免疫細胞の一種である「virtual memory CD8 T 細胞」に着目し、肥満との関係を調べました。その結果、脂肪組織に存在するこれらの細胞を解析する手法を確立しました。今後は、この細胞の働きを明らかにすることで、肥満や糖尿病の新たな予防・治療法の開発につなげたいと考えています。

Development of Dynamic Asymmetric Crystallization for Synthesis of Medicinal Drugs

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Summary Abstract

In this research, we investigate a new synthetic strategy using dynamic asymmetric crystallization to give access to various chiral compounds such as natural products and medicinal drugs. When the common intermediate is a conglomerate, the enantiopure intermediate is successfully synthesized by dynamic asymmetric crystallization under reversible conditions without use of any chiral reagents and catalysts.

Key Words : conglomerate, crystallization, drug discovery

Introduction

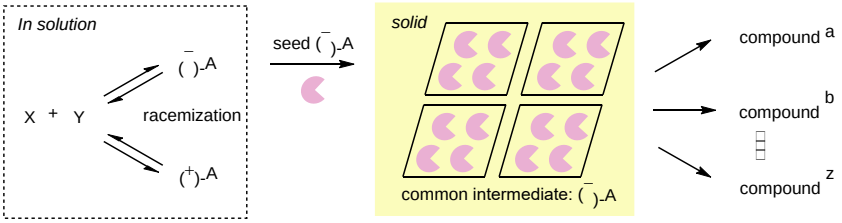
In general, crystallization of racemic compounds produces racemic crystals. However, certain racemic compounds are known to form conglomerate crystals, in which each crystal is composed of a single enantiomer. When products form conglomerate crystals, dynamic asymmetric crystallization is expected as a highly useful method to provide enantiopure compounds from racemic compounds. Unfortunately, practical application of this method to give biologically active compounds such as medicinal drugs is very limited because most compounds form racemic crystals.

Results

In dynamic asymmetric crystallization, racemic product **A** must be formed from reactants **X** and **Y** under reversible conditions. If compound **A** is conglomerate crystals, addition of seed crystal **(−)-A** to a solution of racemic product **A** promotes asymmetric amplification through both racemization and crystallization process. First, **(−)-A** is preferentially crystalized in the presence of seed crystals. Although **(−)-A** in the solution phase temporarily decreases, **(+)-A** is converted to **(−)-A** through racemization process under reversible conditions, leading to further crystallization of **(−)-A**. Thus, dynamic asymmetric crystallization provides products theoretically in 100% yield and with >99%ee without use of any chiral reagents or chiral catalysts. In spite of the high utility of the dynamic asymmetric crystallization, However, direct synthesis of various chiral compounds is infeasible because target products rarely form conglomerate crystals. To solve this limitation, we envisioned a new synthetic strategy, in which a conglomerate crystal is employed as a common intermediate for a variety of chiral biological active compounds. If the common intermediate is enantioselectively synthesized by dynamic asymmetric crystallization, a number of chiral biological active compounds **a**-**z** are available from the common intermediate.

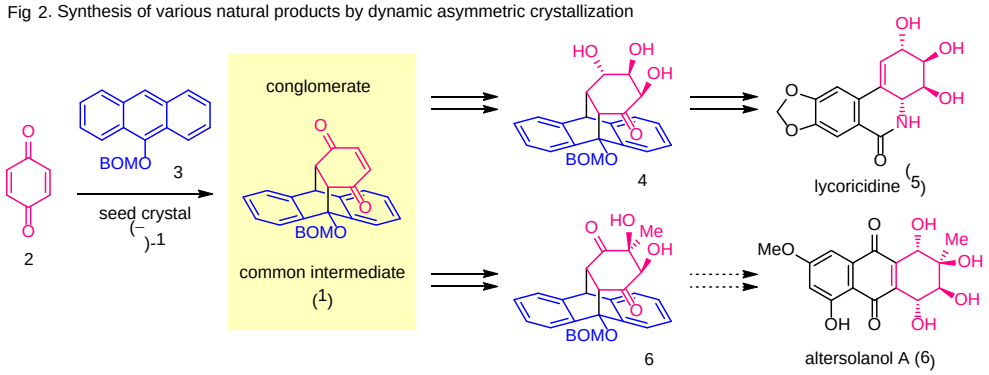
First, we investigated the possible common intermediate for a variety of chiral biological active compounds. The common intermediate must form conglomerate crystals, and be synthesized under reversible conditions. First, we selected

Fig 1. Synthesis of various chiral compounds by dynamic asymmetric crystallization



compound **1** from a list of known conglomerate compounds (about 1800 compounds). Then, reversibility of formation of compound **1** was confirmed. When reactants **2** and **3** were heated, formation of compound **1** through Diels-Alder reaction was observed. On the other hand, heating a solution of the resulting product **1** promoted the retro-Diels-Alder reaction, regenerating starting materials **2** and **3**. These results indicated that product **1** was synthesized under reversible conditions. Thus, compound **1** was found to meet two requisite conditions to perform dynamic asymmetric crystallization. After racemic compound **1** was formed by heating reactants **2** and **3**, addition of 1 mol% of seed crystal **(−)-1** to the solution at that elevated temperature successfully induced asymmetric amplification, giving **(−)-1** in 86% yield with 78%ee. Subsequent recrystallization afforded **(−)-1** in 62% yield with 99%ee from starting materials **2** and **3**. To demonstrate that compound **1** actually becomes a common intermediate for a variety of biologically active compounds, transformation of compound **1** to different types of natural products was attempted. So far, we accomplished the total synthesis of the *Amaryllidaceae* alkaloids, which are expected as drug lead compounds due to their biological properties such as antitumor and antiviral activities.

Fig 2. Synthesis of various natural products by dynamic asymmetric crystallization



Discussion & Conclusion

In this study, we achieved the enantioselective synthesis of common intermediate **1**, which is known to form conglomerate crystals, through dynamic asymmetric crystallization using the Diels-Alder reaction under reversible conditions. The common intermediate was successfully converted to one of the Amaryllidaceae alkaloids. Dynamic asymmetric crystallization itself is a known method since Havinda's report in 1954. However, the practical application has been limited for a long time because conglomerate crystals are very rare. This study demonstrated a new synthetic strategy using dynamic asymmetric crystallization based on the modern organic synthesis, which enables design of multi-step synthesis.

References

Takaaki Sato, Synthesis of natural products and their derivatives using dynamic crystallization. *Chem. Lett.* **2025**, *54* (1), upae244.

一般の皆様へ

一般的に、有機合成化学の反応は溶液中で行われます。しかし、溶液中の反応では、望まない副生成物が同時に生成してしまうことが、しばしばみられます。本研究では、溶液中の反応に結晶化というプロセスを組み合わせた動的不斉結晶化という方法論の開発・応用に取り組みました。コングロメレートという特殊な結晶を形成する化合物を、共通の中間体化合物として設計し、動的不斉結晶化により合成しました。得られた共通中間体は、医薬品など様々な有用物質への変換が可能であることがわかりました。

Molecular mechanisms by which RNA splicing abnormalities induce acute myeloid leukemia

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Summary Abstract

Our goal is to lay the groundwork for a new therapeutic strategy that targets RNA splicing abnormalities to advance the development of treatments for refractory acute myeloid leukemia (AML). The Ahed mutation, which was identified in human AML, exhibits a distinct protein interaction profile from the wild-type form. This suggests that the mutation may play a role in the development of drug resistance.

Key Words : RNA splicing factor, Acute myeloid leukemia

Introduction

We recently identified an uncharacterized gene essential for hematopoiesis and named it *Attenuated hematopoietic development (Ahed)*. Molecular genetic analysis have revealed its critical roles in hematopoiesis by regulating RNA splicing (Nat Commun 2024). This study focuses on elucidating physiological functions of Ahed proteins and clarifying the influence of Ahed mutations in the progression of AML. Systematical analyses were performed for the function of Ahed. Then, we have evaluated how Ahed mutations influences malignant phenotypes of leukemic cells.

Results

1. Effects of Wild-Type and Mutant Ahed on Human Leukemia Cells

We forced the expression of wild-type Ahed and mutant Ahed in the human leukemia cell line K562 to examine their effects on proliferation and drug resistance. Under standard culture conditions, no significant changes in proliferation were observed in either Ahed-expressing cell line. In contrast, under conditions containing imatinib (1 μM), a molecularly targeted drug against the BCR-ABL fusion protein, the parent K562 cells showed no proliferation, whereas cells overexpressing wild-type Ahed maintained their proliferative capacity. Furthermore, cells harboring Ahed missense mutations (S138C and E291D) reported in human AML exhibited higher proliferative capacity compared to wild-type cells in the presence of imatinib.

2. Analysis of Ahed Protein Interaction Partners

We conducted a comprehensive analysis of Ahed's binding partners using the proximal biotin labeling method and mass spectrometry.

・**Proximal biotin labeling method:** The biotinylating enzyme AirID was fused to wild-type or mutant Ahed and introduced into K562 cells using a retroviral vector. This allowed us to biotin-label molecules in direct or indirect proximity to Ahed for analysis. As a result, numerous RNA helicases and RNA-binding proteins were identified in wild-type Ahed. In addition, mitochondrial ribosomal proteins involved in RNA translation within mitochondria were strongly labeled. In mutant Ahed, changes were observed in the labeling levels of various proteins; in particular, a general trend toward reduced labeling was observed among RNA splicing-related proteins.

・**Mass Spectrometry:** Immunoprecipitation was performed using anti-AirID antibodies on K562 cells expressing the Ahed-AirID fusion protein, and a comprehensive analysis of interacting proteins was conducted. As a result, numerous factors involved in RNA maturation were identified as proteins interacting with wild-type Ahed; in addition to RNA splicing-related proteins, these included proteins involved in the export of RNA from the nucleus. Conversely, proteins that specifically associate with mutant Ahed were also detected, including molecules associated with poor prognosis in AML and solid tumors.

Discussion & Conclusion

The results suggest that the expression of wild-type Ahed is involved in maintaining and enhancing proliferative capacity, as well as increasing drug resistance, in human AML cells. Furthermore, cells harboring Ahed missense mutations tended to exhibit even stronger manifestations of these properties, suggesting that Ahed malfunction may contribute to the malignant phenotype of leukemia cells.

Interaction analysis revealed that Ahed not only participates in RNA splicing but also extensively interacts with protein groups involved in nuclear export of RNA and the translation process, suggesting that it is likely to be a molecule involved in the regulation of overall RNA metabolism. In particular, the observation of interactions with mitochondrial-associated proteins is noteworthy as it suggests a link to energy metabolism and the regulation of protein synthesis. In addition, since Ahed mutations identified in human AML exhibited protein interaction profiles different from those of the wild-type Ahed, it is possible that these mutations influence the properties of tumor cells through the reorganization of specific molecular networks.

Moving forward, it will be necessary to verify the reproducibility and physiological validity of these results by conducting analyses using different tagging systems and at endogenous expression levels. Furthermore, by developing molecular-targeted approaches, such as antisense oligonucleotides targeting Ahed function, and verifying their therapeutic efficacy against refractory and relapsed AML in preclinical models, we anticipate the development of new therapeutic strategies.

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A newly identified gene Ahed plays essential roles in murine haematopoiesis.
Nat Commun. 15, Article number: 5090, 2024.

一般の皆様へ

近年、一部の血液がんに対しては特効薬的な分子標的薬が登場し、予後が顕著に改善しました。しかし、急性骨髄性白血病に対しては従来の抗癌剤治療が今なお主役です。その理由の一つに、原因遺伝子が多岐に渡り治療標的を特定できないこと、未解析・未発見の遺伝子異常が存在することが挙げられます。私たちが発見したAhedおよびその変異は、急性骨髄性白血病のみならず神経系の悪性腫瘍にも見出されています。Ahedの異常がどのような機序で悪性腫瘍を引き起こすのかを明らかにすることが、生命科学領域において新たな分野の開拓につながると期待しています。

Development of novel asthma therapy based on the pathophysiology of airway hyperresponsiveness

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Summary Abstract

Airway hyperresponsiveness (AHR) is a key feature of asthma, yet the mechanisms linking inflammation to abnormal airway contraction remain unclear. In a house dust mite–induced asthma mouse model, we observed elevated glutamate levels in the airways. We further show that glutamate activates the umami taste receptor TAS1R1/TAS1R3 expressed in airway smooth muscle, potentiating contraction through Ca^{2+} increase and cAMP inhibition. Pharmacological inhibition of TAS1R3 with lactisole reduces airway resistance and augments β_2-adrenoceptor–mediated airway relaxation in mice. These findings establish a mechanistic link between inflammation-associated glutamate accumulation and airway hyperreactivity, suggesting that targeting TAS1R1/TAS1R3 may offer a new therapeutic approach for asthma.

Key Words : Asthma, Airway hyperresponsiveness, Glutamate, Umami taste receptor

Introduction

Asthma affects over 262 million people globally and remains a leading cause of mortality. Current treatments, such as β2 adrenoceptor agonists, are limited by receptor downregulation and tolerance with frequent use (1,2), highlighting the need for novel therapies (3). Airway hyperresponsiveness (AHR), a hallmark of asthma, correlates with symptom severity and persistence (4,5), yet its underlying mechanisms are not fully understood. This study investigates the role of glutamate, a neurotransmitter and umami taste modulator, in AHR.

Results

Increased in Glutamate Concentrations in Bronchoalveolar Lavage Fluid of Asthmatic Mice

To determine whether glutamate levels are altered in inflamed airways, we measured glutamate concentrations in bronchoalveolar lavage fluid (BALF) from a house dust mite (HDM)-induced asthma mouse model. Glutamate concentrations were significantly elevated in HDM-treated mice compared with control mice ($P < 0.05$, $n = 8$). Consistently, immunohistochemical analysis revealed stronger glutamate staining in the airway epithelium and alveolar regions of HDM-treated lungs than in controls, indicating local glutamate accumulation in inflamed airways.

Expression of TAS1R1/TAS1R3 in Airway Smooth Muscles

RT-PCR confirmed expression of both TAS1R1 and TAS1R3 in primary cultured HASM cells from three independent donors and in freshly isolated human tracheal smooth muscle tissue. Immunostaining further demonstrated TAS1R1/TAS1R3 protein expression in the airway smooth muscle layer of murine trachea. Notably, TAS1R1/TAS1R3 expression levels were not significantly altered in HDM-induced asthmatic mice compared with controls.

Glutamate Potentiates Airway Smooth Muscle Contraction via TAS1R1/TAS1R3

To examine whether elevated glutamate affects airway smooth muscle contractility, we assessed contractile responses in murine tracheal rings and precision-cut lung slices (PCLS). Monosodium glutamate (MSG) alone or in combination with inosine monophosphate (IMP), a positive allosteric modulator of TAS1R1/TAS1R3, did not alter basal airway tone. However, co-administration of MSG and IMP significantly potentiated acetylcholine (ACh)-induced contraction in tracheal rings and methacholine-induced contraction in peripheral airways within PCLS ($P < 0.05$). In contrast, pharmacological blockade of metabotropic glutamate receptors or ionotropic glutamate receptors did not affect ACh-induced contraction in the presence of glutamate, indicating that this effect is mediated specifically through TAS1R1/TAS1R3.

Since actin reorganization is essential for smooth muscle contraction, we next investigated whether activation of TAS1R1/TAS1R3 induces actin reorganization in HASM cells. MSG alone did not induce actin reorganization in HASM cells. However, co-treatment with MSG and IMP significantly enhanced ACh-induced actin reorganization, as demonstrated by fluorescence imaging and quantitative analysis ($P < 0.01$). siRNA-mediated knockdown of TAS1R1 or TAS1R3 markedly attenuated this response, confirming that TAS1R1/TAS1R3 signaling contributes to glutamate-dependent potentiation of airway smooth muscle contraction.

TAS1R1/TAS1R3 Regulates Intracellular Ca^{2+} and cAMP Singaling

Co-treatment with MSG and IMP significantly increased ACh-induced intracellular Ca^{2+} mobilization in HASM cells compared with MSG alone ($P < 0.05$). This effect was inhibited by pretreatment with the Gαi inhibitor pertussis toxin and by knockdown of TAS1R1/TAS1R3. In addition, MSG significantly suppressed forskolin-stimulated cAMP accumulation in HASM cells ($P < 0.05$). This inhibitory effect was reversed by knockdown of TAS1R1 or TAS1R3 and by inhibition of Gαi signaling, indicating that TAS1R1/TAS1R3 predominantly acts through Gαi to reduce cAMP production.

Antagonizing TAS1R1/TAS1R3 Attenuates Airway Hyperresponsiveness

To evaluate the functional relevance of this pathway in vivo, we examined airway responsiveness in A/J mice. Inhalation of the TAS1R3 antagonist lactisole significantly reduced methacholine-induced central airway resistance compared with vehicle-treated mice ($P < 0.05$). In addition, lactisole enhanced β-adrenoceptor agonist–induced relaxation of airway smooth muscle in tracheal ring experiments ($P < 0.01$). These findings indicate that inhibition of TAS1R1/TAS1R3 attenuates airway hyperresponsiveness and enhances bronchodilator responsiveness.

Discussion & Conclusion

Asthma is characterized by airway hyperresponsiveness (AHR), yet the molecular mechanisms driving this feature remain incompletely understood. In this study, we demonstrated that airway inflammation increases glutamate levels in the lung, which in turn activates the umami taste receptor TAS1R1/TAS1R3 in airway smooth muscle. Activation of this receptor potentiates airway smooth muscle contraction by potentiating intracellular Ca^{2+} mobilization and inhibiting cAMP production. These signaling changes promote airway contraction and may contribute to the development of AHR in inflamed airways. Importantly, pharmacological inhibition of TAS1R1/TAS1R3 using the antagonist lactisole reduced airway resistance in vivo. Moreover, lactisole potentiated β-adrenoceptor agonist–induced airway smooth muscle relaxation, suggesting that inhibiting TAS1R1/TAS1R3 may improve the efficacy of existing bronchodilator therapies. Together, these findings identify glutamate–TAS1R1/TAS1R3 signaling as a previously unrecognized pathway regulating airway smooth muscle contractility and AHR, and suggest that targeting this receptor may represent a promising therapeutic strategy for asthma.

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一般の皆様へ

気道過敏性は喘息の病態の 1 つですが、その機序は十分に解明されていません。本研究では、喘息モデルマウスの気道で上昇したグルタミン酸がうま味受容体（TAS1R1/TAS1R3）を活性化することで気管平滑筋の収縮を増強し、気道過敏性が誘発される可能性明らかにしました。また、TAS1R3 拮抗薬の Lactisole はマウスの気道抵抗を低下させるとともに、β_2アドレナリン受容体作動薬による気管平滑筋弛緩作用を増強しました。これらの結果は、炎症に伴い上昇したグルタミン酸が気道過敏性誘発する可能性を明らかにしたもので、TAS1R1/TAS1R3 を標的とすることが喘息治療の新たな治療法となる可能性を示唆しました。

Mutually promoting effects of circadian rhythm center dysfunction and Alzheimer's disease onset/progression

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Summary Abstract

In Alzheimer's disease (AD), neurodegeneration and sleep–wake rhythm abnormalities may mutually exacerbate each other, and dysfunction of the central circadian clock, the suprachiasmatic nucleus (SCN), may be deeply involved in this vicious cycle. This study aimed to contribute to the development of safe and simple dementia-prevention strategies and new therapeutics targeting the SCN. We combined AD model mice with our uniquely developed techniques for manipulating the central clock and mouse models with selective central clock impairment, and examined whether SCN dysfunction appears in AD and whether central clock impairment accelerates AD progression.

Key Words : Sleep, circadian rhythm, suprachiasmatic nucleus, Alzheimer's disease, amyloid β

Introduction

In many patients with Alzheimer's disease (AD), sleep–wake rhythm disturbances precede cognitive impairment, suggesting early dysfunction of the central circadian clock, the suprachiasmatic nucleus (SCN). Sleep deprivation and circadian disruption are also risk factors for AD. Thus, AD onset and progression may involve a vicious cycle between neurodegeneration and sleep–wake rhythm abnormalities, potentially driven by SCN dysfunction. However, this has not been directly demonstrated due to limited research tools. Here, we aimed to test this hypothesis by combining AD model mice with our unique techniques and mouse models for selective manipulation or impairment of the central clock.

Results

We combined our uniquely developed mouse models with selective central circadian clock impairment with AD model mice, and attempted to examine: (1) whether central circadian clock dysfunction is observed in AD model mice, and (2) whether impairment of the central clock accelerates AD progression.

AD model mice

We used *App*$^{NL\text{-}G\text{-}F}$ mice, mutant amyloid precursor protein (APP) knock-in mice developed by Dr. Saido and colleagues at RIKEN (Saito et al., Nat. Neurosci. 2014). These

genetically modified mice carry three familial AD mutations in the *App* gene, develop amyloid plaques from 2 months of age, and show impaired learning and memory at 6 months of age. They are an excellent model that reflects human AD pathology and allows efficient investigation.

1. Age-dependent changes in the circadian rhythm of spontaneous activity in App$^{NL\text{-}G\text{-}F}$ mice

We measured daily fluctuations in spontaneous locomotor activity in the home cages of *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice and examined abnormalities in the locomotor activity rhythm, which reflects the sleep-wake rhythm. Based on symptoms commonly observed in AD patients, such as difficulty falling asleep at night, early morning awakening, and prolonged daytime napping, we expected that *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice might show abnormalities such as a delayed circadian phase, reflected in changes in the timing of activity onset or offset, and/or reduced rhythm amplitude. We examined mice at approximately 15 and 28 weeks of age, when amyloid plaques are sufficiently deposited, but observed no significant differences compared with wild-type mice. We also examined sleep–wake rhythms, but similarly found no significant differences.

2. Effects of central clock impairment on AD pathology progression in App$^{NL\text{-}G\text{-}F}$ mice

We examined whether selective impairment of central clock function enhances amyloid plaque formation and neuroinflammation in *App*$^{NL\text{-}G\text{-}F}$ mice. As a model of central clock impairment, we first used AVP neuron-specific *Bmal1*-deficient mice (*Avp-Bmal1*$^{-/-}$; Mieda et al., Neuron 2015). *Bmal1* is an essential clock gene, and its deletion disrupts the intracellular molecular clockwork. We crossed *Avp-Bmal1*$^{-/-}$ mice with *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, prepared brain sections, and quantified amyloid plaque formation in regions such as the cerebral cortex and hippocampus by immunostaining with an anti-Aβ antibody. Analyses at 8 and 15 weeks of age revealed no significant differences compared with control mice. We also examined activation of astrocytes, assessed by GFAP immunostaining, and microglia, assessed by Iba1 immunostaining, as indices of neuroinflammation, but again observed no significant differences.

We have also found that specific blockade of neurotransmission from SCN Prokineticin 2 (Prok2) neurons, candidate SCN output neurons, by expressing tetanus toxin light chain abolishes circadian behavioral rhythms (manuscript in preparation). In addition to *Avp-Bmal1*$^{-/-}$ mice, we performed similar analyses using this new central clock impairment model. We crossed *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice with transgenic mice that specifically express tTA in Prok2 neurons, and expressed TeNT in Prok2 neurons using a tTA-dependent AAV vector at 15 weeks of age. One month later, we prepared brain sections and quantified amyloid plaque formation and neuroinflammation, but observed no significant differences compared with control mice.

Discussion & Conclusion

Unfortunately, we have not yet obtained positive evidence either for central clock abnormalities in AD model mice or for acceleration of AD progression caused by reduced central clock function. However, we believe it would be premature to reject the hypothesis proposed in this study. Circadian rhythm disruption is unlikely to directly cause AD pathology by itself, but may act as a modifying factor. Therefore, to quantitatively demonstrate its effects, experimental conditions such as the mouse model and age need to be carefully optimized.

In *Avp-Bmal1* $^{-/-}$ mice, central clock function is not completely abolished, and the impairment may be masked, particularly under light–dark conditions. In mice with inhibited Prok2 neuron function, the interval between AAV administration and sampling was only one month, which may have been too short for the effects to become evident. Although *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice are an excellent model, their AD pathology progresses very rapidly and may not be suitable for this purpose. Given the clear association between sleep rhythm disruption and dementia risk, we will continue to test this hypothesis under more appropriate conditions.

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一般の皆様へ

アルツハイマー病（AD）の発症・進行において、神経変性と睡眠覚醒リズム異常との双方向性の促進作用が予想され、この悪循環に中枢体内時計・視交叉上核（SCN）の異常が深く関与する可能性が考えられる。しかし、それを直接示した研究は皆無である。本研究では、中枢時計・SCNを標的とした、安全で容易な新たな認知症予防手法の創出や、新たな治療薬の開発に繋げることを目的した。申請者らが独自に開発した中枢時計の操作技術や特異的障害モデルマウスを、ADモデルマウスと組み合わせ、①AD発症前・初期に中枢時計に異常が現れるか、②中枢時計の障害がADの進行を早めるか、の検証を試みた。

Development of Next-Generation Hedgehog Inhibitors Targeting Drug-Resistant Cancer Cells

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Summary Abstract

Aberrant activation of the Hedgehog (Hh) signaling pathway drives tumorigenesis in several cancers. Although SMO inhibitors have shown clinical efficacy, their therapeutic benefit is frequently limited by the emergence of drug-resistant SMO mutations. In this study, we investigated an alternative strategy targeting regulators of Hh signaling. We identified candidate molecule X as a regulator of Hh signaling and evaluated the efficacy of its inhibition. Our findings demonstrate that inhibition of molecule X suppresses Hh signaling.

Key Words : Cancer, Hedgehog signal, SMO

Introduction

The Hedgehog (Hh) signaling pathway plays a critical role in embryonic development and tissue homeostasis, and its dysregulation is implicated in tumorigenesis. Clinically approved Hh inhibitors, such as vismodegib and sonidegib, target Smoothened (SMO), a G protein–coupled receptor in the pathway. While these inhibitors exhibit strong antitumor activity, their clinical efficacy is often compromised by the emergence of drug-resistant SMO mutations, which can arise during treatment. Therefore, there is an urgent need to develop novel therapeutic strategies that suppress Hh signaling.

Results

We investigated the feasibility of targeting regulators of the Hedgehog (Hh) signaling pathway.

First, we established stable cell lines expressing a drug-resistant oncogenic mutant, which is frequently observed in Hh pathway–dependent tumors. Consistent with previous reports, treatment with the clinically approved SMO inhibitor vismodegib significantly suppressed Hh signaling in cells expressing wild-type SMO. In contrast, vismodegib failed to inhibit Hh pathway activation in mutant SMO -expressing cells, confirming the resistance phenotype and validating this model as a platform to assess alternative therapeutic strategies.

Next, we focused on candidate molecule X, identified through our studies as a potential positive regulator of Hh signaling. To evaluate its functional significance, we performed both genetic and pharmacological inhibition experiments.

Knockdown of molecule X using siRNA resulted in a significant reduction in the expression of Hh target genes, including GLI1, in both SMO^{WT} and mutant SMO cells. These findings

indicate that molecule X appears to be involved in Hh pathway activation. Importantly, this effect was observed under both basal and ligand-stimulated conditions, suggesting that molecule X contributes broadly to pathway activation rather than acting in a context-specific manner.

We further examined the effects of chemical inhibition targeting molecule X. Treatment markedly suppressed Hh signaling activity in both wild-type and mutant SMO-expressing cells, as assessed by quantitative PCR analysis of GLI1 expression. The magnitude of inhibition was observed across different conditions.

To validate these findings in a cancer context, we conducted similar analyses using the human cancer cell line, a representative Hh pathway–dependent tumor model. Stable overexpression of mutant SMO resulted in constitutive activation of the Hh pathway that was resistant to vismodegib, recapitulating clinically relevant resistance. In contrast, chemical inhibition significantly suppressed Hh target gene expression in both SMO^{WT} and mutant SMO-expressing cells, demonstrating that this approach is applicable in a disease-relevant cellular context.

We also assessed the impact of molecule X inhibition on tumorigenic properties using a proliferation assay. Inhibition of molecule X led to a marked decrease in cell number, indicating impaired tumorigenic potential. Notably, this suppressive effect was observed regardless of SMO mutation status, whereas vismodegib failed to reduce proliferation in mutant SMO-expressing cells.

Taken together, these results demonstrate that molecule X functions as a potential regulator of Hh signaling and that its inhibition effectively suppresses both pathway activity and tumorigenic properties. These findings support the feasibility of developing next-generation Hh inhibitors.

Discussion & Conclusion

Our study provides evidence that targeting novel regulators of the Hedgehog (Hh) signaling pathway represents an effective strategy to overcome resistance to SMO inhibitors. In particular, inhibition of candidate molecule X suppresses Hh signaling, addressing a major limitation of current therapies.

Given that drug-resistant SMO mutations frequently emerge during treatment, approaches targeting signaling components are expected to have broad clinical applicability. Furthermore, the observed suppression of tumorigenic properties in cancer cells highlights the translational potential of this strategy.

Future studies will focus on evaluating the efficacy in vivo tumor models and on identifying optimized compounds with improved pharmacological properties.

一般の皆様へ

本研究は、がん治療において問題となる「薬が効かなくなる現象（薬剤耐性）」を克服することを目的としています。

特に、基底細胞がんでは、現在の治療薬が効かなくなるケースがあり、新しい治療法の開発が求められています。

本研究では、がん細胞の増殖に関わる重要な仕組みを別の方法で抑えることで、従来の薬が効かないがんにも効果を示す可能性を見出しました。今後の新しい治療薬の開発につながる成果が期待されます。

Exploration of functional molecules that transmit environmental changes

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Summary Abstract

This study aimed to elucidate how plants perceive environmental changes and enhance stress tolerance through signaling pathways. A highly conserved leucine-rich repeat receptor complex, Dynamic Stress Perception Regulator 1 and 3 (DSPR1/DSPR3), was identified as a mediator of the environmental stress responses. Through phenotypic screening, a peptide called DSPR-interactive Peptide 2 (DSP2) was identified, which directly binds to the DSPR1/DSPR3 receptor complex, a key regulator of environmental stress responses. These findings deepen our understanding of plant environmental sensing mechanisms and suggest the potential strategies for regulating stress tolerance through targeted receptor activation.

Key Words : Peptide, Environnemental Stress, Signal Transduction

Introduction

Since plants lack a central integrative organ, they have evolved a system in which information processing and response functions are distributed across individual tissues and organs. This distributed organizational structure represents an adaptive strategy in sessile organisms, enabling effective responses to fluctuating environmental conditions. However, the molecular mechanisms underlying distributed signal transduction and inter-organ coordination remain poorly understood.

Results

In response to environmental stress, sessile plants close their stomata to reduce water loss through transpiration, thereby maintaining cellular water potential under adverse conditions. This stomatal response represents one of the most important physiological mechanisms for plant survival in fluctuating environments. However, the molecular mechanisms underlying this cascade of responses remain poorly understood. In particular, how plants temporally coordinate external stress perception with intracellular signalling events remains an open question. A detailed understanding of the timing and regulation of these processes is essential for elucidating how plants balance stress adaptation with the maintenance of growth and metabolic activity.

In previous studies, a receptor complex called DSPR1/DSPR3, which exhibits an environmental stress-sensitive phenotype, was identified. This phenotype suggests that the DSPR1/DSPR3 complex plays a crucial role in perceiving environmental stress signals

and initiating downstream signalling cascades that regulate stomatal behaviour. Based on these observations, the present study was designed to further investigate the molecular function of the DSPR1/DSPR3 receptor complex, with particular emphasis on how it detects environmental cues and transduces them into intracellular responses that ultimately contribute to stomatal regulation and stress adaptation.

To address this objective, a systematic screening was conducted using a library of compounds and peptides that were predicted to physically interact with the DSPR1/DSPR3 receptor complex. The purpose of this screening was to identify potential ligands or interacting molecules that may be involved in the early stages of receptor-mediated signal perception. During the screening process, particular attention was given to ensuring the specificity of observed interactions. Therefore, compounds and peptides whose effects were altered by prolonged exposure were excluded, as such behaviour could indicate indirect or secondary effects rather than direct receptor engagement. In addition, molecules that inhibit cellular proliferation were also removed from the candidate pool to avoid confounding physiological effects that could obscure the interpretation of receptor-specific interactions. This strategy enabled a more refined selection of candidates likely to be directly associated with receptor function. Following the initial screening, further biochemical and biophysical analyses were carried out to investigate whether the selected small molecules directly bind to the DSPR1/DSPR3 receptor complex. To this end, binding assays were performed using two independent approaches, referred to as methods thermal shift assay (TSA) and isothermal titration calorimetry assay (ITC). These complementary methods were employed to ensure robustness and reproducibility of the observed interactions. The results of these analyses demonstrated that the small molecule of interest binds with comparable affinity to both components of the DSPR1/DSPR3 receptor complex, therefore we named this compound as DSPR-interactive protein 2 (DSP2). This finding suggests that the ligand interacts with the receptor complex in a coordinated manner, potentially stabilizing or modulating the function of the complex rather than acting on a single subunit independently. In contrast, when the same binding analyses were performed using a mutant version of the receptor complex, no detectable binding was observed. This result indicates that the interaction between the small molecule and the DSPR1/DSPR3 complex is highly dependent on the intact structural configuration of the receptor. The absence of binding in the mutant further suggests that specific structural elements within the receptor complex are essential for ligand recognition and stable interaction. These findings collectively support the idea that the receptor complex possesses a defined and structurally sensitive binding interface required for signal perception.

Discussion & Conclusion

In this study, the applicant identified specific small-molecule DSP2 that binds to receptor complex (DSPR1/DSPR3) involved in environmental responses through screening assay. DSP2 was initially identified through a screening approach as a molecule that specifically associates with the receptor complex under normal conditions, and subsequent analyses indicated that this interaction depends on the structural integrity of DSPR1/DSPR3. These findings imply that DSP2 may constitute an important molecular link between environmental signal perception and downstream stress-response signalling pathways. Furthermore, our

results suggest that DSP2 may function as a potential regulator of stomatal responses to novel environmental stresses mediated by the DSPR1/DSPR3 receptor complex. Taken together, these observations provide a basis for future investigations into the functional role of DSP2 in plant environmental stress tolerance and its contribution to adaptive responses under changing environmental conditions.

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"The role of peptide-receptor modules in transducing environmental stress signals" Shimotohno A.*, *International Plant Growth Substances Association*, *IPGSA*, USA (2025), symposium, oral presentation, invited, 02/07/2025.

一般の皆様へ

本研究を通して、動くことができない植物が周囲の環境変化をどのように感知しているのかについて、分子レベルからその仕組みを解明するための手がかりが得られたと考えられる。これまで十分に明らかにされていなかった環境応答の過程について、極めて微細な分子の働きが関与している可能性が示され、感知機構の理解に新たな視点を与える成果となった。今後は、この仕組みが他の植物種にも共通して存在するのかを検証することが重要となる。もし同様のメカニズムが広く保存されていることが明らかになれば、複数の生物や関連現象における環境応答の理解が大きく進展することが期待される。さらに、その知見は地球温暖化や気候変動、食料問題といった現代社会が直面する環境問題の解決に向けた基盤的知見となり得る。すなわち、本研究は複雑な環境応答機構の理解を深化させ、将来的な応用展開へとつながる重要な出発点となると考えられる。

Omics-driven insights into chemotherapy-induced modulation of tumor immune microenvironment in human biliary tract cancer.

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Introduction

Biliary tract cancer (BTC) has a poor prognosis. While combining immune checkpoint inhibitors (ICIs) with chemotherapy improves survival,[1,2] ICI monotherapy remains ineffective because most BTCs are not inherently "immune-hot".[3] We hypothesized that chemotherapy converts BTCs into ICI-sensitive tumors. Thus, we aimed to elucidate chemotherapy-induced changes in the immune microenvironment by performing omics analysis on rare, surgically resected BTC specimens following neoadjuvant chemotherapy,

Results

We utilized three distinct methodological approaches to systematically investigate how neoadjuvant chemotherapy reshapes the tumor immune microenvironment in biliary tract cancer (BTC).

First, we performed a comprehensive immunohistochemical (IHC) analysis on surgically resected human BTC specimens to evaluate macroscopic spatial immune dynamics. We compared a clinical cohort of 47 patients who received gemcitabine-based neoadjuvant chemotherapy prior to surgical resection against a control group of 20 patients who underwent upfront curative-intent surgery without any prior systemic therapy. Utilizing advanced digital pathology software, we achieved precise spatial quantification and cellular segmentation, allowing us to rigorously evaluate specific immune cell populations within the microenvironment. This quantitative analysis revealed that neoadjuvant chemotherapy significantly decreased the overall density of critical immunosuppressive cellular populations, specifically FOXP3+ regulatory T cells (Tregs) and CD68+ macrophages (**Figure 1**). Importantly, this targeted cytotoxic regimen did not indiscriminately deplete all lymphoid

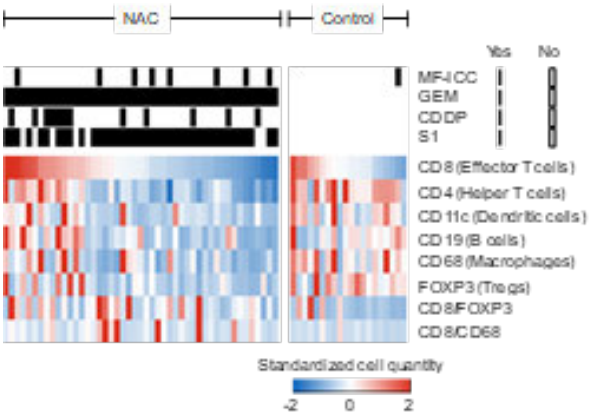
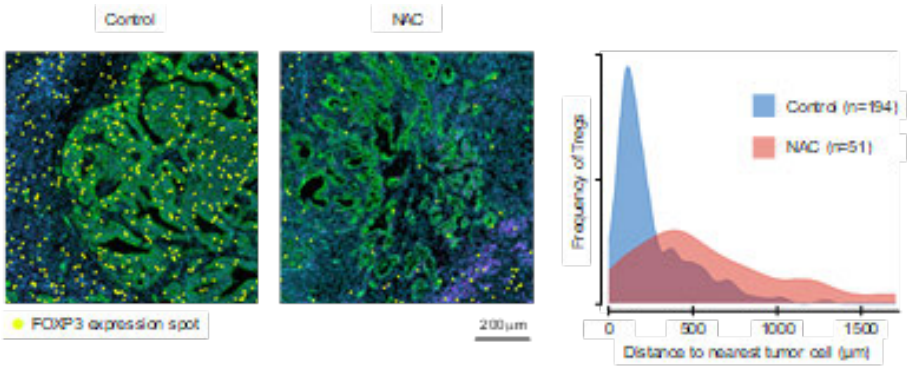


Figure 1. Immune-modulating effects of gemcitabine in human BTC.

lineages; the density of anti-tumor CD8+ cytotoxic T cells was remarkably preserved. Consequently, the spatial ratios of CD8+ T cells to Tregs, as well as to CD68+ macrophages, were significantly elevated in the chemotherapy-treated tissues. These histopathological results strongly indicate that gemcitabine-based neoadjuvant chemotherapy effectively mitigates the highly immunosuppressive baseline microenvironment classically observed in advanced BTC, driving the tumor toward a more "immune-hot" phenotype.

Second, to attain a more comprehensive, high-resolution understanding of these cellular dynamics, we utilized spatial single-cell transcriptomics. We evaluated two representative full-slide tissue samples from both the neoadjuvant and control cohorts using the CosMx Spatial Molecular Imager platform. This state-of-the-art technique allowed us to accurately map complex cellular interactions while preserving the exact topographical architecture of the tissue. Using this high-plex technology, we successfully identified and clustered over 250,000 individual cells into 20 distinct functional subpopulations. Our spatial neighborhood analysis demonstrated that gemcitabine-based chemotherapy visibly reduced both the overall accumulation and the physical proximity of Tregs located immediately adjacent to malignant epithelial cells (**Figure 2**). Furthermore, cell-type-specific differentially expressed gene (DEG) analysis uncovered a crucial qualitative functional shift. We observed that chemotherapy significantly downregulated the transcription of the PD-



1(*PDCD1*) gene specifically within the Treg population.[4] In contrast, baseline PD-1 expression levels in the effector CD8+ T cells remained completely unaltered. This differential regulation suggests that gemcitabine-based regimens prime the BTC microenvironment for ICI efficacy not only by quantitatively depleting Tregs but also by qualitatively impairing their suppressive function.

Third, we developed an *in vivo* syngeneic murine model to experimentally validate these clinical findings and systematically refine our translational treatment strategies. A key objective was to isolate the specific immune-modulating effects of gemcitabine from its primary cytotoxic capability. To achieve this, we first established a gemcitabine-resistant BTC cell line. We derived this line by chronically exposing a murine BTC cell line—originally established from a genetically engineered mouse model driven by the *KrasG12D* mutation, *Tgfbr2* knockout, and *E-cadherin* knockout (termed KTC)[5]—to escalating low doses of gemcitabine over a 30-day period. Subsequently, we transplanted both the wild-type gemcitabine-sensitive KTC and the newly generated gemcitabine-resistant KTC lines into

immunocompetent syngeneic mice to observe their differential immune responses *in vivo*. Remarkably, despite demonstrating diminished direct cytotoxic effects on the tumor cells themselves, gemcitabine treatment in mice harboring gemcitabine-resistant KTC tumors still induced a significant decrease in the number of infiltrating Tregs compared to control groups. This critical finding suggests that the immunomodulatory depletion of Tregs by gemcitabine is an independent mechanism, fundamentally distinct from its traditional role as a direct cytotoxic agent against cancer cells.

Discussion & Conclusion

Collectively, these clinical, spatial, and *in vivo* findings elucidate a paradigm-shifting role for gemcitabine in biliary tract cancer (BTC). Traditionally viewed solely as a cytotoxic agent, our data demonstrates that gemcitabine actively remodels the tumor immune microenvironment from an inherently immunosuppressive state to a more vulnerable phenotype. By selectively depleting FOXP3+ regulatory T cells and CD68+ macrophages while preserving effector CD8+ T cells, gemcitabine favorably alters the spatial immune architecture. Furthermore, the targeted downregulation of PD-1 expression on surviving Tregs highlights a profound qualitative impairment of their suppressive capabilities, an immunomodulatory effect that operates independently of direct tumor cytotoxicity.

In conclusion, gemcitabine-based regimens effectively prime the BTC microenvironment for immune checkpoint inhibitor efficacy by both quantitatively depleting Tregs and qualitatively impairing their suppressive function. By neutralizing this Treg-mediated barrier, neoadjuvant chemotherapy sensitizes BTC to anti-PD-1 therapies. This provides a robust, mechanistically grounded rationale for combination strategies, offering a highly promising therapeutic avenue to overcome baseline immunotherapy resistance and ultimately improve patient survival outcomes.

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一般の皆様へ

胆道がんは治療が難しく、期待される新しい免疫治療薬も単独では効果が出にくいという大きな課題がありました。 本研究では、一般的な抗がん剤をうまく活用することが、この課題を克服する鍵になることを発見しました。 患者さんの組織や動物モデルを解析した結果、抗がん剤はがんを攻撃する「味方の免疫細胞」を保ったまま、 がんを守ろうとする免疫細胞だけをピンポイントで減らし、がんのバリアを壊すことがわかりました。 つまり、抗がん剤が免疫治療薬の効きやすい環境をあらかじめ整え、両者の相乗効果を引き出してくれるのです。抗がん剤が免疫のブレーキを解除するというこのメカニズムの解明は、画期的な複合免疫療法の基盤となります。 この成果が、難治性である胆道がんに対する極めて効果的な新しい治療戦略の確立につながり、 一人でも多くの患者さんの命を救う希望となるよう、強い使命感を持って引き続き研究に邁進してまいります。

Development of novel therapeutic strategies for chronic GVHD post allogeneic hematopoietic cell transplantation targeting oral microbiota

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Summary Abstract

The oral microbiota is the second in abundance to the gut but its specific role in graft-versus-host disease (GVHD) pathogenesis was unclear. Our study revealed that oral dysbiosis in patients after bone marrow transplantation (BMT) was associated with increased chronic GVHD (cGVHD). In murine BMT models, oral dysbiosis exacerbated cGVHD and increased bacterial load in the oral cavity and gut, with Enterococcaceae significantly increasing in both organs. Pathogenic bacteria increase in the oral cavity might not only exacerbate local inflammation but also enhance systemic inflammation throughout the HCT course. Our findings highlight the importance of oral dysbiosis in cGVHD.

Key Words : Oral microbiota, dysbiosis, chronic GVHD

Introduction

Polysulfide functions as a universal regulator of physiological processes across diverse organisms through protein polysulfidation. In plants, proteomic analyses in *Arabidopsis thaliana* have revealed that more than 2,000 proteins undergo polysulfidation, highlighting its significance in plant physiology.

Notably, several key regulators of chloroplast biogenesis have been identified among polysulfidated proteins. Because the chloroplast is a defining organelle of plant cells, focusing on its biogenesis provides a framework for understanding how polysulfide signaling influences plant physiology. This study therefore aims to clarify its role in chloroplast development and its broader physiological significance in plants.

Results

I. Relationship Between Oral Dysbiosis and Intestinal Dysbiosis in Mouse Models.

The applicants performed BMT using a mouse model of oral dysbiosis induced by placing suture thread between the maxillary first and second molars of naive mice. It was revealed that the oral dysbiosis model mice exhibited poorer post-transplant survival rates compared to wild-type mice. Analysis of the oral microbiota in pre-transplant oral dysbiosis model mice showed reduced diversity and increased proportions of Enterobacteriaceae and Enterococcaceae. Analysis at 21 days post-transplant revealed that in oral dysbiosis mice, the proportion of Enterococcaceae was increased not only in the oral cavity but also in the

intestine compared to wild-type mice, contributing to worsened GVHD. These results suggest that oral dysbiosis may be a trigger for intestinal dysbiosis following BMT.

II. Mechanisms of Immune Cell and Tissue Factors of chronic GVHD Exacerbation in Oral Dysbiosis

Analysis of immune cells revealed elevated levels of inflammatory cells such as T cells, dendritic cells, and macrophages, along with increased cytokines in the oral dysbiosis group. Notably, activation of dendritic cells and enhanced T cell-stimulating capacity were observed in the cervical lymph nodes, which drain the oral cavity. This was considered a cause of worsening GVHD. The cause of these changes was identified: in oral dysbiosis mice, bacterial translocation of oral bacteria (specifically Enterococcaceae in this analysis) was detected in the cervical lymph nodes even before transplantation. This translocation worsened post-transplantation, leading to chronic stimulation of antigen-presenting cells and subsequent T cell activation. These results indicate that oral dysbiosis contributes to chronic GVHD by causing chronic stimulation and activation of immune cells through bacterial translocation originating from oral dysbiosis.

IV. GVHD Improvement Through Oral Dysbiosis Correction

As a method to improve GVHD by correcting oral dysbiosis, approaches such as (i) silk thread removal and (ii) antibiotic application were investigated. We compared wild-type mice with oral dysbiosis mice to determine whether these interventions improved post-transplant survival rates and the severity of clinical and pathological GVHD. (i) Removal of silk filaments in oral dysbiosis mice resulted in improved chronic GVHD and increased survival rates. Mice with silk filaments removed showed improved oral bacterial diversity and reduced levels of Enterococcaceae and Enterobacteriaceae. Conversely, (ii) intervention with antibiotic application also improved chronic GVHD and increased survival rates in oral dysbiosis mice. However, oral bacterial diversity decreased; while Enterococcaceae decreased, Enterobacteriaceae increased. The mechanism for GVHD improvement was thought to be largely attributable to the reduction in the absolute quantity of oral bacteria. Thus, while improvement in GVHD was achieved through different intervention methods for oral dysbiosis, the effects on the oral microbiota differed significantly, indicating that caution is required in clinical practice.

V. Basic Data Using Human Patient Specimens and Consideration of Clinical Applications

Based on these findings, we are currently collecting oral and stool samples before, immediately after, and one year following BMT. We are conducting a prospective study to investigate the association between oral dysbiosis and the risk of developing or experiencing severe chronic GVHD.

Discussion & Conclusion

We showed an association between oral dysbiosis and development of cGVHD. Based on our preclinical mechanistic studies, we speculate that oral dysbiosis in patients undergoing BMT promotes oral mucosal damage and expansion of pathological bacteria that subsequently translocate to the draining lymph nodes in which they augment antigen presenting cell and donor T-cell allogeneic immune responses. These activated allo-immune cells may then damage local and distal tissues thereby further promoting bacterial dysbiosis, translocation, and allo-immune responses that establishes a systemic positive feedback loop

aggravating cGVHD. The second entails pathogenic bacteria from the oral cavity seeding the intestinal tract after BMT, thereby worsening cGVHD. Addressing oral inflammation that leads to dysbiosis could foster immunological tolerance and become a therapeutic target for cGVHD.

References

We would like to express our deepest gratitude to all those involved with the Novartis Foundation for Science for their support of this research.

一般の皆様へ

白血病の根治治療として行われる骨髄移植には病気が治った後にQOLの低下や晩期合併症として時に致命的となる慢性移植片対宿主病（慢性GVHD）が併発します。その予防・治療には免疫抑制剤を用いた治療が行われますが過剰な免疫抑制は白血病の再発や感染症などの合併症が増加します。近年腸内細菌叢の乱れがGVHDの増悪に関連していることが明らかとなりましたが、その原因は不明であり、また、その他部位の細菌の影響はさらに不明です。本研究では、骨髄移植患者のうち50−60%の方が有する歯周病＝口腔細菌叢異常が慢性GVHDの発症リスク上昇、悪化に関連することを見出しました。今後は、口腔ケアや歯周病治療による慢性GVHD発症予防・軽減法を確立すことを目標に、研究を続けて参ります。

Does DNA methylation cause skeletal muscle aging?

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Summary Abstract

The age-related decline in skeletal muscle mass and function is known as "sarcopenia." The underlying cause of sarcopenia, skeletal muscle aging, has not been well understood. In this study, we found that increased DNA methylation modifications, a type of epigenetic information, are characteristic of aged skeletal muscle. These findings highlight the significance of DNA methylation in regulating skeletal muscle aging and imply that disruptions in skeletal muscle epigenetic information, particularly the accumulation of DNA methylation, are closely linked to the onset of sarcopenia.

Key Words : Sarcopenia, DNA methylation, aging, epigenetics

Introduction

Sarcopenia, the age-related decline in skeletal muscle mass and function, represents a significant medical and social challenge. Recently, it has been suggested that changes in the epigenomic information of tissues and organs are a hallmark of aging. It is known that global DNA methylation increases with age in human skeletal muscle. However, there has been no evidence of a causal relationship between DNA methylation and muscle aging.

To induce the increase in DNA methylation characteristic of muscle aging, we generated genetically modified (Dnmt3a Tg) mice that overexpress the DNA methyltransferase Dnmt3a specifically in skeletal muscle. Based on these preliminary findings, this study aims to answer the following question: "Does increased DNA methylation in skeletal muscle lead to muscle aging?"

Results

In this study, we generated a new epigenetically modified mouse model in which DNA methylation was specifically accumulated in skeletal muscle (Dnmt3a-Tg: a mouse overexpressing Dnmt3a, a de novo DNA methyltransferase) and investigated the effects of epigenetic information disruption caused by increased DNA methylation on skeletal muscle. The results showed that in epigenetically modified mice with accumulated DNA methylation, there was 1) fast-twitch-specific muscle atrophy (a characteristic of sarcopenia), 2) an increase in the proportion of Myh7+ (Type I) muscle fibers, 3) an increase in the number of perinuclear-positive muscle fibers (a characteristic of disrupted muscle homeostasis), 4) enhanced expression of a group of genes related to cellular senescence and inflammation, including senescence-associated secretory phenotype (SASP) factors, 5) decreased

metabolic resilience, 6) autophagy dysfunction, and 7) decreased mitochondrial protein expression. Furthermore, analysis of motor function via treadmill testing and measurement of muscle weight revealed that age-related declines in skeletal muscle mass and function were accelerated in epigenetically modified mice with accumulated DNA methylation. Interestingly, we found that chronic inflammation in skeletal muscle is synergistically enhanced by aging and the accumulation of DNA methylation. While these analyses suggest that the disruption of epigenetic information due to the accumulation of DNA methylation induces aging-like changes in skeletal muscle, we could not rule out the possibility that, in the aforementioned Dnmt3a-Tg model mice, the increase in DNA methylation from the early stages of muscle development may have directly affected muscle formation itself. Therefore, as a next step, to clarify the causal relationship between increased DNA methylation and skeletal muscle aging-like changes, we conducted an experiment to induce postnatal Dnmt3a overexpression in adult mice using a highly muscle-targeted AAV variant (MyoAAV). The results showed that even when Dnmt3a was overexpressed in the muscle fibers of adult mice using MyoAAV, various aging-like changes were induced in skeletal muscle, similar to those observed in Dnmt3a-Tg model mice. These changes included a decrease in skeletal muscle mass and muscle cross-sectional area, an increase in the number of perinuclear muscle fibers, autophagy dysfunction, and increased expression of genes associated with cellular aging and inflammation. Based on these results, we have demonstrated that the disruption of epigenetic information due to the accumulation of DNA methylation induces aging-like changes in skeletal muscle. These findings highlight the importance of DNA methylation in the regulation of skeletal muscle aging and suggest that the disruption of epigenetic information in skeletal muscle (particularly the accumulation of DNA methylation) is closely associated with the onset of sarcopenia.

Discussion & Conclusion

Mammalian aging is reportedly driven by the loss of epigenetic information; however, its impact on skeletal muscle aging remains unclear. This study shows that aging mouse skeletal muscle exhibits increased DNA methylation, and overexpression of DNA methyltransferase 3a (Dnmt3a) induces an aging-like phenotype. Dnmt3a overexpression resulted in reduced muscle mass and strength and impaired endurance exercise capacity with age, accompanied by an enhanced inflammatory signature. In addition, Dnmt3a overexpression reduced not only sensitivity to starvation-induced muscle atrophy but also the restorability from muscle atrophy. These findings suggest that increased DNA methylation disrupts skeletal muscle homeostasis, promotes an aging-like phenotype, and reduces muscle metabolic elasticity.

一般の皆様へ

加齢に伴う骨格筋の量および機能の低下は「サルコペニア」と呼ばれ、寝たきりや要介護、医療費の増大などにつながることから、社会的・医学的問題となっています。しかし、サルコペニアの原因となる骨格筋老化がどのようにして引き起こされるのか、これまで十分な知見が得られていませんでした。

今回、私たちが行ったマウスを用いた研究から、エピゲノム情報の一つである遺伝子のDNA メチル化修飾の増加が、老化した骨格筋の特徴であることがわかりました。本研究成果は、骨格筋老化制御におけるDNAメチル化の重要性を明らかにし、骨格筋のエピゲノム情報の破綻（特に、DNA メチル化の蓄積）がサルコペニアの発症と密接に関わっていることを示唆するものです。

Elucidation of the signaling mechanism for chloroplast biogenesis regulated by polysulfide

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Summary Abstract

Polysulfides function as signaling molecules by modifying cysteine residues of proteins through polysulfidation. In this study, we investigated how polysulfide signaling affects chloroplast biogenesis in Arabidopsis thaliana. Polysulfide treatment during dark-to-light transition suppressed chlorophyll accumulation and repressed light-induced expression of photosynthesis-associated genes. Transcriptome analysis further revealed broad downregulation of chloroplast-related gene expression. Genetic and molecular analyses suggested that the light signaling regulators HY5 and COP1 participate in this response, indicating that polysulfide signaling modulates chloroplast biogenesis through redox-sensitive regulatory mechanisms.

Key Words : Polysulfide, Chloroplast biogenesis, Photosynthetic gene regulation, Photomorphogenesis, Cysteine modification

Introduction

Polysulfide functions as a universal regulator of physiological processes across diverse organisms through protein polysulfidation. In plants, proteomic analyses in Arabidopsis thaliana have revealed that more than 2,000 proteins undergo polysulfidation, highlighting its significance in plant physiology.

Notably, several key regulators of chloroplast biogenesis have been identified among polysulfidated proteins. Because the chloroplast is a defining organelle of plant cells, focusing on its biogenesis provides a framework for understanding how polysulfide signaling influences plant physiology. This study therefore aims to clarify its role in chloroplast development and its broader physiological significance in plants.

Results

To clarify the role of polysulfide signaling in chloroplast biogenesis, we first examined its effect on photomorphogenesis. Dark-grown seedlings were exposed to light in the presence or absence of polysulfide. Under control conditions, illumination induced normal greening, as evidenced by a rapid increase in chlorophyll accumulation and the activation of photosynthesis-related gene expression. In contrast, polysulfide-treated seedlings exhibited a clear delay in greening, with markedly reduced chlorophyll levels during the early phase of light exposure. This suppression was particularly evident within the initial hours after illumination, suggesting that polysulfide interferes with the early steps of chloroplast differentiation. These observations indicate that polysulfide negatively affects the

establishment of photosynthetic capacity during de-etiolation.

To determine whether this physiological phenotype was associated with transcriptional regulation, we analyzed the expression of representative light-inducible nuclear genes encoding photosynthetic components. Quantitative expression analyses revealed that the light-dependent induction of multiple photosynthesis-associated genes was significantly attenuated in the presence of polysulfide. This effect was consistently observed across genes involved in photosystem assembly, light harvesting, and carbon fixation, indicating that the influence of polysulfide is not restricted to a specific pathway. These results suggest that polysulfide broadly modulates the transcriptional network underlying chloroplast development.

To further assess the global impact of polysulfide on gene expression, we performed transcriptome analysis using light-exposed seedlings treated with or without polysulfide. Comparative analysis revealed widespread transcriptional reprogramming, with a prominent downregulation of genes associated with photosynthesis, chloroplast function, and plastid organization. Functional categorization showed that genes involved in chlorophyll biosynthesis, photosynthetic electron transport, tetrapyrrole metabolism, and chloroplast protein import were particularly sensitive to polysulfide treatment. In addition, several genes encoding chloroplast-localized proteins required for thylakoid membrane formation and plastid gene expression were also significantly repressed. These data demonstrate that polysulfide acts as a negative regulator of the coordinated transcriptional program required for chloroplast biogenesis.

We next sought to identify the regulatory components mediating this polysulfide-dependent response. Given that chloroplast biogenesis is tightly controlled by light signaling pathways, we focused on key regulators of photomorphogenesis. Genetic and molecular analyses indicated that the bZIP transcription factor HY5 and the E3 ubiquitin ligase COP1 are involved in this process. HY5 functions as a central positive regulator that promotes the expression of genes required for chloroplast development, whereas COP1 acts as a negative regulator in darkness by targeting HY5 and related factors for proteasomal degradation. Our results suggest that polysulfide affects this regulatory module, thereby influencing downstream transcriptional responses and chloroplast formation.

Notably, COP1 has been reported to undergo redox-dependent regulation through cysteine modifications, such as S-nitrosylation, which alter its activity. Because polysulfidation also targets cysteine residues and can affect protein conformation and function, COP1 represents a plausible target of polysulfide signaling. The observed suppression of light-induced chloroplast development is consistent with a model in which polysulfide modulates COP1 activity, either directly or indirectly, thereby altering the balance between positive and negative regulators of photomorphogenesis.

Taken together, these results demonstrate that polysulfide suppresses chloroplast biogenesis at both physiological and transcriptional levels. This suppression involves widespread inhibition of photosynthesis-related gene expression and is mediated, at least in part, through key components of the light signaling pathway, including HY5 and COP1.

Discussion & Conclusion

This study demonstrates that polysulfide negatively regulates chloroplast biogenesis by suppressing chlorophyll accumulation and inhibiting the light-induced expression of photosynthesis-related genes during photomorphogenesis. Transcriptome analysis further revealed that this effect extends broadly to chloroplast- and photosynthesis-associated genes, indicating that polysulfide modulates the transcriptional program required for chloroplast development.

The involvement of HY5 and COP1 indicates that polysulfide signaling intersects with the canonical light signaling pathway. Rather than acting simply as an inhibitory factor, polysulfide likely functions as an additional regulatory layer that modulates chloroplast biogenesis. The presence of polysulfide signaling alongside light-dependent regulation may contribute to the robustness of chloroplast development.

However, the molecular mechanisms underlying this regulation remain largely unclear. In particular, it is unknown whether COP1 activity is directly regulated by polysulfidation. In addition, the origin, intracellular dynamics, and transmission mechanisms of polysulfide signals have yet to be elucidated. Addressing these questions will be essential for understanding how polysulfide signaling is integrated into the regulatory network controlling chloroplast biogenesis.

一般の皆様へ

ポリスルフィドは、硫黄がつながった分子で、さまざまな生体機能を調節します。本研究では、植物とポリスルフィドの関係を明らかにしました。暗所で育てた黄化芽生え（もやし）に光を当てると葉緑体ができます。この制御にポリスルフィドが関わることを明らかにしました。さらに遺伝子解析　から、この影響が光合成に関わるしくみに広く及ぶこともわかりました。本研究の成果は、ポリスルフィドによる葉緑体や光合成機能の高機能化という新たな農業応用の可能性も秘めています。

Unraveling the Mechanism of Cardiac Fibrosis through the Cellular Lineage of Macrophages

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Summary Abstract

Key Words : Cardiac fibrosis, tissue macrophage, endocardial hematopoiesis, monocyte

Introduction

The immune system plays a pivotal role in maintaining tissue homeostasis, with macrophages serving as central mediators across diverse organs. In the heart, two major macrophage lineages coexist: embryonically derived tissue-resident macrophages and macrophages replenished postnatally by bone marrow–derived monocytes. We previously identified a distinct population of macrophages derived from endocardial hematopoiesis through an Nkx2-5-dependent pathway (Liu et al., Nat Commun, 2023). During embryonic heart development, these endocardial-derived macrophages contribute to endocardial cushion remodeling. Although these cells persist long-term in the adult heart, their postnatal function has remained largely unclear.

Results

In this study, we investigated whether cardiac macrophages exert lineage-dependent functions, focusing on endocardial-derived macrophages and monocyte-derived macrophages. To examine the function of endocardial-derived macrophages, hereafter referred to as Endo-Mac, we analyzed Nkx2-5 lineage-tracing mice (Nkx2-5-Cre; R26-tdTomato) and Endo-Mac–deficient mice (Nkx2-5-Cre; Csf1r-fl/fl). Consistent with our previous reports, Endo-Mac were detected in the endocardial layer of cardiac valves (Shigeta et al., Dev Cell, 2019; Liu et al., Nat Commun, 2023). In addition, we found that Endo-Mac were also localized in the adventitial region of coronary vessels, in close association with vascular smooth muscle cells. Based on this localization, we hypothesized that Endo-Mac contribute to the maintenance of coronary vessel homeostasis.

To test this hypothesis, we subjected control and Endo-Mac–deficient mice to transverse aortic constriction (TAC), a model that induces pressure overload and perivascular fibrosis. Endo-Mac depletion exacerbated perivascular fibrosis and impaired cardiac function after TAC, suggesting that Endo-Mac exert a cardioprotective role by suppressing excessive perivascular fibrotic remodeling. Notably, detailed histological analysis revealed that fibrotic lesions in Endo-Mac–deficient hearts contained fewer Ccr2-positive profibrotic macrophages

after TAC. These findings suggest that the protective function of Endo-Mac is not simply mediated by preventing the accumulation of Ccr2-positive macrophages, but rather by directly modulating the fibrotic response in the perivascular niche.

In parallel, we generated a Cre-dependent Csf1 overexpression mouse model to increase tissue macrophage populations in a lineage-specific manner. Crossing this model with Nkx2-5-Cre increased Endo-Mac in the heart, whereas crossing it with aMHC-MerCreMer induced the accumulation of monocyte-derived macrophages, hereafter referred to as Mono-Mac. Strikingly, Mono-Mac induction caused a marked progression of cardiac fibrosis, whereas Endo-Mac expansion did not result in overt pathological remodeling. These results further support the concept that cardiac macrophages have distinct functional properties depending on their cellular origin. Moreover, when Mono-Mac induction was performed in Ccr2-deficient mice, the cardiac fibrosis phenotype was significantly attenuated, demonstrating that Ccr2-positive Mono-Mac are a direct driver of cardiac fibrosis in this model.

Importantly, long-term observation of Mono-Mac–induced hearts revealed that the fibrosis underwent spontaneous resolution by six months after tamoxifen administration. This finding indicates that cardiac fibrosis induced by Ccr2-positive Mono-Mac is not necessarily irreversible, but can be resolved under sustained Csf1 stimulation. To identify macrophage state transitions associated with fibrosis resolution, we performed single-nucleus RNA sequencing of whole hearts during both the fibrosis progression phase and the resolution phase. This analysis identified a unique macrophage cluster that was increased during fibrosis resolution. Further transcriptomic analysis suggested that this macrophage population may promote lymphangiogenesis. Given that lymphangiogenesis has been implicated in cardiac repair after injury (Vieira et al., J Clin Invest, 2018), these findings suggest that macrophage-mediated lymphangiogenesis may contribute to the resolution of cardiac fibrosis. Notably, a similar macrophage population was also detected in an independent cardiac injury model, TAC, suggesting that this population is not specific to our genetic model but may represent a more general reparative macrophage state involved in cardiac tissue remodeling.

Together, these findings reveal that cardiac macrophages exert lineage-dependent functions in fibrosis progression and resolution, and identify a previously unrecognized reparative macrophage state that may promote cardiac tissue remodeling through lymphangiogenesis.

Discussion & Conclusion

This study demonstrates that cardiac macrophages exert distinct functions depending on their cellular origin and tissue state. While macrophages have often been broadly categorized as inflammatory or reparative cells, our findings suggest that their roles in the heart are more precisely defined by their developmental origin, local tissue niche, and state transition during disease progression and repair.

We found that endocardial-derived macrophages contribute to perivascular homeostasis and protect the heart from excessive fibrotic remodeling after TAC. In contrast, Ccr2-positive monocyte-derived macrophages directly promoted cardiac fibrosis in a Ccr2-dependent manner. Importantly, this fibrosis underwent resolution over time, suggesting that monocyte-derived macrophages are not fixed pathogenic cells but can transition toward tissue-

reparative states within the cardiac environment.

Together, these findings reveal a lineage- and state-dependent framework for understanding cardiac macrophage function. This study further challenges the conventional view that cardiac fibrosis is an irreversible pathological process and suggests that macrophage state transition, potentially coupled with lymphangiogenic remodeling, may actively contribute to fibrosis resolution.

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一般の皆様へ

本研究では、心臓のマクロファージという免疫細胞が、線維化にどう関わるのかを調べました。その結果、胎児期の心臓で生まれたマクロファージと、成体後に血液から入り込むマクロファージでは、働きが大きく異なることが分かりました。この成果は、心臓病の進行や修復に、胎児期の細胞環境が成体後も関わる可能性を示しています。

また、従来「元に戻らない」と考えられてきた心臓の線維化が、免疫細胞の働きで修復され得ることも示唆されました。将来的には、マクロファージの由来や状態を制御することで、新しい心不全治療につながることが期待されます。

Novel inhibitory signaling mechanism of meristematic activities in plants

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Summary Abstract

This study investigates the molecular mechanisms and evolutionary conservation of the TPD1–EMS1 signaling pathway, a novel negative regulator of plant meristem activity. Using *Marchantia polymorpha*, we established a robust chemical induction system for TPD1 expression. Ectopic TPD1 activation suppressed thallus branching, supporting the hypothesis that TPD1–EMS1 counterbalances positive growth regulators. This induction platform provides a critical foundation for future transcriptomic analyses to identify downstream target genes.

Key Words : Meristem regulation; Negative regulator; TPD1-EMS1 signaling; *Marchantia polymorpha*; Evolutionary conservation

Introduction

Plant growth depends on meristematic activity, whose regulation has been partially uncovered through the discovery of several positive regulators. In contrast, negative regulators remain elusive, although intercellular signaling is thought to play a key role in meristem homeostasis. The LRR-RLK X receptor sub-family perceives chemically diverse ligands (1). Among them, the EMS1 receptor that recognizes the TPD1 peptide is highly conserved, yet its developmental role in non-vascular plants remains poorly understood. Using *Marchantia polymorpha,* we show that the TPD1–EMS1 pathway acts as a negative regulator of meristem activity and thallus branching and investigate its underlying mechanisms and evolution.

Results

The primary objective of this research was to identify the downstream target genes of the TPD1–EMS1 signaling pathway (*Targets of TPD1*; *TOT*) in *Marchantia polymorpha*. To narrow down *TOT* candidates, we aimed to capture the immediate, primary transcriptional responses triggered by TPD1 activation while bypassing growth arrest and severe developmental pleiotropy often associated with constitutive gene overexpression. We successfully utilized a two-component chemical induction system for TPD1 (Mp*E2F>>TPD1*) (2). This line allows chemical induction of the TPD1 ligand gene in dividing cells under the spatial control of a β-estradiol-dependent transcription system.

To achieve high resolution in the identification of differentially expressed genes from transcriptome data, the chemical induction conditions were meticulously examined and optimized. Our induction system can be influenced by the timing and method of chemical application, as well as by the precise concentration of β-estradiol. These parameters strongly affect both the sensitivity of gene expression and the specificity of downstream transcriptional profiles, and inappropriate settings can generate high background noise that may mask subtle changes in lowly expressed genes. Therefore, we conducted extensive optimization experiments by varying β-estradiol concentrations across a broad range and systematically evaluating the duration of exposure under standardized growth chamber conditions.

Thorough examination of these parameters defined a spectrum from strong to weak induction conditions. Strong induction led to pronounced suppression of thallus branching by inhibiting meristematic activity, whereas weak induction caused only modest morphological alterations without completely suppressing branching. These different strengths of signal induction can be used in combination and will be extremely useful for targeting genes with varying potencies and expression levels in future transcriptome analyses.

Specifically, under the optimized strong induction conditions, Mp*E2F>>TPD1* plants exhibited marked suppression of thallus branching at the apical notch. This phenotype is the exact opposite of the tpd1 loss-of-function mutant, which is characterized by hyper-branching. To validate this observation, we are currently conducting a quantitative analysis of branching frequency and the total number of apical meristems per thallus over a defined cultivation period.

In parallel, we analyzed preliminary transcriptome data obtained under strong induction conditions and mock controls. These data were further compared with transcriptome profiles from wild-type and *tpd1* mutant plants. This cross-comparison yielded a set of genes whose expression was altered in response to TPD1 activity, which we defined as part of the *TOT* candidate set. Reporter lines for these genes were generated, and we are currently examining the effects of the *tpd1* mutation on their expression patterns.

To better analyze the effects of the *tpd1* mutation on the expression patterns of *TOT* candidates, we examined the spatial and temporal expression of *TPD1* and *EMS1*, focusing on thallus meristems. Preliminary analyses revealed broader *EMS1* expression. Therefore, we concentrated on further characterizing *TPD1* expression patterns. These analyses revealed highly dynamic changes in *TPD1* expression during thallus branching.

These clear phenotypic observations, together with the expression dynamics, strongly support our hypothesis that the TPD1–EMS1 signaling pathway functions as a key negative regulator that suppresses meristem activity and limits branching in *M. polymorpha*. Furthermore, successfully capturing this distinct, reproducible phenotype demonstrates that chemically induced TPD1 protein is fully functional, properly secreted, and capable of activating the downstream intracellular signaling cascade within the designated timeframe, thereby establishing a solid experimental foundation for the subsequent steps of this project

Discussion & Conclusion

Our phenotypic analysis of the Mp*E2F>>TPD1* line demonstrated that ectopic activation of the TPD1–EMS1 pathway induces clear suppression of thallus branching. This critical finding provides strong support for our hypothesis that this signaling system functions as a novel negative regulator of meristem activity in *Marchantia polymorpha* and effectively counterbalances positive regulators such as auxin and CLE2 to fine-tune plant development (3).

Although large-scale transcriptomic sequencing is currently in preparation due to our meticulous optimization of chemical induction conditions, the robust experimental platform established during this funding period ensures high-quality data generation. In the immediate next phase, we will rapidly validate the *TOT* candidate genes. Ultimately, deciphering this molecular mechanism in *M. polymorpha* will pave the way toward understanding evolutionarily conserved mechanisms governing plant stem cell regulation.

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一般の皆様へ

道端や庭先で見かける植物「ゼニゴケ」は規則正しく枝分かれして成長しますが、そのプロセスは謎に包まれています。これまでの研究では主に「成長を促すアクセル」のシステムが注目されてきましたが、私は植物自身が成長を抑える「ブレーキ」の役割に注目しました。このブレーキ遺伝子を人工的に働かせたところ、ゼニゴケの枝分かれをピタリと止めることに成功したのです。

この発見は、植物がアクセルとブレーキの絶妙なバランスで成長する生命の不思議を紐解く大きな一歩です。今後は他の植物でもこの共通のメカニズムを調べ、将来の作物増産や緑化技術の開発へとつなげます。

Elucidation of the molecular recognition mechanism of PFAS by human heart-type FABP

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Summary Abstract

PFAS are persistent pollutants that bioaccumulate through interactions with fatty acid-binding proteins (FABPs). However, the molecular basis of their recognition remains unclear. Here, we report ultra-high-resolution crystal structures of human heart-type FABP (FABP3) complexed with two perfluoroalkyl acids (PFAAs) and their corresponding medium-chain fatty acids (MCFAs). Fluorescence displacement assays revealed that PFAAs bind FABP3 with higher affinity than MCFAs. Structural analyses showed that PFAAs adopt conformations resembling MCFAs but engage in distinct dipole-mediated interactions between CF_2 chains and water molecules in the binding pocket. These interactions suppress fluctuations of the carboxyl moiety and stabilize the ligand. Our findings indicate that FABP3 recognizes PFAAs through a mechanism partially shared with fatty acids, yet driven by uniquedipole-interactive effects. This work provides structural insight into PFAS recognition and highlights their potential to competitively inhibit fatty acid binding *in vivo*.

Key Words : fatty acid binding protein, FABP3, PFAS, perfluoroalkyl acid, protein structure

Introduction

Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent pollutants associated with growing concern regarding human exposure and toxicity [1–6]. Perfluoroheptanoic acid (PFHpA) and perfluorooctanoic acid (PFOA) are representative perfluoroalkyl acids (PFAAs) in which all hydrogen atoms of a fatty-acid hydrocarbon chain are replaced by fluorine (Fig. 1) [7–9]. Because of their structural similarity to fatty acids, PFAAs can bind fatty acid-binding proteins (FABPs) and may interfere with fatty-acid transport and metabolism [10–13]. FABP3 is highly expressed in muscle and neuronal tissues [14–17] and recognizes fatty acids via two internal water clusters within a U-shaped binding pocket [18,19]. A central question is how a perfluoroalkyl chain, which does not participate in classical hydrogen bonding, can contribute binding free energy distinct from a CF_2 alkyl chain, and how ordered solvent is involved [22]. Here we combine ANS displacement assays with sub-Å/near-atomic crystal structures of FABP3 bound to PFAAs (PFHpA and PFOA) and matched MCFAs (C7:0 and C8:0).

Results

ANS displacement assays established clear differences in FABP3 binding between medium-chain fatty acids (MCFAs) and perfluoroalkyl acids (PFAAs). Consistent with chain-length effects for hydrocarbon ligands, C8:0 inhibited ANS binding more effectively than C7:0, whereas both MCFAs were far weaker competitors than an LCFA reference (C16:0) [18]. In striking contrast, full fluorination enhanced binding at identical carbon counts: PFHpA and PFOA displayed substantially lower IC_{50} values than the corresponding MCFAs, demonstrating that hydrogen-to-fluorine substitution converts weakly binding MCFA-length ligands into markedly higher-affinity FABP3 ligands. Notably, the magnitude of affinity enhancement was greater for PFHpA than for PFOA, indicating that fluorination contributes an additional stabilizing component beyond hydrophobic surface area alone.

To define the structural basis of these trends, we determined FABP3 complex structures with C7:0, C8:0, PFHpA, and PFOA at sub-Å/near-atomic resolution. The overall FABP3 fold was conserved in all complexes, and superposition showed that binding of PFAAs and MCFAs did not induce significant global conformational changes in the protein scaffold (Fig. 4). This observation is important because it indicates that the fluorination-dependent affinity gain is not explained by large-scale induced fit.

For the MCFA complexes, electron density was well defined at sub-angstrom resolution (0.90 Å for C7:0 and 0.86 Å for C8:0). In both structures, the carboxylate head group was anchored by Arg126 and two conserved waters (W5001 and W5008), and the hydrocarbon chain adopted the characteristic U-shaped conformation within the pocket [18,19]. The internal water architecture was also conserved: Cluster-1 formed a stable hydrogen-bond network, whereas Cluster-2 was less ordered, consistent with established FABP3 chain-length selectivity [18,19]. Thus, for MCFAs, both ligand placement and internal solvent organization closely matched those observed in LCFA–FABP3 complexes.

For the PFAA complexes, structures were determined at ultra-high/near-atomic resolution (1.00 Å for PFHpA and 1.05 Å for PFOA) with clear omit electron density (Fig. 5). Structural analysis revealed that, although PFAAs are chemically distinct from MCFAs, they bind at nearly identical locations within the FABP3 pocket. The anchoring geometry of the head group was preserved: the carboxylate interacted with Arg126 and the same conserved waters (W5001 and W5008), indicating that differences in affinity do not arise from a different head-group binding mode. Compared with the close similarity between the two MCFAs, the two PFAAs exhibited a larger ligand RMSD, primarily reflecting conformational distortion of PFOA near the pocket terminus, consistent with steric constraints around the E/F loop region (e.g., proximity to Asp76).

We next performed matched active-site comparisons (PFHpA vs C7:0; PFOA vs C8:0) to evaluate how fluorination affects the internal solvent environment. Despite the distinct physicochemical properties of perfluoroalkyl versus hydrocarbon chains, the membership and overall architecture of Cluster-1 were largely preserved in the PFAA complexes. Importantly, however, Cluster-1 water oxygen positions were reproducibly displaced outward relative to the MCFA complexes, and the calculated pocket volume increased (approximately 1.2-fold) upon PFAA binding. Average contact distances between Cluster-1 waters and the chain region remained similar for CH_2 and CF_2 segments (~3.1 Å), consistent with subtle geometric adjustments that maintain preferred packing distances while accommodating differences in chain chemistry and size. Together, these results show that PFAAs and MCFAs occupy

the same binding site with conserved head-group anchoring and without global protein rearrangement, while PFAA binding is accompanied by small but consistent solvent-coupled shifts within the pocket.

Discussion & Conclusion

This study provides matched, solvent-inclusive comparisons of PFHpA versus C7:0 and PFOA versus C8:0 in human FABP3. Fluorescence competition assays show that full fluorination markedly enhances binding at identical carbon counts, converting weakly binding MCFA-length ligands into higher-affinity FABP3 ligands. The sub-Å/near-atomic structures explain why this occurs without invoking a different binding site or major induced fit. PFAAs and MCFAs occupy nearly identical positions and share the same head-group anchoring motif (Arg126 with W5001 and W5008), and the overall FABP3 scaffold remains globally unchanged [18,19,28]. The key difference is observed in the pocket solvent environment: although perfluoroalkyl chains are chemically distinct from hydrocarbon chains, the internal Cluster-1 water-network architecture is largely retained, while water positions and the cavity geometry shift subtly but reproducibly. The accompanying pocket expansion and small water displacements indicate that FABP3 accommodates perfluoroalkyl chains primarily through fine, solvent-coupled adjustments rather than large protein rearrangements. This framework rationalizes the strong fluorination effect on affinity and the weaker chain-length dependence for PFHpA versus PFOA relative to C7:0 versus C8:0. Overall, our results establish a structural basis for PFAA recognition by FABP3 in which conserved internal waters remain an integral part of the binding pocket, supporting the possibility that PFAAs can competitively affect fatty-acid binding in vivo and motivating extension to longer-chain PFAS and other FABP subtypes [23].

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一般の皆様へ

PFAS（ピーファス）は分解されにくく、環境中に長く残るため「永遠の化学物質」とも呼ばれます。私たちは、PFASの一種（PFHpA、PFOA）が、ヒト体内で脂肪酸を運ぶタンパク質FABP3にどう結合するかを調べました。実験の結果、同じ長さの脂肪酸よりもPFASの方が、FABP3に強く結合することが分かりました。

さらに、非常に高い精度の結晶構造解析で、PFASは脂肪酸とほぼ同じ場所に結合することを確認しました。重要なのは、タンパク質が脂肪酸よりも強くPFHpA、PFOAと結合し、さらに脂肪酸とPFASをほぼ同じものと認識している可能性が高いことです。 今後は、より安全だとされているPFAS代替物で研究を進める予定です。

Breath biopsy for nonalcoholic steatohepatitis

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Summary Abstract

Ferroptosis, an iron-dependent cell death mechanism characterized by excessive lipid peroxidation, has been implicated in numerous human diseases and organ pathologies[1,2.] In this study, we employed oxidative volatolomics to comprehensively characterize the volatile oxidized lipids (VOLs) produced during ferroptosis. Polyunsaturated fatty acid-derived VOLs were generated via iron-dependent lipid peroxidation (LPO) and released extracellularly as ferroptosis progressed[3]. These VOLs were specifically generated during hepatic ferroptosis in Non-Alcoholic SteatoHepatitis (NASH) and were also detectable in the exhaled breath of patients with NASH. Specific VOLs released upon iron-dependent LPO are potential markers of ferroptosis in vivo and may facilitate noninvasive monitoring of cellular health in humans.

Key Words : nonalcoholic steatohepatitis, Ferroptosis, Breath analysis, Volatile oxidized lipid

Introduction

NASH is a serious disease with a high prevalence—progressing to cirrhosis in over 20% of cases—and a high risk of advancing to hepatocellular cancer. “Lipid peroxidation-dependent cell death: ferroptosis,” a form of cell death believed to involve iron, is closely associated with the progression of NASH. During ferroptosis induction, lipid peroxides with various chemical structures are produced; some of these are low-molecular-weight compounds that are “volatile” at human body temperature and are exhale. Therefore, we hypothesized that if we could detect the “volatile lipid peroxides” released by hepatocytes undergoing ferroptosis (ferroptotic hepatocytes) in exhaled breath (breath biopsy), we could detect NASH early and non-invasively. The objective of this study is to target ferroptotic hepatocytes in the liver and establish a breath-based technology for the early screening of NASH.

Results

1. Identification of VOLs released from hepatic ferroptotic cells

In cellular experiments, we attempted to identify the VOLs released from hepatocytes undergoing ferroptosis. We induced ferroptosis in human hepatocellular carcinoma-derived cells (HepG2) using elastin and collected the volatile oxidized lipids released into the gas phase during this process. We performed the analysis of volatile oxidized lipids by connecting Shimadzu Corporation’s (Kyoto, Japan) TD-30R thermal desorption system to a GCMS-QP2010 Ultra mass spectrometer.

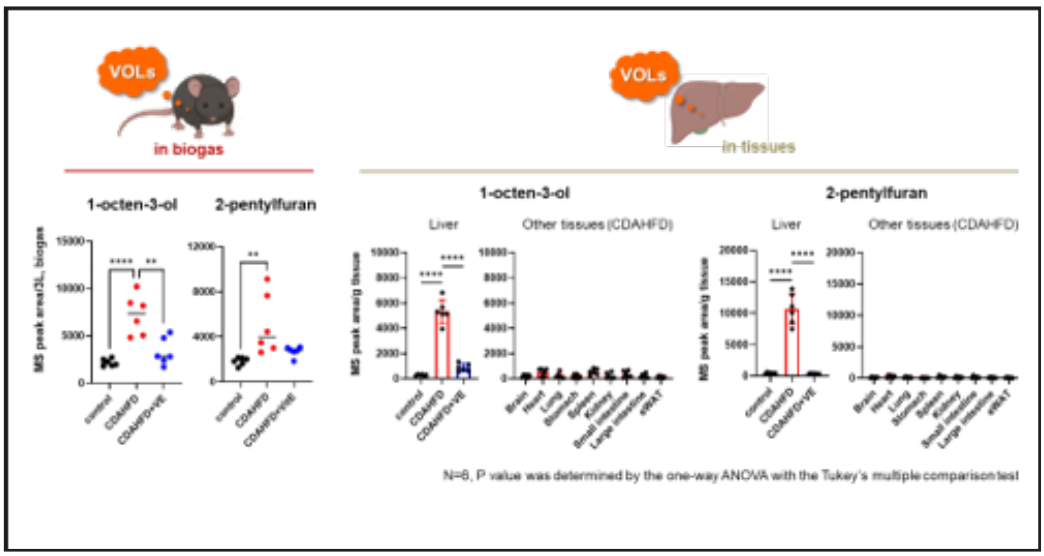
The results revealed a significant increase in several types of oxidized lipids (1-octen-3-ol, and 2-pentylfuran) in the gas phase above the cells. Importantly, these molecules were not observed when other forms of cell death were induced.

2. Validation using NASH model mice

Next, we monitored the levels of VOLs in exhaled breath using diet-induced NASH model mice. We generated diet-induced NASH model mice and collected their exhaled breath using a proprietary experimental system. The levels of VOLs in the exhaled breath were analyzed using a Shimadzu Corporation (Kyoto, Japan) TD-30R thermal desorption system connected to a GCMS-QP2010 Ultra mass spectrometer.

The results revealed a significant increase in the level of the oxidized lipids (1-octen-3-ol, and 2-pentylfuran) in the exhaled breath of NASH model mice. The level of these molecules decreased after the administration of the antioxidant drug vitamin

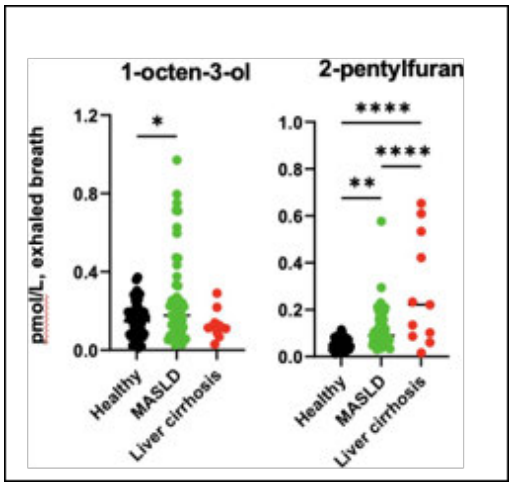
E. Furthermore, these molecules were only released from the liver and not from other organs.



3. Feasibility Verification of NASH Breath Markers (Human Clinical Study)

We investigated whether the identified VOLs were elevated in the exhaled breath of patients with fatty liver, including NASH. Breath VOL analysis was conducted on healthy individuals (n = 48), patients with fatty liver (n = 56), and cirrhosis (LC) patients (n = 11) following the collection of breath samples in thermal desorption tubes and TD GC/MS analysis. Since the analysis of exhaled breath gas is subject to pitfalls—such as VOCs in the ambient air acting as noise, leading to misinterpretation of potential biomarkers, or the high likelihood of overlooking markers useful for low-level biological processes—it is necessary to devise methods for collecting samples while minimizing environmental noise. In this study, we employed a high-performance breath collection device to implement two key strategies: (1) inhalation of air filtered through a HEPA filter, and (2) collection of end-tidal VOCs while monitoring carbon dioxide (to minimize the collection of breath gases present in the dead space). These measures enabled the collection of breathVOCs suitable for highly accurate analysis

Patients with fatty had significantly higher ferroptosis-related VOLs levels. Specifically, 1-octen-3-ol levels were elevated in patients with fatty liver compared to those in healthy individuals ($p = 0.0143$); however, this increase was suppressed in patients with LC. Meanwhile, 2-pentylfuran levels were elevated in fatty liver and LC ($p = 0.0022$ for healthy vs. fatty liver, $p < 0.0001$ for healthy vs. LC, and $p < 0.0001$ for fatty liver vs. LC). In addition, 2-ethylfuran was significantly elevated in the LC group ($p < 0.0001$ for healthy vs. LC and $p = 0.0008$ for fatty liver vs. LC).



Combining these markers enabled discrimination between healthy individuals and those with fatty liver with area under the curve (AUC) values of 0.94.

Discussion & Conclusion

Until now, liver biopsy has been the only method for diagnosing NASH, but non-invasive methods are currently being developed. Liver stiffness measurements using the “Fibroscan” device have been reported to correlate with histopathological findings and are listed in guidelines as one method for distinguishing NASH. However, this technology has drawbacks, such as the need for medical personnel and significant measurement errors depending on the operator. Furthermore, while liver stiffness assesses the late phase of NASH and reflects advanced fibrosis, our technology is suggested to capture the pre-pathological state and early phase of NASH; if clinically applied, it could lead to early detection and therapeutic intervention. Because this technology is non-invasive and easy to perform, it can be applied to all patients with asymptomatic fatty liver disease, facilitating early diagnosis. This will significantly reduce the number of cases overlooked due to undiagnosed conditions and help prevent cases from progressing to irreversible cirrhosis and liver cancer.

Conclusions

We employed an innovative analytical platform, oxidative volatolomics, to identify VOLs (1-octen-3-ol, and 2-pentylfuran) specifically released during ferroptosis. Using these markers, we developed a technology for the noninvasive detection of ferroptosis in vivo. We anticipate that the markers and detection technologies developed in this study will be utilized and further developed, contributing to the

noninvasive and early detection of ferroptosis-related diseases.

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一般の皆様へ

非アルコール性脂肪肝炎（NASH）は、肝臓に脂肪が蓄積し炎症を起こしている状態で、放置すると20%を超える高頻度で肝硬変や肝癌に進行する重要な病態です。ところが、現状 NASH の唯一の診断方法が肝生検であるため、容易には実施できず多くの症例が不可逆的な肝障害の出現まで見落とされているのが現状です。NASH の病態進行には、酸化脂質依存的細胞死（フェロトーシス）が密接に関与します。我々は、フェロトーシス誘導時に産生される酸化脂質の一部は「揮発性」を有し、呼気として体外へ排出されることを見出してきました。本研究では、これまでに申請者が開発した酸化脂質解析技術を拡張・応用し、「呼気による NASH 早期スクリーニング技術」を確立することです。

Effect of rRNA modification on ribosome rescue system

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Summary Abstract

We investigated the role of rRNA pseudouridine modifications in ribosome rescue using biochemical and structural approaches. We found that three pseudouridines in 23S rRNA are specifically required for stop codon-independent peptidyl-tRNA hydrolysis without affecting ribosome binding. Kinetic and structural analyses suggest that these modifications play a critical role in post-binding steps of this rescue pathway. These findings reveal a previously unrecognized contribution of rRNA modification to ribosome rescue mechanisms.

Key Words : Ribosome, Pseudouridine, rRNA modification

Introduction

Ribosome stalling frequently occurs during translation due to truncated mRNAs lacking stop codons. In bacteria, stalled ribosomes are rescued by specialized systems such as tmRNA and ArfA[1,2]. In addition to these pathways, a distinct ribosome rescue factor has been identified that functions independently to catalyze peptidyl-tRNA hydrolysis[3]. Unlike other systems, this factor does not require partner molecules, suggesting a unique mechanism of action. Although structural studies have provided insights into ribosome–factor complexes, the contribution of ribosomal RNA modifications to this process remains unclear. In this study, we investigated the role of pseudouridine modifications in ribosome rescue.

Results

To investigate the role of rRNA modifications in ribosome rescue, we performed a combination of biochemical and structural analyses using a reconstituted in vitro translation system. This system, in which all translation components are purified and individually controlled, allows precise reconstruction of defined ribosome complexes and enables quantitative analysis of specific reaction steps that are difficult to isolate *in vivo.*

Previous structural studies of ribosome rescue complexes have shown that rescue factors bind in proximity to several modified nucleotides in 23S rRNA. In particular, three pseudouridine residues located near the factor-binding site were identified as potential contributors to function. These pseudouridines are clustered in functionally important regions of the ribosome, suggesting that they may play a role in fine-tuning ribosome-associated reactions.

To assess their functional significance, we prepared ribosomes lacking these pseudouridine modifications. Using *in vitro* assays, we first examined general translation activity and found

that the absence of these modifications does not significantly affect canonical translation. We also tested other ribosome rescue pathways and observed no substantial defects, indicating that the overall structural integrity and basic functions of the ribosome are largely preserved in the absence of these modifications.

In contrast, the activity of a specific ribosome rescue factor was selectively impaired. Further biochemical analyses using a reconstituted system demonstrated that these pseudouridine modifications are required for efficient peptidyl-tRNA hydrolysis in this pathway. In these assays, stalled ribosome complexes were assembled under defined conditions, and factor-dependent hydrolysis was monitored as a readout of rescue activity. Importantly, binding assays indicated that the absence of pseudouridine does not significantly affect the affinity of the rescue factor for the ribosome.

These results suggest that the modifications are not required for the initial binding of the factor but instead contribute to a subsequent step required for productive ribosome rescue. This functional distinction indicates that rRNA pseudouridine modifications play a specific role in facilitating post-binding events, such as the proper positioning or activation of the catalytic center. Together, these findings indicate that rRNA pseudouridine modifications play a specific and essential role in this rescue pathway, distinguishing it from other ribosome rescue mechanisms that rely on different molecular strategies.

To gain structural insight into this effect, we are currently performing cryo-electron microscopy analyses of the corresponding ribosome–factor complex using ribosomes lacking these modifications. These analyses are ongoing and are expected to reveal how the absence of pseudouridine affects the positioning and functional engagement of the factor on the ribosome.

In parallel, we have developed a site-specific hydroxyl radical probing approach using cysteine-substituted variants of the rescue factor. A large set of mutants has been generated, and their biochemical activities are being evaluated. This approach enables residue-level mapping of the spatial relationship between the factor and ribosomal RNA and provides complementary information to structural analyses.

Taken together, our results establish that specific rRNA modifications are selectively required for a subset of ribosome rescue pathways and provide a framework for understanding how ribosomal RNA contributes to translation quality control mechanisms. These findings highlight a previously underappreciated role of RNA modification in regulating factor-dependent ribosome rescue.

Discussion & Conclusion

Our results demonstrate that pseudouridine modifications in 23S rRNA play a specific role in ribosome rescue. The three target residues (ψ1911, ψ1915, and ψ1917) are highly conserved across species, although ψ1911 is not conserved in archaea, suggesting both conserved and lineage-specific contributions. In bacteria, ψ1915 is further modified by methylation, highlighting the functional importance of this region. Previous studies have shown that these modifications also influence translation termination efficiency by RF2, particularly at UGA codons such as in the rpsG gene.

Together with these findings, our results suggest that this rRNA region constitutes a

critical functional hub that supports multiple translation-related processes. Notably, our data indicate that these modifications are specifically required for post-binding steps in a subset of ribosome rescue pathways, revealing a previously unrecognized role of rRNA modification in translation quality control.

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一般の皆様へ

私たちの細胞では、タンパク質を作る過程で時々「翻訳の停滞」というトラブルが起こります。このような異常が起きると、細胞の働きに大きな影響を与えるため、細胞にはこれを解消する仕組みが備わっています。本研究では、この「リボソームレスキュー機構」に着目し、RNAのわずかな化学修飾がこの仕組みに重要な役割を果たしていることを明らかにしました。これまで見過ごされがちであったRNAの修飾が、タンパク質合成の精度や効率に深く関わっている可能性が示されました。今後はこの仕組みをさらに詳しく解明することで、生命の基本原理の理解や、新たな創薬の基盤につながることが期待されます。

Elucidation of the pathogenesis of Parkinson's disease and development of curative compounds

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Summary Abstract

Parkinson’s disease is a neurodegenerative disorder caused by the degeneration of dopaminergic neurons in the midbrain. However, its pathophysiological mechanisms remain unclear, and no curative treatment has been established. In this study, I demonstrated that TMD-pd1, a newly identified compound, binds to the target molecule CH2BP1 and alleviates the abnormal localization of the CHCHD2 mutant. Furthermore, TMD-pd1 is effective in neurons derived from PD-familial patient iPSC. The generation of such a drug candidate has not been previously reported, and this finding suggests the potential for drug development not only for familial Parkinson’s disease but also for sporadic Parkinson’s disease.

Key Words : Parkinson's disease, curative compound

Introduction

PARK22 was identified through genetic analysis of large families with Parkinson’s disease, in which multiple genetic mutations were detected. However, no method has yet been established to suppress the protein abnormalities caused by PARK22/CHCHD2 mutations. In previous studies, I demonstrated that [1] while CHCHD2 is expressed in mitochondria, the mutant CHCHD2 T61I is expressed in a dot-like pattern in the cytoplasm outside the mitochondria. [2] Dopaminergic neurons are reduced and motor function is impaired in the brains of CHCHD2 mutant mice. [3] α-synuclein accumulates and colocalizes with mutant CHCHD2 in patient brain tissue and iPS cell-derived neurons (Ref.1).

Results

Through large-scale compound screening, we had previously discovered that some TMD compounds alleviate the localization abnormalities associated with the CHCHD2 mutation and neuronal cell death. Therefore, in this research project, I set the following five objectives: 1. Identification of the target molecules of the TMD-pd1 compound using chemical biology techniques. 2. Validation of the relationship between TMD-pd1 and its target molecules in cells. 3. To verify the pharmacological effects of TMD-pd1 administration in CHCHD2 mutant mice. 4. To further verify the effects of TMD-pd1 compounds in neurons derived from iPSCs of CHCHD2 mutant patients. 5. To identify more potent analogues by structurally modifying TMDs for future drug discovery.

Regarding point 1, we employed a novel experimental technique called photoaffinity labeling. This method allows for the simple covalent binding of the compound to the target molecule via UV light stimulation, thereby stabilizing the bond. We decided to identify candidate intracellular target proteins for the TMD-pd1 compound using IP-MS analysis, which combines this technique with immunoprecipitation and mass spectrometry. The results of the experiment identified a single protein as a clear target of TMD-pd1 using the photoaffinity labeling method, namely CH2BP1, a CHCHD2-binding protein.

Regarding point 2, we verified the binding between the identified target candidate molecule, CH2BP1, and the TMD-pd1 compound using the photoaffinity labeling method again. In the result, we confirmed binding between CH2BP1 and TMD-pd1. Furthermore, we found that the efficacy of the TMD-pd1 compound was reduced in cultured cells in which CH2BP1 was knocked down using siRNA duplexes. Regarding the relationship between the target molecule and CHCHD2, analysis of their intracellular localization and direct binding revealed that CHCHD2 and CH2BP1 colocalize in the same organelle.

Regarding point 3, we administered TMD-pd1 compounds into the brains of CHCHD2 mutant knock-in mice and evaluated their effects on motor abnormalities through behavioral analysis and neuropathology. In CHCHD2 mutant knock-in mice, loss of dopaminergic neurons in the substantia nigra and impaired motor function typically begin to appear around 30 weeks of age. We found that long-term, continuous intracerebral administration of TMD-pd1 via an osmotic pump alleviated the loss of dopaminergic neurons, α-synuclein accumulation, and motor abnormalities observed in CHCHD2 mutant knock-in mice.

Regarding point 4. Furthermore, we verified the effects of TMD-pd1 compounds using neurons derived from iPS cells of CHCHD2 mutant patients. It was previously known that abnormal CHCHD2 localization, α-synuclein accumulation, and neuronal cell death are induced in neurons derived from iPS cells of CHCHD2 mutation patients. We found that TMD-pd1 administration alleviates the abnormal localization of CHCHD2 in dopamine neurons derived from CHCHD2 mutation iPS cells, halts α-synuclein aggregation, and suppresses neuronal cell death.

Regarding point 5. Through collaborative research with a structural analysis group, we conducted structural development of the TMD-pd1 compound, synthesized several new analogues, and analyzed their effects to develop even more potent compounds. As a result, we were able to develop several new compounds, including the highly potent CBT-17, through structural development of TMD-pd1. These compounds are expected to be effective at lower concentrations and reduce side effects.

Discussion & Conclusion

In this study, the efficacy of the TMD-pd1 compound against familial Parkinson's disease caused by CHCHD2 mutations was demonstrated, and the target molecule for the TMD-pd1 compound was identified, revealing its mechanism of action. Since CH2BP1, a CHCHD2-binding protein, was identified as the target molecule for these small-molecule compounds, this discovery is expected to lead to a better understanding of the mechanism underlying the onset of Parkinson's disease. The creation of such drug candidate compounds has not been reported previously, and this finding suggests the potential for drug development not only for familial Parkinson's disease but also for a broader range of sporadic Parkinson's disease cases.

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一般の皆様へ

パーキンソン病は、中脳黒質神経の変性脱落に起因する神経変性疾患の一つですが、その病気の発症メカニズムは未解明でのため、対処療法が行われており、根本治療は未確立なままです。私が注目している遺伝子は、複数の遺伝子変異がパーキンソン病の大規模家系の遺伝子解析から同定されました。

しかしこの変異によるタンパク質異常を抑制する方法も確立できていません。本研究において、私は新規化合物TMD-pd1のパーキンソン病への有用性の解明と、この化合物の標的分子を含めた分子メカニズムを解明しました。このような創薬候補化合物の創出はこれまで報告が無く、今後家族性のみならず孤発性パーキンソン病を含めた創薬に繋がる可能性が示される。

Establishment of the molecular basis for systemic health care based on the identification of exerkines

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Summary Abstract

This research proposal aims to elucidate the pathology of age-related diseases associated with motor aging and develop innovative therapeutic and preventive strategies, based on a deeper understanding of homeostasis, through the demonstration and molecular mechanisms of exerkines that are responsible for motor aging and exercise benefits.

Key Words : 1. Motor aging 2. Exercise benefits 3. Exerkine 4. Inter-organ crosstalk 5. Age-related diseases

Introduction

The decline and loss of motor function, which is increasing with the super-aging, is an extremely serious problem in the human society, as it leads to requiring nursing care, being bedridden, and death. The locomotor organs, bones and muscles, achieve an integrated motor function while maintaining dynamic homeostasis. “Motor aging” such as osteoporosis and sarcopenia leads not only to a decline in physical activity, but also metabolic and neuropsychiatric diseases, which significantly reduce healthspan and longevity. Meanwhile, exercise as therapeutic intervention is effective in improving and preventing these systemic diseases and exerting anti-aging effects. Therefore, understanding the health benefits of exercise “Exercise benefits” will be a game changer in unraveling the mysteries of the dynamic homeostasis and the pathological mechanisms of age-related diseases associated with its dysfunction, improving healthy life expectancy and quality of life, and ultimately reducing the ever-increasing burden of healthcare costs. However, the underlying molecular mechanisms have not yet been elucidated. The concept of “Exerkines” have been proposed as molecules responsible for this inter-organ crosstalk.

Results

This research proposal aims to elucidate the pathology of age-related diseases and develop innovative therapeutic and preventive strategies based on a deeper understanding of systemic homeostasis through molecular mechanisms of exerkines which are responsible for motor aging and exercise benefits. Three project goals will be established: (1) Identification of exerkines that regulate motor aging and exercise benefits (2) Elucidation of mechanisms of

homeostasis and disruption by exerkines.

As a research strategy, we will conduct a single-cell analysis of cells from locomotor organs during the exercise from young to aged mice, identification of target organs/proteins derived from locomotor organs by *in vivo* labeling and proteomic analysis from exercised and/or aged mice to profile the information of expressed genes and proteins. We attempt to construct of “Exerkine Database” and analyze their functions. We also identify novel sensing molecules of exercise from the construction of a comprehensive functional screening system that uses an altered expression of exerkines. By demonstrating the molecular mechanisms of maintenance and disruption of systemic homeostasis *in vivo* by generating genetically modified mice with selected exerkines, their target molecules, intracellular signals, and exercise sensing molecules, as well as locomotor cell-ablated mice, we unravel the mechanisms of spatio-temporal motor aging and exercise benefits. Furthermore, a large human cohort study based on wearable devices will be combined to explore a distinct “Biomarker of motor aging and exercise benefits” in humans. In addition, large-scale screening using chemical libraries and the search for activators and inhibitors of exerkines pave the way for the establishment of emerging therapeutic and preventive strategies of aging-related diseases

To identify the exerkines that regulate motor aging and exercise benefits, we collected tissues and cells from mouse models of aging, exercise and motor dysfunction, and accumulate information on the expression of genes that are differentially expressed during cellular differentiation of locomotor cells, as well as mechanical stimuli, through single-cell RNA-seq analysis and other methods. Furthermore, we characterize all proteins that originate from locomoter organs, reach multiple targets, and are altered in expression with aging and exercise by proteome analysis using non-locomoter organ derived from locomoter-specific *in vivo* labeling mice. By integrating this all genetic and protein information, we performed construct the world's first “Exerkine database”

In this study, we performed an enhancement of motor function (i.e., voluntary exercise, forced treadmill exercise, and reinforced mastication) in young and aged mice. We also generate mouse models of decreased mastication and immobility that simulate bedridden. Postmenopausal model mice (ovariectomy) used because estrogen deficiency in aging women not only leads to osteoporosis and obesity, but also threatens healthy life expectancy by causing various systemic diseases, such as impaired cognitive, memory and learning functions. In addition, high-fat diet-induced obesity and diabetes models will be used, and tissues and cells collected to reveal genes whose exprssion is altered by pathological conditions and exercise stimuli through single-cell RNA-seq, total RNA-seq and epigenetic analyses(unpublished data).

We have identified a novel osteocyte-specific gene (tentative name: osteocyte specific gene 1, OSG1) by comparing osteocyte-expressed genes with the database of gene expression of multiple organs. Subsequently, we have established Cre knock-in mice by taking advantage of OSG1's specificity in osteocytes and have successfully developed the true osteocyte-specific gene modification system. Osteocyte-specific loxP recombination was confirmed in osteocyte-specific labeled mice generated by crossing these novel OSG1-Cre mice with tdTomato reporter mice (which express red fluorescence after Cre-mediated recombination), without nonspecific recombination in non-bone organs (unpublished data). Isolation of osteocytes from these mice enables single-cell RNA-seq analysis and changes in gene expression with aging are being elucidated. The development of osteocyte-specific gene modification system is extremely significant and will be a pioneering and powerful driving force in this area of research.

Discussion & Conclusion

Humankind have believed for centuries that “physical activity and exercise are the basis for maintaining a healthy body and a healthy mind”. However, we have not yet obtained answers to questions such as what mechanisms are driven by locomotor organs and exercise, and act on remote multiple organs to improve health and anti-aging; and what mechanisms can improve and prevent systemic diseases caused by their dysfunction. The scientific evidence on these questions is eagerly awaited at the global level.

This research proposal aims to elucidate the pathology of age-related diseases associated with motor aging and develop innovative therapeutic and preventive strategies, based on a deeper understanding of homeostasis, through the demonstration and molecular mechanisms of exerkines that are responsible for motor aging and exercise benefits.

The outcome of this research proposal is expected to realize not only innovative treatment strategies for age-related diseases, but also a healthy and long-lived society with longevity in which diseases are prevented at an early stage through exercise benefits, and will become intellectual property that should be shared by all humankind.

References

The results of this research are currently being submitted as a scientific paper.

一般の皆様へ

骨粗鬆症やサルコペニアなどの運動器疾患は身体活動の低下を導くだけでなく、精神神経疾患や代謝性疾患を高頻度に合併することで健康寿命を著しく低下させます。一方、身体活動の強化や運動習慣が、全身性疾患の改善や予防、死亡率の低下に繋がることが世界レベルで現在知られています。従って、身体活動や運動による健康への恩恵、すなわち“運動ベネフィット”の理解が、生体恒常性の維持とその破綻に伴う様々な全身性疾患の病因メカニズムの謎を紐解き、健康寿命の向上のゲームチェンジャーになると考えられています。

Publication - FY2024 Grant Recipients

Title of the research project	Self-organization mechanism mediated by control of perivascular mechanical field in angiogenesis
Recipient (Institution)	Koichi Nishiyama (University of Miyazaki, Laboratory for Vascular and Cellular Dynamics, Faculty of Medicine)
Journal article / other material	Nature Communications | (2025) 16:6788 doi.org/10.1038/s41467-025-61804-z
Title of the paper	Biomechanical control of vascular morphogenesis by the surrounding stiffness

Title of the research project	Novel function and regulation of neutrophil-producing IL-23a in the modulation of autoimmune diseases
Recipient (Institution)	Keiji Hirota (Kyoto University, Lab. Integrative Biological Science)
Journal article / other material	Nature immunology 3 March 2026 doi.org/10.1038/s41590-026-02447-0
Title of the paper	Tissue-restricted secondary TCR engagement drives the transition from stem-like to CD200+ Egr2hi arthritogenic Th17 cells
Journal article / other material	Mukoyama et al., Sci. Adv. 12, eaec0986 (2026) 25 March 2026 doi: 10.1126/sciadv.aec0986
Title of the paper	Pathogenic GM-CSF drives functional diversification of inflammatory macrophages in autoimmune arthritis

Title of the research project	Blockage of multi-drug resistance of cancer cells by inhibition of bitter taste receptors
Recipient (Institution)	Motonao Nakamura (Okayama University of Science, Department of Life Science, Graduate School of Science,)
Journal article / other material	Scientific Reports | (2025) 15:27451 doi.org/10.1038/s41598-025-12889-5
Title of the paper	Chemosensory role of intracellular TAS2Rs, the activation of which triggers drug excretion by ABCB1 in cancer cells

Publication - FY2024 Grant Recipients

Title of the research project	Development of In Vivo Antibody-based Drug Production Technology for Dementia Prevention
Recipient (Institution)	Keiichiro Suzuki (Osaka University, Suzuki laboratory)
Journal article / other material	Communications Medicine | (2025) 5:269 doi.org/10.1038/s43856-025-00959-8
Title of the paper	Targeted in vivo gene integration of a secretion-enabled GLP-1 receptor agonist reverses diet-induced non-genetic obesity and pre-diabetes

Title of the research project	Understanding of bone formation mechanisms by genome-wide association study (GWAS) using the hybrids of ornamental medaka
Recipient (Institution)	Yoshihiro Omori (Hiroshima University, Laboratory of Functional Genomics, Graduate School of Integrated Sciences for Life)
Journal article / other material	Molecular Biology and Evolution, 2026, 43, 1.22 doi.org/10.1093/molbev/msag021
Title of the paper	Genomic consequences of domestication and the diversification of body coloration and morphology in ornamental medaka strains

Title of the research project	Elucidation of the molecular recognition mechanism of PFAS by human heart-type FABP
Recipient (Institution)	Sugiyama Shigeru (Kochi University, Research and Education Faculty, Natural Sciences Cluster)
Journal article / other material	International Journal of Biological Macromolecules 2026 doi.org/10.1016/j.ijbiomac.2026.152710
Title of the paper	Intermolecular interactions of perfluoroalkyl acids with human heart-type fatty acid-binding protein

＜論文掲載＞ 2024 年度

助成タイトル	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
受賞者	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）
論文掲載誌・書誌事項	Nature Communications	(2025) 16:6788 doi.org/10.1038/s41467-025-61804-z
論文タイトル	Biomechanical control of vascular morphogenesis by the surrounding stiffness

助成タイトル	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
受賞者	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）
論文掲載誌・書誌事項	Nature immunology 3 March 2026 doi.org/10.1038/s41590-026-02447-0
論文タイトル	Tissue-restricted secondary TCR engagement drives the transition from stem-like to CD200+ Egr2hi arthritogenic Th17 cells
論文掲載誌・書誌事項	Mukoyama et al., Sci. Adv. 12, eaec0986 (2026) 25 March 2026 doi: 10.1126/sciadv.aec0986
論文タイトル	Pathogenic GM-CSF drives functional diversification of inflammatory macrophages in autoimmune arthritis

助成タイトル	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
受賞者	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）
論文掲載誌・書誌事項	Scientific Reports	(2025) 15:27451 doi.org/10.1038/s41598-025-12889-5
論文タイトル	Chemosensory role of intracellular TAS2Rs, the activation of which triggers drug excretion by ABCB1 in cancer cells

＜論文掲載＞ 2024 年度

助成タイトル	認知症予防に向けた抗体薬生体内産生技術の開発
受賞者	鈴木 啓一郎 (大阪大学 鈴木研究室)
論文掲載誌・書誌事項	Communications Medicine | (2025) 5:269 doi.org/10.1038/s43856-025-00959-8
論文タイトル	Targeted in vivo gene integration of a secretion-enabled GLP-1 receptor agonist reverses diet-induced non-genetic obesity and pre-diabetes

助成タイトル	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
受賞者	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)
論文掲載誌・書誌事項	Molecular Biology and Evolution, 2026, 43, 1.22 doi.org/10.1093/molbev/msag021
論文タイトル	Genomic consequences of domestication and the diversification of body coloration and morphology in ornamental medaka strains

助成タイトル	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
受賞者	杉山 成 (高知大学 教育研究部自然科学系理工学部門)
論文掲載誌・書誌事項	International Journal of Biological Macromolecules 2026 doi.org/10.1016/j.ijbiomac.2026.152710
論文タイトル	Intermolecular interactions of perfluoroalkyl acids with human heart-type fatty acid-binding protein

Ⅲ.

Reports from the Recipients of Grants for International Meetings

Report on Research Meeting

Date:30th Sep 2025

1. Name of Research Meeting / Conference
   7th International Workshop on SOX transcription factors

2. Representative
   Masami Kanai-Azuma

3. Opening period and Place
   8th to 11th Sep, 2025

4. Number of participants
   Prince Resort MICE Karuizawa, Nagano, Japan

5. Number of participating countries and areas
   83 people from 17 countries in Europe, North America, Asia, and Oceania

6. Total cost
   10,461,780 yen

7. Main use of subsidy
   Of the total venue setup cost of 730,884 yen for the second day (9th Sep) of the meeting, **400,000 yen** was subsidized by the Novartis Foundation.

8. Result and Impression

   The 7th International SOX Conference (SOX2025) was held at the Prince Resort MICE venue in Karuizawa, Nagano, from September 7 (Day 0) to 11, 2025. This conference is the international meeting dedicated to SOX gene research, and since its inception in 2005, it has continued for 20 years as a valuable forum bringing together leading researchers from around the world. This year marked the 20th anniversary of the conference, and it was the second time it had been held in Japan, the first being in 2008.

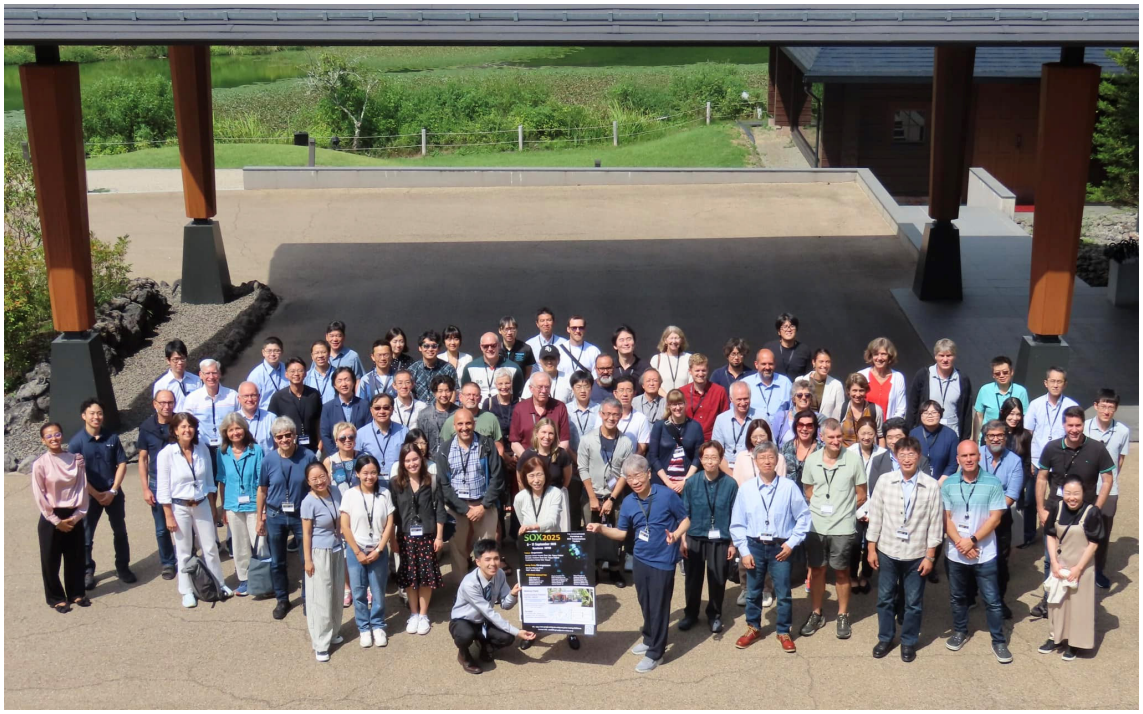
   A total of 83 participants attended, including 46 researchers from overseas, representing more than 17 countries across Europe, North America, Asia, and Oceania. The program featured two plenary lectures, one special lecture, 43 oral presentations, and 22 poster presentations. The realization of such a diverse and international meeting was made possible in large part thanks to the generous support of your foundation.

   The Larysa Pevny Award was presented to two young SOX researchers, and travel awards (six recipients) as well as presentation incentive grants (three recipients) were provided to graduate students and postdoctoral fellows from overseas. These measures greatly encouraged the participation of international doctoral students and early-career postdoctoral researchers. As a result, young scientists from diverse backgrounds were brought together, fostering opportunities for networking that will lead to future international collaborations.

   We would like to express our sincere gratitude to your foundation for the generous support extended to the successful organization of SOX2025.

Additional description

SOX research not only deepens our fundamental understanding of developmental biology but also holds broad potential applications in diverse medical fields, including cancer, regenerative medicine, and genetic disorders. The international networks and emerging collaborations fostered at this meeting will directly contribute to future academic progress and strengthen the research foundation from Japan to the world. The generous support of the foundation has been indispensable in laying this groundwork, and we are confident it will generate far-reaching impact in the long term. It has also been decided by the organizing committee that the next meeting will be held in Hong Kong in 2028.



Report on Research Meeting

Date:August 12th, 2025

1. Name of Research Meeting / Conference
   7th International Conference on Plant Vascular Biology

2. Representative
   Koh Aoki

3. Opening period and Place
   From July 7th to July 12th, 2025, at KKR Hotel Osaka, Osaka

4. Number of participants
   169

5. Number of participating countries and areas
   20

6. Total cost
   19,055,000 JPY

7. Main use of subsidy
   Travel Award (support for travel by undergraduate students, graduate students and early-career professionals)

8. Result and Impression

The main objective of this conference was to provide a forum for researchers to share and discuss new research findings on the structure and function of plant vascular systems. This objective was highly achieved thanks to the high-level presentations and fruitful Q&A sessions by the participants.

In the opening remarks on the first day, Dr. Lucas of the University of California pointed out that "it is important to utilize plant vascular biology in agriculture," and this comment was echoed in the presentations made by the participants that followed.

The highlights of the conference were the sessions on Single Cell Biology and Vascular Stem Cell. In these sessions, approximately 10 research results were presented using the recently developed single-cell gene expression analysis method. There was an in-depth exchange of information on the technical aspects of the latest methods.

In addition to these new attempts, many groundbreaking research results were shared in traditional sessions such as Xylem & Wood, Phloem, and Nutrition & Transport, including the use of recent genome editing techniques and real-time imaging of molecular transport.

Plant vascular biology is deeply related to plant disease resistance. At this conference, much information was shared on topics such as the role of vascular communication throughout the plant in interactions with fungi and plant viruses, and the molecular mechanisms that locally control intercellular communication.

In particular, when there was a presentation on integrated research into molecular transport to increase the nutritional value of cassava in Africa, it was a moment when all participants were able to share an image of the outcomes of Plant Vascular Biology for Agriculture.

Unlike many international conferences, there was no tour for all participants this time, but instead, several small group tours to Dotonbori and Kyobashi were organized, and everyone who attended seemed to enjoy Osaka culture to the fullest. The banquet was also held at a restaurant in the Honmaru Plaza of Osaka Castle, where birthday parties were held to celebrate two people who had made significant contributions to the community and the only high school student participant, making it a harmonious and memorable banquet. Summing up the whole event, on the final day the international advisors gave it high praise, saying it was "one of the most wonderful international conferences I have ever attended."

9.Additional description

Travel Award and Best Poster Award were given to graduate students and early-career researchers.

Travel Award
Marilou Camboue, INRAE, ISVV, France
Yookyung Lim, Chungbuk National U., Korea
Michelle Heeney, Cornell U. USA
Qi Yang Ngai, John Innes Centre, UK
Wang Xu, The University of Queensland, Australia

Best Poster Award
Khadija Rombi, Heinrich-Heine University, Düsseldorf, Germany
Hui Cao, University of Potsdam, Germany
Matias F. Gleason, Cold Spring Harbor Laboratory, USA
Yagmur Hasbioglu, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany
Lucas Müller, Heinrich Heine University Düsseldorf, Germany

Group Photo



Opening Remark



Reception



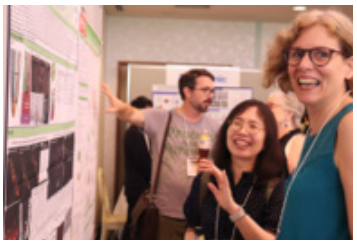
Oral sessions







Poster Session





Lunch Dinner Banquet Celemony






Report on Research Meeting

Date:25/July/2025

1. Name of Research Meeting / Conference
   The 43rd Sapporo International Cancer Symposium

2. Representative
   Seishi Ogawa, Kyoto University
   Yasuhito Nannya, The University of Tokyo

3. Opening period and Place
   5/July/2025 ~ 6/July/2025, Grand Mercure Sapporo Odori Park

4. Number of participants
   113

5. Number of participating countries and areas
   UK 2, Korea 2, US 1, Canada 1, Japan 107

6. Total cost
   8,620,000 yen

7. Main use of subsidy
   2,479,140 Venue usage fees
   1,740910 Overseas speakers'' travel expenses
   419,100 Overseas speakers''accommodation costs
   2,075,935 Administrative expenses
   845,878 Brochure, website
   1,020,540 Speaker honorarium

8. Result and Impression

The 43rd Sapporo International Cancer Symposium took place over two days, from Saturday, July 5, to Sunday, July 6, 2025. The event was co-hosted by the Sapporo Cancer Seminar Foundation and held at the Grand Mercure Sapporo Odori Park.

Seishi Ogawa, from the Department of Pathology and Tumor Biology at Kyoto University, served as the representative organizer this year. The main theme was "Cancer Evolution Revealed by New Technology." The development of clones has been observed in normal tissues prior to carcinogenesis, which enables a detailed description of the process. These advancements were made possible by improvements in micro-sampling, single-cell colony analysis, organoid analysis, single-cell omics analysis, and spatial genomics. The symposium showcased the latest cancer research fueled by these technological breakthroughs.

A total of 113 people attended the symposium, including six from overseas.

On the 5th, at the beginning of the symposium, Seishi Ogawa expressed gratitude to the Sapporo Cancer Seminar Foundation and related parties for hosting the event. Masanori Hatakeyama, Council Member, then delivered a welcome address on behalf of the Sapporo Cancer Seminar. He introduced the history of the Sapporo Cancer Seminar and this symposium, as well as their influence on cancer research in Japan.

The symposium included two keynote lectures, one special lecture, seven sessions, two luncheon seminars, and a poster session.

The keynote lectures featured Dan Landau from the New York Genome Center and Gyoti Nangalia from the Wellcome Sanger Institute. Landau presented research linking genomes and phenotypes using single-cell multiomics, and Nangalia presented research on methylation using single-cell-derived colonies.

For the special lecture, Safia Danovi, editor of Nature Genetics, provided a detailed explanation of what leading academic journals expect from authors.

The sessions were organized around the following themes:Gastrointestinal tumors, Clonal evolution and normal tissue, Hematological malignancies 1, Breast and brain cancer, Hematological malignancies 2, Cancer environment, Spatial omics. Twenty speakers presented their latest research in these sessions..

The luncheon seminar featured presentations by Dr. Yoshiyuki Nagumo (University of Tsukuba) and Dr. Hiromichi Suzuki (National Cancer Center). Dr. Nagumo presented on research into cfDNA methylation in urothelial cancer, and Dr. Suzuki presented on single-cell research in brain tumors.

During the poster session, 28 presentations were given. Young researchers presented their current studies and received valuable feedback and advice.

All presentations were followed by active Q&A sessions. Discussions continued even after the scheduled time had ended and spilled over into the social gathering outside the venue.

On July 4, a welcome party was held for presenters and symposium organizers. After the first day's sessions on July 5, a social gathering was held for all participants. These events provided an opportunity to build relationships with leading researchers, including international invited speakers. Discussions continued beyond the scheduled session times.

Through this symposium, we hope to contribute to the advancement of cancer research by fostering new research ideas and opportunities for collaborative studies.

9. Additional description

This symposium provided an opportunity for researchers from Japan and overseas to engage in direct discussion, get to know each other personally, and expand the possibilities for new research. We would like to express our deepest gratitude for your financial support.



Report on Research Meeting

Date: July 18th, 2025

1. Name of Research Meeting / Conference
   Plant Reproductive Development and Genomics

2. Representative
   Tetsuya Higashiyama, Professor, Yuki Tsuchikane, Project Assistant Professor,
   Graduate School of Science, The University of Tokyo

3. Opening period and Place
   May 18–22, 2025
   Awaji Yumebutai International Conference Center, Hyogo, Japan

4. Number of participants
   137

5. Number of participating countries and areas
   16 countries (Japan, China, Switzerland, USA, Pakistan, Germany, Australia, Austria, India, South Korea, Taiwan, Canada, France, Italy, Saudi Arabia, UK)

6. Total cost
   20,441,513 JPY

7. Main use of subsidy
   The subsidy covered travel expenses for seven early-career researchers (JPY 50,000 each) and partially funded six poster awards (USD 200 each).

8. Result and Impression
   The CSHA2025 international conference “Plant Reproductive Development and Genomics” was a resounding success, gathering 137 participants from 16 countries across Asia, Europe, North America, and the Middle East. Held over five days, the conference provided a unique platform for discussing key biological questions in plant reproductive systems, spanning topics such as ecological and evolutionary aspects, domestication and breeding, sex determination, germline development, and gametophyte–sporophyte transitions.

   The program included 20 invited talks by internationally recognized leaders in the field, as well as 27 short talks selected from general abstract submissions. In addition, 59 poster presentations were featured, the majority of which were delivered by graduate students and postdoctoral fellows. These posters formed a vital part of the scientific exchange, with active discussions during both scheduled and informal sessions.

   The conference placed strong emphasis on supporting early-career researchers. Six Best Poster Awards were presented (four to Japanese and two to Chinese participants), and Travel Awards were granted to seven researchers from India, Germany, Italy, Switzerland, Saudi Arabia, and China. Feedback from participants highlighted the scientific quality of the sessions, the stimulating discussions, and the value of opportunities for networking and collaboration.

Beyond the formal sessions, evening gatherings and relaxed social events allowed for meaningful exchanges between senior and junior researchers, fostering a truly international and interdisciplinary atmosphere. The conference strengthened Japan's leadership in plant reproductive biology and is expected to catalyze new international collaborations in the years to come. CSHA founder and CEO Dr. Maoyen Chi also joined the meeting and delivered a talk, underscoring the event's significance.

Thanks to generous funding support, the conference was held to a high standard and received strong positive feedback from attendees. It is expected to catalyze future collaborations and contribute to the development of a robust international research community in this field.

9. Additional description
Website: https://www.csh-asia.org/?content/2653



Photo 1. Presentation in progress



Photo 2. Poster presentation session



Photo 3. Participants during the lunch excursion

39th Grant Report (FY2025)

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 2025 are as follows.

39th Novartis Research Grant:	35 Researchers (JPY 1 mil.),	Subtotal JPY 35.0mil.
Research Meeting Grant:	5 Meetings (JPY 0.4 mil.),	Subtotal JPY 2.0mil.
		Total JPY 37.0mil.

39th Novartis Research Grant (FY2025)

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	Takehiro Takahashi	Hospital, Department of Dermatology, Tohoku University	Lecturer	Elucidation of the pathogenic mechanisms determining the severity and treatment responses of alopecia areata.
2	Daisuke Koshiyama	Department of Neuropsychiatry, The University of Tokyo Hospital	Assistant Professor	Application of Event-Related Potentials and Diffusion Tensor Imaging to the Study of Neural Network Abnormalities in Schizophrenia
3	Manao Kinoshita	Department of Dermatology, University of Yamanashi	Lecturer	Comprehensive profiling of lesional cell types in bullous pemphigoid with the analysis of blister-originated granulocytes
4	Toshinori Hayashi	Graduate School of Integrated Sciences for Life, Hiroshima University	Professor	Establishment of a Comparative Disease Model for Cardiac Pressure Overload Response in a Mammal and an Amphibian
5	Wataru Kakegawa	Laboratory for Neurophysiology, Department of Life Science, Faculty of Science, Gakushuin University	Professor	Optogenetic Control of Acidic Organelles for Unraveling Learning and Memory Mechanisms
6	Richiro Ushimaru	Institute for Advanced Study, Kyushu University	Associate Professor	Development of radical biocatalysts for structural editing of bioactive nucleosides
7	Masahiro Sonoshita	Institute for Genetic Medicine, Hokkaido University	Professor	Targeting intracellular metabolism for treating pancreatic ductal adenocarcinoma
8	Ryuji Kitano	Faculty of Humanity-Oriented Science and Engineering, Food bioscience laboratory, Kindai University	Associate Professor	Deciphering Sex-Dependent Mechanisms of Atherosclerosis Progression via GPR30 toward Next-Generation Preventive Medicine
9	Masayasu Taki	Institute for Glyco-core Research, Gifu University	Professor	Single-Cell Analysis of Fatty-Acid Flux Using Environment-Sensitive Fluorescent Probes

#	Name	Institution	Title	Research Project
10	Takayuki Katoh	Bioorganic Chemistry lab, Department of Chemistry, Graduate School Of Science, The University of Tokyo	Associate Professor	Development of novel antibiotics targeting bacterial ribosome by means of macrocyclic β-sheet foldamer peptide library
11	Takeshi Ichinohe	International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo	Associate Professor	Integrated analysis of temperature, microbiota, and aging in viral infection and host defense
12	Takahiro Osada	Department of Neurophysiology, School of Medicine, Juntendo University	Associate Professor	Multi-level mechanisms of ultrasound neuromodulation on human cognitive function
13	Yuki Kinjo	Department of Bacteriology, The Jikei University School of Medicine	Chairman and Professor	Elucidation of mechanism of augmentation and persistence of antibody production
14	Tatsuya Morishima	Morishima Laboratory, International Research Center for Medical Sciences (IRCMS), Kumamoto University	Lecturer, Wakakusu Researcher	Hematopoietic anti-aging by taurine through mitochondrial tRNA modification
15	Naoko Fujito	Brain Research Institute, Laboratory for Evolutionary Brain Pathology, Niigata University	Associate Professor	APOBEC3B Deletion Polymorphism and Cancer from an Evolutionary Perspective
16	Mahito Sadaie	Department of Applied Biological Science, Graduate School of Science and Technology, Tokyo University of Science	Professor	Elucidating the Molecular Mechanisms of ATRX Deficiency to Establish a Diagnostic Platform for Telomerase-Independent Cancers
17	Daisuke Ono	Research Institute of Environmental Medicine, Nagoya University	Lecturer	Elucidation of the aging mechanism of the circadian clock via cAMP
18	Makoto Saito	Pioneering Research Institute, RIKEN	RIKEN ECL Team Leader	Biological characterization of the novel RNA-guided system TIGR-Tas
19	Masato Enomoto	Faculty of Medical Sciences, University of Fukui	Professor	Elucidating the Mechanism of Cancer Sex Differences Mediated by Systemic Signaling
20	Haruya Kawase	Department of Cardiology, Nagoya University Hospital	Assistant Clinical Professor	Atypical Chemokine Receptors as Novel Regulators in Ischemic Heart Disease
21	Hiroshi Takikawa	Faculty of Pharmaceutical Sciences, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University	Professor	Generation of Organic Azide-Derived Reactive Species for Biomolecular Modification
22	Mizuki Honda	Graduate School of Integrated Sciences for Life（School of Science）, Hiroshima University	Assistant Professor	Molecular network mechanisms underlying stress-dependent brain alterations in psychiatric disorder onset
23	Rawin Poonperm	Graduate School of Bioresources, Laboratory of Molecular & Cellular Biology, Mie University	Assistant Professor	Simultaneous DNA copy number and gene expression analysis using a new multi-omics method, scRR-seq
24	Hiroyoshi Kunimoto	School of Medicine, Department of Hematology and Clinical Immunology , Yokohama City University	Senior Lecturer	Exploring molecular basis and individualized therapy of high-risk myelodysplastic/myeloproliferative neoplasms based on cancer genome alterations

#	Name	Institution	Title	Research Project
25	Kentaro Jingushi	Laboratory of Stem Cell Regeneration and Adaptation, The University of Osaka	Associate professor / Lecturer	Exploring Liver-Derived Extracellular Vesicles as Immune Modulators for Enhancing Cancer Immunotherapy
26	Noriyuki Miyoshi	Lab. of Biochemistry, School of Food and Nutritional Sciences, University of Shizuoka	Professor	Comprehensive inhibition analysis of gut microbial uremic toxin-producing enzymes and AI-driven discovery of novel CKD control strategies
27	Takuya Osakada	School of Life Science and Technology, Institute of Science Tokyo	Specially appointed associate professor	Identification of interaction between lateral habenula neurons and oxytocin network in the brain to control social behaviors
28	Sakurako Suma	Department of Hematology, University of Tsukuba Hospital	Assistant Professor	Development of Innovative Therapeutic Strategies Targeting Extranodal Niche Formation and Molecular Interactions in T-cell Lymphomas
29	Eiji Morita	Department of Biochemistry and Molecular Biology, Faculty of Agriculture and Life Science, Hirosaki University	Professor	Crohn's Disease Pathogenesis Caused by Xenophagy dysfunction
30	Keisuke Hamada	Department of Clinical Biochemistry, Tokyo University of Pharmacy and Life Sciences	Assistant Professor	Development of specific extracellular protein degradation inducer and their application to drug discovery
31	Yasutomi Higashikuni	Department of Cardiovascular Medicine, Jichi Medical University	Assistant Professor	Elucidating pathogenic mechanisms of cardiac aging and heart failure through post-translational modification modulated by RNA metabolism and modification
32	Hayato Ishikawa	Graduate School of Pharmaceutical Sciences, Chiba University	Professor	Unified Total Synthesis of Dimeric Indole Alkaloids for Medicinal Chemistry Applications
33	Takayoshi Yamauchi	Department of Microbiology, Division of Infectious Diseas, Asahikawa Medical University	Assistant Professor	Overcoming Cancer Immunotherapy Resistance by Targeting Immuno-inflammatory Pathways
34	Yukako Oda	Graduate School of Biostudies, Kyoto University	Professor	Regulation of cancer progression and metastasis based on cell-cell adhesion
35	Mitsunori Fukuda	Laboratory of Membrane Trafficking Mechanisms, Graduate School of Life Sciences, Tohoku University	Professor	Elucidation of a novel removal mechanism of damaged lysosomes by Rab small GTPases

FY2025 Research Meeting Grant

(JPY 400 thousand x5 = 2.0 million)

No.	Name	Institution	Title	Place/Date	Meeting
1	Masayuki Yamamoto	Department of Biochemistry and Molecular Biology, Tohoku University Tohoku Medical Megabank Organization	Professor	Sendai/ 2027.3.27-28	The Environmental Response VII
2	Kazuhiro Hirayama	Research Center for Food Safety, Graduate School of Agricultural and Life Sciences, The University of Tokyo	Director	Tokyo/ 2026.10.14	Food Safety in Asia-Pacific Region – In Progress and Outlook
3	Yoshihisa Uenoyama	Laboratory of Animals Reproduction, Nagoya University	Associate Professor	Nagoya/ 2026/7/23-25	The 5th World Conference of Kisspeptin (Kisspeptin 2026)
4	Shinjiro Yamaguchi	Institute for Chemical Research, Kyoto University	Professor	Kyoto/ 2026.11.24-25	International Symposium on Gibberellin
5	Koji Ikegami	Department of Anatomy and Developmental Biology, Graduate School of Biomedical and Health Sciences, Hiroshima University	Professor	Okayama/ 2027.3.17-19	12th Asia Pacific International Congress of Anatomists (APICA2027)

＜ Return of a FY2024 Research Meeting Grant ＞

The JPY 400,000 grant awarded for “Minisymposium on the blood-brain barrier: from basic to clinical research” (Yoichi Morofuji, Associate Professor, Department of Neurosurgery, Nagasaki University) was returned to the Foundation because invited speakers from overseas were unable to travel to Japan.

第39期（2025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。2025年度は、下記の総額3,700万円の助成事業を実施しました。

第39回ノバルティス研究奨励金	35件（1件100万円）	3,500万円
研究集会助成	5件（1件 40万円）	200万円
		総額 3,700万円

第39回ノバルティス研究奨励金（2025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	髙橋　岳浩	東北大学 病院 皮膚科	講師	円形脱毛症の重症度と治療応答性を規定する背景因子と病態機序の解明
2	越山　太輔	東京大学 医学部附属病院精神神経科	助教	事象関連電位および拡散テンソル画像を応用した統合失調症の神経ネットワーク異常の解明研究
3	木下　真直	山梨大学 医学域皮膚科学講座	学部内講師	水疱性類天疱瘡の水疱内顆粒球の多様性と皮疹部の全細胞種解析による病態解明
4	林　利憲	広島大学 大学院統合生命科学研究科	教授	哺乳類と両生類における心圧負荷応答の比較解析モデルの構築
5	掛川　渉	学習院大学 理学部生命科学科神経生理学教室	教授	酸性オルガネラを標的とした光操作技術の開発と記憶・学習機構の解明
6	牛丸理一郎	九州大学 高等研究院	准教授	ラジカル生体触媒を用いた生物活性ヌクレオチド構造編集法の開発
7	園下　将大	北海道大学 遺伝子病制御研究所	教授	細胞内代謝を標的とする新規膵がん治療法の開発
8	北野　隆司	近畿大学 産業理工学部 生物環境化学科 食品生命機能学研究室	准教授	GPR30 を標的とした性差依存的動脈硬化進展メカニズムの探索と次世代予防医学への展開
9	多喜　正泰	岐阜大学 糖鎖生命コア研究所	教授	環境応答性蛍光プローブ群を用いた脂肪酸フラックスの1細胞解析
10	加藤　敬行	東京大学 理学系研究科化学専攻・ 生物有機化学研究室	准教授	環状βシート型フォルダマーペプチドライブラリを基盤とした細菌リボソーム阻害抗生物質の開発
11	一戸　猛志	東京大学医科学研究所 感染症国際研究センター ウイルス学分野	独立准教授	体温、腸内環境、細胞老化が制御するウイルス感染時の炎症応答と生体防御機構の統合的解析
12	長田　貴宏	順天堂大学 医学部生理学第一講座	准教授	ヒト認知機能への超音波ニューロモデュレーション：多階層メカニズムの解明
13	金城　雄樹	東京慈恵会医科大学 細菌学講座	主任教授	抗体産生の増強と持続をもたらす免疫機構の解明
14	森嶋　達也	熊本大学 国際先端医学研究機構 森嶋研究室	特任講師、若楠研究者	タウリンによるミトコンドリア tRNA 修飾を介した造血老化予防の試み

No.	氏　名	所　属	職位	研究課題
15	藤戸　尚子	新潟大学 脳研究所　進化脳病態研究室	准教授	進化的視点から見る APOBEC3B 欠失多型とがん
16	定家　真人	東京理科大学大学院 創域理工学研究科 生命生物科学専攻	教授	ATRX 発現不全の分子機構解明によるテロメラーゼ非依存型がんの診断基盤の構築
17	小野　大輔	名古屋大学 環境医学研究所	講師	cAMP を介した概日時計の老化メカニズムの解明
18	齋藤　諒	国立研究開発法人理化学研究所 開拓研究所	チームリーダー	新規 RNA 誘導性システム TIGR-Tas の生物学
19	榎本　将人	福井大学 学術研究院医学系部門	特命教授	遠隔シグナルによるがん性差の形成メカニズムの解明
20	川瀬　治哉	名古屋大学 医学部附属病院　循環器内科	病院助教	非定型ケモカイン受容体による虚血性心疾患の病態制御機構
21	瀧川　紘	金沢大学 医薬保健研究域薬学系 生命有機分子化学研究室	教授	生体分子修飾を指向した有機アジド由来高反応性化学種発生法の開発
22	本田　瑞季	広島大学 大学院統合生命科学研究科 (理学部生物科学科)	助教	精神疾患発症を規定するストレス依存的脳変化の分子ネットワーク解析
23	プーンパーム　ラウィン	三重大学 大学院生物資源学研究科 分子細胞生物学教育研究分野	助教	新しいマルチオミクス法 scRR-seq を用いた DNA コピー数と遺伝子発現の統合解析
24	國本　博義	横浜市立大学 医学部 血液・免疫・感染症内科	講師	がんゲノム異常に基づく高リスク骨髄異形成・骨髄増殖性腫瘍の分子基盤と個別化治療の研究開発
25	神宮司健太郎	大阪大学 大学院 薬学研究科 再生適応学分野	講師	癌免疫応答を高める肝臓由来細胞外小胞の分子機構解明と革新的免疫治療の開発
26	三好　規之	静岡県立大学 食品栄養科学部　生化学研究室	教授	腸内細菌由来尿毒症物質産生酵素の網羅的阻害解析と AI 創薬による新規 CKD 制御戦略の構築
27	小坂田拓哉	東京科学大学 生命理工学院	特任准教授	外側手綱核がオキシトシンネットワークを介して社会性を制御する回路基盤の解析
28	須摩　桜子	筑波大学附属病院 血液内科研究室	病院助教	T 細胞リンパ腫における毛包とクローン性造血由来炎症細胞による節外ニッチ形成機構の解明と革新的治療戦略の開発
29	森田　英嗣	弘前大学 農学生命科学部分子生命科学科	教授	ゼノファジーの破綻に起因するクローン病発症機構の解析
30	濵田　圭佑	東京薬科大学 薬学部　病態生化学教室	助教	特定の細胞外タンパク質を分解に導く化合物の創製と創薬への応用
31	東邦　康智	自治医科大学 自治医科大学附属病院 循環器センター内科部門 循環器内科	講師	RNA 代謝・修飾機構による翻訳後修飾調節を介した心筋細胞老化及び心不全発症メカニズムの解明
32	石川　勇人	千葉大学 大学院薬学研究院	教授	二量体型インドールアルカロイドの網羅的全合成と医薬化学への展開
33	山内　隆好	旭川医科大学 医学部 感染症学講座	助教	免疫炎症経路を標的としたがん免疫治療耐性の克服
34	小田裕香子	京都大学 生命科学研究科	教授	細胞間接着の人為的制御によるがんの進行・転移制御
35	福田　光則	東北大学 大学院生命科学研究科 膜輸送機構解析分野	教授	低分子量 G 蛋白質 Rab による損傷リソソームの新規除去機構の解明

2025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。2025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	山本　雅之	東北大学 東北メディカル・ メガバンク機構 分子医化学分野	教授	仙台／ 2027.3.27-28	第7回環境応答 国際シンポジウム
2	平山　和宏	東京大学大学院 農学 生命科学研究科附属 食の安全研究センター	センター長	東京／ 2026.10.14	アジア太平洋地域の 食の安全の現在と未来
3	上野山賀久	名古屋大学 大学院生命農学研究科 動物生殖科学研究室	准教授	名古屋／ 2026/7/23-25	第5回世界キスペプチン会議 (Kisspeptin 2026)
4	山口信次郎	京都大学 化学研究所・ 生体触媒化学研究室	教授	京都／ 2026.11.24-25	ジベレリン国際シンポジウム
5	池上　浩司	広島大学 大学院医系科学研究科 解剖学及び発生生物学 教室	教授	岡山／ 2027.3.17-19	第12回アジアパシフィック国際 解剖学会議（APICA2027）

＜2024年度研究集会助成金返還＞

「血液脳関門シンポジウムー基礎から臨床までー」（諸藤陽一 准教授、脳神経外科、長崎大学）については、国外招待演者の来日が困難となったため、助成金40万円の返還を受けた。

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
2025	35	3,500	4	160	0	0	0	0
Total	1,614	189,760	274	12,480	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
2025	0	0	0	0	39	3,600
Total	13	2,600	14	582	2,069	233,673

助成金実績一覧表

（単位：万円）

年号	研究奨励金		研究集会		日欧研究交流		海外出張助成	
	人数	助成額	人数	助成額	人数	助成金額	人数	助成金額
1987	18	1,800	8	400	0	0	0	0
1988	39	3,900	8	400	7	1,740	0	0
1989	42	4,200	8	400	9	2,260	0	0
1990	51	7,650	10	500	8	2,440	0	0
1991	55	11,000	11	550	9	2,710	9	250
1992	50	10,000	10	500	10	3,315	8	265
1993	50	10,000	10	500	11	3,511	9	300
1994	50	10,000	10	500	8	2,530	6	155
1995	50	6,500	10	500	7	2,020	6	170
1996	45	5,850	10	500	6	1,600	4	120
1997	41	4,920	10	500	6	1,610	2	55
1998	41	4,920	10	500	4	1,070	8	160
1999	41	4,920	10	500	4	710	8	160
2000	41	4,100	8	400	3	660	0	0
2001	41	4,100	7	350	2	440	0	0
2002	40	4,000	8	400	0	0	0	0
2003	40	4,000	4	200	0	0	0	0
2004	45	4,500	5	200	0	0	0	0
2005	45	4,500	5	200	0	0	0	0
2006	46	4,600	6	240	0	0	0	0
2007	50	5,000	6	240	0	0	0	0
2008	45	4,500	7	280	0	0	0	0
2009	30	3,000	6	240	0	0	0	0
2010	38	3,800	5	200	0	0	0	0
2011	41	4,100	6	240	0	0	0	0
2012	40	4,000	6	240	0	0	0	0
2013	42	4,200	5	200	0	0	0	0
2014	42	4,200	6	240	0	0	0	0
2015	35	3,500	6	240	0	0	0	0
2016	35	3,500	5	200	0	0	0	0
2017	41	4,100	5	200	0	0	0	0
2018	37	3,700	5	200	0	0	0	0
2019	37	3,700	5	200	0	0	0	0
2020	39	3,900	9	360	0	0	0	0
2021	40	4,000	5	200	0	0	0	0
2022	39	3,900	5	200	0	0	0	0
2023	41	4,100	5	200	0	0	0	0
2024	36	3,600	5	200	0	0	0	0
2025	35	3,500	4	160	0	0	0	0
Total	1,614	189,760	274	12,480	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
2025	0	0	0	0	39	3,800
Total	13	2,600	14	582	2,069	233,673

39th Financial Report

Balance Sheet

As of March 31, 2026

(Unit：JP Yen)

Account	Amount
Ⅰ **Assets**	
1. Current Assets	
Current Assets Total	20,702,181
2. Fixed Assets	
(1) Basic Fund	
Basic Fund Total	1,100,000,000
(2) Other Long - term Assets	
Other Long - term Assets Total	85,040,537
Fixed Assets Total	1,185,040,537
Assets Total	1,205,742,718
Ⅱ **Liabilities**	
1. Current Liabilities	
Current Liabilities Total	44,102,731
Liabilities Total	44,102,731
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	161,639,987
（Amount Appropriating to）	(100,000,000)
Equity Total（**Net Assets**）	1,161,639,987
Liabilities & Equity Total	1,205,742,718

Statement of Net Assets

From April 1, 2025 to March 31, 2026

(Unit：JP Yen)

Account	Amount
Ⅰ **General Net Assets Changes**	
1. Ordinary income & Expenditure	
(1) Ordinary income	
Interest from basic fund	13,255,898
Donation	40,000,000
Other Income	730,918
Ordinary Income Total	53,986,816
(2) Ordinaty Expenditure	
Project Expense	17,055,226
Grant Expense	36,600,000
Novartis Research Grant	35,000,000
Research Meeting Grant	1,600,000
Administrative Expense	6,320,831
Ordinary Expenditure Total	59,976,057
Ordinary Balance without Appraisal Profit or Loss	△ 5,989,241
2. Nonrecurring Profit & Loss	
Nonrecurring Balance of Current Period	0
General Net Assets Ending Balance	161,639,987
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**	1,161,639,987

第39期（2025年度）財務報告

貸借対照表

2026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	20,702,181
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	85,040,537
固定資産合計	1,185,040,537
資産合計	1,205,742,718
Ⅱ負債の部	
1．流動負債	
流動負債合計	44,102,731
負債合計	44,102,731
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	161,639,987
（うち基本財産への充当額）	(100,000,000)
正味財産合計	1,161,639,987
負債及び正味財産合計	1,205,742,718

正味財産増減計算書

2025年4月1日から2026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	13,255,898
受取寄付金	40,000,000
雑収益	730,918
経常収益　計	53,986,816
⑵ 経常費用	
事業費	17,055,226
支払助成金	36,600,000
ノバルティス研究奨励金	35,000,000
研究集会助成金	1,600,000
管理費	6,320,831
経常費用　計	59,976,057
当期経常増減額	△ 5,989,241
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	161,639,987
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	1,161,639,987

List of Trustees, Auditors, Councilors and Grant Selection Committee Members

[Board of Trustees] 6 trustees, 2 auditors As of July 1, 2026

Post	Name	Title
Chairman	Kuniaki Takata, Ph.D.	Professor Emeritus, Gunma University
Trustee	Sadayoshi Ito, M.D., Ph.D.	Special Administrator, Katta General Hospital Professor Emeritus, Tohoku University
	Masahide Takahashi, M.D., Ph.D.	Designated Prof., Director of Academic Programs. Fujita Health Univ. Prof. Emeritus, Nagoya 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, 2026

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 Neuro-intelligence, 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
	Koichi Nishijo, M.D., Ph.D.	Director, Head of Development Japan, Novartis Pharma K.K.

[Grantee Selection Committee] 20 members As of July 1, 2026

Post	Name	Title
Chairman	Yasuyuki Fujita, M.D., Ph.D.	Professor, Kyoto University Graduate School of Medicine
Member	Norihiko Takeda, M.D., Ph.D.	Professor, University of Tokyo Graduate School of Medicine
	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
	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
	Shosei Yoshida, M.D., 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.	Professor, Kyushu University Medical Institute of Bioregulation
	Takeshi Sakaba, Ph.D.	Professor, Doshisha University Graduate School of Brain Science
	Yukari Fujimoto, Ph.D.	Professor, Department of Chemistry, Faculty of Science and Technology, 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
	Motoharu Awazawa, M.D., Ph.D.	Division Chief, Department of Molecular Diabetic Medicine, National Institute of Global Health and Medicine, Japan Institute for Health Security
	Takashi Shichita, M.D., Ph.D.	Professor, Medical Research Laboratory, Institute of Science Tokyo
	Tetsuhiro Nemoto, Ph.D.	Professor, Graduate School of Pharmaceutical Sciences, Chiba University
	Kaori Hayashi, M.D., Ph.D.	Professor, Department of Internal Medicine, School of Medicine, Keio University

公益財団法人ノバルティス科学振興財団

［理事・監事］

任期2026年6月18日～ 2028年6月　　2026年7月1日現在（敬称略）

職 名	氏 名	現　　職
代表理事	高田　邦昭	群馬大学 名誉教授
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
	高橋　雅英	藤田医科大学 統括学術プログラムディレクター 特命教授 名古屋大学　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　　2026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
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＊2026 年 6 月 18 日～ 2028 年 6 月

［選考委員］

2026年7月1日現在（敬称略）

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	林　　香	慶應義塾大学 医学部 教授

事 務 局 便 り

事務局より

2026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。

近年、科学技術を取り巻く環境は大きく変化しています。AI技術の急速な進歩は研究活動そのものの在り方にも大きな影響を与えており、研究者のみならず私たち研究助成財団事務局にとっても、新たな時代の到来を実感する一年となりました。しかし、こうした時代だからこそ、新たな知を生み出す基礎研究の重要性はますます高まっているように感じます。また、世界各国の研究力が急速に向上する中、日本の基礎研究の国際競争力を維持・発展させていくことの重要性を改めて感じます。

そのような中、昨年のノーベル化学賞を受賞された北川進先生に続き、本年は東京理科大学の秬合憲三先生がノーベル化学賞を受賞されました。北川先生は第7回チバガイギー研究奨励金（当時）の助成者のお一人であり、また秬合先生は第10回チバガイギー研究奨励金受領者として、今年のノーベル賞につながる研究を推進されました。両先生の受賞は、日本の基礎研究の層の厚さを示すとともに、独創的な研究の芽を育む研究助成の意義を改めて示す出来事であったと感じています。基礎研究は必ずしも直ちに成果が見えるものではありませんが、未来の科学技術や社会の発展を支える源泉であることを改めて実感いたしました。

また、日本においても基礎研究の重要性に対する認識が高まりつつあり、研究力強化に向けたさまざまな取り組みが進められています。当財団も民間助成財団の一つとして、自然科学分野における独創的な研究への支援を通じ、日本の学術発展に微力ながら貢献してまいりたいと考えております。

本年報では、2024年度に助成を行い、2026年3月までに研究を完了された第38回ノバルティス研究奨励金受領者の研究成果をご紹介しております。研究者の皆様による創意工夫に富んだ研究の成果を拝見し、将来の大きな発見や新たな学問分野の創出につながる可能性を感じるとともに、本年報としてその成果をご紹介できることを大変嬉しく思います。基礎研究の成果が社会に広く評価されるまでには、数十年にわたる地道な研究の積み重ねが必要です。当財団も、将来どのような花を咲かせるか分からない研究の新芽を支援し続けたいと考えています。

当財団は1987年9月の設立以来、助成件数2,070件、総額約23.4億円の研究助成を行ってまいりました。これまで事業を継続できておりますのも、ひとえに財団活動をご理解・ご支援くださる皆様、助成を受け研究を推進された研究者の皆様、そして財団関係者の皆様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
2026年10月7日

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。

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優遇措置の概略

個人：個人のご寄附については、税法上の要件を満たす場合、寄附金控除の対象となります。寄附金控除額は、「その年に支出した特定寄附金の額の合計額」と「総所得金額等の40％相当額」のいずれか少ない金額から2,000円を差し引いた額となります。

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