Annual Report (2022)

No.34
The NOVARTIS Foundation (Japan) for the Promotion of Science

2022年度

財団年報 第34号

公益財団法人 ノバルティス科学振興財団

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Introduction

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

This annual report includes research reports from the 34th Novartis Research Grant recipients (research from April 2021 to March 2022: 39 research grants and 6 research meetings).

The novel coronavirus (COVID-19) infection that began in 2019 has raged around the world, infecting a cumulative total of more than 600 million people worldwide and killing more than 6 million as of this writing in early October, shaking the very foundations of the world. In Japan, the seventh wave of infection by the Omicron strain that began this summer saw an unprecedented number of infections spread, and the cumulative number of infected people exceeded 20 million. On the other hand, vaccination and experience in treatment have been accumulated, and it can be said that society's awareness of this infectious disease has reached a turning point. Universities and research institutes are gradually returning to normal operations. Restrictions on personnel transfers to and from overseas have been eased, and the holding of academic conferences in the usual face-to-face format is resuming. I hope that the day will come soon when the COVID-19 infections will no longer be something special and researchers will be able to devote themselves to research without restrictions.

The Novartis Foundation (Japan) for the Promotion of Science was established in 1987 with a donation of 1 billion yen from Ciba-Geigy AG (now Novartis AG), Switzerland. The purpose of the Foundation is clearly stated in its Articles of Incorporation as "to promote science by encouraging creative research in the natural sciences, thereby contributing to the improvement of the health and welfare of the people. In addition, the document entitled "Prospectus for Establishment of the Foundation," which was written in the year of the foundation's establishment, states that the foundation "seeks to promote and support creative research in the natural sciences, which will be the axis of science in the coming 21st century, and thereby contribute to the welfare of mankind. The document also states that the foundation will "provide financial support for research and opportunities for cross-border exchanges. Based on this policy, over the past 35 years, our Foundation has provided a total of 1,899 grants, amounting to approximately 2.18 billion yen. In this time of turmoil due to the COVID-19 disaster, the Foundation is determined to return to this starting point and support excellent research that will pave the way for the next era.

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

はじめに

代表理事　高田　邦昭

本年報には、第34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（2021年4月～2022年3月の研究：研究奨励金39件、研究集会6件）を収録しました。

2019年に始まった新型コロナウイルス（COVID-19）感染症は世界中で猛威をふるい、この原稿を執筆している10月初旬では世界中で累計6億人以上の人が感染し、600万人を超える人が亡くなり、世界の根幹を揺るがす事態となりました。日本でも、本年の夏からのオミクロン株による第7波ではかつてない数の感染の広がりを見ることとなり、累計感染者数は2,000万人を超えました。一方で、ワクチン接種や治療上の経験の蓄積も進み、この感染症に対する社会の認識は転換期に来ていると言えます。大学や研究機関においても、徐々に通常の大学運営に戻って来ています。海外との人的異動制限緩和も進み、通常の対面形式での学会開催が再開されつつあります。新型コロナウイルス感染症が特別のものではなくなり、研究者が制約なく研究に没頭できる日が一日も早く訪れることを祈っています。

本財団は1987年に、スイス、チバガイギー社（現ノバルティスファーマ社）からの10億円のご寄附をもとに設立されたものです。財団の目的は、定款に「自然科学における創造的な研究の奨励等を行うことにより、学術の振興を図り、もって国民の健康と福祉の向上に寄与する」と明記されています。また、財団設立の年に記された「財団設立の趣意」と題する文書には、財団が「来るべき21世紀の科学の軸となる自然科学の創造的研究の振興助成をはかり、以って人類の福祉に寄与できれば」とあり、「研究のための資金的な助成、並びに国境を越えた交流の場の提供」がうたわれています。このような方針のもと、35年間で総計1,899件、金額にして約21.8億円の助成を行ってきました。コロナ禍で混迷する現在こそ、財団は改めてこの原点に立ち返り、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

Roles of memory helper T cells in vivo

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Summary Abstract

In this project, we have examined the roles of bone marrow and splenic memory helper T (Th) cells in the recall response. Bone marrow memory Th cells mainly play an essential role in the recall response and the role of splenic memory Th cells will be further analyzed.
Key Words : Immune memory, T helper cells, recall response.

Introduction

Memory Th cells are a key player of immune memory and recall response. We have shown that memory Th cells mainly reside and rest in the bone marrow and are also detectable in the spleen. Recall response is performed in the spleen. It remains unclear whether bone marrow memory Th cells migrate into the spleen in the recall response. We here show the effects of depletion of memory Th cells in the bone marrow and/or spleen in the recall response.

Results

We have examined the roles of bone marrow and splenic memory Th cells in the recall response. In an immune reaction to antigenic peptide, mice were transferred with the antigen-specific T cell receptor transgenic CD4 T cells and immunized with the peptide and adjuvant. On day 40 or later after immunization, memory Th cells were detected in the bone marrow (70-80%) and spleen (20-30%). Injection of anti-integrin alpha2 and inhibitors of integrin alpha2 or the downstream, Focal adhesion kinase (FAK) in the memory phase reduced the number of bone marrow memory Th cells. The depletion of bone marrow memory Th cells induced a 2-3 days-delay of T cell expansion following boosting. The appearance of T follicular helper (Tfh) cells, germinal center B cells and antigen-specific antibody titers was also delayed or decreased. FAK-condition deficient mice are generating and will be analyzed in near future. In contrast, injection of anti-IL-7 antibody reduces the number of splenic memory Th cells. The depletion of splenic memory Th cells did not affect the recall response. However, we assume that an inhibition of IL-7, by remaining anti-IL-7 antibody, may affect the expansion of reactivated memory Th cells. Thus, we now try to inject with anti-IL-7 antibody 2-3 weeks before boosting and analyze the effect in the recall response. Finally, we will investigate the role of all memory Th cells in the recall response by depletion of both bone marrow and splenic memory Th cells. The result will be obtained soon, but we can suggest that memory Th cells contribute to the quick recall response.effects on cells in tumor biology and could be exploited for targeted cancer therapies.

Discussion & Conclusion

We show that bone marrow memory Th cells play an essential role in the recall response. It is still unknown whether splenic memory Th cells contribute to the recall response. The delayed recall response by reduction of bone marrow memory Th cells suggests two possibilities; (1) bone marrow memory Th cells work for a quick response in the early phase of recall response or (2) they totally work for recall response. In case of (2), the depletion of bone marrow memory Th cells (60% reduction) may be incomplete. We will further establish a method to deplete more bone marrow memory Th cells and obtain the data when splenic memory Th cells are depleted.

一般の皆様へ

ワクチンは免疫記憶を誘導するためのものであり、記憶ヘルパー T 細胞は免疫記憶や二次応答の中枢を担う細胞です。我々は、記憶ヘルパー T 細胞は主に骨髄で維持されていることを示してきましたが、脾臓にも少数残っていることもわかっています。そこで、本研究では、骨髄と脾臓のどちらが二次応答時に主に働くのかを解明することを目的としています。これがわかることにより、ワクチン作製において、どちらの組織の記憶ヘルパー T 細胞の形成・維持を指標にすれば良いのかが明らかになり、大変価値のある研究になります。本研究期間中には、骨髄の記憶ヘルパー T 細胞が少なくとも非常に重要であることが明らかになり、大変有意義な研究になりました。

Targeting Spliceosomal Mutant Leukemias by a Novel ASO Therapy

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Summary Abstract

Mutations affecting splicing factors are frequently identified in a variety of hematologic malignancies. Based on our understanding of how these spliceosomal mutations contribute to leukemogenesis, we have developed a novel antisense oligonucleotide-based therapy for leukemias bearing a recurrent mutation in spliceosome.
Key Words : RNA splicing; Leukemia; Antisense oligonucleotide

Introduction

There has been accumulating evidence that RNA splicing is frequently dysregulated in a variety of cancers and that hotspot mutations affecting key splicing factors, SF3B1, SRSF2 and U2AF1, are commonly enriched across cancers, strongly suggesting that aberrant RNA splicing is a new class of hallmark that contributes to the initiation and/or maintenance of cancers. There has been a growing interest in targeting altered splicing in the treatment of cancers, which promotes a wide variety of investigations including genetic, molecular and biological studies addressing how altered splicing promotes oncogenesis and how cancers bearing alterations in splicing can be therapeutically targeted.

Results

SRSF2 is an auxiliary splicing factor, which binds ESEs to recruit the core spliceosome to promote splicing. While wild-type SRSF2 physically binds CCNG and GGNG sequences equally,[1] hotspot mutations in *SRSF2* affecting Proline 95 alter this preference.[2-5] As a result, mutant SRSF2 promotes splicing of exons with C-rich sequences over G-rich sequences (Figure 3C). Although the frequency of mutations in *SRSF2* was originally estimated as low (<2%) in AML,[6] our group reanalyzed The Cancer Genome Atlas (TCGA) dataset and identified that 95% (18/19) of patients with *SRSF2* mutations were missed in the previous TCGA publication,[4] which makes *SRSF2* one of the most frequently mutated genes in AML. This was probably due to the markedly GC-rich sequence around the *SRSF2* mutational hotspot, leading to low coverage around this region in the next-generation sequence result. Interestingly, genomic analysis of the TCGA AML cohort using the refined *SRSF2* genotyping revealed the frequent and significant co-occurrence in mutations affecting *SRSF2* and *IDH2* in AML. Further functional and biological studies clarified that aberrant RNA splicing and mutant IDH2-mediated DNA hypermethylation closely cooperate with each other to drive leukemogenesis. One of the most robust splicing changes in *IDH2/SRSF2* double-mutant AML is the combined intron retention and exon skipping events in *INTS3* (Integrator 3), whose loss results in dysregulated gene expression programs associated with hematopoietic cell differentiation and multiple

signaling pathways, and blockade of myeloid differentiation leading to the development of myelodysplastic/myeloproliferative neoplasms in vivo.[4] Based on these observations, we aimed to “correct” the mis-splicing event in INTS3 in SRSF2 mutant leukemias using an antisense oligonucleotide (ASO). ASO therapy is a novel approach to therapeutically target aberrant splicing in cancers (this is also referred to as splicing switching antisense oligonucleotide or SSO) that binds complementarily to RNA through base pairing. This class of therapies aims to target the RNA for degradation or to be used to affect splicing via hybridization to RNA, and has been recently approved in the United States for the treatment of neurodegenerative diseases such as spinal muscular atrophy[7] and Duchenne muscular dystrophy.[8] Technologically, synthetic oligonucleotides composed of subunits with a morpholine ring (termed morpholino) was developed to improve stability of ASOs.[9] The morpholino lacks the negatively charged backbone of traditional ASOs that may nonspecifically bind to other components of the cell and therefore may reduce the toxicity of ASOs. This technology is also suitable to targeting splicing because it is not recognized by RNAse H and thus does not cause direct degradation of the targeted pre-mRNA. To achieve this goal, we utilized K562 leukemic cell lines that endogenously express a SRSF2 P95H hotspot mutation. We confirmed that the endogenous knock-in of this hotspot mutation causes global alterations in RNA splicing by using our pipeline which enables us to specifically identify complicated splicing changes. Using our novel pipeline that predicts target sequences for ASO therapy associated with a splicing change, we designed two ASOs targeting the intron retention events in INTS3. Interestingly, one of the ASOs significantly “corrected” the intron retention event while the other was not effective at all. In cells treated with the effective ASO but not the other one, protein expression of INTS3 was almost completely restored to the level of that in SRSF” wild-type counterpart. We also confirmed that SRSF2 P95H expression significantly blocks myeloid differentiation of HL60 leukemia cells upon treatment with all-trans retinoic acid. Treatment with the ASO targeting INTS3 released the myeloid differentiation block in vitro as expected.

Discussion & Conclusion

Although our understanding of the genomics, molecular biology, and therapeutic implications of altered RNA splicing in cancer has been greatly improved since the frequent SF mutations in cancers were identified in 2011, standard treatment strategies targeting cancers bearing splicing alterations have not been established yet. In addition, the full contribution of aberrant RNA splicing to cancer pathogenesis has not been elucidated. The major stream of the field so far is the use of splicing modulators aiming synthetic lethality in cancers with SF mutations. The tactics to inhibit regulatory proteins such as CLKs, SRPKs and PRMTs and RBPs including RBM39 are also being explored as therapeutic avenues. Although emerging technologies and the rapid development of treatment strategies using ASOs still have many challenges to be resolved before clinical use, these therapeutic strategies will expand the treatment options for cancers with aberrant splicing. There still remains a plenty of unsolved problems in terms of the pathogenesis of spliceosome mutant cancers and the development of therapeutic avenues for cancers with aberrant splicing. In parallel, the ongoing efforts to modulate splicing will hopefully address the molecular and clinical questions of whether pharmacologically intervening the global splicing is efficacious and safe in patients with cancers.

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一般の皆様へ

DNA の情報は mRNA に転写され、その後蛋白質に翻訳されることによってその蛋白質が細胞の中で様々な役割を果たします。未熟な mRNA は当初 Intron と Exon という2種類の配列を含みますが、スプライシング因子の働きにより Intron 部分が除去され、成熟した mRNA になりますが、Intron を除去するこの働きをスプライシングと呼びます。近年、がんにおいてはスプライシングの異常が発がんに重要な働きをすることがわかってきました。本研究ではスプライシング異常を持つがんに対して、COVID-19ワクチンでも知られるようになった核酸医薬という新しいクラスの薬剤を開発する試みです。私たちの研究グループは、がんに対する核酸医薬を患者さんにお届けするため、今後も研究開発を続けて参ります。

Significance of acquired immune cells during embryonic and developmental stages in brain formation

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Summary Abstract

Mouse models of Alzheimer's disease, autism, and schizophrenia at developmental stages such as embryonic and juvenile stages will be used. We will comprehensively analyze and compare the overall picture of immune responses in the brain by single cell analysis, identify brain antigens and antibodies recognized by brain T cells and B cells, and clarify their significance in the development of the nervous system.
Key Words : Immune cells, Alzheimer's disease, Autism

Introduction

The linkage between the nervous system and the immune system has been the focus of much attention in recent years. The central nervous system (CNS), including the brain and spinal cord, begins to form early in embryonic life and develops into the central nervous system through the complex and dynamic interaction of a wide variety of cells. In addition to microglia and macrophages of the innate immune system, a wide variety of immune cells such as T cells and B cells of the acquired immune system are involved in the brain during development[1-3].

Results

We performed single-cell RNA sequencing analysis of immune cells in the developing brain. Analysis of fetuses and newborns in the maternal immune activation model, as well as fetuses and newborns in the genetic autism model, revealed different cellular increases in MIA and genetic autism. In the genetic autism model, the mothers were wild-type, and despite being in littermate, there were increases in several types of immune cells in the offspring with the genetic duplication. We are currently analyzing whether administering antibodies to eliminate those immune cells improves autism-like behavior or neuronal shedding. The localization of immune cells is also not known, so by using cytokine reporter mice and transparency techniques, they are trying to determine the localization of the immune cells of interest. In the genetic autism model, we will use bone marrow chimera mice to determine whether changes in the central nervous system are triggering the infiltration of immune cells into the brain, or whether changes in the immune cells are causing them to affect the nervous system.

Discussion & Conclusion

In the future, by identifying the time point at which immune cells in the brain increase, which is important for the development of autism, it is expected that the temporary control of immune cells during the developmental period will become a novel treatment for maternal immune activation during pregnancy and genetic autism.

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一般の皆様へ

発達期の脳形成における免疫細胞の影響について研究を行った。母体のウイルス感染などを模している母体免疫活性化モデルや、遺伝的な自閉症モデルの胎児や新生児の解析から、正常の胎児や新生児とは脳内免疫細胞の数や種類が異なることが分かってきた。今後は、この免疫細胞を制御することで、治療法の開発を目指していきたい。

Functional Analysis of Subplate Layer in Neocortical Formation of the Developing Mouse Cortex

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Summary Abstract

Subplate neurons (SpNs) are essential for the formation of thalamocortical networks, the first neural circuits in the developing cortex; SpNs have spontaneous neural activity and signal via synaptic transmission to newborn neurons during radial neuronal migration, facilitating migration mode conversion. Transcriptome analysis has suggested that SpN dynamics are associated with developmental disorders, as autism-related genes are highly expressed in the subplate and residual SpN is frequently observed in autism spectrum disorder (ASD) cases. This study approaches the elucidation of the mechanisms of brain construction by understanding the characteristics and subtypes of SpN molecular expression.
Key Words : Cerebral cortex, Subplate, Development, Brain formation

Introduction

In the developing cortex, neurons are born sequentially from neuroepithelial stem cells and then migrate to their destination. We have discovered that SpNs promote radial neuronal migration by sending signals via synaptic transmission to neurons born later (***Science*** 2018). In addition to this role, SpNs play essential roles in brain development, such as establishing thalamus-cortical connections and the formation of brain sulci. However, the details of the subtypes SpN cell population and their differing roles remain unclear.

Results

We aim to understand the physiological significance of SpNs in brain development by analyzing their subtypes and roles. SpNs will be isolated by FACS, and single-cell RNA-seq followed by clustering analysis will be used to identify novel markers of embryonic SpNs.

① Identification of molecular markers of SpNs

First, fluorescently labeled SpNs from two strains of mouse embryonic brains (E17), Lpar1-GFP and Neuro D1-CreERT2 /Ai14 (E10Tmx), were isolated by FACS and subjected to single-cell RNA seq analysis. The integrated analysis revealed six clusters, of which cl3 was a cell population containing SpN. We confirmed the expression regions of the genes in cluster 3 by in situ hybridization and performed Visium spatial transcriptome analysis simultaneously.

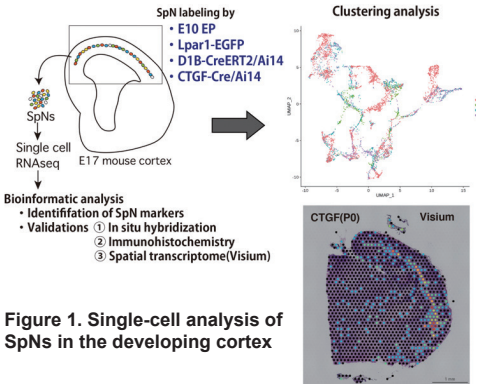
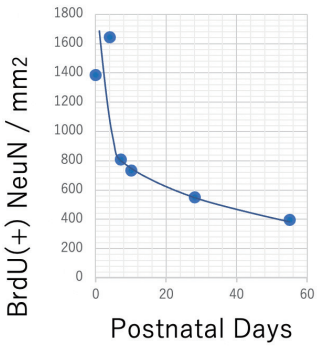


Figure 1. Single-cell analysis of SpNs in the developing cortex

We used in situ hybridization, Immunostaining, and data from spatial gene expression analysis (Visium) to validate molecular markers, which are already under analysis. The identified early marker candidate genes will be analyzed in detail in the expression regions to identify the developmental origin of SpN (Fig.1).

② Dynamics od SpNs during brain development

In the developing human brain, the thickness of the SP layer is known to decrease rapidly after birth. It has also been reported that this loss is less pronounced in autistic patients, suggesting a link between the residual state of SpN and developmental disorders. However, its long-term dynamics has not been analyzed, especially in mouse models. Therefore, we labeled SpN by injecting BrdU and EdU.in mouse E10-12 and confirmed its postnatal dynamics by immunostaining. We found that the number of NeuN-positive SpNs decreased dramatically by postnatal day 8 and then slowly decreased (Fig.2). However, some NeuN-positive SpNs were still present in the adult brain at postnatal day 56, suggesting that the SpNs also has some functions in adult brain.



Interestingly, it has been reported that the subplate layer (6b) is the only neuronal layer in the cortex that responds to orexin, which causes the sleep disorder disease narcolepsy. SpN may also be involved in the regulation of sleep and consciousness (Fig.3).

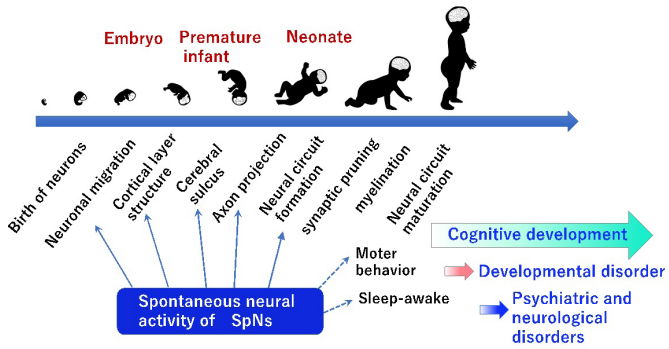


Figure 3.
The neural activity of SpN is involved in various brain developmental processes from the fetal period to adulthood. Defects in brain construction during fetal life have lifelong effects.

Discussion&. Conclusion

We intend to elucidate the fundamental characters of SpNs using the mouse model. In the future, we will examine the effects of reducing or increasing SpNs in genetically modified mice on cortical architecture, and response selectivity and plasticity of cortical neurons and SpNs. Thus this project advances our understanding of how SpN dysregulation affects neural network activity and contributes to the onset of developmental disorders such as ASD and mental retardation. As a critical player, SpN promotes brain development and function, and its defects can have lifelong consequences, such as the development of autism. Through this project, we aim to clarify the unanswered questions regarding the application of SpN to human brain development and diseases

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一般の皆様へ

胎児期に脳ができる際、数百億のニューロンの移動、配置や、神経回路形成は正確に制御されており、サブプレートニューロン（SpN）はそのキープレイヤーとして脳構築を促します。SpN 活動の不具合は発達障害等の原因になると考えられますがメカニズムは不明なままです。本研究は、マウスモデルを用いて SpN の役割を明らかにすることで、SpN の神経活動ダイナミズムによる脳発達過程の全容解明を目指しています。そのために SpN の分子発現の特徴とサブタイプについてシングルセル解析を行い、新規の分子マーカーを同定しました。

Molecular basis for early dendritic cell lineage specification during pathogen infection

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Summary Abstract

In this study, we analyzed the mechanism of early hematopoietic lineage commitment and identified a new dendritic cell (DC) primed multipotent progenitor population. Our results indicate that the activation of early DC fate specification might contribute to the rapid production of DCs during infection.
Key Words : Dendritic cells, hematopoiesis, infection, transcription factor

Introduction

The hematopoietic system produces more than 20 blood cell types and plays diverse roles such as immune responses, oxygen supply, and tissue homeostasis. In the classical model, hematopoietic stem cells (HSCs) differentiate into intermediate progenitor cells, which then undergo a multi-step differentiation program to eventually produce mature hematopoietic cells. Recently, various technologies have been developed to analyze the differentiation potential of hematopoietic cells in vivo at the single-cell level, and it has become clear that a part of HSCs and upstream progenitor cell populations can produce only a few cell types in a single cell level. These results suggest that hematopoietic cell fate decisions may occur even in early hematopoietic stages close to HSCs. However, the molecular mechanisms of lineage determination in early hematopoiesis are largely unknown.

Results

Dendritic cells (DCs), essential for induction of innate and acquired immunity, are bone marrow-derived cells. DCs are subdivided into three subpopulations: cDC1, cDC2, and pDC. In particular, cDC1s induce the differentiation of naïve $CD8^+$ T cells into cytotoxic T cells and plays a central role in the induction of acquired immunity against intracellular pathogens and tumors. There are several progenitor populations capable of producing DCs. In a multistep differentiation model, DCs are believed to be generated from HSCs by passing through LMPPs (lymphoid primed multipotent progenitors), GMPs (granulocyte monocyte progenitors), MDPs (monocyte DC progenitors), and CDPs (common DC progenitors), and pre-cDCs.

To understand the molecular mechanisms of hematopoietic differentiation, we have been analyzing the role of transcription factors in the regulation of gene expression during the differentiation of mononuclear phagocytes including monocytes, macrophages, and DCs (Kurotaki et al. *Blood* 2013; *Nat Commun* 2014; *Cell Rep* 2018; *Bone* 2020 [review]). To identify DC lineage primed multipotent progenitors and understand the molecular mechanisms of early fate determination, we performed single-cell RNA-seq analysis in mouse multipotent progeni-

tor cells. As a result, we found the transcription factor IRF8-expressing LMPP subpopulation (Kurotaki et al. *Blood* 2019). We further analyzed IRF8-GFP chimeric knock-in mice, in which IRF8 protein expression could be detected by flow cytometry, and found that IRF8 was not expressed in HSCs and MPPs, but detected low expression of IRF8 in LMPPs. Interestingly, $IRF8^+$ LMPPs preferentially differentiate into DCs *in vivo*, especially cDC1s, but not monocytes, neutrophils, or lymphocytes. Furthermore, we performed ATAC-seq analysis on $IRF8^+$ LMPPs and showed that epigenetic regulation by IRF8 is important for the early fate determination to DCs. However, the immunological significance of the early DC specification is totally unknown.

It is known that cytokine secretion is enhanced during infection, and stimulates DC expansion. We administered various types of cytokines to mice and examined the expression of IRF8 in HSCs and multipotent progenitor populations. As a result, the number of DCs increased in mice treated with some cytokines, and IRF8 expression was induced not only in multipotent progenitor cells but also in HSCs. Consistent with these results, induction of IRF8 expression was also observed in pathogen infection. Furthermore, analysis of *Irf8* enhancer deficient mice revealed that these enhancers may not be involved in the induction of IRF8 expression by the cytokines. On the other hand, we identified a *cis*-regulatory element that is involved in the induction of IRF8 expression by the cytokine administration. We next performed ATAC-seq in HSCs and multipotent progenitor cells from cytokine-injected mice and found some transcription factor binding motifs are significantly enriched in open chromatin regions newly formed by the cytokine injection. These results suggest that cytokines secreted by pathogen infection stimulated HSCs and multipotent progenitors to induce IRF8 expression through activation of the *cis*-regulatory region we identified, resulting in epigenetic changes and emergency DCpoiesis.

We have also analyzed the chromatin structure of infection-responsive gene loci during DC differentiation using Hi-C. The higher-order chromatin structures of these genomic regions change significantly during differentiation.

Discussion & Conclusion

From our results, we concluded that the cDC1-lineage priming occurs at a very early progenitor stage. The transcription factor IRF8 is required for this DC lineage priming. Our analysis also revealed that pathogen infection or cytokine administration induces IRF8 expression in HSCs and multipotent progenitor cells. In the future study, we will analyze the differentiation potential of HSCs and multipotent progenitor cells isolated from mice after pathogen infection or cytokine administration. We will also try to identify transcription factors involved in the induction of IRF8 expression and elucidate its role in early DC fate determination. Furthermore, we will analyze immunological significance against pathogens using mice lacking IRF8 and the *cis*-regulatory element of the *Irf8* gene.

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一般の皆様へ

私たちは『どのようにして造血幹細胞から数十種類もの血球細胞が産生されるのか』ということについて研究を行っています。最近の私たちの研究によって、造血幹細胞の上流の前駆細胞集団の中には、樹状細胞と呼ばれる免疫細胞のみを産生する亜集団が含まれることがわかってきました。私たちは、この造血早期における樹状細胞産生の仕組みが感染時に活性化することで樹状細胞のより素早い産生を可能にし、効果的に病原体を除去することに貢献しているのではないかと考えています。

Elucidation of the mechanisms of new tubulointerstitial nephritis with IgM positive plasma cells and comprehensive analysis of IgM-type autoantibodies in serum

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Summary Abstract

In 2017 we discovered a "new nephritis" in which many IgM-producing cells are present in the kidney. We analyze the characteristics of this disease and report it in JASN. However, since this is a new concept of disease, it has not been determined what requirements must be met to consider this disease. Therefore, we collected laboratory data from patients and kidney biopsy tissue from collaborating institutions to complete diagnostic criteria that could diagnose IgMPC-TIN with considerable precision. Furthermore, we had no idea why and how this nephritis occurs. This time, analysis of the patient's blood has begun to indicate the possible presence of autoantibodies and characteristic changes in mRNA. More detailed studies, including reproducibility, are expected to help elucidate the pathogenesis of the disease.
Key Words : IgM, TIN, plasma cells

Introduction

In late 2017, we pioneered the discovery of tubulointerstitial nephritis (TIN) with IgM positive plasma cells (IgMPC). The discovery of IgMPC-TIN was reported in J Am Soc Nephrol, a leading journal in the field of nephrology [1].

IgMPC-TIN has been overlooked due to nonspecific fluorescence microscopic findings that are usually seen on renal biopsy. The clinical characteristics of IgMPC-TIN include high blood IgM levels, renal tubular acidosis, urinary glucose, and primary biliary cholangitis (PBC), and histological characteristics of TIN caused by a large number of IgMPCs and T cells.

Since IgMPC-TIN has only been proposed for a short time, diagnostic criteria do not yet exist. Therefore, diagnostic criteria for IgMPC-TIN with high sensitivity and specificity are needed and we aimed to create them. Furthermore, since the pathological mechanism of this disease is still largely unknown, we planned to conduct in vitro experiments using patient blood.

Results

For histological diagnostic criteria, the average number of IgMPCs in the three most densely populated areas and the percentage of IgMPCs among the total PCs were classified in importance order. For comprehensive diagnostic criteria, which consisted of tissue and clinical parameters, serum IgM concentration, glucose presence in urine, and the maximum number of IgMPC infiltrates in a populated area were classified in importance order. The sensitivity and specificity of both diagnostic criteria were above 90%.

Image analysis software was implemented for quantitative evaluation of the tissue. As shown in Figure 1, the quantitative evaluation of the infiltrating cells of the kidney was possible by dividing them into four groups. IgM-CD138-, CD138+IgM-, CD138+IgM+, and CD138-IgM+. Other cases should be analyzed in the same manner to confirm that there is no difference between the analysis performed by the human eye and the analysis performed by the software.

Using a human protein microarray (Fuji Film Wako), we comprehensively analyzed serum autoantibody profiling in patients with IgMPC-TIN. Eighty-three autoantibody candidates were found to be significantly more abundant in the serum of the patient than in healthy subjects. We plan to quantify blood autoantibody titers by direct ELISA using HRP-conjugated anti-human IgM antibody.

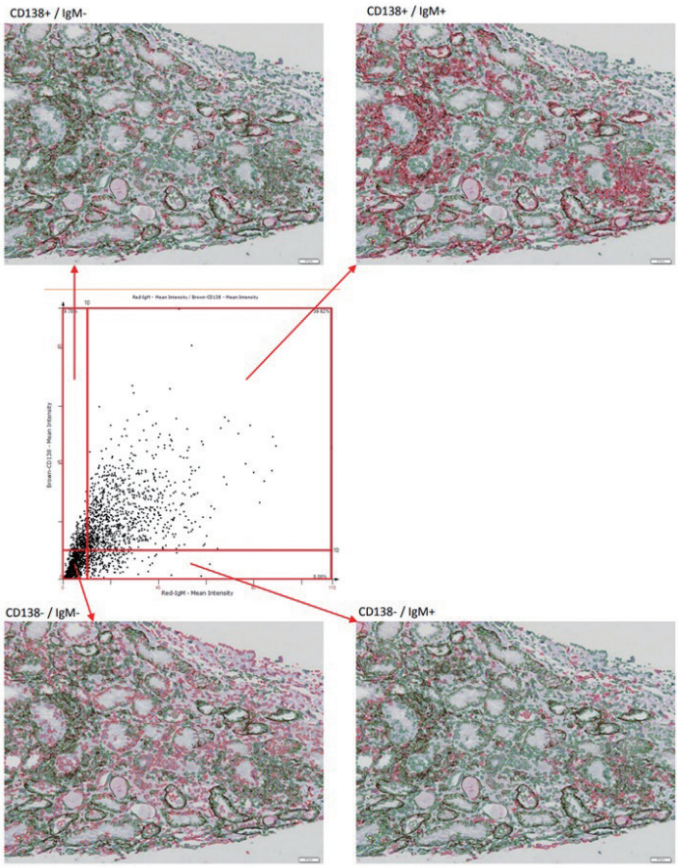

Figure 1 An example of quantitative tissue analysis in a patient with IgMPC-TIN

The patient serum was passed through an IgG and albumin desorption column, two-dimensional electrophoresis was performed, and western blotting with an anti-μ-chain antibody was performed. Although the isoelectric point of the μ-chain positive band shifted slightly in some cases, it was not statistically significant, and reproducibility should be examined with more cases in the future.

Total RNA was extracted from peripheral blood from patients with IgMPC-TIN and healthy subjects using the Leucosep and Qiagen RNA extraction kit. Comprehensive analysis of expressed genes was performed using Affymetrix Gene Chip Arrays (Clariom S). Genes with less than 2-fold expression differences were excluded from the scatter plot using Subio and the KEGG pathway analysis and the Venn analysis were performed. Many genes were found to vary between the two groups, but contrary to expectations, there was little significant variation in genes for immunoglobulin differentiation and many genes related to B and T cell differentiation were variable. In the future, quantitative analysis of genes related to B and T cell differentiation will be carried out.

Discussion & Conclusion

The comprehensive diagnostic criteria for IgMPC-TIN, including histological and clinical parameters, are now largely complete. Although the pathological significance of the disease is unknown, the possibility of the presence of IgM antibodies that react with human proteins in the serum of the patient (autoimmune aspect) has been identified. Although the analysis of the mechanism of this disease is still in its infancy, the molecules that are important in pathogenesis are gradually being identified and future developments can be expected.

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一般の皆様へ

2017年私たちは、免疫グロブリンM（IgM）を作り出す細胞が多く腎臓内に存在する「新腎炎」を発見しました。その特徴を明らかにし、腎臓病学雑誌に報告しました。しかしこれは新しい病気のため、どういった要件が揃うとこの病気と言えるのかがまだ定まっていません。そこで、私たちは全国の施設から患者さんの検査情報や腎組織を集め、かなりの確率で診断できるような基準を作成しました。この結果、この腎炎が全国のどの施設でも診断できるようになります。また、この腎炎がなぜ、どのように起こってくるのかが全く分かっていません。今回、患者さんの血液を用いた解析から、本疾患の患者さんにのみ多く存在する分子の情報が分かり始めました。今後さらなる検討を行い、病気のメカニズムを明確にし、メカニズムに応じた治療法を考えて行きたいと思います。

Synthesis and development of flavan-derived polyphenol with protective effect against amyloid-β-induced toxicity

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Summary Abstract

Oligomeric proanthocyanidins is an attractive class of polyphenols due to their potential inhibitory effects of amyloid polypeptide aggregation. In this study, we developed an efficient synthetic method, enabling access to oligomeric proanthocyanidins by the coupling and annulation method that enables to construct a complex oligomeric molecular architecture. We also evaluated bioactivities of several synthetic compounds.

Key Words : polyphenol, flavonoid, catechin, oligomer, amyloid-β

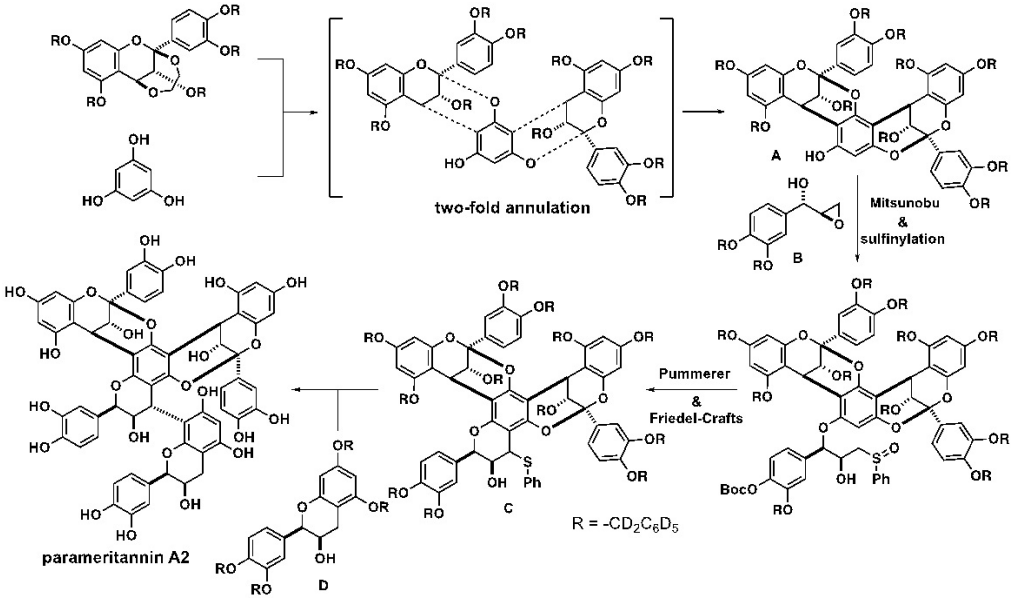
Introduction

Amyloid polypeptide aggregation is thought to trigger the onset of Alzheimer's disease. Thus, inhibiting amyloid polypeptide aggregation and disaggregation of existing amyloid aggregates are promising approaches in the therapy and prevention of the disease. Recently, several research groups reported promising protective effects of oligomeric flavonoids against amyloid-β-induced toxicity. Stimulated by these studies, we developed an efficient synthetic protocol accessing various flavan-derived polyphenols, particularly oligomeric proanthocyanidins, which would serve as a new chemical space for searching for promising pharmaceutical candidates.

Results

1. Synthesis of parameritannin A2, a doubly-annulated epicatechin tetramer

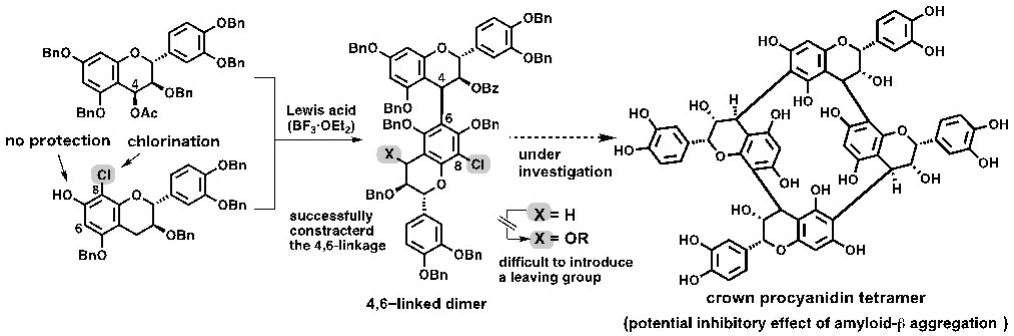
We successfully achieved the first total synthesis of parameritannin A2,[1] a doubly-annulated epicatechin (EC) tetramer with a branched structure.[2] Two-fold annulation of EC units with phloroglucinol successfully gave the key synthetic intermediate **A**, which was then linked with the half unit **B** of the middle EC unit. The newly-developed three-carbon flavan annulation method fully constructed the middle EC unit via Pummerer/Friedel–Crafts cascade reaction, giving the trimeric intermediate **C** with a C4-thio leaving group, ready for the final coupling with the bottom EC unit. Activation of the phenylthio group by TMSOTf allowed the regioselective installation of the bottom EC unit **D** in rigorous stereocontrol. Final deprotection successfully gave parameritannin A2. A bioassay of the synthetic sample is underway in our laboratory.



2. Synthetic study on crown procyanidins, a representative family of macrocyclic oligomeric flavans.

Crown procyanidins (CPs), distributed in grape skins, constitute a class of epicatechin oligomers, sharing a unique macrocyclic structure.[3] The tetrameric derivative (CPT) shows protective effects against amyloid-β induced toxicity. Intrigued by these unique structural features and potential bioactivity, we embarked on a synthetic study on these natural products.

We first investigated a regioselective construction of the hemispherical unit of the CPT, exploiting regioselective condensation of two epicatechin units. Although the formation of an interflavan linkage between the C4 and C6 position in flavan units is generally problematic because of the poor nucleophilicity of the carbon center at the C6 position, we found that the use of the C8-chlorinated flavan unit with the free phenol at the C7 position realizes regioselective interflavan formation, giving the C4-C6 linked dimeric product in high yield. However, the subsequent installation of a leaving group, which is necessary for the following annulation protocol, was unsuccessful due to the instability of the substrate. Other synthetic approaches to changing the order of coupling and functionalization are underway in our laboratory.

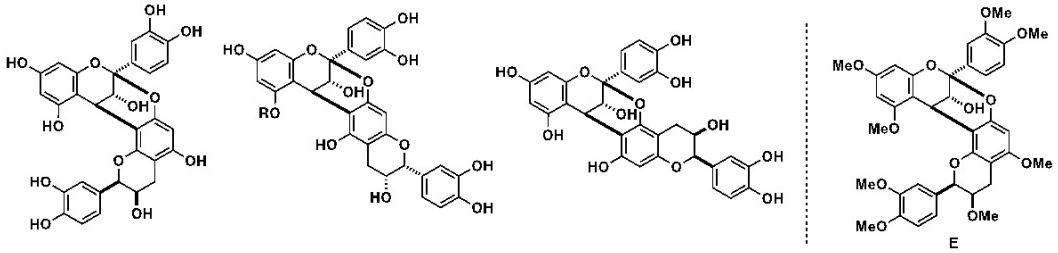


3. Evaluation of the Ab/hIAPP anti-aggregation and disaggregation activities of oligomeric proanthocyanidins.

We evaluated the Ab/hIAPP anti-aggregation and disaggregation activities of A-type procyanidins, which were synthetically obtained in our laboratory.[4] We conducted the thioflavin-T

assay, which quantifies the degree of aggregation of amyloid polypeptides based on fluorescence intensity, and transmission electron microscopy, employed to observe amyloid polypeptides directly, were used to evaluate the activity. The results showed remarkable Ab/hIAPP anti-aggregation and disaggregation activities. In contrast, permethylated compound **E** with no free catechol moiety had no activity.

These results suggest that catechol moiety in a compound is crucial to expressing both activities. Detail study of the biological action of these compounds will be investigated in our research group.



Discussion & Conclusion

We developed a concise synthetic method of oligomeric proanthocyanidins. The first total synthesis of parameritannin A2, a complex natural proanthocyanidin tetramer sharing a branched oligomeric structure and two double-linkage between epicatechin units, has been achieved. We used the two-fold annulation of monomeric epicatechin units with phloroglucinol and Pummerer/Friedel–Crafts cascade reaction to construct the central flavan unit.

We also investigated a regioselective interflavan linkage between two monomeric flavan units to obtain a key annulation precursor, which would be utilized for synthesizing crown procyanidins, a unique macrocyclic OPA showing protective effects against amyloid-β induced toxicity.

Furthermore, Ab/hIAPP anti-aggregation and disaggregation activities of several synthetic OPAs were evaluated. These results would be valuable for discovering a new pharmaceutical candidate to prevent Alzheimer's disease.

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一般の皆様へ

緑茶やワイン等に多く含まれるポリフェノール類には、様々な生理作用が認められている。例えば、複雑な環状構造を持つフラバンオリゴマーには、最近、アミロイドβの凝集を抑制することが明らかにされている。このことはポリフェノールの科学に新たな可能性を示すものであるが、より詳細な研究を進めるためには、多種多様な類縁化合物を高純度サンプルとして得、それらを用いて系統的に生理活性評価を行ってゆく必要がある。

本研究では、これらの一連の化合物の合成研究を行い、これまで天然から得ることの難しかった複雑なポリフェノール類の供給の道を拓いた。さらに、これまで未知であったある種のフラバンオリゴマーが顕著なアミロイドβ凝集抑制作用を示すことを明らかにした。

Molecular genetic analysis of plant sub-stomatal chamber development

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Summary Abstract

The photosynthesis factory, plant leaves have internally porous structure made up of green mesophyll cells and intercellular air space. Despite its importance to enable carbon dioxide uptake and absorption, how leaf mesophyll forms intercellular air space remains unsolved since no genes involved in this process was known. By using *Arabidopsis thaliana* as a molecular genetic model, I discovered multiple components of a novel inter-cell-layer signal transduction that guides the formation of mesophyll air space specifically under stomatal pore in the epidermis. Knockout mutants of these genes altered air space formation.
Key Words : Leaf development, Signal transduction

Introduction

The photosynthesis factory, plant leaves are flattened organs that simultaneously achieve absorption of light and gas molecules including carbon dioxide. For the latter purpose, the outer surface (epidermis) of a leaf has numerous stomata as microscopic air valves, and the inner tissue of a leaf has porous structure made up of mesophyll cells and intercellular air space. Molecular mechanism of stomatal formation has been extensively studied through genetic analysis of mutants with abnormal stomatal development. By contrast, how leaf inner tissue form intercellular air space has remained completely unknown, because of the lack of any genetic clues.

Results

By using *Arabidopsis thaliana* as a model system, I serendipitously discovered a gene named *SUBSTOMATAL CHAMBER* (*SSC*). Transgenic Arabidopsis plants expressing beta-glucuronidase (GUS) reporter under the control of *SSC* gene promoter (*SSCpro::GUS*) visualized a striking patchy expression pattern in numerous small clusters of mesophyll cells located under every stoma. Each clustered mesophyll cells formed an air space in-between, known as the substomatal chamber, which facilitates gas exchange through stomatal pore. The unique expression pattern of the *SSC* gene implied that the mesophyll cells could sense the presence of stomata at close proximity, and that a specific transcription factor should be activated in such cells to target the *SSC* promoter. Thus, the Arabidopsis *SSC* gene is the first, long-sought-for genetic clue of mesophyll air space formation.

I made a series of *SSC* promoter constructs with various deletions, fused with GUS reporter and introduced into Arabidopsis. This narrowed down the regulatory region required for substomatal expression, and I finally pinpointed a short *cis* element that is known as the

binding core sequence of a family of transcription factors. Then I found that a member of the transcription factor family (hereafter transcription factor X, or TFX) is specifically expressed in the clustered mesophyll cells under stomata, very similar to *SSC*. Knockout mutant of *TFX* disrupted the patchy *SSCpro::GUS* expression pattern. Because TFX is expected to target multiple downstream genes other than *SSC*, I constructed a chemically-inducible expression system of TFX fused with GFP, to search for differentially regulated genes upon TFX-GFP induction. Upon chemical induction of TFX-GFP ubiquitous over-expression (confirmed by GFP fluorescence), the *SSCpro::GUS* showed a ubiquitous reporter expression irrespective of the distance between stomata and mesophyll cells, validating that the *SSC* gene promoter is one of the targets of TFX. I am planning to perform transcriptome analysis of chemically induced TFX-GFP by RNA-seq.

SSC encodes a transmembrane protein predicted to function as a receptor of secreted hormones. Because SSC is expressed in mesophyll cells, it is tempting to speculate that SSC receives the secreted signal from stomatal guard cells. Remarkably, the *ssc* knockout mutant disrupted the patchy expression pattern of *SSCpro::GUS*, consistent with the idea that the SSC receptor is required for feedback regulation of its own expression, possibly as a component of stomata-to-mesophyll signaling. Then I focused on a pair of paralogous hormone-related genes specifically expressed in stomatal cells, as the candidate of SSC ligand. I created the double mutant of these genes by crossing respective single mutants generated by CRISPR/Cas9 genome editing. As the result, the hormone double mutant severely disrupted the expression pattern of *SSCpro::GUS* and even abolished air space formation in certain tissue, although the mutant plants were still viable.

The *SSC* gene homolog is widely conserved in land plants even in bryophytes, suggesting the ancient origin of SSC function that accompanied land colonization of plants and adaptation to gaseous atmosphere. The *cis* element targeted by TFX is highly conserved in the promoter region of *SSC* homologs of Brassicaceae family, which includes important crop and ornamental species such as canola, cabbage, radish, wasabi, etc. I cloned the promoter region of *SSC* homolog from Chinese cabbage, fused with GUS reporter and introduced into Arabidopsis. This transgenic line showed patchy substomatal expression of GUS reporter. This result supports the idea that the signaling pathway that I revealed in this study is acting in crop species, which could be utilized for future engineering of more efficient air space structure for carbon dioxide sequestration.

Discussion & Conclusion

Here I discovered a novel and physiologically important signal transduction pathway in plants that guides the formation of mesophyll air space specifically under stomatal pore in the epidermis. Genetic alteration of this pathway led to disruption of air space formation at least in certain tissue, but not in whole leaf possibly because of other redundant pathways. Evolutionary conservation of this mechanism in crop species (as shown in Chinese cabbage) opens a possibility to engineer leaf internal air space to enhance carbon dioxide absorption and hence photosynthetic productivity.

A drawback of substomatal chamber is that the air space is utilized as the entry point of pathogenic microbes to invade inside the leaf tissue. The SSC promoter could be used to drive pathogen resistance genes specifically in substomatal chamber to protect plants from such infection risks. This concept should be experimentally tested in the near future.

Overall, my study is solving an important question in basic plant biology and will also provide clues to improve agricultural production and to provide a solution to global environmental issue.

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[Conference oral presentation]

Yoshida, Y. and Sawa, S. (2022) Intercellular signaling that drives substomatal chamber formation. *In*: Abstract book of the 63rd Annual Meeting of the Japanese Society of Plant Physiologists. pp. 490

一般の皆様へ

植物の葉は光合成の際に二酸化炭素を吸収し、その取り入れ口である気孔については研究が進んでいます。気孔から取り込んだ二酸化炭素を、光合成を行う葉肉細胞に行き渡らせるためには葉の内部にも隙間が必要ですが、隙間を形成する仕組みは全く不明でした。私は、気孔からホルモンの一種が分泌され、それを受け取った気孔近くの葉肉細胞だけが活性化され、細胞間に隙間を作り出す指令となることを発見しました。この仕組みは実験植物だけでなく野菜でも共通しているらしく、より二酸化炭素を吸収する品種が将来的に作れると期待されます。

Highly Efficient Synthesis of Rare Macrolide, Directing Drug Discovery

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Summary Abstract

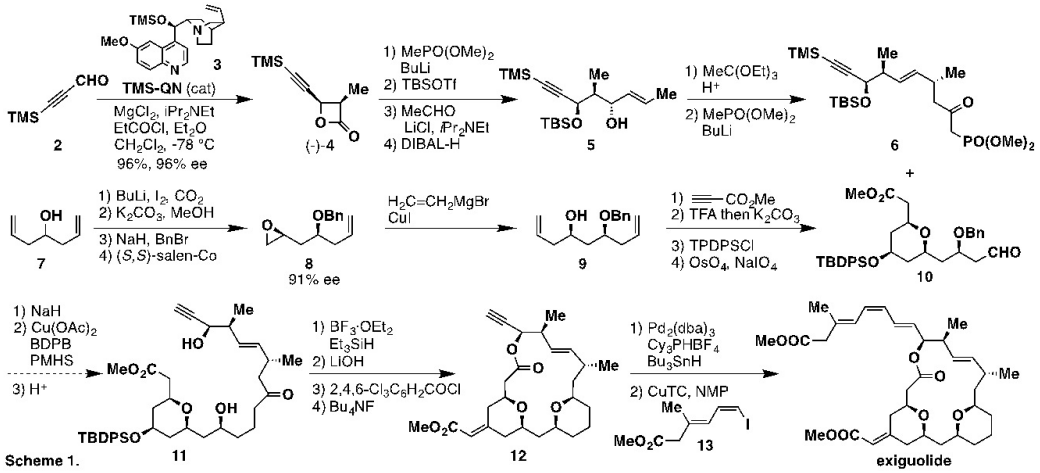
Aiming to develop the synthesis of promising middle-molecular compounds as drug leads and biological tools, highly efficient synthesis of rare macrolide, exiguolide, was studied. The practical and concise strategy provides natural exiguolide and the related compounds and their biological properties were investigated.
Key Words : natural product synthesis, macrolide, antitumor activity

Introduction

Recent rise of antibody and nucleic acid drugs has reached a new phase in the development of low-molecular drugs, which had played a pivotal role for drug development. In contrast, cyclic middle-molecular compounds can interact with protein-protein relationship and have excellent intracellular translocation. In this context, it is urgent to develop a highly efficient synthesis of promising middle-molecular compounds as drug leads and biological tools in a short time. In this work, we focused on the efficient synthesis of polycyclic macrolide natural products such as exiguolide and bryostatins.

Results

Exiguolide is a complex 20-membered macrolide embedded with a bis(tetrahydropyran) motif. This natural product shows inhibition against the growth of various cancer cell lines such as NCI-H460 A549, MB-231, and BxPC3. Exiguolide is thought to be a structurally simplified analogue of bryostatins, which are potent activators of protein kinase C and show remarkable activities against a range of cancers. The significant biological properties and structural challenges have made this molecule attractive targets for synthesis. We have recently achieved the synthesis of a polycyclic macrolide natural product, exiguolide,[1] however there remain room for improvement and challenges from the viewpoint of quantitative supply. Thus, our endeavor in this work focus on more efficient and practical synthesis of these molecules (Scheme 1). Aiming to shorten the synthesis of the C15/C21 segment, we attempted to review our strategies. A [2+2] cycloaddition reaction using asymmetric organocatalyst TMS-QN was performed to obtain the chiral β-lactone (-)-**4** in high yield and in a highly stereoselective manner. Phosphonate **6** was synthesized from (-)-**4** via a six-step process, which involves Horner-Emmons reaction and Johnson-Claisen rearrangement. This synthetic method enabled to avoid a reduction of alkyne to alkene which was less reproducible in our previous work, and also to shorten the step.

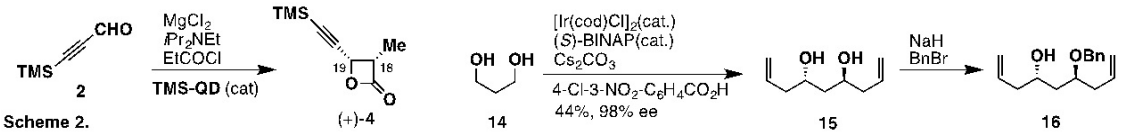


Scheme 1.

In the C1/C14 segment, we found a method that can supply large quantities. That is, after synthesizing a chiral epoxide **8** from a symmetric alcohol **7** via kinetic resolution, the nucleophilic addition of a vinyl group afforded compound **9**, which was readily transformed to aldehyde **10** *via* Prins cyclization. Horner-Emmons reaction of **6** and **10** can be converted to compound **11**, which is a promising intermediate of our previous synthesis of exiguolide.

On the other hand, it has been found that the use of TMS-QD as an asymmetric organocatalyst in the synthesis of β-lactone provides (+)-**4** and the diastereomer (**16**) of **9** is easily obtained in two steps from the diol **14** (Scheme 2). The synthesis of isomers of exiguolide using (+)-**4** and **16** is currently under investigation. Since these synthetic methods enable to provide both enantiomers of segments, a wide variety of exiguolide analogues can be created.

To examine the biological properties, antitumor activities of synthetic exiguolide and the related compounds against A549 cancer cell were examined. The synthesis of 15-*epi*-exigu-



Scheme 2.

olide was carried out according to our synthetic method of exiguolide. Ketone **17**, compound **18** without side chain, exiguolide, 15-*epi*-ketone **19**, compound **20** without side chains and 15-*epi*-exiguolide (**21**) were subjected to the antitumor activity test. It was proved that only exiguolide exhibit cancer cell growth inhibition while the others showed no activity at all. Notably, 15-*epi*-exiguolide showed no antitumor activity.

We next calculated the optimal conformation of exiguolide and 15-*epi*-exiguolide based on MMFF force field. The molecular mechanics studies showed that the conformation of 15-*epi*-exiguolide has a drastic conformational change in macrolide with the side chain covering macrocyclic ring. The conformation change would be attributed to the inversion of C16/C17 alkene adjacent C15 to avoid the A1,3 distortion.

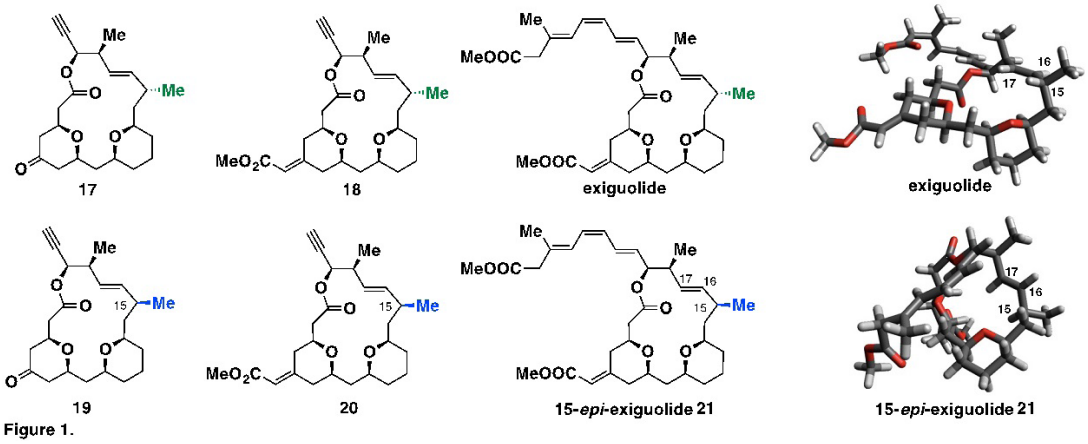


Figure 1.

Discussion & Conclusion

In summary, highly efficient synthesis of rare macrolide, exiguolide and the related compounds was performed with the aim of establishing and creating drug leads. Improved synthesis of exiguolide was developed and antitumor activities of the resulting several analogues were investigated. We carried out conformational calculation studies of the compounds, which shows that the macrolide conformation is very much significant for the biological properties. Further syntheti studies on the related compounds of exiguolide and biological activities are currently under investigation.

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Oka, Kengo; Fuchi Shunsuke; Komine, Keita; Fukuda, Hayato;
Hatakeyama, Susumi; Ishihara, Jun
Chemistry-A European Journal, 2020, 25(26), 12862-12867

一般の皆様へ

今回、我々はイグジグオリドという未来の抗がん剤の候補化合物に着目し研究を行いました。本化合物は天然界から極微量しか得らず、材料不足で創薬研究が進まないという問題がありました。今回、我々は簡便で大量供給可能な合成方法を開発しました。同時に基礎研究に必要な様々な類似化合物を作り出す方法を見出し、ガン細胞に対する活性を調べました。残念ながら、新薬に結びつく結果はまだ得られておりませんが、今後も、より活性が強く、ガン特異的に効く新しい化合物の合成を検討していきます。いつか新しい医薬品を皆様に提供できる日が来ることを望んでおります。

Catalytic Enantioselective α-Halogenation of Acylpyrazoles Directed towards Synthesis of Chiral Drugs

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Summary Abstract

Novel chiral π−copper(II)−π complex catalyzed enantioselective α-chlorination and -bromination of *N*-acyl-3,5-dimethylpyrazoles are described. The π−copper(II)−π complexation of $Cu(OTf)_2$ with 3-(2-naphthyl)-L-alanine-derived amides greatly increases the Lewis acidity and triggers the in situ generation of enolate species without an external base, which has a suppressing effect for α-chlorination and -bromination due to undesired halogen bonding. This strategy provides facile access to α-halogenated compounds in high yield with excellent enantioselectivity. X-ray crystallographic and ESR analyses of the catalyst complexes suggest that the release of two counteranions ($2TfO^-$) from the copper(II) center might be crucial for the efficient activation of *N*-acyl-3,5-dimethylpyrazoles.

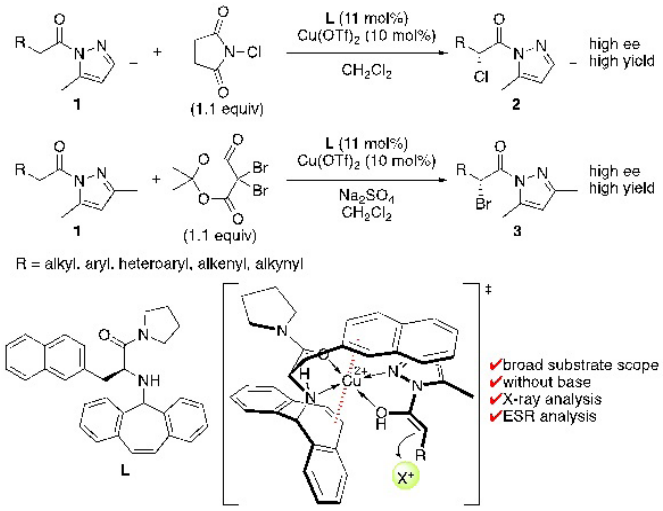
Key Words : enantioselective, α-halogenation, copper(II) catalyst, π−copper(II) interaction, asymmetric catalysis

Introduction

Enantioselective carbon−halogen bond formations are particularly important due to their potential as synthetic intermediates as well as marine natural products and pharmaceuticals. Among the various methods available to build carbon−halogen bonds, the enantioselective electrophilic α-halogenation of carbonyl compounds is one of the most common. Although α-halogenation reactions using 1,3-dicarbonyl compounds, aldehydes, and ketones have been well established, few reports are available on catalytic enantioselective α-chlorination for carboxylic acid derivatives with pK_a values that are relatively high. Most importantly, there have been no successful examples of asymmetric direct α-bromination reactions for amides or esters.

Results

Here we report enantioselective α-chlorination and -bromination reactions using a novel type of catalytic system. We found that the newly designed π−Cu(II)−π catalyst prepared in situ from $Cu(OTf)_2$ and chiral ligand **L** was a superior chiral Lewis acid catalyst because two counteranions were released from Cu(II), thus providing the corresponding halogenated carboxamides in high yield with high enantioselectivity without an external base. *N*-chlorosuccinimide and 5,5-dibromomeldrum's acid were used as halogen sources. α-Halogenated products could be transformed into α -amino esters and epoxides. Successfully, we obtained a single crystal of the 1:1:1 complex of phenylalanine-derived analogous ligand•$Cu(OTf)_2$•*N*-acetyl-3,5-dimethylpyrazole. X-ray crystallographic analysis indicated that one side of the aryl moiety of the *N*-5-benzosuberyl-substituted group seems close to the copper center and its



distance was around 3Å, which is considered to be due to π–copper interaction. We believe this interaction is important for both catalytic activity and asymmetric induction.

Discussion & Conclusion

In summary, we have developed the catalytic enantioselective α-chlorination and bromination of *N*-acyl-3,5-dimethylpyrazoles. With a newly designed highly active π−Cu(II)−π complex, the halogenation reaction of *N*-acyl-3,5-dimethylpyrazoles can be performed using carboxylic acid derivatives with or without an electron-withdrawing group at the α-position without an external base, which has a suppressing effect due to undesired halogen bonding. X-ray crystallographic analysis of copper complexes and ESR analysis revealed the existence of π−Cu(II)−π interaction, which is essential for increasing the reactivity. Thus, a halonium ion predominantly approaches to the *re*-face of the *in situ*-generated enol-form of **1** to give **2** or **3**.

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一般の皆様へ

カルボン酸誘導体のα位の水素をハロゲンで置換し、光学活性α－ハロカルボン酸誘導体を触媒的に不斉合成する方法を開発した。本法で生成するα－ハロカルボン酸誘導体からα－アミノ酸誘導体に化学変換することができることも確かめられており、様々なα－アミノ酸が不斉合成できることになる。α－アミノ酸は医薬品の原料としての用途価値があることから、本研究成果は医薬品の製造や創薬研究に有効である。

Invasive evolution of pathogenic bacteria and cryptic gene activation

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Summary Abstract

In recent years, we have developed a co-culture method for pathogenic actinomycetes and animal cells, and succeeded in activating the criptic gene of actinomycetes to obtain new compounds. This new method reproduces the situation when a pathogenic microorganism infects an animal and imitates a pseudo-infectious state. It is the first example in Japan and overseas, and is original and highly novel. In this study, we investigated how the cryptic gene is activated in the co-culture. Also, co-culture of pathogenic fungi and animal cells gave the compound by activating the cryptic gene. These phenomena are cryptic gene activations based on heterologous interactions. We consider such compound production to be the result of "invasive evolution" and are working to elucidate the mechanism.
Key Words : Natural products, co-culture, cryptic genes, bacteria

Introduction

Actinomycetes and fungi have provided many useful compounds. However, only about 20% of the gene works, and it is known that the biosynthetic gene that will produce a new natural product are cryptic genes. Recently, we have developed a co-culture method for pathogenic actinomycetes and animal cells, and succeeded in activating cryptic genes. This new method reproduces the situation when a pathogenic microorganism infects an animal and imitates a pseudo-infectious state. It is the first example in Japan and overseas, and is original and highly novel. The purpose of this study is to apply this co-culture method to pathogenic fungi, to find new co-culture specific compounds, and to approach their production mechanism.

Results

1. Analysis of Nocarjamide production mechanism

Co-culture of the pathogenic actinomycete *Nocardia tenerifensis* and the mouse macrophage J774.1 produces nocarjamide. By analysis by HPLC, compound peaks that do not appear in single culture alone appear in co-culture. We examined what *N. tenerifensis* responded to in macrophages. Pathogenic actinomycetes are known to be preyed on by macrophages [1]. Then, is it necessary for actinomycetes to come into contact with cells? First, nocarjamide was not obtained by culturing with *N. tenerifensis* after crushing the cells under lysate conditions or autoclave, suggesting that the cells need to be alive. Next, when a culture solution of co-culture of *N. tenerifensis* and mouse macrophage J774.1 or a culture solution of J774.1 alone was added to a single culture of *N. tenerifensis*, nocarjamide was produced

interestingly. This suggests that physical contact between *N. tenerifensis* and the mouse macrophage J774.1 is not required for the production of nocarjamide. It is possible that *N. tenerifensis* is reacting with the proteins and compounds produced by J774.1. Then, when *N. tenerifensis* and J774.1 were shielded and cultured with a dialysis membrane (pore size 5 Å) (compounds and small proteins can pass through), nocarjamide was not produced. Therefore, it was suggested that *N. tenerifensis* may be responding to a relatively large protein produced by macrophages.

2. Co-culture of the pathogenic fungus Aspergillus and macrophages

Next, we attempted co-culture of the pathogenic fungus *Aspergillus* with macrophages. Among the pathogenic fungi *Aspergillus*, the highly pathogenic *Aspergillus fumigatus* strain and mouse macrophage-like cell RAW264 were co-cultured under various medium conditions. Produced compounds were compared with the compounds produced in the single culture of microorganisms by LC-MS. Co-culture-specific compounds were found in Czapek-Dox (CD) medium, static culture, and 1-week culture. After statically culturing in a large-scale culture to obtain a culture solution, various chromatographies are performed. From HRMS (m / z 360.0690 [M + Na] +, calcd for $C_{15}H_{15}NO_8Na$ 360.0691, the molecular formula of the compound was determined to be $C_{15}H_{15}NO_8$. The structure was determined using various NMR (nuclear magnetic resonance apparatus). NMR suggested the existence of a trans-type double bond, and two-dimensional NMR suggested the existence of a benzene ring, γ-lactone skeleton, amide bond, and ester bond with a pyrogallol-type oxidation mode. Compound **1** has a structure similar to the known compound (-)-fumimycin [2] isolated from *A. fumisynnematus* F746. A *Fumigatus* strain, which does not have azole resistance, was similarly co-cultured, but no peak of **1** was found.

We also attempted to identify genes whose expression is increased or decreased specifically in co-culture by RNA-seq. As a result, the expression of SidE, which is a biosynthetic gene cluster of fumarylalanine, was significantly increased. Very interestingly, it has been reported that when *A. fumigatus* is infected from the nasal cavity of mice, the expression of SidE, a biosynthetic cluster of fumarylalanine, is increased and fumarylalanine is produced [3]. If you look closely at the structure of compound **1**, there is a unit of fumarylalanine. That is, it was speculated that co-culture of *A. fumigatus* and mouse macrophage-like cell RAW264 also increased the biosynthesis of fumarylalanine, and that compound **1** was biosynthesized using fumarylalanine. Thus, this co-culture is considered to imitate the actual infection state in vivo. Currently, we are working on the evaluation of the biological activity of compound **1**.

Discussion & Conclusion

Pathogenic fungi isolated from clinical specimens held by medical mycology research center, Chiba University and immune cells (mouse macrophage-like cells J774.1, RAW264) were co-cultured under various conditions. The compound production during cell-only culture, fungus-only culture, and co-culture was compared by HPLC, and co-culture-specific compounds were found.

Various studies were conducted to elucidate the production mechanism of nocarjamide produced by co-culture of pathogenic actinomycete *Nocardia tenerifensis* and mouse macrophage J774.1, and physical contact between *N. tenerifensis* and mouse macrophage J774.1 is not necessary for the production of nocarjamide. It was speculated that *N. tenerifensis* may

be responding to a relatively large protein produced by macrophages.

The pathogenic fungus *Aspergillus fumigatus* strain and mouse macrophage-like cell RAW264 were co-cultured, and compound **1** produced specifically for co-culture was isolated and structurally determined. The co-culture was carried out in the same manner with the *A. fumigatus* strain having no azole resistance, but no peak of **1** was found. We also found that the expression of fumarylalanine biosynthetic cluster SidE was increased in a co-culture-specific manner by RNA-seq. Co-culture of *A. fumigatus* and mouse macrophage-like cell RAW264 increased the biosynthesis of fumarylalanine, and it was speculated that compound **1** was biosynthesized using fumarylalanine.

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一般の皆様へ

我々は近年、病原放線菌と動物細胞の共培養法を開発し、放線菌の休眠遺伝子活性化に成功し新規化合物を得ている。この新規手法は、病原微生物が動物に感染する際の状況を再現し、疑似感染状態を模倣したもので、国内外でも初めての例であり独創的で新規性が高い。本研究では、どうやって休眠遺伝子が活性化されるのかを探るとともに、新たに病原真菌と動物細胞の共培養に取り組み、休眠遺伝子活性化による化合物の取得に成功した。これらの現象は、異種生体間相互作用が基盤となる休眠遺伝子活性化であり、大変に興味深く、我々はそのような化合物生産を「浸潤進化」の結果であると考え、メカニズム解明に取り組んでいる。

Development and evolution of an optogenetic gene expression system with red-light

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Summary Abstract

In this research, based on optogenetics, in which protein engineering was applied to red photoreceptors derived from fungi based on genetic engineering techniques, I aimed on the development of a new red light-controllable gene expression system that combines a signal amplification method to realize highly efficient optical manipulation of gene expression in mammals. Based on the red photoreceptor bacteriophytochrome DrBphP derived from *Deinococcus radiodurans*, I have been developing chimeric proteins that fuse the LexA DNA-binding domain with the transcription factor VP 16. As a result, gene expression was successfully induced several times in a red light-dependent manner. In the future, based on this developed technology, I will develop a basic technology for optogenetics that can realize highly efficient red light manipulation of gene expression in mammalian organisms by improving light induction efficiency by combining signal amplification methods.
Key Words : Optogenetics

Introduction

Optogenetics has been emerging as a technology that uses light-responsive photoswitching proteins to freely control protein activity, localization, and gene expression in a light-dependent manner. However, as many existing optogenetic technology systems that have been developed so far are based on blue light-responsive photoswitching proteins, the tissue permeability of blue light in living organisms, especially mammals, is a serious problem to use them in vivo. A technology to control protein functions and gene expressions by using the light of long-wavelength that is more suitable for tissue permeability is required.

Results

In this study, I worked on the development of a novel red light-controllable gene expression system combined with a signal amplification method to realize highly efficient optical manipulation of gene expression in mammalian organisms, based on an engineered photoreceptor, which is protein engineering of red light photoreceptors. I have developed a novel red light-controllable gene expression system using the bacterial phytochrome DrBphP[1], a red light photoreceptor from a *Deinococcus radiodurans* that binds the bilin cofactor bilveridin as a light-senstive factor. First, based on information on the homodimeric structure of the photoreceptor and the LexA DNA-binding domain structure[2], I found that the fusion protein of DrBphP and LexA binds to DNA only when irradiated with red light. Based on the information on the homodimeric structure of the receptor and the LexA DNA-binding domain structure, I rationally designed a molecule that can bind to DNA only when illuminated with red light. Based on this

principle of operation, a multi-chimeric protein was created by further fusing the transcription factor VP16 to the DrBphP-LexA fusion protein. When the construct was transfected into a mammalian cell line and irradiated with red light, changes in bioluminescence by firefly luciferase, a reporter gene, were observed in a light-dependent manner, indicating that gene expression can be freely controlled by starting or stopping red light illumination (Figure 1). This result suggests that a chimeric protein fusing the LexA DNA-binding domain with the transcription factor VP16, based on the bacterial phytochrome DrBphP can induce several-fold increase in gene expression in a red light-dependent manner. On the other hand, induction of gene expression in the dark condition (dark-leakiness) remained a major issue. Therefore, I developed a chimeric protein that combines a DNA-binding domain and a transcription factor based on bacterial phytochrome, a red light receptor from cyanobacteria. As a result, the induction of gene expression in the dark (dark-leakiness) was greatly improved in the absence of light. On the other hand, however, the light-dependence of gene expression was greatly reduced, and as a result, the improvement of light-induction efficiency remains a major issue to be addressed. These results indicate that they will be used as the basis for the development of a basic technology for optogenetics to realize highly efficient red light manipulation of gene expression in mammalian cells by improving the efficiency of light induction through the combination of the signal amplification method based on the developed technology.

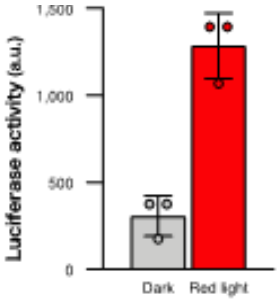

Figure 1_Luciferase assay of gene expression induction of red-light photoswitch system with red light or in the dark condition.

Discussion & Conclusion

In this study, I developed a novel basic technology to manipulate gene expression by red light. By combining this technology with signal amplification[3], dramatic amplification of signal gene expression is expected in the future. This will enable to realize optogenetic manipulation of gene expression in mammals with high light induction efficiency as a basic technology with protein engineering based on genetic engineering methods. This is also expected to greatly improve the current basic technology of optogenetics and to have a large ripple effect on the entire field of optogenetics. In addition, as a practical optogenetic technology in living mammals, it is expected to be applied to unexplored research areas where optogenetics has been difficult to apply so far, such as organ regeneration medicine and various disease therapies.

References

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一般の皆様へ

本研究では、遺伝子工学的手法を基にタンパク質工学を施した光遺伝学を基盤技術として、哺乳動物生体内で遺伝子発現の光操作を高い光誘導効率で実現するために、シグナル増幅法を組み合わせた赤色光作動性の遺伝子発現光操作技術の開発に取り組んだ。放射線耐性細菌由来の赤色光受容体バクテリアフィトクロム DrBphP を基に、赤色光による遺伝子発現の操作を実現する新規基盤技術を開発した。本技術はシグナル増幅法とさらに組み合わせることによって、遺伝子発現の劇的な増幅を実現することが期待される。それにより現在の光遺伝学が抱える青色光の組織透過性の問題に対して、赤色光受容体を用いることでその克服が期待される。また同時に既存技術の実用性の問題に対して、高い光誘導効率を実現する。本研究の成果は、現在の光遺伝学の基盤技術を大きく底上げし、当該領域全体に大きな波及効果が得られることが期待される。さらに、生きた哺乳動物生体内で実用的な光遺伝学の技術として、臓器再生医療や様々な疾患治療法など、これまでの光遺伝学が応用困難であった未開拓の研究領域に応用する展開が期待される。

Study on germ cell-specific genes that are crucial for fertility

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Summary Abstract

Meiosis is a crucial process for production of sperms and oocytes. During meiotic prophase, chromosomes are organized into axis-loop structures, which underlie the meiosis-specific events such as meiotic recombination and homolog synapsis. Meiotic prophase is accompanied by robust alterations of gene expression programs and chromatin status for subsequent sperm production. Recently, we discovered MEIOSIN that directs the switch from mitosis to meiosis. In our studies, we discovered the downstream genes activated by MEIOSIN, which are crucial for meiotic prophase-specific events and gene expression program.
Key Words : Meiosis, infertility

Introduction

Meiosis is a specialized cell cycle for germ cell development. Recently, we identified MEIOSIN (Meiosis initiator) as a germ cell-specific factor that activates numerous meiosis-related genes (Ishiguro et al. ***Dev Cell* 2020**). Our discovery of MEIOSIN led to other findings of numerous MEIOSIN-binding targets that were yet to be characterized. Notably, some of those targets were encoded by hypothetical genes whose annotation had not been registered on the database. Given that MEIOSIN binds and activates genes that are essential for meiosis, we reasoned that those uncharacterized MEIOSIN target genes may play a crucial role in meiosis.

Results

We have been extensively studying those MEIOSIN-downstream genes, and identified new factors for miosis and germ cell development. Among the MEIOSIN target genes, we newly identified genes that play essential roles in meiotic prophase-events, of which disruption led to male infertility (Takemoto et al, ***Cell Reports* 2021**, Horisawa-Takada et al., ***Nat. Commun.* 2021,** Tanno et al, ***iScience* 2022**).

ZFP541 is expressed in spermatocytes and round spermatids, and interacts with HDAC1/2 and germ-cell specific KCTD19. Disruption of *Zfp541* leads to defects in the completion of meiotic prophase, with severe impact on male fertility. Thus, ZFP541 plays a critical role in promoting developmental progression of meiotic prophase towards completion in spermatocytes. Chromatin binding analysis of ZFP541 combined with transcriptome analysis demonstrates that ZFP541 binds to and represses a broad range of genes whose biological functions are associated with the processes of transcriptional regulation and covalent chromatin modification (Figure 1). It is worth noting that most of these genes were generally expressed in broad cell types rather than being germ-cell specific. For example, these include *Dnmt1, Dnmt3A* (DNA methyl transferase), *Ino80D, Chd1, Chd2, Chd3, Chd6* (chromatin remodel-

ing), *Kat6A, Kmt2C, Kmt2B, Kdm2A, Kdm5B, Ash1L, Bmi1, Jarid2, Ezh1, Ezh2, Ehmt1, Rnf2, Suz12* (Histone modification), and *Ctcfl* (chromatin binding) (Figure 7B). Notably, expressions of those ZFP541-bound genes are overall declined in prophase spermatocytes and post-meiotic round spermatids, compared to spermatogonia. This, at least in part, leads to reconstruction of chromatin status for spermiogenesis. Thus, HDAC1/2-containing ZFP541-KCTD19 complex represses the transcription of a subset of critical genes that are involved in transcriptional regulation and chromatin modification prior to the completion of meiotic prophase. ZFP541 may trigger the reconstruction of the transcription network to promote the completion of prophase, finalize meiotic divisions, and proceed into spermatid production.

For another example, we identified Fbxo47 that acts for homolog synapsis during meiotic prophase (Tanno et al, ***iScience* 2022**). Our finding clarified that FBXO47 is the stabilizer of synaptonemal complex during male meiotic prophase I. Disruption of FBXO47 shows severe impact on homologous chromosome synapsis, meiotic recombination and XY body formation, leading to male infertility. Notably, in the absence of FBXO47, although once homologous chromosomes are synapsed, the synaptonemal complex is precociously disassembled before progressing beyond pachytene. Remarkably, *Fbxo47* KO spermatocytes remain in earlier stage of meiotic prophase I and lack crossovers, despite apparently exhibiting diplotene-like chromosome morphology. We proposed that FBXO47 plays a crucial role in preventing synaptonemal complex from premature disassembly during cell cycle progression of meiotic prophase I.

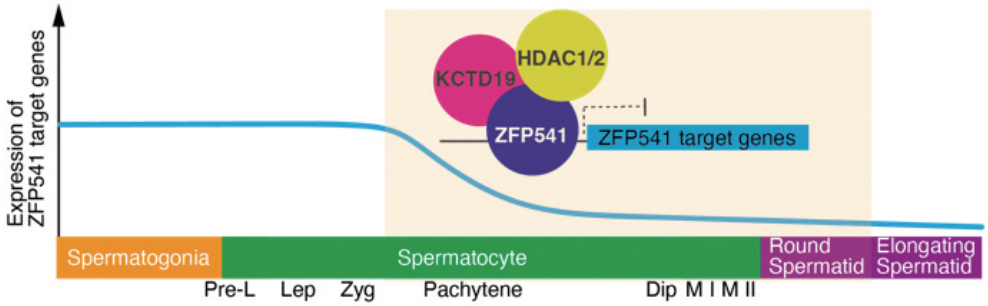

Figure1. ZFP541 represses chromatin-related genes during meiotic prophase

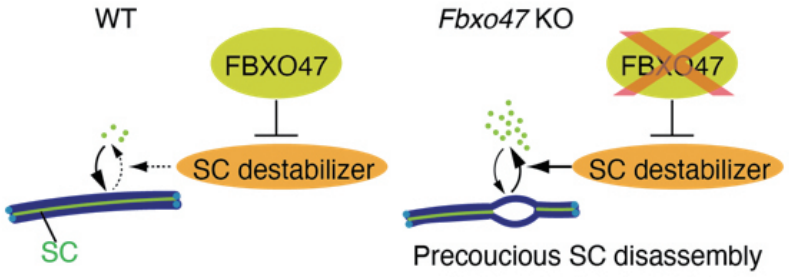

Figure2. FBXO47 plays a role in preventing synaptonemal complex from premature disassembly

Discussion & Conclusion

We identified new players of meiosis by investigating the downstream target genes of MEIOSIN. Our finding clarified the new mechanism of how gene expression and chromosome dynamics are regulated during meiosis I. Our preliminary data suggests that many of those potential MEIOSIN target genes are specifically expressed in the testis and/or ovary, and conserved only in mammals or vertebrates. Since regulation of meiosis is accompanied by the processes of germ cell development, we hypothesize that those MEIOSIN target genes may play previously unforeseen roles that link meiosis and germ cell development in mammals. Further identification of new factors that involve in regulation of meiosis in the context of germ cell development will facilitate our understanding of genetic mechanisms of human infertility.

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一般の皆様へ

我々が同定した減数分裂開始のマスター転写因子 MEIOSIN によって直接制御される標的には多くの機能未解析の遺伝子が含まれている。これら MEIOSIN の転写制御下に置かれている未解析の遺伝子には、減数分裂の進行に必要とされる未知のものが含まれる可能性があり、一挙に減数分裂の仕組みの解明に迫る足掛かりを得ることが期待される。本研究によって、このうちの一つである Fbxo47遺伝子や Zfp541遺伝子が減数分裂において極めて重要な働きをすることや、これらの欠損によって雄性不妊を示すことを明らかにした。減数分裂開始因子の親玉を押さえたことで、体細胞分裂と減数分裂との本質的な違いを決定付けるメカニズムの全容解明に向けて、国際的にも圧倒的に有利な状況で今後の研究を推進することができる。また、疾患モデル動物による検証を組み合わせた研究により、減数分裂の基礎研究とともに不妊に関連する原因因子の特定に資することが期待される。

Multidimensional regulation of DNA replication by a genome-epigenome-transcriptome (GET) network during cellular senescence

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Summary Abstract

I found that histone H2B mono-ubiquitination (H2Bub), a transcription associated histone mark, is highly enriched in rapidly proliferating early-passage (P2) primary mouse embryonic fibroblasts (pMEFs). Strikingly, in late-passage (P5) non-proliferative MEFs, H2Bub levels are drastically reduced, showing that H2Bub could be a previously unappreciated histone mark that distinguishes rapidly proliferative cells from non-proliferative ones. Loss of H2Bub in non-proliferative MEFs is accompanied with increased deposition of the insulator protein CTCF on the chromatin. My study therefore indicates that reduction of H2Bub may cause re-wiring of the 3D genome by excess binding of CTCF in non-proliferative cells.
Key Words : Primary MEF, H2Bub, Transcription, CTCF, 3D genome

Introduction

Senescence plays a role in aging, and disruption of senescence is frequently associated with diseases such as cancer that show uncontrolled proliferation of transformed cells. However, the molecular mechanisms that regulate senescence are not well understood. Here, I have focused on the role of genome-epigenome-transcriptome (GET) to gain insight into cellular senescence.

Results

Mouse pMEFs (see above) rapidly proliferate during early-passage (passage 1-3), but stops proliferation in late-passage (passage 5). Early-to-late passage pMEFs, therefore, presents a nice model to study cellular senescence without using artificial experimental schemes such as RAS-induced senescence.

From my preliminary studies in mouse embryonic stem (ES) cells, I have found that depletion of H2Bub (see above) causes attenuation of cell proliferation, indicating that H2Bub might suppress senescence. To reveal whether H2Bub plays a role in pMEF senescence, I performed ChIP-seq and determined the level of H2Bub in early (P2) or late (P4, P5) passage pMEFs.

H2Bub is deposited directly downstream of RNA polymerase II (Pol2) mediated transcriptional elongation. Consistent with this notion, I observed that in early-passage pMEFs, highly transcribed (and therefore Pol2-bound) genes were also enriched in H2Bub. Strikingly, how-

ever, the level of H2Bub at the same genes were dramatically reduced in late-passage (P4 or P5) pMEFs (see Figure 1A, 1B). This indicated that H2Bub is a feature of rapidly proliferating cells and that loss of H2Bub accompanies attenuation of cell proliferation in pMEFs, supporting my observations in mouse ES cells that loss of H2Bub leads to attenuation of proliferation.

From my analysis in mouse ES cells, I have also found that H2Bub is enriched over thousands of short interspersed nuclear elements (SINEs) in the mouse genome. Interestingly, the insulator protein CTCF that plays a key role in 3D genome organization, also bind SINEs. Furthermore, depletion of H2Bub in mouse ES cells led to over-accumulation of CTCF, indicating that H2Bub suppresses CTCF binding. Given that H2Bub antagonizes CTCF, I wondered whether CTCF binding was increased in non-proliferative pMEFs (P4 or P5). Indeed, ChIP-seq analyses showed that there was genome-wide increase of CTCF binding in the non-proliferative pMEFs.

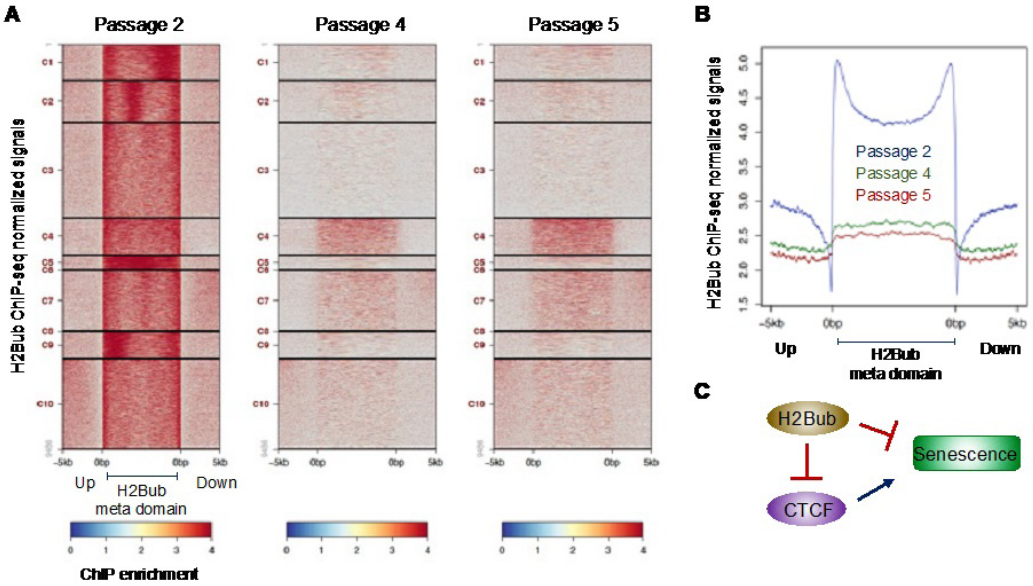

Figure 1. (A) Heatmap showing that H2Bub enrichment over genes is high in early-passage (passage 2) pMEFs but is dramatically depleted in late-passage (passage 4 or 5) pMEFs. (B) Metaplot representation of the results shown in (A). (C) Model showing the mechanistic relationship between H2Bub and CTCF regulating cellular senescence.

Taken together, these results indicate a model by which H2Bub counteracts over-enrichment of CTCF in rapidly proliferative cells (i.e. early-passage pMEFs or mouse ES cells). However, in non-proliferative cells (i.e. late-passage pMEFs) H2Bub levels are reduced, in parallel with a reciprocal gain of CTCF binding to the chromatin. The same molecular antagonism is also observed in mouse ES cells in which depletion of H2Bub leads to increase of CTCF binding.

Cellular senescence is often characterized by drastic changes in the nuclear architecture such as SAHF (senescence associated heterochromatin foci). This indicates that 3D re-wiring of the 3D nuclear architecture may play a role in cellular senescence. From recent studies, it has also become clear that CTCF, in association with cohesin proteins such as RAD21 and SMC1A, regulates the 3D genome. CTCF binding is required to form loops that insulate neighboring domains from one another. However, it is not known whether CTCF also plays a

role in 3D genome re-wiring during senescence. My study, by linking H2Bub and CTCF during cell proliferation, provides a model that could integrate transcription (Pol2 elongation), epigenome (H2Bub) and genome (CTCF mediated re-wiring at SINE repeats) during cellular senescence (see Fig 1C).

Discussion & Conclusion

Here, I utilized a primary MEF (pMEF) model that recapitulates cellular senescence in cell culture. Early-passage (P2) pMEFs proliferate rapidly, but late-passage (P4 or P5) almost stop proliferation. Using this model, I was able to show that H2Bub, a histone mark deposited directly downstream of RNA polymerase II (Pol2) mediated transcriptional elongation, is strongly enriched over highly transcribed genes. However, H2Bub levels are dramatically reduced in late-passage pMEFs that stop proliferation, indicating that decrease in H2Bub is associated with attenuation of cell proliferation. Intriguingly, reduced H2Bub led to increase of binding of the insulator protein CTCF to chromatin, especially in the genomic regions that harbor the SINE repetitive elements. H2Bub, therefore, might play a role to regulate correct 3D genome organization in rapidly proliferating cells by counteracting CTCF. Reduction of H2Bub, leading to a reciprocal gain of CTCF, may re-wire the 3D genome in non-proliferative cells and facilitate cellular senescence. In summary, my study provides a previously unknown link between histone modification and chromatin insulation, mediated by H2Bub and CTCF, during cell proliferation and senescence (Fig 1).

一般の皆様へ

細胞老化は免疫応答などを司る幹細胞の増殖を制御し、健康寿命に密接に関わっている。さらに、細胞老化機構の異常は細胞の無限増殖を誘導することで癌などの原因にもなり得る。そのため、健康状態の維持や癌抑制などの観点から、細胞老化の分子機構の解明は重要な意味を持つ。本研究では、初代マウス胎児線維芽細胞（pMEF）と用い、活発に増殖する細胞とほとんど増殖を止めている老化細胞を比較した。この比較から、活発に増殖する細胞ではヒストン H2B ものユビキチン化（H2Bub）レベルが高く、一方で増殖をほとんど止めている細胞では CTCF という分子の結合が強くなることを見出した。今後、本研究で明らかになった H2Bub や CTCF などの因子に着目していくことで、細胞老化の分子メカニズムをより深く理解し、さらにその先にある医薬品開発などにも結びつけたいと考えている。

A chemical genetics approach for the identification of direct substrates of Alzheimer's disease-associated kinase MARK4 in neurons

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Summary Abstract

Abnormal activation of microtubule affinity regulating kinase (MARK4) has been linked to the pathogenesis of Alzheimer's disease and cancer, while the molecular details have not been elucidated. The aim of this research is to systematically identify direct substrate of MARK4 by using chemical genetics screen. The outcome of this study enhance our understanding of these diseases and may reveal novel therapeutic targets.
Key Words : Alzheimer's disease, cancer, phosphorylation, chemical genetics, microtubule affinity regulating kinase 4

Introduction

Ser/Thr kinases that belong to Par-1 /Microtubule affinity regulating kinase (MARK) family are conserved among species and play essential roles in cell proliferation, development, polarity, and energy metabolism (Wang and Mandelkow, 2016). Mammals have four members including MARK4, whose upregulation has been proposed to play a role in the pathogenesis of Alzheimer's disease (AD) (Lund et al., 2014; Pathak et al., 2020; Rovelet-Lecrux et al., 2015). Accumulation of the microtubule-associated protein tau causes neuronal death in AD, and MARK4 enhances tau-induced neurodegeneration. MARK4 is well known for its role in phosphorylating tau, while additional mechanisms seem to contribute to the enhancement of tau toxicity via MARK4 (Oba et al., 2020). Systematic identification of MARK4 substrate would reveal the cellular processes associated with tau toxicity. In this project, we used a chemical genetic screen to identify direct substrates of MARK4.

Results

Preparation and characterization of an analog-specific mutant of MARK4 (AS-MARK4)

We first prepared analog-specific MARK4 (AS-MARK4). We identified the 'gatekeeper residue' in the ATP-binding pocket of MARK4 via alignment of the amino acid sequence of AMPK, a closely related kinase and successfully used for chemical genetics (Banko et al., 2011). This residue will be in close contact with the N6 position of the adenine ring of ATP. We replaced it with a smaller amino acid so that MARK4 with this mutation (AS-MARK4) can use ATP analogs containing bulky groups at the N6 position. When it uses N6-modified ATPγS nucleotides to phosphorylate substrates, the transferred thiophosphate can be alkylated and recognized by a ThioP-specific monoclonal antibody (Banko et al., 2011). AS-MARK was subcloned into

an expression vector and sequenced. We expressed AS-MARK4 in HEK293 cells by transient transfection, and AS-MARK expression was confirmed with Western blot.

To test if AS-MARK can label the substrate, HEK293 cells expressing AS-MARK was incubated with N6-modified ATPγS. Free N6-modified ATPγS were removed and the thiophosphate were alkylated. The lysate were spotted on the membrane, and probed with anti-ThioP antibody. We detected alkylated thiophosphate signals that lysate from AS-MARK transfected cells, but not that from mock- transfected cells. Next, we carried out SDS-PAGE followed by western blotting with ThioP antibody. We detected several bands specifically labeled with AS-MARK4 by Western blotting with ThioP antibody. We carried out a similar experiment with HeLa cells, which showed an overlapping but different set of bands detected by ThioP antibody. These results suggest that AS-MARK4 can be used to label MARK4 substrates in cultured cells.

Immunoprecipitation of substrate

To systematically identify MARK4 substrate, the cell lysate was subjected to immunoprecipitation via ThioP antibody to concentrate them for LC-MS/MS. However, we encountered many technical problems. The proteins should be visible on the gel after CBB staining, while we could not detect clear bands other than IgG. We tried to improve the immunoprecipitation efficiency by changing buffer and IP conditions. Higher expression of AS-MARK may help; thus we plan to try a virus-mediated expression system.

Enhancing kinase activity of AS-MARK4 maybe another strategy to improve labeling efficiency. While MARK4 has significant basal activity, it can be activated by upstream kinase, LKB1 (Timm et al., 2006). We will test if co-expression of LKB1 with AS-MARK4 improves the labeling efficiency. From another line of experiments, we identified a novel autoinhibitory domain in MARK4, and deletion of this domain may result in a constitutively active form (unpublished data). We will make AS-version of this MARK4 to label substrate more efficiently.

Discussion & Conclusion

We successfully established analog-sensitive MARK4 (AS-MARK4) as a tool to identify direct MARK4 substrates. Western blotting results suggest that AS-MARK4 labels its substrates with ATP analogs. To identify those substrates, we are in the process of finding the conditions for immunoprecipitation. Once the condition is optimized, we will scale it up to obtain sufficient proteins for LC-MS/MS. In addition to Alzheimer's disease and related neurodegenerative disorders, MARK4 is associated with several diseases, including cancer and diabetes (Heidary Arash et al., 2017). AS-MARK4 may contribute to understanding the molecular mechanisms underlying disease pathogenesis.

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一般の皆様へ

私たちは、アルツハイマー病など認知症の原因となる神経変性疾患の発症メカニズムを研究しています。これらの疾患では、記憶など認知機能を担う脳の細胞が死んでいきますが、それを止める手立ては今のところありません。なぜ脳の細胞が死んでしまうのかが分かれば、根本的な予防法、治療法の開発につながります。この研究では、アルツハイマー病脳で増加し、細胞死に関わると考えられている酵素について調べました。細胞死に関わる経路を解明することで、新しい薬や治療法の開発につながることが期待されます。

Establishment of in vivo β-cell Ca^{2+} imaging method for analysis of insulin dynamics

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Summary Abstract

Using transgenic mice expressing a calcium sensor protein in pancreatic β-cells, we tried to establish intracellular calcium activity of β-cells for further understanding of insulin dynamics in living animals. We produced the methods to monitor Ca^{2+} activities in multiple islets simultaneously, and obtained some unprecedented findings in β-cell Ca^{2+} activities.
Key Words : β-cells, Ca2+ signal, in vivo imaging

Introduction

Pancreatic β-cells release insulin to control glycemia in a Ca^{2+}-dependent manner. Spatio-temporal dynamics and function of Ca^{2+} signaling in β-cells have been studied by experiments using in vitro and ex vivo preparations. However, in vivo study remains challenging. Unlike the case of in vitro/ex vivo preparations, β-cells in vivo are under the influence of the autonomic nervous system, hormones and other bioactive substances. Thus, β-cell Ca^{2+} activities under physiological conditions have not been clarified.

Results

In current study, we tried to establish a method to analyze in vivo β-cell Ca^{2+} signals using a transgenic mouse line expressing a ratiometric Ca^{2+} indicator protein, yellow cameleon-Nano50 (YC-Nano50). Using the tetO-mouse line which was produced in our previous study (Kanemaru et al., Cell Rep 2014) and β-cell-specific tetracycline transactivator mouse line, we succeeded to produce a mouse line with abundant YC-Nano50 expression in pancreatic β-cells. We visualized β-cell Ca^{2+} signals in laparotomized mice under anesthesia, and observed synchronized Ca^{2+} oscillations in β-cells within individual islets. Furthermore, we succeeded in monitoring Ca^{2+} activities in multiple islets simultaneously. Now, we are challenging to observe Ca^{2+} activities in β-cells under awake mice with chronic attachment of glass cover slips as an optical imaging window. Preliminary data indicating Ca^{2+} signals just after feeding was obtained. Further confirmation with proceeding of the experiments is required for clarification of relationship between glycemia to blood insulin level.

Discussion & Conclusion

Our current study provided unprecedented findings which may be clues to understand the regulation mechanism of intravital insulin dynamics. Although further confirmations and repeated experiments are required, our ongoing study supported by the Novartis Research Foundation will be published in near future. Moreover, further studies in living animals using our new method are expected to help elucidate the mechanism of insulin secretion and the etiology of diabetes. This method can be broadly applied to other organs/cell types.

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一般の皆様へ

観測方法が存在しないために未解明な体内インスリン動態の謎は数多く存在する。本研究では生体内で唯一のインスリン供給源である膵β細胞のインスリン放出動態を、膵β細胞内のカルシウム動態で間接的にモニターするという、これまでは非常に困難であった手法の開発に挑戦した。モデル動物として遺伝子改変マウスを用いた実験により、生きたマウス体内での膵β細胞内のカルシウム動態の可視化解析に概ね成功している。この手法を用いたさらなる研究は、インスリン分泌機構とインスリン体内動態のさらなる理解、ひいては糖尿病の新規治療法のヒントを与える可能性が期待される。

Glial control of behavioral disorders induced by social distancing

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Summary Abstract

Prolonged isolation could result in social behavioral disorders. In this study, we recorded brain activity in mice that is altered by social environmental factors. Among them, we focused on the cerebellum, which has recently been implicated in autism spectrum disorders. We found that the cerebellum, traditionally thought to be exclusively involved in motor learning, is involved in social behavior. Furthermore, we measured the activity of glial cells, which influence the mode of operation of neural activity. A novel treatment strategy that targets the modification of cerebellar glial activity could be sought to overcome socialization deficits caused by social isolation.

Key Words : cerebellum, astrocyte, social interaction, fiber photometry, optogenetics

Introduction

The cerebellum has been thought to be a brain region primarily involved in motor regulation and motor learning. Recently, functional neural connections between the deep cerebellar nuclei (DCN) and the ventral tegmental area (VTA) were identified, revealing part of the neural basis linking the cerebellum to non-motor brain functions (Carta et al., *Science* 2019). Furthermore, inflammatory responses by microglial cells in the cerebellum have been shown to lead to depressive-like symptoms and decreased sociability (Yamamoto et al., *Cell Reports* 2019). In this study, we decided to test the hypothesis that cerebellar Bergmann glial cell activity is linked to social behavior.

Results

Human fMRI studies have shown that cerebellar activity is associated with social behavior, emotion, and addiction. Abnormal cerebellar activity has also been linked to autistic spectrum disorders and schizophrenia. It was also discovered that oxytocin, which is closely related to social behavior and is called the affection hormone, is also present in the cerebellum (Lipari et al., *Cerebellum Ataxias* 2015).

We have previously shown the presence of the signal exchange between cerebellar Bergmann glial cells and Purkinje cells (Matsui and Jahr, *Neuron* 2003). During infarction, rapid acidification occurs in cerebellar Bergmann glial cells, which triggers the release of excess glutamate and the destruction of brain tissue due to excitatory neurotoxicity (Beppu et al., *Neuron* 2014). Cerebellar-dependent fine motor control and motor learning are thought to be achieved by long-term depression (LTD) of synaptic transmission between parallel fibers and Purkinje cells. However, artificial photostimulation of the Bergmann glial cells covering these synapses to release glutamate alone was sufficient to induce LTD and promoted cerebellar-

dependent motor learning (Sasaki et al., *PNAS* 2012). Furthermore, blocking this glia-to-neuron signaling pathway reduced the activity of mGluR1, which is suggested to be the key receptor for the expression of LTD (Beppu et al., *J Physiol* 2021). Therefore, we propose that glia mediated synaptic signal amplification mechanisms is required for the proper functioning of the cerebellum. We have also analyzed the existence and function of signals that control glial cell activity, which changes on a second-by-second basis, and bridge the gap between glial cells and neurons through neuron-glia-vascular coupling as well as through crosstalk between metabolism and neuronal information processing (Masamoto et al., *Sci Rep* 2015; Takata et al., *Glia* 2018).

The use of genetic manipulation tools is essential to the advancement of modern biology, and mice are exclusively used as animal models. In addition, "behavior" is the final phenotype of brain function that can be objectively evaluated. However, in conventional brain science research, many methods have directly linked gene and molecular functions to behavior, leaving the crucial circuits in the brain as a black box. This is especially true in the analysis of social behavior, where the influence of the brain circuits of each individual on social behavior has not been well investigated. In addition, when analyzing social behavior, it is necessary to distinguish between multiple individuals for quantitative analysis, which has been difficult with existing image analysis systems. Therefore, this study aimed to elucidate the brain circuitry that generates social behavior by simultaneously measuring the brain activity in individuals. Specifically, simultaneous discrimination of multiple individuals was accomplished using the next-generation behavior analysis platform with the deep learning method (DeepLabCut). At the same time, glial cell activity was measured optically using the fiber photometry method.

We have previously generated genetically engineered mice expressing sensor protein that changes fluorescence in response to glia-specific Ca^{2+}, pH, lactate, and pyruvate levels (Kanemaru et al., *Cell Rep* 2014). By implanting an optical fiber into the cerebellum, irradiating it with excitation light, and measuring the returned fluorescence spectroscopically using a photomultiplier tube, the dynamics of various molecules in the living brain was measured in real time. One parameter of interest was pH. It has been reported that several pathological mouse models such as schizophrenia have abnormal brain pH (Hagihara et al., *Neuropsychopharmacology* 2018). First, the chronic changes in pH, Ca^{2+}, and local brain blood volume were measured in novel cage environment and then transient changes in these parameters were analyzed when the mouse encountered other individuals. Traditional social interaction experiments were done while placing one individual in a small enclosure and placing the other freely moving. However, since we were able to distinguish the individuals by DeepLabCut method, in our case, we let both individuals freely interact. Under such condition, unexpectedly, we were able to bring out a wide variety of social interaction behaviors, including aggressive behavior. Such behavior could not have been studied if one of the individual is placed separately in a small enclosure. Difference in the cerebellar astrocyte activity was observed depending on the directionality of social behavior. Further statistical analysis will need to be devised; however, correlation of social behavior and cerebellar glial activity was confirmed. In future, we aim to manipulate the glial activity with optogenetics to understand the causal relationships between the glial influence of the brain circuit and the social behavior outcome.

Discussion & Conclusion

Through a series of experiments, we have come to the conclusion that glial cells not only support the nervous system but also have the ability to influence the operation of neural circuits, and that targeting glial cells may enable the treatment of brain pathologies (Shimoda et al., *Neurobiol Dis* 2022). In future studies, we aim to form or inhibit sociality through pharmacological or optogenetic interventions. Through these techniques, we will be able to elucidate the mechanisms in the brain that shape social functions.

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一般の皆様へ

新型コロナウイルスによる外出禁止は、社会との隔離がされている状態です。本研究では、社会的環境要因によって変化する脳内活動をマウスから記録しました。中でも、自閉スペクトラム症との関連が指摘されている小脳に注目しました。さらに、本研究では、神経細胞ではなく、神経活動の動作モードを左右するグリア細胞の活動を計測しました。従来、もっぱら運動学習に関わるとされてきた小脳が社会性行動に関わることが示唆されました。今後、小脳グリア細胞活動をターゲットにした創薬等によって、社交障害を克服する治療戦略が立てられる可能性が期待されます。

Genome-wide analysis of DNA methylation and transcription dynamics in mesenchymal stem cell models of Immunodeficiency, Centromere Instability, and Facial anomalies (ICF) syndrome.

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Summary Abstract

Craniofacial development is a complex process requiring the intricate co-ordination of multiples tissues, molecular cues and signaling pathways. Mutations in ZBTB24 result in craniofacial malformations. Little is known about the molecular function of ZBTB24. In this study we use genome-wide ZBTB24 chromatin localization maps, ZBTB24 protein interaction networks and gene expression profiles of ZBTB24 deficient mesenchymal stem cells to determine the molecular functions of ZBTB24.
Key Words : Zbtb24, Osteoblast, Bone, Methylation, ICF

Introduction

Mutations in the zinc-finger transcription factor *ZBTB24* occur in patients with Immunodeficiency-centromeric instability-facial anomalies syndrome 2 (ICF2) [1][2]. Clinical features of ICF2 include facial malformations such as micrognathia (small jaw), depressed nasal bridge, flat face, hypertelorism (wide-set eyes) and fused teeth [1][2][3][4]. At the molecular level, ICF2 is also characterized by abnormal DNA hypomethylation and chromatin decondensation [2]. Exactly how mutations in a single transcription factor results in such dramatic consequences remain unclear. Even less is known about ZBTB24-associated protein complexes and how these intrinsic protein interactions dictate ZBTB24's critical functions and genomic occupancy.

Results

CRISPR/Cas9 gene editing was used to disrupt *Zbtb24* expression in C3H10T1/2 mesenchymal progenitor cells. Deletion of *Zbtb24* inhibited osteogenic differentiation and reduced osteoblast marker expression and alkaline phosphatase staining. *Zbtb24* knockout cells also recapitulated the significant DNA hypomethylation at minor satellite centromeric regions observed in ICF2 patients. By immunofluorescence imaging we found that whereas wildtype ZBTB24 colocalized with HP1α, a heterochromatin protein, ZBTB24 constructs harboring patient-variant (C382Y or R456X) mutations failed to colocalize with heterochromatin and were instead mislocalized to fibrillarin-positive nucleolar foci. Affinity purification and mass spectrometry analysis of wildtype and patient-variant ZBTB24 protein complexes identified several novel interactants important for regulation of cell differentiation. Notably, we found that wildtype ZBTB24, but not C382Y or R456X, was associated with multiple chromatin-

remodeling complexes and DNA methylation regulators. One such wildtype-specific interactant was the VPRBP-DDB1-CRL4 complex, a nuclear ubiquitin ligase implicated in SMAD signaling and TET2-mediated DNA demethylation [5]. Consistent with this, *Zbtb24* knockout cells exhibited defective SMAD signaling and abnormal TET2 ubiquitination. Since ZBTB24 is a transcriptional regulator, we sought to identify downstream targets using an integrated approach of RNA-Seq and ChIP-Seq analysis. Using RNA-seq, we examined the transcriptional profile of cells with *Zbtb24* overexpression and *Zbtb24* knockdown. By focusing on genes that showed coordinated inverse regulation in both gain-of-function and loss-of-function systems we could identify "true" transcriptional targets. Candidate genes were increased upon *Zbtb24* overexpression and also decreased upon *Zbtb24* knockdown. Combining this data with our genome-wide chromatin binding map of ZBTB24 generated by ChIP-Seq, we could confirm that ZBTB24 occupies the promoter region of several candidate genes including *Cdca7* and *Zfp57*. Interestingly CDCA7 and ZFP57, like TET2 are strongly linked to DNA methylation. CDCA7 is a component of the HELLS/CDCA7 chromatin-binding and remodeling complex [6]. This complex is required for efficient DNA methylation. Mutations in HELLS or CDCA7 result in hypomethylation of satellite repeats and are responsible for ICF4 and ICF3, respectively. ZFP57 is a co-factor in the recruitment of DNA methyltransferases to maintain DNA methylation in progenitor cells [7]. *ZFP57* mutations underlie variable DNA hypomethylation patterns in patients with transient neonatal diabetes [8]. Therefore, we hypothesize that ZBTB24 is an upstream regulator of major DNA methylation maintenance factors. To explore the implications of altered DNA methylation on bone cell function, we examined the role of a novel cytosine-based selective TET enzyme inhibitor, Bobcat339 (BC339) [9]. Methylated DNA immunoprecipitation and bisulfite sequencing showed that inhibition of TET with BC339 led to increased 5mC at specific CpGrich regions including the promoter of *Sp7*, a key osteoblast transcription factor. Consistent with promoter 5mC marks being associated with transcriptional repression, luciferase activity of an *Sp7*-promoter reporter construct was repressed by in vitro DNA methylation or BC339. Chromatin immunoprecipitation analysis confirmed that TET2 does indeed occupy the promoter region of *Sp7*. Accordingly, forced overexpression of SP7 rescued the inhibition of osteogenic differentiation by BC339 [9].

Discussion & Conclusion

Taken together, our data suggests that ZBTB24 is required for osteoblast differentiation and that a disrupted protein-protein interaction basis underlies multiple defective pathways which contribute to the differentiation and epigenetic abnormalities underlying ICF2 syndrome. Furthermore, we demonstrated that DNA methylation plays an important role in bone cells during differentiation. TET-mediated DNA demethylation of genomic regions, including the *Sp7* promoter, is required for the initiation of osteoblast differentiation. We also show that BC339 is a novel pharmacological tool for the modulation of DNA methylation dynamics for research and therapeutic applications.

These findings are published in our new paper, Dusadeemeelap *et al.*, [9].

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一般の皆様へ

出生時にみられる奇形のうち頭蓋顔面領域に発生するものが3分の1以上を占めています。この頭蓋顔面奇形において、手術などによる奇形の改善には大きな進歩が見られますが、奇形の発生に関わる分子を標的としたような治療法はほとんどありません。

その理由として、頭蓋顔面奇形の発生は複数の転写因子、成長因子および受容体などが複雑な交絡していることが挙げられます。したがって、頭蓋顔面奇形の病態生理を分子レベルで理解するための研究が必要です。

最近、頭蓋顔面奇形が出現するICF症候群においてZBTB24という遺伝子に変異があることがみつかりました。そして今回この助成金をいただいたことで、ZBTB24の変異が頭蓋顔面奇形を引き起こす分子メカニズムの一端を明らかにすることができました。

今後、本研究を発展させ、疾患のより深い理解、ひいては治療法の開発につなげていきたいと考えています。

Elucidating the 3D genome structure and its related protein composition in human senescent cells

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Summary Abstract

The disorganization of the 3D genome structure is connected to human diseases, including cancer. This study aims to understand how the specific 3D genome structure is formed upon cellular senescence, an important anti-cancer mechanism. Our hypothesis is that the 3D genome structure, including enhancer-promoter contacts, is involved in the up-regulation of senescence-related genes. To test the hypothesis, we have performed genomics and proteomics experiments and determined the 3D genome structure at a high resolution and a series of chromatin proteins enriched in human senescent cells.
Key Words : Cellular senescence, 3D genome organization, genomics, proteomics

Introduction

Cellular senescence is an important tumor suppression mechanism that limits the propagation of cells subjected to insults such as oncogene activation. Senescent processes are known to drastically alter the expression of many genes, especially genes encoding the Senescence-Associated Secretory Phenotype (SASP) factors. Senescent processes also entail significant alterations to the global 3D genome structure, such as the formation of Senescence-Associated Heterochromatic Foci (SAHF) and the distension of centromeric satellites. In this study, we proposed to employ genomics and proteomics approaches to elucidate how the 3D genome structure is organized during senescent processes.

Results

The Hi-C method combines a molecular biological procedure, referred to as chromosome conformation capture (3C), with next-generation sequencing. Hi-C genomics experiments were performed with IMR-90 oncogene-induced senescence (OIS) and propagating cells. By sequencing several billion reads, we successfully captured the 3D genome structures in senescent and propagating cells at a very high resolution (~10 kb). The Hi-C data clearly revealed heterochromatin aggregation (SAHF) in senescent cells but not in propagating cells, indicating that our genomics experiments worked well. Moreover, we improved our bioinformatics pipelines to detect enhancer-promoter contacts, and the new algorithm detected a large number of enhancer-promoter contacts specifically formed in senescent cells.

In addition, we performed RNA-seq transcriptome analysis to determine expression profiles in senescent and propagating cells. We reproducibly observed that senescence-related genes are up-regulated in senescent cells compared to propagating cells. Moreover, by comparing the Hi-C and RNA-seq genomics data, we found a positive correlation between enhancer-pro-

moter contacts and the up-regulation of senescence-related genes, including SASP genes. These results strongly suggest that enhancer-promoter contacts formed in senescent cells contribute to the activation of senescence-related genes.

We also performed in situ Hi-C experiments using senescent cells with and without condensin knockdown, and the same cells were applied to RNA-seq experiments. We are now analyzing these data to investigate how the condensin knockdown affects the 3D genome structure and gene expression in senescent cells. We expect to find that condensin binds to enhancers and/or promoters and promotes enhancer-promoter contacts, although these analyses are yet to be conducted.

Moreover, we employed a proteomics approach that combines stable isotope labeling by amino acids in cell culture (SILAC) and mass spectrometry (MS) to quantitatively measure chromatin proteins in senescent and propagating cells. Please note that proteomics approaches are often recognized as less quantitative, but the SILAC-MS-combined proteomics approach can overcome the pitfall. As a result, we successfully obtained reproducible proteomics data and observed that many chromatin proteins were more abundant in senescent cells than propagating cells. For instance, the amounts of HMGA1, HMGA2, and histone macro H2A.1 proteins were elevated in senescent cells compared to propagating cells, which is consistent with the previous observations in the senescence research field. Interestingly, we could also identify potentially important chromatin proteins, which have not previously been linked to senescent processes.

Discussion & Conclusion

Our genomics results suggest that many senescence-related genes, including SASP genes, are up-regulated by enhancer-promoter contacts. Moreover, proteomics data imply that many chromatin factors, which were previously not connected to senescent processes, are enriched in senescent cells compared to propagating cells. Based on these results, our current hypothesis is that those chromatin factors bind to enhancers and/or senescence-related genes and promote enhancer-promoter contacts, thereby up-regulating senescence-related genes. To test this hypothesis, we will next knockdown several chromatin proteins elevated in senescent cells and perform Hi-C and RNA-seq genomics experiments. These analyses allow us to understand how the chromatin factors contribute to the formation of the specific 3D genome structure (enhancer-promoter contacts) and gene regulation in senescent cells.

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一般の皆様へ

細胞老化は、細胞の異常な増殖を防ぐことを通じて、極めて重要ながん抑制メカニズムとして機能しています。申請者グループは、ゲノミクスとプロテオミクスの技術をヒト細胞に適用し、細胞老化の各段階における3D ゲノム構造の経時変化とその背景にあるタンパク質群の挙動を包括的に理解する事を目的に実験を行いました。その結果、老化細胞の3D ゲノム構造とそれを形成するクロマチンタンパク質の組成を明らかにすることができました。

Elucidation of the hyper-adaptation mechanism by reconstruction of synapse-formations and AI motion capture systems - Challenging for the hyper-amelioration from spinal cord injury-

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Summary Abstract

We demonstrated the unprecedented early recovery from acute and subacute phase of spinal cord injury(SCI) by a synthetic synapse organizer protein and the anti-sense oligo(ASO) of N-acetylgalactosaminyltransferase-1(T1), a key gene in SCI. We constructed the AI motion capture system to rigorously evaluate the recovery from SCI.
Key Words : Spinal cord injury

Introduction

We attempt to establish the super-recovery mouse from SCI by inducing artificial synapse connect and providing extra cellular matrix field suitable for regeneration. This mouse will allow us to dissect the neural basis of adaptive circuits during recovery. To evaluate how the adaptive neural circuits generate the locomotory outputs, we are constructing the novel motion capture analysis system operated with AI algorithm. This system could detect and extract the behavioral elements specific to the super-recovery mouse. We aim that our AI motion capture system will become powerful and rigorous system for high-throughput analysis of rodent behaviors.

Results

CPTX is a novel synthetic protein that crosslinks a presynaptic molecule (Nrx: neurexin) and a postsynaptic molecule (AMPAR: AMPA receptor)(Fig1). It was created as an artificial chimeric protein (CPTX) in which Nrx binding domain of Cbln1 and AMPAR binding domain of NP1 are joined (Science. 2020) (Fig. 1). CPTX induces de novo synaptogenesis both in vitro and in vivo. we tried if CPTX is effective to chronic phase of SCI. It is well known that recovery of chronic phase of SCI is extremely difficult. Despite the difficulty, majority of patients suffering from SCI are clinically chronic phase. Thus, overcoming the chronic phase of SCI is an important mission. CPTX restored the chronic phase of SCI(Fig 2). CPTX injection activated the hind leg movement within a few days, which otherwise showed the permanently poor movement.

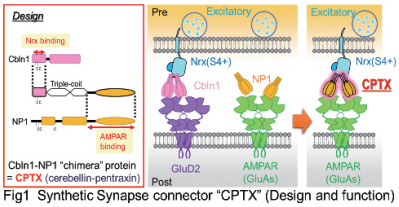

Fig1 Synthetic Synapse connector "CPTX" (Design and function)

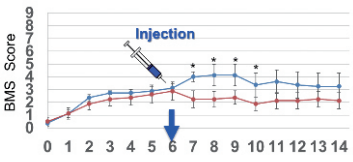

Fig 2 Administration in the chronic phase (6 weeks after SCI). Physiological function improves after SCI (BMS score)

We reported that chondroitin sulfate (CS) KO mice, which are the strongest inhibitors of nerve regeneration after spinal cord injury, show dramatic post-injury recovery. We developed and applied an antisense oligo (ASO) for knocking down of CS expression in a tissue specific manner. We applied ASOs to SCI animals and found that our oligos restored the locomotory function of acute and subacute phase of SCI.

We are promoting the introduction of AI into the evaluation of functional recovery to obtain the correlation between the quantitative motor function, nerve reorganization, and motor function of the super-recovery model after spinal cord injury. We promoted the system construction and the analysis to extract the characteristics of walking during the recovery process (Fig. 3).

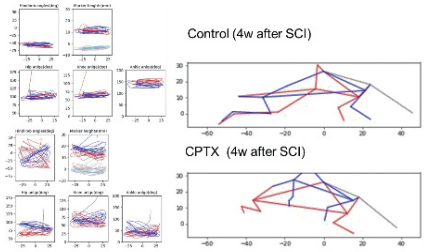

Fig3 Recovery process after spinal cord injury. Analysis by extracting various parameters such as hind leg movement angle and cycle

Discussion & Conclusion

This year, we were able to obtain results of functional recovery from SCI through CPTX application. CPTX restored acute and sub-acute phase of SCI. We obtained the preliminary data that CPTX is also effective to chronic phase, which is beyond the initial plan. Since we have already prepared ASO for CS suppression, we will create a super-recovery model and expand the AI trace system in parallel. Development of next generation synapse connector that bridges inhibitory synapse is also in progress. Such novel tool combined with AI trace system will allow us to study super-adaptation and to conduct artificial intervention for functional recovery.

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一般の皆様へ

現在、根本治療法がない脊髄損傷に対して、我々は損傷部に発現する神経再生阻害因子であるコンドロイチン硫酸の発現を抑制する手法（再生環境の整備）と、新しい人工合成神経シナプスオーガナイザー CPTX 投与による神経再編（人為的な神経回路再構築）の二つの新たな方法論の展開を進めている。今回、これまで治療が特に難しいとされる罹患後慢性期でのこれらの可能性を示すことが出来、さらにヒトでの治療に外挿するためにモデル動物の回復過程をAI機械学習で抽出することが出来るようになった。これらをさらに臨床での応用に向けて展開することを目指している。

Exploration of pharmaceutical resources based on rapid synthesis and machine learning analysis of optically active compounds

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Summary Abstract

A stereoselective synthetic route to chiral C3-spirooxindole- and C2-spiropseudoindoxyl-pyrrolidines was accomplished by an enantioselective organocatalyzed Pictet-Spengler reaction of tryptamines and isotryptamines followed by a diastereoselective oxidative rearrangement of the generating tetrahydrocarboline intermediates. This sequential reaction enables rapid access to chiral C3-spirooxindole- and C2-spiropseudoindoxyl-pyrrolidines in a one-pot process. Towards the machine learning analysis of optically active compounds, a molecular descriptor of the prepared enantioenriched spiro compounds exhibited Wnt signaling inhibitory were also considered.

Key Words : Wnt signaling inhibitory activity; Spiro compound; One-pot synthesis; Green oxidant; Machine learning

Introduction

Chiral C3-spirooxindole-pyrrolidines **1** and C2-spiropseudoindoxyl-pyrrolidines **2** have received increasing attention in the fields of organic chemistry and drug discovery.[1] Various synthetic methods have been reported for the construction of their scaffolds,[2] however, a facile, ecofriendly, and highly stereoselective one-pot synthetic strategy is still required. Herein we report a practical synthetic route to **1** and **2** *via* an enantioselective PS reaction of commercially available tryptamine using the chiral squaramide catalyst, and a subsequent diastereoselective oxidative rearrangement without any racemization.[3] The ability of the newly obtained enantioenriched spiro compounds to inhibit the Wnt signaling pathway is also examined.

Results

Initially, to obtain the enantioenriched tetrahydro-β-carboline intermediates **3**, we tested the organocatalyzed enantioselective PS reaction of commercially available tryptamine with isovaleraldehyde. Following a comprehensive screening of the reaction conditions, the *tert*-leucine-derived squaramide catalyst[4] (10 mol%) allowed the reaction to proceed in toluene at 35 °C to yield the desired product **3** in 89% yield and 90% ee following Boc protection of the PS product. Subsequently, based on this result, we investigated the catalytic enantioselective synthesis of the tetrahydro-γ-carboline intermediates **4** using isotryptamine and isovaleraldehyde. The reaction using 10 mol% of the organocatalyst in addition to succinic acid provided the corresponding product **4** in 90% yield and 79% ee. Replacement of the acidic additive

with benzoic acid significantly improved the enantioselectivity to 92% ee, and after trituration with *n*-hexane, **4** was obtained in 70% yield and 98% ee. The highly enantioselective PS reactions of both tryptamine and isotryptamine were successfully catalyzed by organocatalyst, which combined the steric bulks of the *tert*-butyl and benzhydryl groups with squaramide as a strong hydrogen-bonding donor.

Next, to achieve the highly diastereoselective oxidative rearrangement of **3** and **4** without any loss in enantiopurity, a range of oxidative conditions was examined. Among the various oxidants subjected to screening, NaOCl · $5H_2O$, which is a bench-stable and easily removable green oxidant, efficiently promoted the oxidative rearrangement of the Boc-protected **3**. More specifically, in a DMF/H_2O (2:1) mixed solvent system, the desired spirooxindole **1** was obtained in 93% yield and in a highly diastereoselective manner with no reduction in the enantiopurity being observed (17:1 dr, 88% ee). Although the oxidative rearrangement of **4** with NaOCl · $5H_2O$ gave **2** with acceptable diastereoselectivity, the yield was only 12% due to the low reactivity, which contrasted to the use of Oxone®, wherein was obtained in 75% yield with a good diastereoselectivity (10:1), and no reduction in enantiopurity.

After the successful development of the organocatalyzed PS reaction/oxidative rearrangement sequence, we focused our attention on the asymmetric one-pot syntheses of chiral C3-spirooxindole- and C2-spiropseudoindoxyl-pyrrolidines from tryptamines and isotryptamines. For this purpose, each two-step reaction was attempted in a single vessel. To our delight, the enantioselective PS reaction of tryptamine with isovaleraldehyde, and the subsequent NaOCl · $5H_2O$-mediated oxidative rearrangement of **3** proceeded smoothly to afford the desired **1** in a moderate yield (55%) with a high stereoselectivities (15:1 dr, 88% ee). In addition, the one-pot transformation of isotryptamine with *o*-tolualdehyde into **2** was achieved in 47% yield, >20:1 dr, and 86% ee under the optimized conditions. To the best of our knowledge, this is the first successful example of an asymmetric one-pot syntheses of chiral C3-spirooxindole- and C2-spiropseudoindoxyl-pyrrolidines from tryptamine and isotryptamine.

To evaluate the biological activities of chiral spirooxindoles and spiropseudoindoxyls, a Wnt signaling inhibitory assay was employed because oxindole derivatives exhibited potential to inhibit the Wnt signaling pathway.[5] The Wnt signaling pathway plays a key role in the motility, morphology, differentiation, and proliferation of cells. In addition, various human cancers, such as colon cancer, aberrantly activate the Wnt pathway.[6] Therefore, compounds that inhibit the Wnt signaling could be considered potential candidates for anticancer agents. Thus to survey the abilities of spiro products to inhibit the Wnt signaling pathway, we employed a TOP-Flash assay, which is a cell-based reporter luciferase assay measuring TCF/β-catenin transcription in the cell line STF/293 with LiCl as a GSK-3β inhibitor to induce β-catenin accumulation.

Discussion & Conclusion

Among our assay, the newly synthesized *tert*-Butyl (2*S*,2'*S*)-3-oxo-2'-(o-tolyl)spiro[indoline-2,3'-pyrrolidine]-1'-carboxylate (>99% ee) inhibited TCF/β-catenin transcription with an IC50 value of 2.2 μM, and was found to exhibit a low cytotoxicity, in addition to low inhibition of the FOP-Flash activity. Toward ML-driven exploration of pharmaceutical resources having Wnt signaling inhibitory, particularly in the limit of small data set sizes[7-10], expert-guided descriptors encode the nuances of spiro structures in a predictively useful and interpretable way. The development of new descriptors and evaluation for different ML prediction tasks will be

enhanced by the sustained development with the Transformative Research Areas (A) 21A204 Digitalization-driven Transformative Organic Synthesis. Further application of this organocatalytic/oxidative rearrangement sequence for the stereoselective construction of complex heterocycles and their AI-driven investigations as well as their bioassay are ongoing in our group, and the results will be presented in due course.

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一般の皆様へ

本研究では、有毒な遷移金属を必要としない官能基選択性に優れた有機分子不斉触媒を活用して、「ドミノ倒しゲーム」の様な連続反応を積極的に合成プロセスにデザインすることで、医薬品候補迅速合成を達成しました。抗癌剤への応用が期待される新規 Wnt シグナル阻害活性シードの効率的な探索とその作業工程でのコスト（資源・時間・廃棄物等）削減を両立すべく、剛直な骨格を有するスピロオキシインドールをファーマコフォアとするデジタルデータベースを構築し、機械学習研究と融合することで、TCF 転写阻害活性試験結果と光学活性スピロオキシインドール構造の相関解明を目指しました。

Development of Protein Ligand Library for Stereodivergent Synthesis

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Summary Abstract

Our cupin-system is applied into asymmetric transfer hydrogenation of ketones and proved to be useful as chiral ligands that is promising for application as a catalyst with organometallic metal center. We rationally designed the first coordination sphere based on the number of donors and coordination geometry, and succeeded in obtaining a metal ligand for the TM1459 mutant by varying the metal binding site through site-directed mutagenesis. The metal ligand thus obtained successfully binds ruthenium, rhodium, or iridium complexes and catalyzes asymmetric transfer hydrogenation of ketones in water with high selectivity.
Key Words : Artificial metalloenzymes, Stereodivergent synthesis, Protein metal ligands

Introduction

We have recently developed artificial metalloenzymes by employing a TM1459 cupin-type protein as an advanced metal ligand. TM1459 gene from *Thermotoga maritima* encoded a Mn-binding protein with a molecular weight of 12,977 Da.[1,2] This cupin protein from hyperthermophile involves a 4-His motif as metal binding site in the active site. We have constructed well-defined Os-cupin complex system for regio- and stereoselective *cis*-1,2-dihydroxylation reaction by using this protein as the ligand instead of tris(2-pyridylmethyl)amine ligand, and modified the second coordination sphere to enhance the reactivity.[1] Furthermore, our cupin metal ligands with Cu(II) ions were found to catalyze the Michael addition reaction of 2-azachalcones to nitroalkanes with high enantioselectivity and diastereoselectivity.[2]

Results

To expand the versatility of TM1459 as metal ligands toward organic synthesis as described above, the metal complex with TM1459 was explored as a catalyst for asymmetric transfer hydrogenation of aromatic ketone compounds. In the previous reports, the wild-type TM1459 protein has a six-coordinate octahedral structure coordinated to four histidines when Os is introduced. Here, on the basis of the previous results, we designed TM1459 mutants,1-His mutants, which have the metal binding sites consisting of single histidine residue.

First, the concentration of sodium formate was examined using the mutant (No. 52) complexed with the Ru compound. In consequence, the yield increased as the concentration of sodium formate increased. However, the selectivity clearly decreased when the concentration of sodium formate exceeded 0.15 M. Therefore, 0.10 M was determined to be the optimum concentration of sodium formate. Secondly, pH dependence was investigated in the same manner as described above. The reaction provided highest yield at pH 5.0. Finally, a variety of buffer salts were tested, acetate-phosphate buffer, acetate buffer, barbiturate buffer, and

water without buffer salts. Consequently, we decided on phosphate-acetic acid buffer as the optimal buffer because it exhibited the highest yield and selectivity.

Then, asymmetric transfer hydrogenation of aromatic ketone compounds was carried out using 1-His mutants and organometallic complexes under optimized conditions. The reaction hardly proceeds without TM1459 mutant. We found that the Ru-bound mutants can catalyze the reaction. The Rh-TM1459 tend to exhibit higher yield than Ir-TM1459. In particular, No. 52-Ru complex showed very good enantioselectivity although the yield was somewhat poor. On the other hand, C106A mutation increase the selectivity of No. 52 mutant from 6.7 to 85 % (R) and the yield also got higher. It is noteworthy that this mutant showed very good enantioselectivity, whereas No. 54 mutant showed inverted enantioselectivity. Thus, we succeeded not only in improving the selectivity but also in reversing it by modifying the second coordination sphere.

In order to further improve the ligand exchange rate, the metal complexes that chloride ligands are replaced by acetonitrile ligands were employed in place of complexes that we used so far. We hypothesized that when the latter metal complexes were dissolved in buffer, chloride ligands were not released but remained bound to the metal complexes, making it difficult for the substrate to approach to the metal center. Namely, acetonitrile complex would be converted into aqua complex by exchanging by water molecules. In the context, asymmetric transfer hydrogenation reactions were performed using the former metal complexes under optimized conditions. As a result, No. 52-Ru complex and No.58-Ir showed very good enantioselectivity (*ee* = 90 % (*R*) and *ee* = 82 % (*R*)). It should be noted that the selectivity was found to be improved within other mutants that we employed. Thus, we has succeeded in incorporating a metal complex with an organometallic moiety into the TM1459 protein in anticipation of further diversification of chemical reactivity, and have shown that it is a useful chiral ligand with potential application as a catalyst for asymmetric hydrogenation transfer reactions.

Discussion & Conclusion

We took the opportunity to expand their system into increase the diversity of reactions using the TM1459 protein as a ligand. The 1-His ligands with Ru complex were found to catalyze the asymmetric transfer hydrogenation reaction of aromatic ketone compounds with high enantioselectivity in water. Thus, we succeeded in developing an artificial metalloenzyme that can control the enantioselective formation of *R*- and *S*-forms in asymmetric transfer hydrogenation reaction. The use of such a small cupin library system facilitates a promising approach for creating artificial metalloenzymes that can catalyze enantiodivergent reactions. Hopefully, this system based on the cupin superfamily protein will be applied various reactions that can be used in synthetic organic chemistry.

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一般の皆様へ

新たな物質を作り上げる合成化学の究極的な目的の一つは原子配置を自在に制御することです。すでに科学が発展し、そんなことは当然のようにできると思われているかもしれません。しかし、非対称分子と呼ばれる複雑な化合物を合成することはいまだに極めて難度が高いとされています。そういった化合物は医薬品や農薬などの有用物質になるため、自在に高難度化合物を簡便に作り分ける手法、立体分岐型合成システムの構築が今なお活発に研究されています。そのため、今後、本研究で得られた結果が発展することで究極の触媒が完成し、効率的な有用物質生産へとつながると期待されます。

Revealing the intra-cellular dynamics of SARS-CoV2.

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Summary Abstract

Live cell imaging is a powerful and useful technique to analyze the intracellular replication mechanisms of pathogens, e.g. highly pathogenic viruses. In this study, we attempted to reveal the mechanism for viral replication of the novel coronavirus (SARS-CoV2), which is a biggest and urgent international public health concern, by using live-cell imaging analyses.
Key Words : SARS-CoV2, intracellular dynamics, live-cell imaging, RNP

Introduction

The basic structure of filoviral RNP, NP-RNA complex has been revealed recently, enabling to analyze RNA-NP and NP-NP interaction m,./

s[1]. Although, the structure of ribonucleoprotein complexes (RNPs) has been largely concealed for years. In our previous study, we constructed a live cell imaging system to visualize the intracellular transport pathways of RNPs and viral particles. It enabled to reveal the regulating factors for filoviral RNP assembly and transport[2, 3]. The aim of this study is to apply this system to SARS-CoV2 and to reveal the intracellular dynamics of it including host factors using live cell imaging systems.

Results

We previously established the infectious and non-infectious live cell imaging systems to visualize filoviral intracellular dynamics. In this study, we aimed to apply this system to the SARS-CoV2 intracellular replication mechanisms. Specifically, we would like to visualize each process of viral life cycle, i) entry into the host cell and decapsulation of RNP, ii) viral genome replication, iii) RNP assembly and transport, iv) viral particle assembly, and v) release of progeny particles. Moreover, we hope to elucidate the molecular mechanisms of virus-host interactions during each of these processes. To achieve this, viral particles and RNPs will be labelled with fluorescent proteins and visualized together with host factors, which were labelled with different colors to elucidate their subcellular localization and interactions.

1) Firstly, we produced vector-plasmids for mammalian cells expressing each SARS-CoV2 structural protein. The SARS-CoV2 has four structural proteins, N protein: directly encapsulating viral genome, E protein: the envelope protein, M protein: the membrane protein, and S protein: the surface antigenomic protein. Of interest that a single expression of M protein demonstrated a long-distance transport, whereas the other proteins did not demonstrate long-distance transport in single expression. Next, co-expression of each viral protein in the SARS-CoV2 infected cells was performed. As expected, M protein demonstrated with a long distant transport, and interestingly N protein as well demonstrated long distant

transport together with M protein. The long-time course experiments demonstrated that M and N proteins co-transported from cellular periphery to the perinuclear region to form kind of viral inclusion bodies. To reveal the molecular mechanisms of N-M proteins interaction, we are preparing the series of deletion mutants of N and M proteins.

2) In another approach, we focused on the cellular organelles co-localized or co-transported with viral proteins. We prepared florescent labelled markers for either ER, ERGIC: ER Golgi intermediate compartment, Golgi, Mitochondria, Endosomes, Lysosome, and cytoskeletons such as microtubules and actins. It is well known that the cytoskeletons are the major transport pathway for cellular and exogenous expressing proteins. To reveal which pathway is used for the target protein transport, polymerization inhibitors were applied to live cell imaging. The microtubule depolymerizing drug Nocodazole stopped the M proteins movement as well as Endosomes, Mitochondria, Lysosomes. Of interesting that microtubules are the major transport pathway of SARS-CoV2 viral proteins, which is similar to the cellular transport-competent organelle. On the other hand, actin depolymerizing drug Cytochalasin D did not influence their transport. Noteworthy, N and M proteins sometimes co-localized with ER membrane and co-transported with ERGIC, Golgi.

   On the other hand, Mitochondria and Lysosomes were rarely observed co-localization and co-transport with viral proteins both in the viral proteins transfected cells and SARS-CoV2 infected cells.

3) Next, we combined 1) and 2) approaches to reveal the viral and host proteins interactions. The produced deletion mutants were co-expressed together with the cellular partners, especially focusing on ERGIC and Golgi. It is interesting that the N-M proteins interaction and viral and cellular proteins associations were not in the same line. Now we are researching the molecular machinery of SARS-CoV2 protein complexes and cellular organelle association.

   After identification of regulating domain of the simplest RNP-like structure, we will introduce mutations into these domains to reveal the molecular mechanisms of RNP assembly and transport in SARS-CoV2. Finally, it is expected that our study contributes to elucidate the foundation of the novel therapeutics which targeting SARS-CoV2 replication.

Discussion & Conclusion

By using live cell imaging systems, we would like to produce a simple model for SARS-CoV2 RNP assembly and transport. And, which host proteins can mediate the transport of SARS-CoV2 proteins are clarified. As a method, we are considering to analyze the protein-proteins interaction, several possibilities can be considered. In addition to the live-cell imaging microscopy, immunofluorescent microscopy, and immunoprecipitation analyses are utilized. Host factors with particularly strong interactions will be controlled in expression by function-blocking compounds, gene silencing methods and dominant-negative proteins, and their effects on the intracellular traffic of RNP-like structures will be assessed. Finally, how host proteins interacting with RNPs and viral particles regulate SARS-CoV2 replication will be assessed using infected cells.

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一般の皆様へ

ライブセルイメージングは病原体の細胞内複製機構を理解するための非常に強力で有用な技術である。本研究では、喫緊の国際公衆衛生上の重要課題である新型コロナウイルス:SARS-CoV2を研究対象とし、ウイルスの複製機構を解明することを目指す。SARS-CoV2の細胞内動態については不明な点が多く、移動に関わる宿主因子もわかっていない。病原体の細胞内動態を解明することは、侵入経路・輸送経路や介在タンパク質、出芽制御因子を介した治療法を開発するための基盤となることが期待される。

Molecular and cell biological approach to understanding the mechanisms of fatigue by studying the cellular stress response and the inflammatory response

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Summary Abstract

In this research we are focusing on the relationship between fatigue and the functions of molecules and cells that function in the cellular stress response and inflammatory response. The findings from this research will not only contribute to basic life sciences, but may also provide new clues to solving health and social problems caused by fatigue.
Key Words : Fatigue, Sleep, Exercise, Cellular Stress, Inflammation

Introduction

From ancient times to the present, fatigue research has been conducted from various perspectives, including psychosomatic medicine, nutrition, infectious diseases, immunology, and neuroscience. Therefore scientific methods for evaluating fatigue have been established, and oxidative stress levels in the blood and herpes virus counts in saliva are now used as indicators of fatigue. On the other hand, we still do not understand the reality of fatigue, and the mechanism of fatigue that everyone feels is not well understood. Based on the aforementioned background and problems, the goal of this research is to elucidate the mechanisms of the body during the generation of fatigue and recovery from fatigue at the level of molecular and cellular biology.

Results

The sleep disorder model was created using a commercially available device (#SW-15-SD from Merquest). Before using this device, mice were previously kept alone for 7 days in the acclimation device (#SW-15S by Merquest) and then kept alone in the sleep disorder device for 5 days.

The mice utilized in the free-rotating sleep disorder device are ERAI-LUC mice [1], OKD48-LUC mice [2], and UMAI-LUC mice [3] with a C57BL6 genetic background. Each of these mice express luciferase genes at sites of endoplasmic reticulum stress, oxidative stress, and integrative stress, in that order. Therefore, when luciferin, a luminescent substrate, is administered to the body, each stress can be detected as a luminescent signal.

For in vivo analysis in living mice, luciferin (150 mg/kg body weight) was administered intraperitoneally to mice 10 minutes before luminescence signal imaging. For ex vivo analysis of excised organs, luminescence signal imaging was performed while the organs were immersed in luciferin (300 mg/mL) solution. IVIS (Perkin Elmer #Lumina) was used for luminescence signal imaging.

Tissue pieces for analysis were lysed by ISOGEN (Nippon Gene #311-02501) and RNA was extracted. From the RNA, cDNA was prepared by reverse transcription kit (Invitrogen #11904-018), and quantitative PCR analysis was performed using various Taqman probes (see below). Probes purchased from ABI for each gene were as follows (ATF3; Mm00476033_m1, BiP; Mm00517691_m1, CHOP; Mm01135937_g1, CReP; Mm00551747_m1, GADD34; Mm00435119_m 1, GAPDH; Mm999999915_g1, NQO1; Mm01253561_m1, HO-1; Mm00516005_m1, SOD1; Mm01344233_g1). GAPDH was used as an internal standard. Probes for XBP1 had been previously made by ourselves [4].

In this study, we attempted for the first time to perform fatigue-loading experiments using a sleep-disrupting device. We did not know whether the mice actually felt fatigue or not, but we were able to confirm that their activity cycle was disrupted and the time that should be allocated for sleep was shortened. However, the degree of sleep disturbance varied greatly from one individual to another, and we felt that it would be desirable to use a stable sleep disturbance device in the future. In addition, we believe that the use of a method to evaluate fatigue should be considered.

Imaging analysis using stress visualized mice revealed that the ER stress response mediated by IRE1 and the integrated stress response mediated by ATF4 are activated in the adrenal gland by sleep disturbance. The activation of the endoplasmic reticulum stress response and the integrative stress response in the adrenal gland was also confirmed by the expression dynamics of endogenous stress response genes. Many autonomic nerves project to the adrenal glands, which also secrete hormones such as cortisol. Both autonomic nerves and cortisol are closely related to fatigue and stress, and it is very interesting that the endoplasmic reticulum stress response and the integrated stress response of the adrenal glands were activated by sleep disturbance. Although not shown in the figure, the adrenal glands of the sleep-deprived mice were slightly larger than those of the control mice, suggesting that further research on the relationship between fatigue and cellular stress, including this enlargement, is warranted.

On the other hand, the results of the imaging and quantitative PCR analyses differed with respect to the oxidative stress response. However, oxidative stress has been studied in relation to fatigue, and the results of the quantitative PCR analysis are probably correct, suggesting that sleep disturbance also activates the Nrf2-mediated oxidative stress response.

In addition, we have not been able to confirm activation of the endoplasmic reticulum stress response, integrated stress response, or oxidative stress response outside of the adrenal gland, but of course, bioimaging is not a universal analysis method, so it is possible that we missed any effects, if any. We would like to further fatigue research while taking these issues into account in future analyses.

Discussion & Conclusion

A sleep disturbance model was employed as a means of fatigue loading. Specifically, mice were kept in a special device using a rotating cage to disturb stable sleep. The following three types of mice were used for analysis (ERAI-LUC mice, OKD48-LUC mice, and UMAI-LUC mice). Mice exposed and unexposed to sleep disturbance (control group) were subjected to in vivo imaging analysis. In addition, RNA was extracted from organs removed from the mice, and changes in expression of cellular stress response genes were evaluated by quantitative PCR analysis.

Sleep disruption by the employed device generally functioned as expected, and mice were awake at times when they would normally be asleep. Bioimaging analysis revealed that sleep deprivation activated the endoplasmic reticulum stress response and the integrative stress response in the adrenal gland. Quantitative PCR analysis confirmed that the oxidative stress response is also activated in the adrenal gland.

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一般の皆様へ

「疲労」は「発熱」や「痛み」と共に生体3大アラームと言われ、私たちの生命や健康を維持する上でカラダが発する重要なシグナルである。しかしその研究と理解は発熱や痛みに比べて遅れており、科学が進んだ現代でも疲労の実態は掴めていない。そこで本研究では疲労が生じる際や疲労が回復する際のカラダの仕組みを分子生物学および細胞生物学のレベルで解明することに目標を定めている。特に細胞ストレス応答で機能する分子や細胞の働きと疲労との関連性に着眼して研究を進めている。この研究は単に基礎的な生命科学へ貢献するだけでなく、疲労が生む社会問題（健康障害から事故・自殺に至るまで）に対する解決への新たな糸口になる可能性を十分に含んでいる。

Sirt1-NAD$^+$ pathway represses fibrosis in inflammatory bowel disease and colitis-associated colorectal cancer

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Summary Abstract

Inflammatory Bowel Disease (IBD) might be prevented by repressing fibrosis in the gut. Here, I show that nicotinamide mononucleotide (NMN) administration could improve dysbiosis in the gut microbiota in IBD mouse model. Then, boosting NAD$^+$ levels could attenuate tumor formation in IBD-associated cancer model. Furthermore, the extracellular flux analysis shows that mitochondrial respiration ould be improved by boosting NAD$^+$ in wild-type mouse embryonic fibroblasts (MEFs) but not Sirt1 deficient MEFs. These studies suggest that boosting NAD$^+$ levels could contribute to homeostasis in the gut and activate mitochondrial functions dependent on Sirt1.

Key Words : NMN, Sirt1, Fibrosis

Introduction

Fibrosis is characterized by excessive extracellular matrix protein deposition and a consequence of local chronic inflammation in organ and tissue. It is highly associated with most of diseases, including cancer and aging, as well as chronic inflammatory diseases [1,2]. Recently, I have shown that increasing the activity of Sirtuin and NAD$^+$ metabolic pathway could attenuate the progression of fibrosis in liver [3,4]. Here, I demonstrate that Sirt1 and NMN-mediated metabolic pathway could attenuate fibrosis and tumor development through mitochondrial function in the gut of IBD-associated cancer mouse model.

Results

I have already shown that the administration of nicotinamide mononucleotide (NMN), which is a Sirtuin activator and a metabolic intermediate on NAD$^+$ synthetic pathway, could attenuate fibrosis and fibroblast activation in colon of wild-type C57BL6/J mice in IBD model using RNA sequencing. And, NMN administration increased the intracellular NAD$^+$ level and attenuated fibrosis-associated molecular pathways in TGFβ-stimulated MEFs. Here, I demonstrated how boosting NAD$^+$ levels could regulate fibrosis and its-associated tumor development in IBD mouse model and in cell culture.

First, I investigated whether Sirt1-NAD$^+$ pathway could regulate tumor development in IBD (colitis) -associated cancer mouse model using azoxymethane (AOM) and dextran sulfate sodium (DSS). The experimental model was established by Dr. Okayasu I et al. [5]. After intraperitoneal injection of AOM, C57BL/6J mice were subjected to three cycles of DSS in drinking water for 7 days and normal water for 2 weeks. At the end of each DSS treatment period, the mice had diarrhea and occult blood in the stools and these signs disappeared after the mice drank normal water for the next 2 weeks. Tumor tissue samples were collected from mice 15-

16 weeks after AOM injection. In NMN-treated mouse group, NMN administration started a week before AOM injection and continued until the end of the experiment. As a result, tumors were developed in the gut of mice regardless of NMN administration although there were the difference of the size and number of tumor size between both groups. I tried to examine the difference of the gene expression pattern in the tumors from NMN-treated and -untreated mice. RNA sequencing analyses showed that molecular pathways, including Th17 cell function, VDJ recombination, arginine metabolism, were up-regulated in tumors of the gut in mice treated with NMN, while molecular pathways, such as B cell apoptosis, negative regulation of organic acid transport, and DNA methylation dependent heterochromatin assembly, were down-regulated by NMN administration. These results suggested that NMN administration could attenuate tumor maturation in the gut of IBD mouse model.

NMN administration could attenuate LPA and TLR4 -related pathway in the gut of IBD mouse model. Next, I examined whether the gut microbiota could be influenced by NMN administration using a new type of next generation sequencing technology, Ion Torrent PGM [(6)]. Bacterial genomic DNA was extracted and purified from mouse stool. Bacterial 16S ribosomal RNA gene was amplified using Ion 16S Metagenomics kit. 16S ribosomal RNA gene sequencing analyses showed that the proportion of the phylum Firmicutes in DSS-treated mice was relatively increased compared with both DSS and NMN-treated mice, while the proportion of the phylum Bacteroidetes was decreased. The phylum Firmicutes/Bacteroidetes ratio is in the interaction between microbiota and diet. The balance is thought to be involved in aging, obesity and inflammation [(7,8)]. These results indicated that NMN administration could attenuate fibrosis and inflammation in the gut by improving the phylum Firmicutes/Bacteroidetes ratio in the microbiome.

Previously, I have shown that Sirt1 and NMN metabolic pathway could attenuate fibrosis through mitochondrial activities [(3,4)]. Here, I investigated how NMN regulates mitochondrial function using the Seahorse XF analyzer. MEFs from wild-type and Sirt1 deficient mice were cultured under standard conditions and treated with or without NMN for 24 hours. The extracellular flux analyses showed that cellular respiration, especially the spare respiratory capacity which is a measure of the ability to produce the energy under stress, was improved by NMN administration in wild-type cells, but not Sirt1 deficient cells. Taken together, these results suggest that boosting NAD^+ levels could increase mitochondrial activity through Sirt1.

Discussion & Conclusion

Fibrosis development is highly associated with aging and aging-associated diseases, including IBD and cancers. Sirtuin and NAD^+ metabolic pathway is also strongly involved in fibrosis, aging, and its related diseases. In this study, I tried to investigate novel molecular pathway on repressing fibrosis through the activation of Sirt1 and NAD^+ metabolism in IBD and IBD associated cancer. NAD^+ administration could not completely suppress tumor development in the gut, but might attenuate the progression of tumor maturation in IBD model. Therefore, anti-tumor activities might be enhanced by boosting NAD^+ in the gut. In this study, it still remains unknown whether NMN affect microbiota homeostasis directly or indirectly, but NMN is absorbed by its transporter, Slc12a8, in small intestine and might affect the immune function while circulating throughout the body [(9)]. Furthermore, boosting NAD^+ levels activated mitochondrial function thought Sirt1 in the extracellular flux assay. Sirt1 deacetylates peroxisome proliferator-activated receptor gamma co-activator 1 alpha/beta (PGC-1α), co-activator

that drive mitochondrial biogenesis and function. Previously, I have shown the forced overexpression of PGC-1α only drives the expression of mitochondrial sirtuins without affecting the other sirtuins [3,4]. Therefore, Sirt1 activates mitochondrial activities, especially spare respiratory capacity, through PGC-1α and mitochondrial sirtuins.

Taken together, these studies demonstrate that boosting NAD+ leads to the activation of mitochondrial function and the attenuation of fibrosis and tumor development through Sirt1-dependent mechanisms.

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一般の皆様へ

今回申請者は、炎症性腸疾患のモデル実験において、生体内で NAD^+ 量を高めることで腸内細菌叢のバランスが回復することや、炎症性腸疾患に関連した大腸がんの成長を弱めることを示した。そして、細胞内の NAD^+ 量を高めるとSirt1の機能を介して、ミトコンドリア機能が活性化することが明らかになった。

Targeting the host factors essential for breast cancer metastasis in PDX mouse models

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Summary Abstract

Development of treatment for metastatic breast cancer is the important clinical challenge. Since I found that host-derived FSC(lo)SSC(-) cells increased in blood and pulmonary metastatic sites of patient-derived xenograft (PDX) mice by flow cytometry analysis, I hypothesized that these cells might promote lung metastasis and thus inhibition of their activated factors might suppress the metastasis as a novel microenvironment-targeted therapy. I identified the population mainly as neutrophils in lungs and found some overexpressed genes in pulmonary neutrophils. I want to demonstrate that inhibition of the important factors can suppress metastasis.

Key Words : neutrophil, metastasis, PDX mouse model

Introduction

Metastasis is the main cause of death from breast cancer and development of prevention and treatment is the important clinical challenge. Some reports show that the tumor microenvironment is essential for metastatic progression and important for treatment targets [1-3]. I established three orthotopic patient-derived xenograft (PDX) mouse models promoting lung metastasis and found that host-derived FSC(lo)SSC(-) cells increased in blood and lungs of the PDX mice compared to healthy mice by flow cytometry analysis. I hypothesized that these cells might promote lung metastasis and inhibition of their activated factors might suppress the metastasis as a novel microenvironment-targeted therapy. In the past year, I identified FSC(lo)SSC(-) cells and sought important factors.

Results

To identify the FSC(lo)SSC(-) cell population, I first analyzed the cells from blood and lungs of my three PDX mouse models by flow cytometry using anti-CD11b, Ly6G, Ly6C, F4/80, and vimentin antibodies. As a result, those cells from blood contained 28.2±31.5% of CD11b(+) Ly6G(+) neutrophils, 47.4±24.0% of CD11b(+)Ly6C(+) monocytes, 2.7±3.0% of CD11b(+) Ly6C(-)F4/80(+) macrophages, and 6.3±4.9% of Vimentin(+)CD11b(-) fibroblasts. Also, the FSC(lo)SSC(-) cell population of the lung cells contained 40.9±13.3% of CD11b(+)Ly6G(+) neutrophils, 17.3±9.4% of CD11b(+)Ly6C(+) monocytes, 0±0% of CD11b(+)Ly6C(-)F4/80(+) macrophages, and 12.1±7.0% of Vimentin(+)CD11b(-) fibroblasts. These data indicate that the most common cells in the FSC(lo)SSC(-) cell population are different between blood and lungs. Among the three PDX mouse models, neutrophils most increased both in blood and lungs in one model, and monocytes and neutrophils most increased in blood and lungs respectively in two models. Interestingly, since neutrophils most increased in lungs of all the

three models, I suggest that activated lung neutrophils can promote lung metastasis. According to previous reports, neutropenia in early breast cancer patients who receive chemotherapy is strongly associated with increased survival [4], and increased neutrophils in locally advanced or metastatic breast cancer patients decrease chemotherapeutic effects and overall survival [5]. These reports support my suggestion, although increased neutrophils in peripheral blood need to be demonstrated to promote tumor growth locally in the metastatic sites.

Next, to determine the factors activated by increased neutrophils, I investigated expression genes in lung neutrophils of the PDX mice by using DNA microarray. I found many upregulated genes in the lung neutrophils of PDX mice with large tumors (>20mm) that were supposed to have aggressive lung metastasis compared to those with small tumors (<8mm) that were supposed to have no or early lung metastasis. Among these genes, one is associated with the regulation of neutrophil differentiation during hematopoiesis and another one is reported to promote tumor progression. I will choose some genes that are supposed to promote metastasis and are reported to be inhibited by some agents to determine (1) whether expression of the genes is upregulated in blood neutrophils or serum of the PDX mice, (2) whether lung metastasis is inhibited by their inhibitors *in vivo*, and finally (3) whether expression of the genes is upregulated in peripheral blood neutrophils or serum/plasma of metastatic breast cancer patients and is associated with their prognosis.

To examine whether FSC(lo)SSC(-) cells promote lung metastasis, I tried to inject PDX tumor cells with or without FSC(lo)SSC(-) cells into tail veins of immunodeficient mice, but neither of them induced lung metastasis. Since PDX tumor cells can orthotopically grow, I will next inject those cells into mammary glands to determine whether FSC(lo)SSC(-) cells promote tumor growth. Or I will choose cell lines (4T1, MCF7, or MDA-MB231 cells) instead of PDX tumor cells for tail vein injection.

Discussion & Conclusion

It has been recently reported that in the mouse mammary tumor virus-polyoma middle tumor-antigen (MMTV-PyMT) mouse model of breast cancer, pulmonary neutrophil infiltration correlates with lung metastatic progression, neutrophil-derived leukotrienes aid the colonization of metastatic tumor cells by stimulating leukotriene receptors expressed on the tumor cells, and treatment with a leukotriene inhibitor reduces lung metastasis [6]. Neutrophils accumulated in lungs of the PDX mouse models that I had established, too. However, my DNA microarray analysis indicated elevation of neither leukotriene nor leukotriene receptor gene expressions. I will focus on other promising genes. As a medical oncologist, I have found an increase in peripheral blood neutrophils at the terminal stage of metastatic breast cancer patients. I expect some important factors activated in the neutrophils of both distant metastatic sites and peripheral blood of the patients. I want to demonstrate that activation of neutrophils at the metastatic tissues in the PDX mouse models correlates with activation of peripheral blood neutrophils in metastatic breast cancer patients and suggest that inhibition of their activation can suppress distant metastasis to contribute to the development of a novel breast cancer treatment.

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一般の皆様へ

乳癌の死亡の主な原因は転移であるため、転移の予防や治療が重要です。今回の研究では、乳癌マウスが転移を起こす肺において、好中球が増殖しており多くの遺伝子を活性化させていることがわかりました。この好中球が肺転移を促進する可能性があり、標的薬剤があれば転移の予防や治療に役立つかもしれません。抗癌剤やホルモン剤だけでは困難な転移の予防・治療に新しい選択肢を提供したいと考えています。

Identification of RNA-binding proteins that regulate miRNA processing by integrated computational and biochemical analysis

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Summary Abstract

In this study, we analyzed how miRNA processing is regulated by RNA binding proteins by computationally analyzing the cancer cell line transcriptome data. The results were verified by gene knockdown and biochemical experiments, and suggested that many RNA binding proteins are involved in regulation of mRNA processing such as splicing and 3' cleavage are also involved in regulation miRNA processing. The study emphasizes the importance of coordinated regulation of mRNA and miRNA processing mechanisms through RNA binding proteins in human health and diseases.
Key Words : miRNA, gene regulation, RNA binding protein

Introduction

miRNAs are important gene regulators whose misregulation often leads to human disease. To regulate miRNA expression accurately, cells have layers of mechanisms to regulate miRNA biogenesis, including transcriptional and post-transcriptional regulatory mechanisms. Post-transcriptional regulatory mechanisms of miRNA biogenesis are not fully understood on a genome-wide scale. This study aims to identify novel RNA-binding proteins that regulate processing of miRNAs.

Results

Through bioinformatics analysis of published transcriptome data from a panel of ~1,000 cancer cell lines (CCLE: Cancer Cell Line Encyclopedia; ref 1), we have shortlisted candidates of RNA binding proteins that might regulate processing of individual miRNAs. By using miRNA expression data and mRNAseq data in the cell lines, we looked for miRNA-RNA binding protein pairs that show significant expression correlations, assuming that positive regulators of miRNA processing would show positive expression correlations with the regulated miRNAs, while negative regulators would show negative correlations. This analysis yielded novel miRNA-RNA binding protein combinations in addition to many combinations that were previously shown to be in regulatory relationships or have physical interactions between the RNA binding protein and the miRNA transcripts. To validate these bioinformatic predictions, selected candidates that were previously studied as regulators of mRNA processing including splicing and mRNA 3' processing factors (ref 2) were knocked down in several human cancer cell lines from various origins and the effects of their knockdown on the mir-17-92a oncomir cluster miRNAs were analyzed by Northern blotting. Knockdown of most of the selected RNA-

binding proteins caused changes in the mature miRNA levels for at least one of the 5 miRNA species produced from the mir-17-92a cluster. To test whether the RNA-binding protein regulate processing of the miRNAs through physical interaction, we performed immunoprecipitation experiments using antibodies against the RNA-binding proteins followed by the detection of co-precipitated miRNA processing intermediates by quantitative RT-PCR. We found that distinct parts of the mir-17-92a cluster transcript were bound by different RNA-binding proteins in HEK293T cells, suggesting that the RNA-binding proteins directly regulate processing of this miRNA cluster at different processing steps. In some cases, the trend of the changes of mature miRNA species upon knockdown were the opposite of the prediction based on the CCLE analysis. We hypothesized that expression levels of RNA-binding proteins might be quantitatively important, and the reduction, not the complete removal, of them by siRNA-mediated knockdown may have produced confounding results. To avoid this potential problem by completely getting rid of the protein in the cell, we knocked out some of the top candidates in cell lines. Knockout of the genes showed reduced proliferation rates or in some cases, knockout cell lines could not be established presumably because they were required for cell growth or viability in some cell lines. These knockout cell lines will be used in future to test how the amount of the protein in the cell affects processing of particular miRNAs. Furthermore, we have also established knock-in cell lines in which the RNA binding proteins are tagged with the DNA sequence encoding a fluorescent protein at their endogenous loci. These cell lines will allow us to quantify the expression levels of the RNA binding protein in each cell, analyze the dynamic localization of the protein in live cells and sort cells based on the levels of the RNA binding protein, in addition to providing useful materials for conventional biochemical experiments for further characterization. These experiments will help us understand how mRNA processing and miRNA processing are coordinately regulated, and may potentially lead to a further understanding about the physiological significance of regulatory mechanisms in the disease.

Discussion & Conclusion

The results suggested that more RNA binding proteins were involved in regulation of miRNA processing than previously recognized. The fact that many of the identified RNA-binding proteins regulating miRNA processing also play roles in mRNA splicing and 3' processing reinforces the hypothesis that miRNA and mRNA processing mechanisms are coordinately regulated. In future, how this coordinated regulatory mechanisms contribute to regulation of developmental processes under natural conditions and how malfunction of the regulatory mechanisms leads to human diseases should be studied.

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一般の皆様へ

癌は遺伝子の病気と言われるように、癌組織では多くの遺伝子の異常が見られます。最近のゲノム解析技術の進歩により、遺伝子に異常やそれぞれの遺伝子の活性化状態を定量的かつ網羅的に解析できるようになりました。今回の研究ではそのような網羅的な定量情報の数理解析により miRNA と呼ばれる遺伝子群の活性調節に関わる遺伝子を推定し、その推定結果を実験的に検証しました。その結果、これまでは mRNA という別のタイプの遺伝子の調節を行うと知られていた遺伝子が、miRNA の調節も行うことを見出しました。タイプの異なる遺伝子が協調的に制御される仕組みを理解することで、なぜ癌が起きるのかという根本的な疑問への答えへと繋がると期待しています。

Development of novel GVHD mechanism and therapy targeting metabolic disturbance of intestinal epithelial cells that leads to dysbiosis.

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Summary Abstract

Intestinal epithelial cell (IEC) damage by T cells contributes to alloimmune, autoimmune and iatrogenic diseases such as graft-versus-host disease (GVHD). Despite significant advances in understanding the aberrant biology of T cells in these diseases, little is known about how the fundamental biological processes of the target IECs influence the disease severity. Here, through analyses of metabolic pathways of IECs, we identified disruption of oxidative phosphorylation without a concomitant change in glycolysis of mitochondria using in vivo models of T cell mediated gastrointestinal damage of GVHD.
Key Words : Dysbiosis, intestinal epithelial cell, BMT, GVHD, mitochondria

Introduction

Recent advances unveiled that the dysbiosis in the gut augmented GVHD. Various pathophysiological insights are suggested but still the precise mechanism is unknown. Emerging data have brought focus into the central role of immune cell metabolism in the regulation of intestinal inflammatory diseases. However, whether the metabolism of the targets of these pathogenic T cells in GVHD, the IECs, are perturbed or reprogrammed, and if so, whether this has an impact on the disease severity with dysbiosis remains unknown. Combining these ideas, in this study, we aimed to determine the metabolic changes in IECs that could affect the microbial composition when they are targeted by pathogenic T cells.

Results

Analysis of mitochondrial respiration in IECs

We explored the impact of immune mediated attack on the bioenergetics of intestinal target cells, IECs. IECs from naive B6 animals, the syngeneic, and allogeneic animals post HCT were analyzed bio-energetic profiles. Compared with IECs from naïve and syngeneic animals, IECs from allogeneic animals demonstrated significantly lower oxygen consumption rates (OCR), but similar extracellular acidification rates (ECAR, an indicator of glycolysis), which dramatically reduced the OCR/ECAR ratio.

TCA cycle metabolite profiling in IECs

Because oxidative phosphorylation (OXPHOS) and mitochondrial respiration are linked, and complex II is critical for both processes, we hypothesized that alterations in the bioenergetics of the IECs would also result in alteration of the metabolites from oxidative phosphorylation. We focused on the TCA cycle on IECs, and profiled for all of the TCA cycle-related me-

tabolites using Liquid chromatography mass spectrometry (LC/MS). Levels of succinate were significantly elevated, whereas the levels of malate, and fumarate were decreased in the IECs harvested from allogeneic recipients when compared with naive and syngeneic recipients. We next explored the mechanisms for succinate accumulation in the IECs from allogeneic recipients. IECs utilize fatty acids supplied from β-oxidation as their key sources of energy. Therefore, high levels of succinate could be the result of enhanced β-oxidation or anaplerosis. We treated transplant recipients with ^{13}C-glucose, ^{13}C-glutamine and analyzed for incorporation of ^{13}C. There were no enhanced anaplerosis from glucose or glutamine that did not contribute to greater levels of succinate in the allo-IECs.

Validation of reduction in SDHA of mitochondrial complex II

We therefore hypothesized that high level of succinate is a function of reduction in complex II subunit, the enzyme succinate dehydrogenase A (SDHA), an enzyme that catalyzes the oxidation of succinate to fumarate. We evaluated SDHA activity and protein levels in the IECs. We stained cryosections of GVHD target organs (colon and ileum) post HCT to assess for SDHA enzymatic activity and protein level. SDHA activity and protein levels was significantly reduced in the colon and the ileum post allo-HCT. These data demonstrate that the accumulation of succinate is secondary to reduction of mitochondrial complex II component, SDHA. To definitively demonstrate that the reduction in SDHA is not a consequence of reduction in mitochondria, we stained SDHA with gold particles post allo-HCT and visualized the gold particles using transmission electron microscopy (TEM). Mitochondria in allogeneic IECs had significantly fewer SDHA gold particles than naïve or syngeneic animals post HCT. These data thus collectively demonstrate that mitochondrial complex II component, SDHA, protein levels were reduced in allogeneic IECs.

SDHA in IECs regulates severity of intestinal GVHD

We next examined the role of SDHA in the enterocytes in vivo, in regulating the severity of alloreactive T cell mediated intestinal GVHD. Recipient animals were gavaged either diluent control or itaconate or malonate or atpenin A5 every other day starting on day 0 post BMT. All of syngeneic mice survived regardless of treatment, but the allogeneic mice receiving itaconate or malonate or atpenin A5 demonstrated significantly greater mortality and had more severe GVHD symptoms than vehicle treated allogeneic mice. Because chemical inhibitors could have off-target effects, to further confirm the in vivo role of SDHA in GVHD, we next resorted to genetic deficiency models. We used SDH assembly factor 1 deficient B6 mice (*Sdhaf1*$^{-/-}$) as recipient in allo-HSCT because these animals have unstable complex II with 20–50% the SDH activity compared with WT B6. Both WT and *Sdhaf1*$^{-/-}$ syngeneic animals showed no mortality and. By contrast, allogeneic *Sdhaf1*$^{-/-}$ B6 mice showed greater severity of GVHD and significantly greater mortality when compared with allogeneic WT B6 littermate controls.

Discussion & Conclusion

The tissue intrinsic mechanisms of the immune target cells, the IECs, remain uncharacterized. Importantly, whether the target cell intrinsic metabolic aberrations can regulate the overall disease severity of GVHD remain unknown. Here we demonstrate a specific metabolic aberration in mitochondrial complex II of the IECs that is common to and exclusive for T cell mediated diseases of the intestinal tract. We show that the IECs accumulate succinate as a consequence of reduction in their mitochondrial complex II component, SDHA. This reduc-

tion in SDHA caused an enhanced sensitivity of the IECs to T cell mediated cytotoxicity, thus serving as an IEC intrinsic metabolic checkpoint that regulated disease severity independent of direct effects of immune cells. The inhibition of SDHA in IECs altered their bioenergetics, decreased OXPHOS without a compensatory increase in glycolysis, which led to reduced O^2 utilization and ATP generation. Utilizing combination of metabolic, genetic, chemical loss and gain of function approaches we demonstrated that SDHA reduction in IECs is the critical target tissue intrinsic common pathway for T-cell mediated colitis, specifically in GI GVHD models. These data identify tissue intrinsic SDHA as novel target to mitigate multiple T cell mediated intestinal immunopathologies. The specific, and the critical T cell dependent pathways that mediate SDHA reduction will need to be addressed in future studies.

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一般の皆様へ

白血病の根治治療として行われる同種造血細胞移植には致命的となる移植片対宿主病（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、その原因は不明です。本研究では免疫細胞による腸管上皮細胞のミトコンドリア障害による代謝異常が組織脆弱性と腸内環境の変化をきたすことを見出しました。今後は詳細な機序を明らかにすることで組織代謝を向上させ、免疫抑制剤を減量した効果的な治療を見出すことを目標に、研究を続けて参ります。

Development of supramolecular hydrogels that enable drug delivery into the central nervous system

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Summary Abstract

For the purpose of developing drug delivery systems into the central nervous system, we designed new low-molecular-weight hydrogelators **1** and **2** possessing a saccharide and nucleoside, respectively. These compounds were synthesized and their gelation properties were investigated. We found that **1** undergoes an enzyme-responsive gel-to-sol phase transition and **2** undergoes a reversible phase transition in response to multiple stimuli including heat, mechanical stress, anions, and solvents. Additionally, we investigated the relationship between gelation and alkyl chain length and developed a pH stimuli-responsive supramolecular hydrogel.

Key Words : supramolecular hydrogel, drug delivery system, central nervous system

Introduction

The development of drugs for disorders related to the central nervous system (CNS) is challenging due to the presence of the blood brain barrier (BBB). Although the BBB plays a crucial role in the homeostasis of the CNS, it also limits the entry of drugs into CNS. To overcome this limitation, it is necessary to develop efficient drug delivery systems (DDSs) for crossing the BBB. We focused on supramolecular hydrogels as new DDSs. Supramolecular hydrogels are formed when small molecules, namely, low-molecular-weight hydrogelators (LMWHGs), self-assemble in water through the exploitation of non-covalent interactions. In this study, we designed new compounds possessing a saccharide and nucleoside.

Results

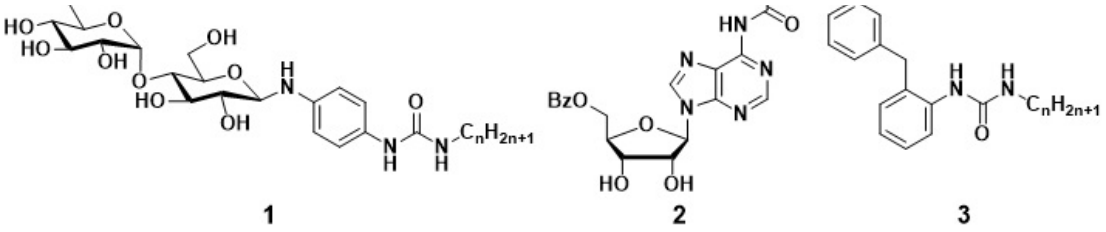
We designed compounds **1** and **2** possessing a saccharide and nucleoside, respectively, as new LMWHGs (Figure 1). In this study, **1** and **2** were synthesized and their gelation properties were investigated.

Amphiphilic ureas **1** bearing a hydrophilic maltose moiety and an alkyl group were synthesized *via* three steps (**Ref. 1**). These amphiphilic ureas functioned as LMWHGs, and the mixtures of the amphiphilic ureas and water formed supramolecular hydrogels. The gelation ability largely depended on the chain length of the alkyl group of each amphiphilic urea. An amphiphilic urea having a decyl group had the highest gelation ability (minimum gelation concentration = 0.4 mM). The physical properties of the supramolecular hydrogels were evaluated by measuring their thermal stability and dynamic viscoelasticity. Additionally, we found that the supramolecular hydrogels underwent a gel-to-sol phase transition upon addition of α-glucosidase as a result of the α -glucosidase-catalyzed hydrolysis of the maltose moiety of the amphiphilic urea.

In the above study, the length of the alkyl group was important in the design of LMWHGs. Although a typical strategy for designing low-molecular-weight gelators (LMWGs) is the introduction of long alkyl groups, there is no uniformity in the appropriate length of the alkyl group. Therefore, the relationship between gelation and alkyl chain length was investigated in this study (**Ref. 2**). Urea derivatives **3**, which were substituted with a 2-benzylphenyl group and an alkyl group, were synthesized as LMWGs for various organic solvents and ionic liquids (Figure 1). Urea derivatives with long alkyl chains were effective for the gelation of polar solvents and ionic liquids. On the other hand, urea derivatives with short alkyl chains were effective for the gelation of non-polar solvents.

The urea derivative **2** bearing an adenosine moiety was synthesized *via* 5 steps (**Ref. 3**). The hydrophobic benzoyl group had been introduced into a hydroxy group of the hydrophilic ribose moiety of **2** to control the solvophilicity of the molecule and its aggregates. Unfortunately, **2** did not form a supramolecular hydrogel in water; however, it selectively formed supramolecular gels in halogenated solvents such as chloroform and 1,2-dichloroethane. Additionally, we found that the supramolecular gel formed from **2** and chloroform underwent multiple stimuli-responsive reversible gel-sol phase transitions. The supramolecular gel showed a reversible phase transition by applying repeated warming–cooling cycles accompanying the gel-sol transitions. The supramolecular gel could undergo 5 repeated mechano-responsive gel-sol transitions. Gel-to-sol phase transitions could also be achieved by adding various anions to the supramolecular gel, such as tetrabutylammonium fluoride. Regelation was realized by adding boron trifluoride etherate to the fluoride-ion-containing sol. Addition of methanol to the supramolecular gel also induced a gel-to-sol phase transition. Regelation was realized by adding molecular sieves 4Å to the suspension.

Furthermore, an amphiphilic tris-urea compound containing hydrophilic resorcinol units was designed and synthesized for the development of a pH-responsive supramolecular hydrogel (**Ref. 4**). The compound formed supramolecular hydrogels in basic buffers. The supramolecular hydrogel formed from this compound and the borate–NaOH buffer exhibited a pH-responsive reversible gel-to-sol phase transition property.



1 2 3

Figure 1. Structures of **1**-**3**.

Discussion & Conclusion

We have synthesized a saccharide-containing LMWHG **1** and a nucleoside-containing LMWG **2** for the purpose of developing DDSs for crossing the BBB into the CNS. We found that **1** underwent an enzyme-responsive gel-to-sol phase transition. In this study, the effect of alkyl groups on gelation was systematically investigated. We also found that **2** underwent a reversible phase transition in response to multiple stimuli including heat, mechanical stress, anions, and solvents. Additionally, we developed a pH stimuli-responsive supramolecular hydrogel. Based on these findings relating to stimuli-responsive phase transitions, it can be concluded that supramolecular hydrogels as DDSs for crossing the BBB into the CNS can be realized.

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一般の皆様へ

アルツハイマー病をはじめとする中枢神経疾患の治療薬開発は非常に困難です。その原因の1つとして、脳に存在する血液－脳関門と呼ばれるバリア機構の存在が挙げられます。血液－脳関門は、脳への異物の侵入を防ぐ役割を担っていますが、脳への薬の移行をも妨げてしまうのです。そこで、薬物送達システムと呼ばれる、薬を患部へ届ける技術が注目されています。本研究では、超分子ヒドロゲルという機能性材料に着目し、新たな薬物送達システムを開発します。

Development of potent non-secosteroidal vitamin D derivatives based on the structure of bile acid

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Summary Abstract

In this study, novel non-secosteroid-type vitamin D derivatives were developed by using lithocholic acid derivative **1** as the lead compound. Two types of lithocholic acid derivatives, that is, amide derivatives and diol derivatives, were designed and synthesized, and their differentiation-inducing activity of human promyelocytic leukemia cells HL-60. All the synthesized compounds showed differentiation-inducing activity, and amide derivatives **2a**, **4** and **5b**, and diol derivatives (24*R*)-**7a** and (24*R*, 25*S*)-**8b** were as active as 1α,25-dihydroxyvitamin D_3. The binding features of some novel compounds with VDR LBD were examined by the X-ray crystallographic analysis of the complex.

Key Words : Vitamin D, lithocholic acid, nuclear receptor, cell differentiation

Introduction

Vitamin D elicits various biological functions, such as calcium and phosphate homeostasis, bone metabolism, and immune regulation, via binding to vitamin D receptor (VDR). Various vitamin D derivatives have been developed, and most of them have the same secosteroid structure as natural vitamin D. Lithoocholic acid was identified as the second endogenous VDR agonist. We have developed potent lithocholic acid derivative (**1**), but it had disadvantage that it was eliminated very quickly from serum in mice. In this study, we designed and synthesized novel vitamin D derivatives by using lithocholic acid derivative **1** as the lead compound.

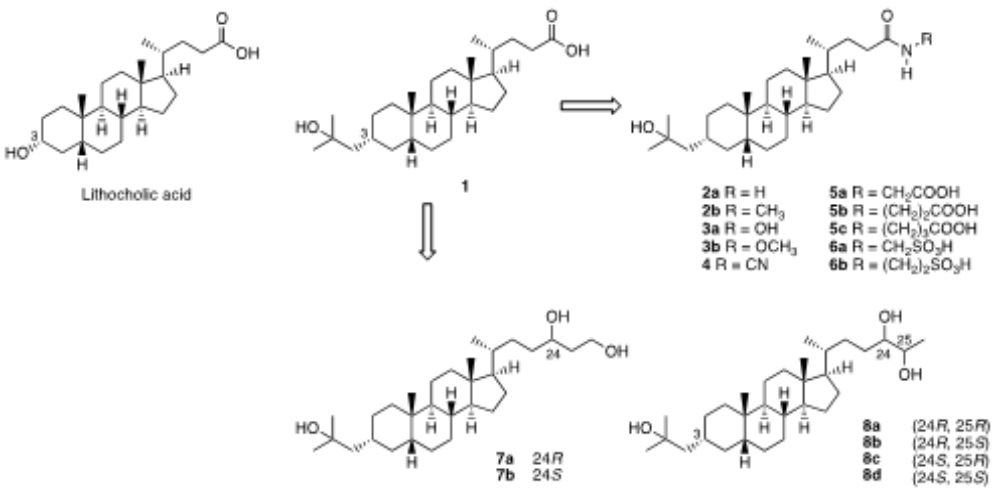
Results

We designed two types of novel derivatives of lithocholic acid derivative **1** with modification of the carboxy potent group at the terminal of the side chain, since it would have important role in the pharmacological properties of **1**. One is the amide derivatives **2** – **6**, and another is 1,3- (**7**) or 1,2-diol compounds (**8**). Amide derivatives were synthesized from compound **1** that was prepared by modification of our reported method. Diol derivatives were also synthesized from lithocholic acid. The isomers at 24 position were separated by using lipase reaction of the synthetic intermediate **9** bearing hydroxy and vinyl group at 24 position, which was prepared from lithocholic acid in 5 steps. Two 1,3-diol compounds **7** were synthesized form each isomer of **9** in 13 steps. Four 1,2-diol compounds **8** were also synthesized form each isomer of **9** in 14 steps.

The biological activity of the synthesized lithocholic acid derivatives was evaluated in terms of cell differentiation-inducing activity toward human acute promyelocytic leukemia cell line HL-60. Compound **2a** with unsubstituted amide group at the terminal of the side chain was

as potent as 1α,25-dihydroxyvitamin D_3 (active vitamin D). The introduction of *N*-substituent affected the activity off the amide derivatives. Compounds **2b**, **3b**, and **4** showed potent activity. In the case of compounds **5** and **6** bearing carboxyalkyl or sulfoalkyl group, respectively, bearing carboxy group, the activity depended on the length of the alkyl group and compound **5** showed potent activity. X-ray crystallographic analysis of the complex of rat VDR LBD with compound **5b** showed that the amide group of **5b** did not form a hydrogen bond with any amino acid residue. Instead, the carboxyl group of **5b** formed hydrogen bonds with Arg270 and the backbone amide bond of Tyr143.

Diol derivatives also showed potent differentiation-inducing activity of HL-60 cells. The activity depended on the stereochemistry of the hydroxy groups in the side chain. In the case of 1,3-diol derivatives, (24*R*)-isomer **7a** was more potent than (24*S*)-isomer **7b**. Among four isomers of 12-diol derivatives, (24*R*, 25*S*)-isomer **8b** was most active, and as potent as 1α,25-dihydroxyvitamin D_3. X-ray crystallographic analysis of the complex of rat VDR LBD with diol derivatives showed that the dihydroxy group interact with the amino acid residues such as Tyr143, Ser233, Arg270 and Ser274 that interact with the carboxy group of lithocholic acid or compound **1** in their complex with VDR LBD.



Discussion & Conclusion

We designed and synthesized two types of lithocholic acid derivatives by using compound **1** as a lead compound. The carboxyl group of compound **3** can be replaced with amide bonds or diol structures. In the case of the amide derivatives, unsubstituted compound **2a** and various *N*-substituted derivatives, such as compound **4** with an *N*-cyano group and **5b** with an *N*-2-carboxyethyl group are as active as 1α,25-dihydroxyvitamin D_3. In the case of diol derivatives, **7a** with 24*R*-hydroxy group and **8b** with (24*R*, 25*S*)-dihydroxy groups showed potent activity. Crystallographic analysis of the complex of the VDR LBD with novel compounds revealed their binding features with VDR LBD, which is helpful for structural optimization of VDR ligands. Compound **1** has potent vitamin D activity and lower calcemic activity than 1α,25-dihydroxyvitamin D_3, while its pharmacological property should be improved. The detailed biological functions and pharmacological properties of novel lithocholic acid derivatives are in progress.

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一般の皆様へ

ビタミンDはカルシウムやリンの恒常性維持、骨代謝、免疫機能制御などの様々な重要な生理機能を司っています。これまで、骨粗鬆症や乾癬などの治療薬開発を目的に何千ものビタミンＤの誘導体が創製されてきましたが、医薬品になった化合物を含めて高活性誘導体はいずれも天然のビタミンDと同じ骨格構造を持っています。私たちは、より幅広い疾患におけるビタミンＤの医薬応用を目的に、新しい骨格構造をもつ化合物の創製を行いました。胆汁酸の1種であるリトコール酸の構造をもとに種々の化合物を設計、合成したところ、非常に高いビタミンＤ活性を有する新規化合物を見いだすことができました。

Understanding of the molecular mechanisms that regulate production of neuronal diversity

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Summary Abstract

Drosophila optic lobe shares the structural features with mammalian cerebral cortex such as layer and columnar structures and it has been used as a model for studying brain development. We previously reported that genes that are temporally expressed in the medulla neural stem cells, they are called temporal transcription factors (TTFs), plays important roles on the specification of neuronal types. However, we do not know what is going on downstream of each TF. Here, we found several candidates of new member of TTFs via Targeted DamID technique. The present results suggests that one of them, Slp-H4, is a direct target of Slp.
Key Words : DamID, stem cells, nervous system

Introduction

Drosophila optic lobe shares the structural features with mammalian cerebral cortex such as layer and columnar structures and contains 100 types of 40,000 neurons. Because of these features, the optic lobe has been used as a model for studying brain development. We reported that genes that are temporally expressed in the medulla neural stem cells, they are called temporal transcription factors (TTFs), plays important roles on the specification of neuronal types. So far, several genes have been identified as TTFs, for example, *eyeless* (Ey), *sloppy paired* (Slp), and *Dichaete* (D). However, the molecular mechanism of what TFs regulate and how they determine the fate of the cells they produce remains unclear.

ResultsT

To identify direct targets of each TF, we conducted DNA binding profiling of each TF and transcriptome analysis in the neural stem cells of optic lobe using Targeted DamID technique.

<DamID Analysis>

Since all TFs are transcription factors, it is necessary to examine the DNA binding pattern of each TF to compare their functions. Using chromatin immunoprecipitation (ChIP), only neural stem cells from the brain need to be removed and collected in large numbers, which is technically very difficult. For this reason, analysis of genes that function downstream of TFs has not been performed for a long time.

In this study, we chose to analyze the DNA binding patterns of TFs using the newly developed DamID method (Southall et al., 2013) instead of ChIP assay.

<Establishment of TF-Dam lines>

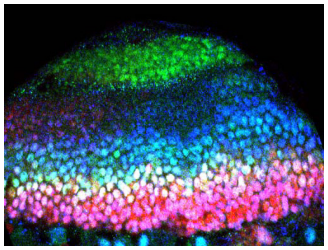
Since DamID analysis requires the establishing transgenic flies expressing TF-Dam fusion proteins, we prepared Dam fusion protein expression constructs for Ey, Slp, and D, which are TFs common to OPC and GPC (Suzuki et al., 2013; 2014; Bertet et al., 2014). The construction of expression vectors for Slp has been completed, and transgenic Drosophila lines have been successfully established.

<Transcriptome analysis in neural stem cells in OPC and GPC>

In order to identify the target gene of each TF, the following two-step analysis was required: (1) Identification of target genes of each TF by investigating the DNA binding pattern of each TF, (2) Confirmation that these candidate target genes are actually expressed in neural stem cells during the relevant TF window.

Since it took about two months after microinjection to generate the TF-Dam lines described above, we decided to perform cell type specific transcriptome analysis for neural stem cells in the OPCs and GPCs during this period for the smooth progress of subsequent analysis. Although single-cell RNAseq is widely used as a central method for cell-specific transcriptome analysis, it is extremely difficult to isolate only neural stem cells from OPCs and GPCs and to collect large numbers of them. We performed transcriptome analysis using DamID. Since we have already identified fly lines that can induce gene expression specifically in OPCs and GPCs, we used these lines to perform transcriptome analysis specifically for each of them. The list of expressed genes in the neural stem cells obtained by DamID included Deadpan and Miranda, which are specifically expressed in neural stem cells, and Slp and D, which are TFs, confirming that our DamID-based transcriptomic experiment worked well.

Expression pattern of SlpTC12



SlpTC12/Slp/NSC

<Target genes for Slp in OPC and GPC>

Using the Slp-Dam line established in this study, we searched for target genes of Slp in the OPC and GPC. Our previous studies have shown that there is a mutual relationship between each TF, and that Slp induces the expression of D, which is then expressed in neural stem cells. Therefore, we focused on the D locus and found that Slp binds to the region around D locus at a high frequency. This suggests that Slp directly binds to the regulatory region of D and regulates its expression. Next, we searched for regions in OPCs where Slp binds strongly, and found that Slp binds highly to the regulatory region of a gene named Slp target candidate 12 (SlpTC12). The transcriptome analysis we performed earlier also showed that SlpTC12 was on the list of expressed genes in OPC. Furthermore, we confirmed expression analysis confirmed its expression in neural stem cells during Slp expression window.

Discussion & Conclusion

Here, we examined the DNA binding pattern of Slp in the neural stem cells in the developing visual center. We identified SlpTC12 as a candidate gene as Slp target in the OPC. Since such specific binding pattern was not observed in GPCs, SlpTC12 is one the specific Slp target genes in the OPC neural stem cells.

Our expression analysis shows that SlpTC12 is also expressed in the subset of neural stem cells younger than Slp+ neural stem cells. This suggests that SlpTC12 is also regulated by the other TFs like Ey. Our future experiment using Ey-Dam may reveal the relationship among those transcription factors.

In the future, functional analysis of SlpTC12 should bring us closer to elucidating the molecular mechanisms that determine the neural fate of the TFs produced.

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一般の皆様へ

神経幹細胞から生み出された多様な神経が、正しい場所に移動し、最適な相手と結合することで正確な神経回路が構築される。神経幹細胞では、Temporal Factors(TFs)という転写因子群が特定の順序で発現することで、多様な神経を作り分けているが、TFsの下流でどのような遺伝子が制御され細胞運命を決定しているのかについては不明な点が極めて多い。

本研究では、TFsの1つであるSlpの下流遺伝子を探索しSlpTC12遺伝子を同定した。今後SlpTC12の機能を解析することによって、神経幹細胞が多種多様な神経を作り分ける分子機構の解明の糸口が得られると期待される。

Elucidating the essential mechanisms underlying AKI to CKD Transition by using Single-cell RNA sequencing and Lipidomics

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Summary Abstract

Acute kidney injury (AKI) elevates the risk of chronic kidney disease (CKD) progression, but the molecular mechanism underlying the AKI to CKD transition remains elusive. Here, the authors show that autophagy is reactivated during both the recovery phase and immediately after AKI, and that induced deletion of autophagy gene Atg5 in proximal tubular epithelial cells (PTECs) after AKI exacerbates CKD progression. Mechanistically, the ATG-conjugation system promotes mitochondrial biogenesis via the autophagy-independent transcription factor EB (TFEB)-peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α) axis as well as autophagy-dependent removal of damaged mitochondria. This novel mechanism may have potential therapeutic implications for preventing the AKI to CKD transition.
Key Words : autophagy, ATG-conjugation system, mitophagy, mitochondrial biogenesis, TFEB

Introduction

Background: For a decade, we have studied the role of autophagy, the process that mediates degradation of intracellular constituents in lysosomes, in the pathophysiology of kidney aging, high-fat diet (HFD)-induced obesity, and diabetes (**Ref.1-6**).

Autophagy in PTECs protects the kidney from AKI. Recently, AKI has been identified as a risk factor for CKD development; however, the mechanism of AKI to CKD transition, especially the involvement of autophagy in this process, remains unclear.

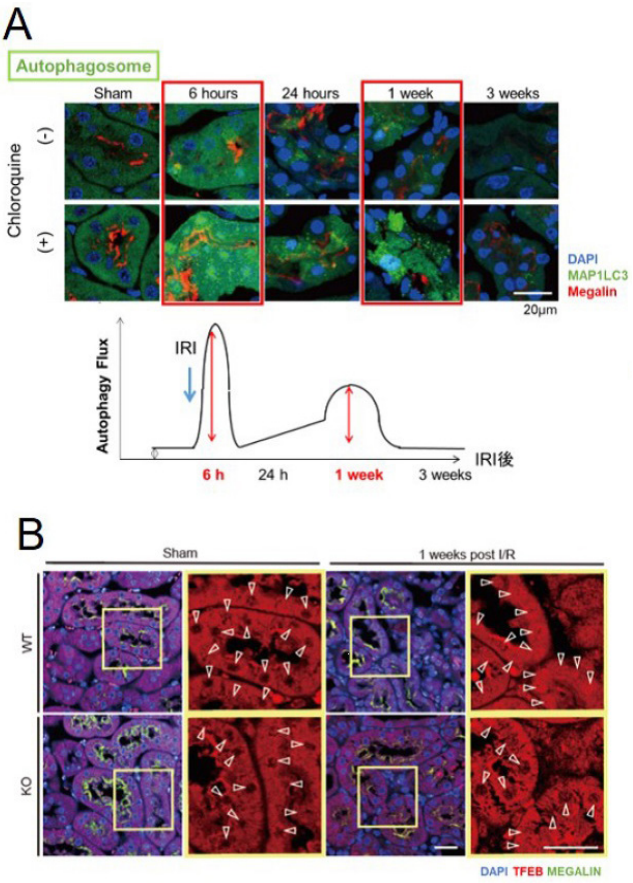
In this study, we aimed to elucidate the mechanisms underlying AKI to CKD Transition by using several genetically modified mice, and to identify new therapeutic targets and biomarker candidates by conducting Single-cell RNA sequencing and Lipidomic analysis.

Results

Methods: First, we subjected PTEC-specific Atg5 (Autophagy related 5)-deficient mice to unilateral renal ischemia reperfusion (I/R) and assessed CKD progression up to 3 weeks. Second, time-dependent autophagic activity after I/R was evaluated using autophagy-monitoring mice. Third, we investigated the role of ATG5 during CKD progression by using tamoxifen-inducible PTEC-specific Atg5-deficient mice. Third, we explored the underlying molecular mechanisms behind the protective role(s) of ATG5 in the AKI to CKD transition. Lastly, we explored conducting Single-cell RNA sequencing and Lipidomic analysis to identify new therapeutic targets and biomarker candidates.

Results: PTEC-specific Atg5-deficient mice exhibited CKD progression with marked fibrosis and inflammation. Autophagy was reactivated 1 week as well as immediately after I/R (**Figure A**). Autophagosomes contained numerous damaged mitochondria during the recovery phase after I/R, suggesting mitophagy activation. Atg5 deletion after I/R significantly exacerbated CKD development with a marked reduction in mitochondrial content and function 3 weeks after I/R.

Mechanistically, ATG5 contributed to the removal of damaged mitochondria by mitophagy and to mitochondrial biogenesis via the transcription factor EB (TFEB)-peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α) axis in an ATG-conjugation system-dependent but autophagy-independent manner (**Figure B**).



In addition, we profiled transcriptomic changes at single-cell level over time in acutely injured kidneys of mice subjected to mild I/R injury. Kidney function, structure, single-cell gene expression analyses were performed 2, 5 and 14d after I/R. We used motif enrichment, trajectory, drug response pattern and cell-cell interaction analyses to define key drivers of failed and successful regeneration (data not shown).

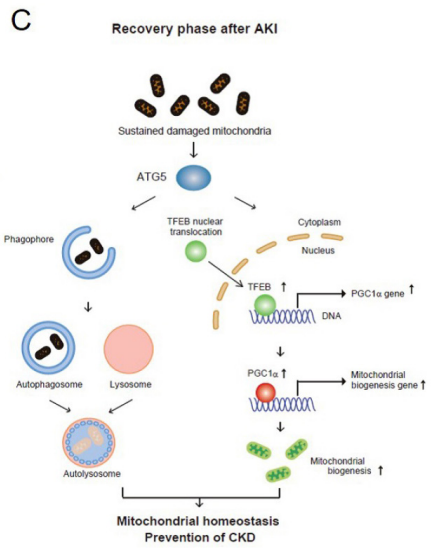
Discussion & Conclusion

Discussion: In this study, we elucidated a previously unknown protective role for the ATG-conjugation system against the AKI to CKD transition: 1) Autophagy, especially mitophagy, is activated during the recovery phase after AKI; 2) In addition to mitophagy, the ATG-conjugation system contributes to mitochondrial homeostasis through upregulating mitochondrial biogenesis via TFEB-PGC1α signaling in an autophagy-independent manner; 3) The ATG-conjugation system plays a protective role in the AKI to CKD transition through these two mechanisms.

A simple schematic drawing of this process is shown in **Figure C**.

During the recovery phase after AKI, ATG-conjugation system could retard AKI to CKD transition by renovating damaged mitochondria toward healthier ones; ATG-conjugation system mediates elimination of damaged mitochondria via mitochondrial autophagy (**Figure C,** left). ATG-conjugation system also facilitates nuclear translocation of TFEB, which increases transcriptional PGC1α expression, leading to the promotion of mitochondrial biogenesis (**Figure C,** right).

Conclusions: The ATG-conjugation system in PTECs prevents CKD progression after AKI by accelerating mitochondrial turnover via autophagic degradation of damaged mitochondria and production of healthy mitochondria. Modulation of the ATG-conjugation system could thus be an attractive therapeutic option for the AKI to CKD transition.



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一般の皆様へ

2016年、大隅良典氏がオートファジー研究によりノーベル医学生理学賞を受賞されました。私たちはこれまで腎疾患におけるオートファジーの研究に取り組んできました。今回、急性腎障害の回復期において、オートファジー関連遺伝子 Atg5は、傷害ミトコンドリアを選択的に除去するオートファジー（マイトファジー）と、同時にミトコンドリア再生を促進することによって、慢性腎臓病への進展を抑制することがわかりました。今後シングルセルとリピドームの統合的な解析を用い、細胞運命決定に関わる因子と病態関連脂質を同定し、創薬・バイオマーカー確立につなげたいと考えています。

Deciphering hepatic feeding-state-dependent metabolic gene regulation coordinated by intercellular crosstalk

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Summary Abstract

Kupffer cells are liver-resident macrophages that play critical roles in innate immune response and iron metabolism. In this study, we investigated the role of Kupffer cells in feeding state-dependent hepatic metabolic gene regulation and found that these cells repressed glucocorticoid receptor target genes in hepatocytes during feeding.
Key Words : liver, Kupffer cell, hepatocyte, cell-cell communication

Introduction

The expression of hepatic metabolic enzymes is regulated at the gene transcription level, mainly by hormones and nutrients, thereby maintaining the systemic homeostasis of glucose and lipid metabolism. Kupffer cells are resident macrophages in the liver and are assumed to affect hepatic gene expression through secreted factors. In this study, we investigated the role of Kupffer cells in the regulation of feeding state-dependent hepatic metabolic genes.

Results

Kupffer cells, which are liver-resident macrophages, are the largest population of tissue macrophages in the body and play critical roles in iron metabolism and clearance of gut-derived microbial products[1]. However, the function of Kupffer cells in the regulation of hepatic glucose and lipid metabolism remains unclear. Therefore, we investigated the role of Kupffer cells in hepatic metabolic gene regulation using inducible Kupffer cell ablation and Kupffer cell-specific genome editing. We have previously developed Kupffer cell-specific cre/reporter mice, namely Clec4f-cre-tdTomato mice[2]. We first crossed these mice with mice in which the diphtheria toxin receptor (DTR) was genetically targeted into the Rosa26 locus behind a LoxP-Stop-LoxP cassette (Rosa26iDTR), resulting in the Kupffer cell-specific expression of the DTR (KC-DTR). We then treated KC-DTR mice with diphtheria toxin (DT) and examined the effect of Kupffer cell depletion on the hepatic transcriptome after 24 h of fasting or 12 h of refeeding. Our RNA-seq studies demonstrated that Kupffer cells are involved in hepatic feeding-dependent gene regulation. Fasting induces the expression of genes involved in gluconeogenesis, fatty acid oxidation, and ketone body synthesis in the liver. Conversely, the expression of these fasting response genes is repressed during feeding. In KC-depleted mice, repression of fasting response genes during feeding was impaired. It has been reported that fasting response genes are induced by transcription factors, forkhead box protein O1 (FOXO1), peroxisome proliferator-activated receptor alpha (PPARα), cAMP response element binding protein (CREB), and glucocorticoid receptor (GR). We analyzed differentially expressed genes in Kupffer cell-depleted livers and found that Kupffer cells repressed GR target genes

in hepatocytes. We are currently planning to perform chromatin immunoprecipitation followed by sequencing (ChIP-seq) for GR to assess the effect of Kupffer cell-derived factors on GR-binding sites in hepatocytes.

To identify Kupffer cell-derived factors regulating hepatocytic GR activity, we explored the secreted factors whose expression was downregulated in the livers of Kupffer cell-depleted mice and found some candidate secreted factors. Among these, we identified several secreted factors whose expression is known to fluctuate in a feeding-dependent manner in Kupffer cells. To examine the role of these secreted factors in hepatic glucose and lipid metabolism, we carried out gene knockout using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-mediated genome editing. To this end, we crossed the Clec4f-cre-tdTomato mice with mice in which Cas9 was genetically targeted into the Rosa26 locus behind a LoxP-Stop-LoxP cassette (Rosa26-LSL-Cas9 knock-in), resulting in the Kupffer cell-specific expression of Cas9 (KC-Cas9). Next, we developed adeno-associated virus (AAV) vectors to express a single guide RNA (sgRNA) in Kupffer cells of KC-Cas9 mice. Capsid gene sequences of AAV are determinants of their tissue tropism. Therefore, we screened various AAV capsid mutants and employed a specific capsid-modified AAV that could efficiently infect Kupffer cells. Successful genome editing via this approach was demonstrated by a substantial loss of T-cell immunoglobulin- and mucin-domain-containing molecule (Timd4) in Cas9 expressing Kupffer cells infected with AAV-Timd4 sgRNA. We are currently analyzing the function of these Kupffer cell-derived secreted factors on hepatic fasting response genes and systemic metabolic homeostasis using our AAV/CRISPR system.

Discussion & Conclusion

We investigated the role of Kupffer cells in hepatic metabolic gene regulation using inducible Kupffer cell ablation and found that Kupffer cell-derived factors suppressed glucocorticoid receptor target genes in hepatocytes during feeding. Recently, it has been reported that Kupffer cell-derived tumor necrosis factor (TNF) suppresses GR-target ketogenic genes in hepatocytes during feeding[3]. However, the loss-of-function of TNF in Kupffer cells has not been investigated. We have previously identified Kupffer cell-derived secreted factors, distinct from TNF, that could affect hepatic feeding-dependent gene regulation. In future studies, we will characterize the functions of these factors in hepatic glucose and lipid metabolism using Kupffer cell-specific genome editing.

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一般の皆様へ

肝細胞の代謝酵素の量は、摂食状態に応じてインスリン・グルカゴンなどのホルモンにより調節されています。一方、糖尿病では、そのような代謝酵素の量を調節する仕組みが破綻し、脂肪肝や高血糖の原因となります。そのため、肝細胞の代謝酵素の量を調節する仕組みの理解は、糖尿病・代謝疾患の研究の重要な課題のひとつです。肝臓は、肝細胞以外にも様々な細胞が集まってできています。本研究で、私たちは肝臓にいる免疫細胞であるクッパー細胞が肝細胞の機能調節をおこなう可能性を考え、検討しました。その結果、クッパー細胞には、肝細胞における摂食状態に応じた代謝酵素の量の調節を助ける働きがあることが明らかとなりました。

Systems approaches towards the non-coding RNA-based production of humanized mice

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Summary Abstract

Expansion in the neocortex is associated with cognitive abilities that distinguish human from other mammalian species. At present, a set of human- or primate-specific genes have been proven that promote cortical expansion. On the other hand, using systems approaches, we have previously reported that promoter-associated non-coding RNAs (pancRNAs) transcribed from bidirectional promoter act on cis-acting elements to differentiate transcription levels of neighboring genes. In this study, we found hundreds of human-specific pancRNAs and detailed analyses for two pancRNAs suggested that evolutionary acquired quantitative difference in the expression of pancRNAs plays a key role in cortical development and folding.
Key Words : non-coding RNA, epigenome, promoter, human-specific, neural stem cells

Introduction

Expansion and folding in the neocortex are associated with unique cognitive abilities that distinguish human from other mammalian species. At present, a set of human- or primate-specific genes have been proven that promote cortical expansion and folding, such as TMEM14B. Since cortical folding emerges progressively during evolution, multiple genes, not only specific genes, but also conserved genes, should be involved in this process by finely tuning their expression levels. Using systems approaches, we have previously reported that promoter-associated non-coding RNAs (pancRNAs) transcribed from bidirectional promoter act on cis-acting elements to differentiate transcription levels of neighboring genes.

Results

Among such pancRNAs, we focused on pancCD63: it is expressed in the human, but not in the mouse neural stem cells (NSCs). CD63, known as an exosomal marker, is also expressed much higher in human NSCs. human NSCs (AF22 cell line) and embryonic (E) 14.5 mouse NSCs were infected by using lentivirus in knockdown and overexpression experiments. Immunostaining detecting active caspase3 and cell cycle labeling assay using EdU were performed to analyze cell apoptosis and proliferation, respectively. As a gain-of-function experiment, in-utero electroporation was performed on E13.5 mouse embryos, and plasmid DNA was microinjected through the uterus into the lateral ventricle. Western blot analysis was performed to explore the downstream target about CD63. Knockdown pancCD63 reduced expression level of CD63, suggesting that pancCD63 can be as a regulatory molecule to affect CD63 expression. Knockdown of either CD63 or pancCD63 resulted in a dramatical decrease in EdU+ cells and an increase in active caspase3+ cells, suggesting that pancCD63-CD63

pair promote human NSC proliferation. Overexpression of CD63 in mouse brain increased number of basal progenitors (BPs) marked by Pax6 and Tbr2 at E15.5. Immunohistochemical and western blot analyses showed that CD63 might increase BPs proliferative ability through ITGB1-Akt pathway. It is to be noted that, at E18.5, CD63 overexpression generated a large number of upper layer neurons and showed a folding-like structure. In parallel, we also assumed that the metabolic pathway has reprogrammed to adapt depending on species-specific modes of stem cell maintenance and functions. For that, we focused on Mitochondrial Uncoupling protein (UCP2), which is the inner mitochondrial membrane protein involved in glycolysis pathway. Using AF22, a human neural stem cell line derived from iPS cells, we searched for molecules that could be involved in gene expression regulation and discovered a promoter-associated non-coding RNA (pancRNA: pancUCP2) as a partner for UCP2. RNA-seq data for human and mouse neural stem cells showed that pancUCP2 was the representative human-specific pancRNA among glycolysis-related gene. Expression of UCP2 was correlated with that of pancUCP2. Comparison of UCP2/Ucp2 expressions in human and mouse neural stem cells indicated that mouse Ucp2 was expressed only 3.13 ± 0.5% of human UCP2. When we inhibited expression of UCP2 using shRNA, EdU uptake experiment and active Caspase3 antibody staining showed that cell proliferation was suppressed by ~70% and apoptosis was increased ~3 times.

Discussion & Conclusion

Evolutionary acquired quantitative difference in the expression of pancCD63-CD63 pair can play a key role in cortical development and folding for developing brain individuality. During neural stem cell development, metabolic reprogramming should occur according to the cell lineage. While in undifferentiated state glycolysis is predominant as metabolic pathway, oxidative phosphorylation is used after differentiation. In line with this idea, we also find that pancUCP2-UCP2 is involved in the maintenance of stem cell properties through human-specific metabolic regulation. Currently, we are introducing human UCP2 overexpression vector into mouse fetal brain using in utero electroporation to see whether human-specific traits can be acquired. Since we have taken systems approaches, we are evaluating the functional significance of ~20 newly identified human-specific pancRNA-gene pairs in parallel. In this way, these comprehensive analyses enable us to realize non-coding RNA-based production of humanized mice for therapeutic purposes.

一般の皆様へ

薬の開発を含む哺乳類の実験は、ネズミを中心に行われてきました。ネズミとヒトの脳は、異なるところも数多くありますが、遺伝子のはたらきは共通なのです。では、共通なのにもかかわらず、脳のかたちやはたらきに違いがあるのはなぜでしょうか？鍵を握るものの1つが「タンパク質に置き換わらない RNA」、ノンコーディング RNA です。すべての遺伝子は RNA に置き換わることで機能しますが、RNA にはタンパク質に置き換わるものと、置き換わらないものがあります。今回たくさんのヒト特異的機能ノンコーディング RNA を発見したことにより、創薬を今よりスピーディに行うことに貢献できます。

Novel mechanism of energy metabolism by Ca^{2+} regulatory proteins

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Summary Abstract

Obesity is a risk factor of life-threatening diseases. Recent evidences suggest that intracellular Ca^{2+} signal is involved in regulation of obesity. We found that a Ca^{2+} sensor protein NCS-1 regulates energy metabolism by increasing mitochondrial respiration and biosynthesis, resulting in decreased energy metabolism without changing in food intake and locomotive activity. These results suggest that NCS-1 can be an important target for metabolic syndrome.
Key Words : Obesity, Intracellular Ca2+ signal, energy metabolism, mitochondrial biosynthesis

Introduction

Obesity is a risk factor of life-threatening diseases; thus, it is important to identify novel mechanisms of its regulation and prevention. Recent evidence suggests that intracellular Ca^{2+} signal is involved in regulation of obesity[1,2]. Neuronal Ca^{2+} sensor-1 (NCS-1) is an EF-hand Ca^{2+} binding protein, which plays important roles in excitable cell functions[3]. We have previously reported that NCS-1 KO mice exhibit lower Ca^{2+} signals, and they became massive obese with ages[4]. However, its molecular mechanism and relationship to Ca^{2+} signals are unknown. The purpose of the present study was to clarify the molecular mechanism of NCS-1-mediated obese regulation.

Results

We first investigated whether this obesity is because of the fat accumulation or water accumulation. MRI analysis shows that both visceral and subcutaneous fats are increased in KO mice.

We next investigated the mechanism of obesity in KO mice in individual levels.

WT and KO mice (n=6 each) were kept individually in CLAMS Comprehensive Lab Animal Monitoring System for 8 days, and food intake, momentum and O_2 consumption/CO_2 emission as an indicator of energy metabolism were monitored. Food intake or momentum were not different between WT and KO mice. However, O_2 consumption and CO_2 emission were decreased in KO mice, indicating that KO mice became obese because energy metabolism was decreased.

Energy metabolism in whole body is regulated by mitochondrial respiration. In the mitochondria, metabolites of glucose and fatty acid are converted into H^+ gradient across the inner membrane of mitochondria. Using this H^+ gradient, ATP is synthesized by ATP synthase.

However, there is a H^+ channel (UCP1) on the inner membrane and if this channel is activated, large amount of H^+ ions are passed through the channel (H^+ leak), and nutrition are converted into heat without being synthesized into ATP. Indeed, it is known that the animals having much UCP1 is thin and animals having little UCP1 is fat.

Thus, we next examined the mitochondrial function in living cells by performing Seahorse XF mito stress test. Using this test, we can measure key parameters of mitochondrial functions, such as basal respiration, ATP production, H^+ leak (UCP1 function) and maximal and minimal respiration levels. The results shows that all these parameters were decreased in KO cells, indicating that mitochondrial respiration is indeed decreased in KO mice. However, normalization to the baseline value produced similar shape of the curves between WT and KO cells, suggesting that mitochondrial biogenesis may be decreased in KO cells rather than specific component of the mitochondrial proteins was affected by NCS-1 deletion. Indeed, Western blot analysis indicated that mitochondria-specific protein levels (Complex I -V) were all decreased in KO cells. These results indicate that Mitochondrial function and biogenesis have been decreased in KO mice.

This was further confirmed by the evidence that the key proteins for mitochondrial respiration UCP1 and PGC1 were both decreased in the KO brown adipocyte tissues (BAT). Furthermore, Ca^{2+}-dependent upstream pathways AMPK and CaMKK2 were both decreased in KO BAT. These results suggest that deletion of NCS-1 resulted in decreased Ca^{2+} signals, which decreased the functions of CaMKK2, AMPK, PGC-1 and UCP1, thus decreased the mitochondrial biogenesis and respiration, leading to decreased energy metabolism in whole body.

Western blot and quantitative RT-PCR analysis showed that NCS-1 are actually expressed in both BAT and while adipocytes WAT. Furthermore, the size of both BAT and WAT were larger in KO mice.

We finally performed metabolome analysis using BAT, WAT and liver in WT and KO mice. In BAT, the metabolites related to energy metabolism such as ATP were decreased, whereas in WAT, the metabolites related to fat accumulation such as acetyl CoA, Malonyl CoA and HMG CoA that are required for synthesis of triglyceride and cholesterols, were increased in KO mice. Thus, both of these pathways (in WAT and BAT) may contribute to the massive obese detected in KO mice.

Discussion & Conclusion

It has recently been reported that NCS-1 KO mice became obese with diabetes mellitus. They also investigated the mechanism of the diabetes mellitus[5]. However, the mechanism of obese observed in KO mice has not been clarified.

In the present study, we found that NCS-1 KO mice become obese with fat accumulation. Analysis in the individual level indicated that this was because energy metabolism was decreased without change in food intake or locomotive activity. Analysis of mitochondria function in the living cells indicated that mitochondrial respiration is indeed decreased KO mice.

Western blot analysis showed that NCS-1 KO BAT exhibited decreased Ca^{2+} signals, which decreased the functions of CaMKK2, AMPK, PGC-1 and UCP1, thus decreased the mitochondrial biogenesis and respiration, leading to decreased energy metabolism in whole body. NCS-1 exists both in the BAT and WAT, and metabolome analysis suggested that the metabolites related to energy metabolism were decreased in the BAT, whereas the metabolites related to fat accumulation were increased in WAT in KO mice, and both of these pathways may contribute to the massive obese observed in KO mice.

In conclusion, NCS-1 is a novel regulator of energy metabolism and can be an important target for metabolic syndrome.

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一般の皆様へ

肥満は糖尿病、心筋梗塞や脳梗塞など命にもかかわるような重大な病気の原因となり得ることから、その調節機構を明らかにすることは重要です。細胞内カルシウムは脳や心臓の働きに極めて重要なことが知られています。今回、そのカルシウムの働きを調節するNCS-1というタンパク質の欠損マウスが顕著な肥満となることから、NCS-1が肥満抑制に関わるのではないかと考え、その原因を探りました。その結果、NCS-1は摂食量や運動量には影響を与えず、基礎代謝を高めることにより肥満防御に働いていることが初めて明らかとなりました。今後はNCS-1関連シグナルを標的として、肥満を抑制する薬物の探索ができることが期待されます。

The Role of fibulin-4 in endothelial functions and application for clinical study

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Summary Abstract

In this study, we used mice with EC- specific deletion of *Fbln4* (*Fbln4*^{ECKO}) to generate EC- and SMC- double knockout mice for *Fbln4* (*Fbln4*$^{ECKO;SMKO}$, termed *DKO*) and investigated the endothelial contribution of Fbln4 in TAA formation. We found that *DKO* mice exacerbated aortic aneurysm with the activation of cardiac valve interstitial cells (VICs) and thickened aortic valves accompanied by turbulent aortic blood flow. Finally, transcriptome analysis detected critical molecules involved in the valvular changes that led to aortic valve abnormality, revealing the protective role of fibulin-4 in valvulo-arterial homeostasis.
Key Words : Aneurysm, Endothelial cells, Smooth muscle cells, Fibulin-4, Aortic valve

Introduction

Thoracic aortic aneurysms (TAAs) refer to an irreversible enlargement of the aortic wall which exceeds 1.5 times the normal aortic diameter. TAAs typically progress asymptomatically and can be life-threatening when they rupture or dissect. Surgical resection or a stent graft insertion is commonly performed as a prophylactic measure against aortic rupture, but effective medications to halt or reverse TAA progression have yet to be established. TAAs can be associated with heritable diseases with syndromic features, such as Marfan syndrome and Loes-Dietz syndrome, and exhibit marked activation of transforming growth factor-beta (TGF-β) and mitogen-activated protein kinase (MAPK) signaling in vascular smooth muscle cells (SMCs). Mutations in extracellular matrix (ECM) proteins and SMC contractile proteins are also related to the development of TAAs. On this basis, therapeutic strategies targeting SMCs with angiotensin type 1 receptors (AT1R) have been developed, but their efficacy over conventional medications such as b-blockers has not been confirmed in human patients. More recently, contribution of vascular endothelial cell (EC) has been reported as a crucial player in the pathogenesis of TAA. For example, endothelial AT1R is responsible for aneurysm formation in *Fbn1*$^{mgR/mgR}$ mice and endothelial ADAM17 contributes to elastase-induced TAAs by disrupting endothelial junctions. In addition, endothelial ROBO4 mutation or deficiency in mice results in TAA formation with bicuspid aortic valve (BAV). BAV, an aortic valve abnormality, has been associated with TAAs, demonstrating the involvement of multiple cell types in TAA formation. However, it is unclear how these cells interact and influence the formation of TAAs.

Results

There is a growing appreciation of the importance of ECs in the formation of TAAs, and discussion has centered around changes in blood flow associated with cardiac hypertrophy, as well as structural changes to the aortic valves such as BAV. As mutations in *FBLN4* in humans are lethal and cause severe vascular pathologies, early diagnosis and intervention are required. All homozygote mutations in *FBLN4* reported thus far have produced symptoms of severe aortic aneurysms, which can be lethal to the early stage of postnatal days, and in many cases lead to death before or after surgery. The development of pathological mouse models and the elucidation of the molecular mechanisms of disease pathogenesis are essential for establishing therapeutic strategies for human diseases. The *SMKO* mouse lines serve as a well-established model for the pathogenesis of this disease, as the mutation does not cause rapid rupture or dissection, making it a suitable model for observing aortic aneurysm development and progression. In this study, we firstly reported that *FBLN4* deletion in HAECs altered its morphological changes and the genetic deletion of *Fbln4* in both ECs and SMCs (*DKO*) progressed the formation of aortic aneurysms. ACE, Thbs1, and Egr1 were highly expressed in the ascending aorta of *DKO* mice, but no clear difference in molecular signaling was found compared to *SMKO* mice. As cardiac hypertrophy and aortic valve thickening were observed in *DKO* mice, we examined the causal relationship between time of aortic aneurysm initiation and aortic valve abnormalities. While the aortic valve was normal prior to the aortic aneurysm (at P7), it began to thicken at the time of aortic aneurysm initiation (P14). In addition, collagen deposition and progressive tissue fibrosis were observed in the aortic valves of *DKO* mice at P60. To explore the molecular significance of the thickened valve in *DKO* mice, RNA-seq analysis was performed, and 36 genes involved in proliferation and tissue fibrosis were identified that were altered in *DKO* valves. We confirmed protein levels of α-SMA, Ccn2 (CTGF) and Gdf15 in *DKO* valves, and showed trans-differentiation (activation) of VICs, leading to tissue fibrosis that results in thickening of the aortic valves. These results indicate the function of fibulin-4 in maintaining vascular wall homeostasis, the disruption of which affects aortic valve thickening and aortic aneurysm progression.

Discussion & Conclusion

Knockdown (KD) of *FBLN4* on HAECs induced mesenchymal-like morphological changes and alteration of gene expressions such as mesenchymal markers *TAGLN* and *MYL9*. *DKO* mice showed severe TAAs with cardiac hypertrophy and turbulent flow in ascending aorta, demonstrating the exacerbated aneurysm phenotype compared to *SMKO* mice. Although aneurysmal signaling such as angiotensin-converting enzyme (ACE), thrombospondin-1 (Thbs1) and early growth response 1 (Egr1) were comparable between *SMKO* and *DKO* aorta, *DKO* mice showed aortic valve thickening from postnatal day 14 (P14), accompanied with aneurysm development. Furthermore, RNA-seq and immunostaining for aortic valve revealed that genes involved in endothelial-to-mesenchymal transition (EndMT), inflammatory response and tissue fibrosis with activation of valve interstitial cells (VICs) are abundant in *DKO* mice. Our study has uncovered a pivotal role of endothelial fibulin-4 in maintaining valvulo-arterial integrity.

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一般の皆様へ

細胞外マトリクス Fibulin-4（Fbln4）の血管内皮細胞における役割は未知であり、その破綻が様々な病態発症に寄与することを見出しているものの、その分子メカニズムは不明です。本研究では、内皮細胞における Fbln4の役割を明らかにするために、ヒト血管内皮細胞やマウス内皮細胞で Fbln4を欠損させ、その機能異常を精査しました。Fbln4欠損内皮細胞は、間葉系細胞に類似した形態変化を示し、その遺伝子発現も内皮細胞とは異なるように変化していました。また、内皮細胞や平滑筋細胞での Fbln4欠損マウスは大動脈弁の肥厚と大動脈瘤の悪化が観察されたことから、内皮細胞における Fbln4が動脈弁－血管の恒常性を保護する役割を担うことが明らかとなりました。Fbln4の役割を補うことが、大動脈瘤の治療に必須であることが示唆されます。今後はヒト臨床検体における検証を重ね、臨床応用に繋げることを期待しています。

Microbes control fly oogenesis development

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Summary Abstract

Commensal bacteria are abundant in the skin and the mucosal surfaces of animals that constitute a bacterial flora (microbiome), especially in the gut, influence and maintain the host's physiological functions such as immunity and metabolism[1,2,3]. Among these functions, we tried to reveal the mechanisms of the oogenesis of *Drosophila* accelerated by microbes. We identified microbe-regulated pathways by the depletion or overexpression of specific genes. In particular, we dissected the molecular basis of the pathways at each stage of ovarian development and explored the mechanisms that regulate germline stem cell development and egg maturation.

Key Words : Drosophila, oogenesis, germline stem cells, microbes, egg maturation

Introduction

It has been reported that commensal bacteria influence and control the host's physiological functions in vertebrates and invertebrates, such as insects. In this study, we focused on the effect of the microbiomes on the fertility of *Drosophila melanogaster*, one of the model organisms. We analyzed the microbe-induced development of oogenesis and its molecular mechanisms. The genetic tools on flies and the histochemical approach revealed that specific genes control oogenesis under microbe-rich conditions. We also identified molecular pathways and chemical compounds that proliferate the germline stem cells and egg maturation during the oogenesis.

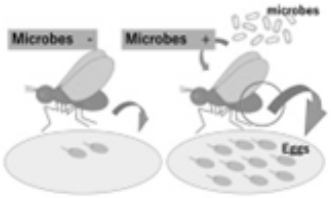

Fig1:Microbes enhance oogenesis and egg maturation.

Results

We found that egg formation was enhanced in the microbe-rich environment and caused by the synergistic effects of three key processes of oogenesis (1. Acceleration of germline stem cells (GSCs) division and proliferation. 2. Inhibition of germ cell death. 3. Enhancement of egg maturation rate.) by using fly genetics and immuno-histochemical approach. We took molecular-based approaches and identified genes and molecular pathways to understand which genes regulate microbe-induced oogenesis.

1. We have identified the hormonal pathways that promote and activate oogenesis development at each stage of oogenesis by somatic RNAi knock-down experiment, which leads more mature eggs and the acceleration of GSCs proliferation. We further confirmed this process by the experiment that excess amount of hormonal ligands in ovaries regulates the oogenesis development even in the microbe-absent condition. Moreover, we visualized

the active hormonal molecules with images of the early and late stages of the ovaries. We found more active molecules in microbe-rich conditions by quantitative image analysis.

2. We identified up- or down-regulated in the microbe-rich environment in the brain and gut, but found no clear differential gene expression in the ovary. Among the differentially expressed genes in the brain and gut, we have identified the most in the gut (366 genes), and nine genes were commonly up-regulated in both the brain and gut compared to the control. We also found the developmental defects in GSCs proliferation and egg maturation in reducing functions of these genes by RNAi knock-down experiments. We will further analyze these genes to define the functions of microbe-regulating pathways.

3. Since we didn't have clear results in the differential gene expression in ovaries, we decided to perform chemical screening to identify the specific pathways that microbes utilize for oogenesis development. We obtained the libraries composed of small molecule compounds that function as inhibitors of the pathways in GSCs. Microbe-sensitized female flies are set together with the chemical compounds and evaluated the GSCs proliferation and egg number enhancement compared to the control. We identified 16 compounds that are supposed to regulate the specific pathway positively and six compounds negatively in GSCs proliferation. At the same time, we also evaluated the nuage formation influenced by these small chemicals under the microbe-relevant activation of oogenesis.

Discussion & Conclusion

We further verified the specific hormonal pathways based on our findings of the microbe-indued oogenesis processes. It has been previously reported that individual microbes specifically act on metabolic pathways and influence oogenesis enhancement and lifespan in a combination-manner[4]. We identified the microbes deposited in the household flies and verified that the specific species activate oogenesis as fly-provided microbes. Since hormonal pathways are known to control the fly oogenesis development at each stage by various environmental factors[5,6], our data is compatible with them and showed microbes activated one of the pathways.

We also clarified that the gene expression in the brain and gut was adjusted to conduct oogenesis development by the transcriptome analysis, though we need further detailed confirmation. To compensate for the transcriptome analysis in the ovary, we set up chemical screening by using the libraries made of low molecular compounds to focus on the effect of the earlier stage of oogenesis. The libraries of inhibitors and drugs are expected to lead to a new approach to drug discovery to enhance oogenesis development. Moreover, these results would provide detailed insights into specific pathways of enhancing the reproductive role in GSCs. Providing compounds and modulating pathways in each tissue will propose the possibility of improving the fertility in mammals, including humans, which will enable us to control the reproductivity of animals.

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一般の皆様へ

体内、特に腸内に多く存在する常在細菌は宿主の免疫、代謝などの生理作用に影響を与えることから、疾患との関連のみならず、健康保持や免疫力向上の面でも注目されています。本研究では微生物の持つ生殖能力の向上機能に着目しました。我々はモデル生物であるキイロショウジョウバエを用いて、微生物叢によって引き起こされる卵巣の発達、それに寄与する分子メカニズムの解析を行いました。その結果、微生物叢により生殖幹細胞が増殖し、卵形成の各発生段階が影響を受けて卵成熟が促進されることがわかりました。さらに微生物叢によって活性化されるホルモン経路を同定し、また別法での低分子化合物のスクリーニングにより生殖幹細胞の増殖作用に特異的に機能する化合物を特定しました。これらの結果は特異的な分子経路の活性化や化合物の供与により生殖機能を向上させることができ、ヒトを含む哺乳類にも適応できることを示唆しています。

Investigating novel treatment strategies for Pseudohypoparathyroidism

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Summary Abstract

Pseudohypoparathyroidism (PHP) is a rare congenital disease that is characterized by reduced serum Ca+ concentration and subsequent symptoms like short stature and seizures which can cause by a mutation in the GNAS gene. One of the symptoms included infraocclusion which is caused by retarded teeth eruption. Recently, we have identified one PHP family that harbored a mutation in the GNAS gene with characteristic craniofacial phenotypes including craniosynostosis and failure of teeth eruption.
Key Words : Pseudohypoparathyroidism, tooth eruption, infraocclusion, craniofacial defect

Introduction

Pseudohypoparathyroidism (PHP) is a rare disease that exhibits multiple symptoms which comprise low serum Ca+ concentration and subsequent symptoms like short stature and seizures which can cause by a mutation in the GNAS gene. One of the symptoms included infraocclusion which is caused by retarded teeth eruption. However, the detailed molecular and cellular mechanisms for causing these symptoms are still largely elusive, especially in the craniofacial area.

Results

In this research, we focused on the mechanism of how mutated GNAS could result in the failure of craniofacial defects such as retarded tooth eruption. The expression of *Gnas* was analyzed by *in situ* hybridization and immunohistochemistry using mice embryos. Interestingly, the expression of *Gnas* exhibits intense expression in the developing frontonasal process at E11.5. Additionally, immunohistochemistry revealed strong expression of *Gnas* at developing alveolar bone and Hertwig's epithelial root sheath cell which represents the cell types that shows the phenotype of the patients with PHP. Interestingly the expression of *GNAS* partially overlapped with *PTHR1* in developing alveolar bone which indicates critical interaction with these two molecules. We have also created a mouse model which harbors the same missense mutation in *GNAS* of one PHP family in our clinic. Homozygous mutation of this mutation caused embryonic lethality around E11.0 with smaller 1st pharyngeal arch. Further analysis revealed there are significant elevation of cell death both in the epithelium and mesenchyme of developing 1st pharyngeal arch. These results indicate *Gnas* play critical roles for cell survival of embryonic epithelial and cranial neural crest cells. We are now trying to establish a cell line from the patients which can monitor the production of cAMP by luciferase assay.

Discussion & Conclusion

The role of GNAS protein in the etiology of PHP is somewhat elusive. In this study we revealed the expression of *Gnas* is also detectable at the frontonasal area during embryonic craniofacial development. This is the first report to show tissue specific expression of *Gnas* in the developing embryo. These results indicate the significance of understanding the embryonic role of *Gnas* for explaining the phenotypes such as craniosynostosis and infraocclusion. From now on, we will continue investigating the model mouse for further understating of molecular and cellular etiology of PHP in a comprehensive way.

一般の皆様へ

偽性副甲状腺機能低下症（Pseudohypoparathyroidism：PHP）は国内での有病率が 3.4 人 /100 万人と推定されている多臓器不全を伴う希少疾患である。PHP の原因遺伝子は G タンパク質共役受容体に属する GNAS とされている。本研究では現在のところ不明な点も多かった PHP の胎生期における病態に着目し新たな知見を得る事が出来た。本研究結果は PHP の新たな診断方法や治療方法の開発が行う上での基盤的データとなる。

Investigation of hepatic metabolic homeostasis and its deterioration via physiological and bioinformatic analyses of vagus nerve-mediated organ crosstalk

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Summary Abstract

Hepatic steatosis and inflammation caused by obesity and insulin resistance impede brain–liver crosstalk mediated by α7 nicotinic acetylcholine receptor (α7nAchR). In the present study, we revealed worsening of hepatic damage, fibrosis, and inflammation in α7nAchR-knockout mice under hepatic steatosis conditions, regardless of whether insulin resistance was present. These findings show that impaired brain–liver crosstalk is critically involved in the exacerbation of obesity and insulin resistance-induced hepatic inflammation.
Key Words : organ crosstalk, inflammation, non-alcoholic steatohepatitis, vagus nerve

Introduction

The brain detects alterations in the systemic energy status and controls hepatic glucose metabolism through the vagal effects of α7 nicotinic acetylcholine receptor (α7nAchR)[1]. This vagus-mediated brain–liver crosstalk is disrupted by obesity and insulin resistance, which increase hepatic glucose production[1]. Although the vagus nerve controls both hepatic inflammation and hepatic glucose metabolism[2], the part played by this crosstalk in obesity and insulin resistance is unclear. Here, we found that α7nAchR impedance of vagal regulation worsens the chronic hepatic inflammation induced by obesity and insulin resistance.

Results

We used α7nAchR-knockout mice (α7KO) to examine the role of brain–liver crosstalk. Hepatic steatosis with chronic inflammation and fibrosis were induced by a 30-week atherogenic high-fat diet (AD), which provides 61% of energy from fat with 1.3 g cholesterol/100 g diet[3]. The AD increased body weight, blood glucose levels, and plasma insulin levels in both control and KO mice versus a normal diet. While the AD was associated with higher plasma ALT and hepatic triglyceride levels than normal diet feeding, α7KO mice fed an AD had higher levels of these factors than control AD-fed mice. Hepatic gene expression of Srebf1, a master regulator of hepatic lipogenesis, was higher in AD-fed α7KO mice than in controls. However, no differences were noted between α7KO mice and controls in the hepatic expression of lipid oxidation enzyme genes (Ppara and Cpt1a), lipogenic enzyme genes (Fasn and Scd1), or a VLDL secretion-associated gene (Mttp).

Next, we investigated hepatic inflammation in AD-fed α7KO mice. Analysis of hepatic gene expression showed that AD feeding upregulated the levels of the proinflammatory genes Tnf, Il6, and Ccl2. The gene expression level of Ccl2, but not that of Tnf and Il6, was significantly higher in α7KO mice than in control mice, suggesting the induction of chronic hepatic inflammation in α7KO mice. No differences were found between the groups in the hepatic expression levels of Itgax, encoding the M1 marker CD11c, Mrc1, encoding the M2 marker CD206, and CD163. As hepatic fibrosis is a characteristic of chronic hepatic inflammation[4], we next examined hepatic fibrosis in AD-fed α7KO mice. Thus, regarding fibrosis-related genes, AD feeding upregulated the gene expression of Col1a1 and Acta2 and showed a tendency for an increase in Tgfb1. Compared with control mice, α7KO mice showed elevated expression of the Col1a1 gene, but not that of Acta2 and Tgfb1. Histological analysis with the collagen fiber marker Sirius Red revealed the worsening of hepatic fibrosis in AD-fed α7KO mice. These findings suggest that α7nAchR deficiency causes severe hepatic inflammation under AD conditions.

Because AD feeding promoted insulin resistance along with hepatic steatosis, we next used a methionine/choline-deficient diet (MCD) to study the effects of brain–liver crosstalk impedance under hepatic steatosis conditions without insulin resistance. MCD feeding for 6 weeks induces severe hepatic steatosis, inflammation, and fibrosis without obesity[5]. Control and α7KO mice showed no significant differences in body weight or blood glucose and plasma insulin levels with MCD feeding. MCD-fed α7KO mice exhibited significantly increased plasma AST levels and a tendency for increased plasma ALT levels. Although MCD feeding augmented hepatic triglyceride levels, the results were similar in control and A7KO mice. The hepatic expression levels of lipogenesis, lipid oxidation, and VLDL secretion genes were not different between control and α7KO mice. In the liver, the gene expression levels of Tnf, Ccl2, Acta2, Col1a1, Tgfb1, and Itgax were higher in MCD-fed mice than in control diet-fed mice and α7KO mice showed higher levels of Tnf and Tgfb1. Sirius Red staining indicated exacerbated hepatic fibrosis in α7KO mice versus control mice.

Discussion & Conclusion

Hepatic steatosis induced by obesity and insulin resistance culminates in chronic hepatic inflammation and fibrosis, a condition called non-alcoholic steatohepatitis (NASH). Obesity and insulin resistance also impair brain–liver crosstalk. Here, we have tried to unravel the role of brain–liver crosstalk in the pathology of NASH. We previously showed an important role in the brain–liver crosstalk for α7nAchR in the vagus nerve, which also controls hepatic inflammation[1,6]. Moreover, we found that α7nAchR deficiency disrupted this crosstalk, affecting hepatic glucose metabolism[1]. Here, we used two different diet-induced NASH models—AD feeding and MCD feeding—to examine the effect of α7nAchR deficiency in obesity and insulin resistance. In both NASH models, α7nAchR deficiency worsened the hepatic inflammation and fibrosis, as indicated by elevated levels of plasma hepatic transaminase and hepatic inflammatory genes. Insulin resistance affects both the brain–liver crosstalk and hepatic inflammation induced by steatosis. Given that MCD feeding promotes hepatic steatosis and inflammation without insulin resistance, defective brain–liver crosstalk worsens steatosis-induced hepatic inflammation independently of insulin resistance. Activated α7nAchR and the brain–liver interaction may be an exploitable target for the prevention and treatment of hepatic inflammation and fibrosis, including NASH.

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一般の皆様へ

脳は、全身のエネルギー状態を感知し、代謝恒常性維持の中心臓器である肝臓を制御する。この脳－肝連関は、肥満・インスリン抵抗性で障害され、その障害は糖代謝異常の原因になる。脳－肝連関は、迷走神経α 7 型ニコチン受容体作用を介している。一方で、α 7 型ニコチン受容体作用は、炎症制御に関与することも知られている。本研究では、α 7 型ニコチン受容体欠損マウスの検討から、脳－肝連関の障害が、脂肪肝に伴う肝慢性炎症の病因となることを見出した。脂肪肝に伴う慢性炎症は、非アルコール性脂肪性肝疾患（NAFLD）として、世界で 4 人に 1 人が罹患する保健上の課題である。本研究は、脂肪肝における脳－肝連関の役割解明により、肥満・インスリン抵抗性の病態理解を進めるものであり、また、新規 NAFLD 治療標的としての迷走神経α 7 型ニコチン受容体作用の可能性を示すものである。

Publication - FY2020 Grant Recipients

Title of the research project	Molecular basis for early dendritic cell lineage specification during pathogen infection
Recipient (Institution)	Daisuke Kurotaki (International Research Center for Medical Sciences, Kumamoto University)
Journal article / other material	PNS August 15, 2022 doi.org/10.1073/pnas.2207009119
Title of the paper	Chromatin structure undergoes global and local reorganization during murine dendritic cell development and activation

Title of the research project	Synthesis and development of flavan-derived polyphenol with protective effect against amyloid-beta-induced toxicity
Recipient (Institution)	Ken Ohmori (Department of Chemistry, School of Science, Tokyo Institute of Technology)
Journal article / other material	Angew. Chem. Int. Ed. 2022, doi.org/10.1002/anie.202205106
Title of the paper	Total Synthesis of Parameritannin A2, a Branched Epicatechin Tetramer with Two Double Linkages

Title of the research project	Molecular genetic analysis of plant sub-stomatal chamber development
Recipient (Institution)	Yuki Yoshida (Faculty of Advanced Science and Technology, Kumamoto University)
Journal article / other material	Microbiol Spectr. 2021 Sep 3;9(1):e0051121. doi: 10.1128/Spectrum.00511-21.
Title of the paper	Entamoeba Chitinase is Required for Mature Round Cyst Formation

Title of the research project	Catalytic Enantioselective α-Halogenation of Acylpyrazoles Directed towards Synthesis of Chiral Drugs
Recipient (Institution)	Kazuaki Ishihara (Graduate School of Engineering, Nagoya University)
Journal article / other material	Synlett 2022, 33, 585–588, DOI: 10.1055/a-1750-8481; Art ID: st-2021-v0465-l
Title of the paper	Thorpe–Ingold Effect on High-Performance Chiral–Copper(II) Catalyst
Journal article / other material	ACS Catal. 2022, 12, 1012−1017 doi.org/10.1021/acscatal.1c05500
Title of the paper	A π−Cu(II)−π Complex as an Extremely Active Catalyst for Enantioselective α-Halogenation of N-Acyl-3,5-dimethylpyrazoles

Title of the research project	Research on infertility
Recipient (Institution)	Keiichiro Ishiguro (Institute of Molecular Embryology and Genetics, Kumamoto University)
Journal article / other material	NATURE COMMUNICATIONS 2021 doi.org/10.1038/s41467-021-23378-4
Title of the paper	Meiosis-specific ZFP541 repressor complex promotes developmental progression of meiotic prophase towards completion during mouse spermatogenesis
Journal article / other material	J-STAGE Advance published date: 25 December 2021 doi.org/10.1266/ggs.21-00054
Title of the paper	MEIOSIN directs initiation of meiosis and subsequent meiotic prophase program during spermatogenesis
Journal article / other material	iScience 25, 104008, April 15, 2022 ª 2022 The Author(s). doi.org/10.1016/j.isci.2022.104008
Title of the paper	FBXO47 is essential for preventing the synaptonemal complex from premature disassembly in mouse male meiosis
Journal article / other material	Sexual Development DOI: 10.1159/000520682
Title of the paper	Sexually Dimorphic Properties in Meiotic Chromosome

Title of the research project	Genome wide analysis of DNA methylation and transcription dynamics in ICF syndrome
Recipient (Institution)	William Addison (Division of Molecular Signaling and Biochemistry, Kyushu Dental University)
Journal article / other material	FASEB J. 2022;36:e22153. doi.org/10.1096/fj.202101402R
Title of the paper	Inhibition of TET- mediated DNA demethylation suppresses osteoblast differentiation

Title of the research project	Development of novel GVHD mechanism and therapy targeting metabolic disturbance of intestinal epithelial cells that leads to dysbiosis.
Recipient (Institution)	Hideaki Fujiwara (Department of Hematology and Oncology, Okayama University Hospital)
Journal article / other material	NATURE IMMUNOLOGY VOL 22 NOV.2021 doi.org/10.1038/s41590-021-01048-3
Title of the paper	Mitochondrial complex II in intestinal epithelial cells regulates T cell-mediated immunopathology
Journal article / other material	Frontiers in Immunolog August 2021, Volume 12 Article 703298 doi: 10.3389/fimmu.2021.703298
Title of the paper	Crosstalk Between Intestinal Microbiota Derived Metabolites and Tissues in Allogeneic Hematopoietic Cell Transplantation

Title of the research project	Development of supramolecular hydrogel that enable drug delivery into central nervous system
Recipient (Institution)	Shinya Kimura (Development of pharmaceutical chemistry, Meiji Pharmaceutical University)
Journal article / other material	Chem Asian J. 2021, 16, 1937 – 1941 doi.org/10.1002/asia.202100376
Title of the paper	Enzymatic Hydrolysis-Responsive Supramolecular Hydrogels Composed of Maltose-Coupled Amphiphilic Ureas
Journal article / other material	Chem Asian J. 2021, 16, 1750 – 1755 doi.org/10.1002/asia.202100433
Title of the paper	Effect of Alkyl Chain Length of N-Alkyl-N'-(2-benzylphenyl) ureas on Gelation
Journal article / other material	Chem. Pharm. Bull. 69, 1131–1135 (2021) doi.org/10.1248/cpb.c21-00539
Title of the paper	Formation of pH-Responsive Supramolecular Hydrogels in Basic Buffers:Self-assembly of Amphiphilic Tris-Urea
Journal article / other material	Chem. Pharm. Bull. 70, 443–447 (2022) doi.org/10.1248/cpb.c22-00134
Title of the paper	Multiple Stimuli-Responsive Supramolecular Gel Formed from Modified Adenosine

＜論文掲載＞ 2020 年度受賞者

助成タイトル	感染防御における樹状細胞系譜早期運命決定の機序解明
受賞者	黒滝 大翼 (熊本大学 国際先端医学研究機構)
論文掲載誌・書誌事項	PNS August 15, 2022 doi.org/10.1073/pnas.2207009119
論文タイトル	Chromatin structure undergoes global and local reorganization during murine dendritic cell development and activation

助成タイトル	アミロイドβ凝集抑制作用を有するフラバンポリフェノール類の合成と機能開拓
受賞者	大森 建 (東京工業大学 理学院化学系)
論文掲載誌・書誌事項	Angew. Chem. Int. Ed. 2022, doi.org/10.1002/anie.202205106
論文タイトル	Total Synthesis of Parameritannin A2, a Branched Epicatechin Tetramer with Two Double Linkages

助成タイトル	植物の気孔腔形成メカニズムの分子遺伝学的解析
受賞者	吉田 祐樹 (熊本大学 大学院先端科学研究部)
論文掲載誌・書誌事項	Microbiol Spectr. 2021 Sep 3;9(1):e0051121. doi: 10.1128/Spectrum.00511-21.
論文タイトル	Entamoeba Chitinase is Required for Mature Round Cyst Formation

助成タイトル	光学活性医薬品合成を指向したアシルピラゾールの触媒的不斉α－ハロゲン化反応の開発
受賞者	石原 一彰 (名古屋大学 工学研究科)
論文掲載誌・書誌事項	Synlett 2022, 33, 585-588, DOI: 10.1055/a-1750-8481; Art ID: st-2021-v0465-l
論文タイトル	Thorpe-Ingold Effect on High-Performance Chiral-Copper(II) Catalyst
論文掲載誌・書誌事項	ACS Catal. 2022, 12, 1012－1017 doi.org/10.1021/acscatal.1c05500
論文タイトル	A π－Cu(II)－π Complex as an Extremely Active Catalyst for Enantioselective α-Halogenation of N-Acyl-3,5-dimethylpyrazoles

助成タイトル	不妊不育に関わる原因遺伝子の解明
受賞者	石黒 啓一郎 (熊本大学 発生医学研究所)
論文掲載誌・書誌事項	NATURE COMMUNICATIONS 2021 https://doi.org/10.1038/s41467-021-23378-4
論文タイトル	Meiosis-specific ZFP541 repressor complex promotes developmental progression of meiotic prophase towards completion during mouse spermatogenesis
論文掲載誌・書誌事項	J-STAGE Advance published date: 25 December 2021 doi.org/10.1266/ggs.21-00054
論文タイトル	MEIOSIN directs initiation of meiosis and subsequent meiotic prophase program during spermatogenesis
論文掲載誌・書誌事項	iScience 25, 104008, April 15, 2022 [a] 2022 The Author(s). doi.org/10.1016/j.isci.2022.104008
論文タイトル	FBXO47 is essential for preventing the synaptonemal complex from premature disassembly in mouse male meiosis
論文掲載誌・書誌事項	Sexual Development DOI: 10.1159/000520682
論文タイトル	Sexually Dimorphic Properties in Meiotic Chromosome

助成タイトル	ICF 症候群の間葉系幹細胞モデルにおける DNA メチル化と転写制御のゲノムワイド解析
受賞者	アディソン ウィリアム (九州歯科大学 分子情報生化学)
論文掲載誌・書誌事項	FASEB J. 2022;36:e22153. doi.org/10.1096/fj.202101402R
論文タイトル	Inhibition of TET- mediated DNA demethylation suppresses osteoblast differentiation

助成タイトル	Dysbiosis に基づく腸管上皮細胞代謝異常を標的とする新規 GVHD 機序の解明と治療応用
受賞者	藤原 英晃 (岡山大学病院 血液・腫瘍内科)
論文掲載誌・書誌事項	NATURE IMMUNOLOGY VOL 22 NOV.2021 doi.org/10.1038/s41590-021-01048-3
論文タイトル	Mitochondrial complex II in intestinal epithelial cells regulates T cell-mediated immunopathology
論文掲載誌・書誌事項	Frontiers in Immunolog August 2021, Volume 12 Article 703298 doi: 10.3389/fimmu.2021.703298
論文タイトル	Crosstalk Between Intestinal Microbiota Derived Metabolites and Tissues in Allogeneic Hematopoietic Cell Transplantation

助成タイトル	中枢への薬物輸送を可能にする超分子ヒドロゲルの開発
受賞者	木村 真也 (明治薬科大学 薬化学研究室)
論文掲載誌・書誌事項	Chem Asian J. 2021, 16, 1937 - 1941 doi.org/10.1002/asia.202100376
論文タイトル	Enzymatic Hydrolysis-Responsive Supramolecular Hydrogels Composed of Maltose-Coupled Amphiphilic Ureas
論文掲載誌・書誌事項	Chem Asian J. 2021, 16, 1750 - 1755 doi.org/10.1002/asia.202100433
論文タイトル	Effect of Alkyl Chain Length of N-Alkyl-N'-(2-benzylphenyl) ureas on Gelation
論文掲載誌・書誌事項	Chem. Pharm. Bull. 69, 1131-1135 (2021) doi.org/10.1248/cpb.c21-00539
論文タイトル	Formation of pH-Responsive Supramolecular Hydrogels in Basic Buffers:Self-assembly of Amphiphilic Tris-Urea
論文掲載誌・書誌事項	Chem. Pharm. Bull. 70, 443-447 (2022) doi.org/10.1248/cpb.c22-00134
論文タイトル	Multiple Stimuli-Responsive Supramolecular Gel Formed from Modified Adenosine

Ⅲ.

Reports from the Recipients of Grants for International Meetings

Report on Research Meeting

Aug. 30, 2022

1. Name of Research Meeting / Conference
   Sapporo Summer Seminar on Natural Product Biosynthesis

2. Representative
   Ikuro Abe, Graduate School of Pharmaceutical Sciences, The University of Tokyo

3. Opening period and Place
   Aug 16, 2022, Faculty of Pharmaceutical Sciences, Hokkaido University

4. Number of participants / Number of participating countries and areas
   Total 100 (On site 30, On line 70)/ Japan, Germany, Switzerland, China, and HongKong

5. Total cost
   2,000,000 Yen

6. Main use of subsidy
   Travel fees including hotel and flight, Conference fees

7. Result and Impression

Seven world-renowned biosynthesis researchers gave lectures at this symposium, and biosynthesis researchers from Germany, Switzerland, Japan, China, and Hong Kong discussed their research in a lively atmosphere. The discussions focused on the following eight areas of biosynthesis: 1. Microbial secondary metabolite synthases - polyketide biosynthesis, 2. Microbial secondary metabolite synthases - terpene biosynthesis, 3. Microbial secondary metabolite synthases - peptide and alkaloid biosynthesis, 4. secondary metabolite synthases from microorganisms - nucleic acids and others, 5. secondary metabolite synthases from filamentous fungi, 6. secondary metabolite synthases from plants - alkaloid biosynthesis, 7. crystal structure analysis and functional modification of enzymes, 8. production of new substances by reconstitution of biosynthetic pathways.

Dr. Kenichi Matsuda presented “Exploring the hydrazine biosynthetic pathways in bacteria”, a new technology for chemical labeling of rare UV-less natural products and their biosynthesis. Dr. Atsushi Minami presented “Development of versatile heterologous expression system for synthesizing fungal natural products”, a construction of versatile platform to express the secondary metabolite biosynthetic genes from higher eukaryotes in heterologous expression host. Dr. Yasushi Ogasawara presented “Two novel types of peptide epimerases to introduce D-amino acid residues in the biosynthesis of ribosomally synthesized and post-translationally modified peptides”, a new biosynthetic knowledge on the epimerization of amino acids in the bioactive peptides from bacteria. Dr. Takayoshi Awakawa presented “β-NAD as a building block in natural product biosynthesis”, a new biosynthetic knowledge on the cofactor-derived natural products and their enzymology. Dr. Yohei Katsuyama presented “Biosynthesis of secondary metabolites using nitrous

acid in actinobacteria", a new methodology on the genome-mining for the heteroatom-heteroatom bond forming secondary metabolite biosynthetic enzymes. Prof. Jeroen Dickschat presented "Terpene synthases - Mechanistic investigations and applications in the synthesis of non-natural compounds", a sophisticated methodology to trace the mechanism of terpene cyclization enzyme in detail. Prof. Jorn Piel presented "New peptide chemistry from exotic and well-known bacteria", a novel biosynthetic knowledge on the anaerobic oxidative enzymes which catalyze the isomerization and methylation on the large peptide sequences.

Intellectual exchange and interaction among numerous researchers in the field of biosynthesis led to further development of this field. Future development of biosynthetic enzyme functions utilizing AI and other technologies and production of new active substances through reconstitution are expected. It is also expected that this symposium will lead to several personal exchanges and collaborations among research groups across national boundaries. This meeting provided an important opportunity to stimulate international research exchanges.

8. Additional description
None

Report on Research Meeting

1. Name of Research Meeting / Conference
   The 39th Sapporo International Cancer Symposium

2. Representative
   Takuro Nakamura

3. Opening period and Place
   July 6 and 7, 2021, Royton Sapporo, Sapporo, Japan

4. Number of participants / Number of participating countries and areas
   Total number of participants: 172/ Number of participating countries: 7

5. Total cost
   ¥6,605,000

6. Main use of subsidy
   Travel costs and AV handling fee

7. Result and Impression

In this symposium, we focused on “cell reprograming”, the functional relationship between oncogenic mutations and epigenetic background in cancer cells. Recend advances in genomic technology identified a number of genetic mutations in cancer, clarifying multi-step processes of carcinogenesis and malignant progression. Aging and DNA damages are major sources of such genetic mutations, and recent accomplishment of this field was discussed at the symposium. Although the multi-step tumorigenesis theory comports with adult-type common cancers, there remain multiple cancers such as pediatric neoplasms that possess only a few mutations with early onset. Therefore, epigenetic modification of cancer is one of the major subjects of this symposium.

The symposium consisted of a keynote address, three special lectures, 18 invited lectures and 37 poster presentations. Takuro Nakamura, the symposium organizer, presented a keynote lecture titled ’Targeting the gene regulatory network in cancer: Modeling leukemia and sarcoma’. For the special lectures, Professor Patrick Tan, National University of Singapore, presented the lecture on genetic and epigenetic heterogeneity in gastrointestinal cancer, and clear stratification of human gastric cancer patients based on molecular and etiological classification. Professor Erwei Song of Sun Yat-sen Unigersity, China, presented the lecture titled ‘Where the wild thigs are: the tumor ecosystem of breast cancer’. In this lecture he talked on the novel findings on tumor microenvironment of breast cancer, and his proposal on the relationship between microenvironment and community of living organisms in the earth was intriguing. Professor Jesus Gil, MRC London Institute of Medical Sciences, UK, presented the lecture titled ‘Strategies to target cellular senescence’. In this lecture, he emphasized the importance of the removal of aging-associated senescent cells from the human body, and his identification of novel compounds to destruct senescent cells. These lectures have

provided the cutting edge information that gave remarkable impression to the audience.

Five sessions consisting of invited lectures were also presented. These include ‘Genetics and modeling’, ‘Epigenetic abnormalities in solid tumors’, ‘Epigenetic abnormalities in hematological malignancies’, ‘microenvironment and cancer stem cell’ and ‘DNA damage and signaling’. Among 18 lectures, Professor David Largaespada, University of Minnesota, USA, presented clarification of important pathways such as SHH, Wnt/beta-catenin and Hippo/YAP in malignant peripheral nerve sheath tumor associated with NF1 mutations. These results were obtained using iPS cells, CRISPR/Cas9 and SB transposon mutagenesis. Professor Ruud Delwel, Erasmus Cancer Institute, Netherlands, exhibited the role of super-enhancer modification in AML associated with EVI1 over-expression. This result will provide useful tools to target EVI1-expressing highly malignant AML. Professor Dmitry Bulavin, INSERM, France, presented relationship between histone modifications and PRC functions in senescent cells. Invited domestic speakers also presented lectures of very high levels of science. A lot of questions were given by audience on site and on-line.

Poster presentation was performed on-line due to pandemic infection of COVID-19. Thirty-seven posters were presented at six different sessions, e.g., Epigenetics (x2), Tumor microenvironment, Hematological malignancies, Cancer Immunology and Modeling and new technology. Discussions were also presented on-line.

Despite the hybrid type symposium consisting of both on site and on-line, we did not have severe troubles that prevented the meeting schedule. Construction of the good network system contributed a lot to the organization of the symposium, and the fund from Novartis Foundation was of great use to this system. We really appreciate the generous funding resource.

8. Additional description
Same as above.

Report on Research Meeting

August 31st , 2022

1. Name of Research Meeting / Conference

The 3rd International Symposium on Hybrid Catalysis for Enabling Molecular Synthesis on Demand（兼）ハイブリッド触媒取りまとめ公開シンポジウム (Official name of the symposium includes the latter part in Japanese.)

2. Representative

Professor Takashi Ooi
Principal Investigator, Institute of Transformative Bio-Molecules (ITbM) /
Professor, Department of Molecular and Macromolecular Chemistry, Graduate School of Engineering, Nagoya University

3. Opening period and Place

Opening period: From Thursday, June 30th to Friday, July 1st, 2022
Place: Sakata and Hirata Hall, Science South Building, Nagoya University

4. Number of participants / Number of participating countries and areas

Number of participants: 102
Number of participating countries and areas: 7

5. Total cost

1,416,540yen

6. Main use of subsidy

Honorarium, printing cost and equipment cost.

7. Result and Impression

The symposium was held in a hybrid manner with the following six invited speakers from overseas and twelve speakers from Japan. Due to concerns over the spread of the COVID-19, one of the invited speakers from overseas cancelled participation and five other invited speakers cancelled the visit to Japan and gave lectures online. However, we could have Dr. Todd Hyster from Cornell University to visit Japan and deliver an inspiring lecture about the recently emerging field of research in bio-catalysis in person at the venue.

After the struggle of not being able to have any face-to-face international meetings for a long time and having to postpone this international symposium last year due to the pandemic, we could finally hold this international symposium in person by implementing infection control measures. The symposium surely provided an excellent forum for in-depth discussion of cutting-edge issues in organic synthesis and catalysis with particular focus on now and the future of hybrid catalysis. More concretely, lectures are categorized into construction and application of hybrid catalyst systems based on (1) metal complex catalysts (Buchwald, Liu, Kanai, and Yamashita); (2) organocatalysts (Radosevich, Gilmour, Terada, Ohmiya, and Ooi); (3) photoredox- and bio-catalyst (Hyster and

Masaoka); (4) heterogeneous catalysis (Shishido); (5) domino reactions (Maimone, Maruoka, Hou, Inoue, and Ouchi); (6) computational analysis (Hatanaka), inducing active exchange of ideas about how these elements of hybrid catalysts could be strategically combined for creating new catalytic systems and innovative molecular transformations. It is also important to emphasize that the program has unparalleled value as a contribution to fostering young researchers who will become the next-generation leaders in the field. The time we shared during the symposium made me realize how important it is to present and discuss research in person, and I believe it had a significant meaning to all the speakers and participants as well. Last but not least, I would like to express my sincere gratitude to the Novartis Foundation again for the support in organizing the symposium.

Invited Speakers:
Todd Hyster (Cornell University, USA)
Alexander Radosevich (MIT, USA)
Guosheng Liu (Shanghai Institute of Organic Chemistry, China)
Ryan Gilmour (WWU Münster, Germany)
Stephen Buchwald (MIT, USA)
Thomas Maimone (U.C. Berkeley, USA)
Hirohisa Ohmiya (Kyoto University, Japan)
Keiji Maruoka (Kyoto University, Japan)
Makoto Ouchi (Kyoto University, Japan)
Masahiro Terada (Tohoku University, Japan)
Masayuki Inoue (The University of Tokyo, Japan)
Miho Hatanaka (Keio Univeristy, Japan)
Motomu Kanai (The University of Tokyo, Japan)
Shigeyuki Masaoka (Osaka University, Japan)
Takashi Ooi (Nagoya University, Japan)
Tetsuya Shishido (Tokyo Metropolitan University, Japan)
Yasuhiro Yamashita (The University of Tokyo, Japan)
Zhaomin Hou (RIKEN, Japan)

8. Additional description

Report on Research Meeting

1. Name of Research Meeting / Conference
   13th AFMC International Medicinal Chemistry Symposium (AIMECS2021)

2. Representative
   Prof. Motomu Kanai

3. Opening period and Place
   November / 29 /2021 ～ December / 2 / 2021
   Graduate School of Pharmaceutical Sciences, The university of Tokyo

4. Number of participants / Number of participating countries and areas
   418 participants / 19 countries, Japan, Asia, U.S.A and Europe.

5. Total cost
   11,600,000 JPY

6. Main use of subsidy
   Honorarium for lectures

7. Result and Impression

As plenary and invited speakers, we invited researchers who are working as the sources of various modalities. Those are, Dr. Shibasaki (Microbial Chemistry) from the development of asymmetric catalysts, Professor Schreiber (Harvard) from the field of chemical biology, Professor Young (MIT) from the field of intracellular liquid phase separation as Plenary speakers, Professor Baran (Scripps) from the field of synthetic organic chemistry, Professor Crews (Yale), the founder of Protac, Professor Cravatt (Scripps), the founder of covalent drugs, Professor Woo (Harvard) from the field of chemical biology, Professor Ting from the field of proteomics (Stanford), Professor Liu (Harvard) from the field of gene editing therapy, Dr. Manoharan (Alnylam Pharmaceuticals) from the field of nucleic acid medicine, Professor Lashuel (EPFL) and Dr. Gissen (Janssen) from the field of central disease treatment as invited speakers.

We were able to hear excellent lectures leading to cutting-edge results. All the lectures left profound impressions. Specifically, Professor Crews' research on Protac started with a curiosity-driven basic research on chemical biology, and at some point he stopped chemical biology and started thinking about disease treatment. The words are impressive. Professor Liu also aims at disease treatment, and was impressed by the direction in which he is seriously working on disease treatment by developing new modality starting from the field of chemistry. Professor Schreiber told that he started a basic research field called molecular glue, which led to the development of various clinical methods such as Protac. We felt a big flow from molecules to treatment.

There were lively questions from the participants, and although it was online, we were able to have sufficient discussions. It was also successful to select excellent chairpersons, mainly young people, who can respond flexibly even in the online format.

There were many applications for general lectures, and finally 45 lectures in a wide range of fields from organic synthesis to machine learning were adopted. There were 146 poster presentations. I think it was good to use EventHub, which is easy to communicate with, as a platform for online academic societies. The total number of participants in the conference was 418. Although it was difficult to interact by Online, such a great success was thanks to the generous funding from various agencies including Novartis Foundation. We would like to express our sincere gratitude. At the same time, we would like to thank the host organization AFMC, the executive committee members, the contractors, students, researchers and secretaries in my lab, and the Pharmaceutical Society of Japan, for their very kind backup.

8. Additional description
None

Report on Research Meeting

1. Name of Research Meeting / Conference
   64th Annual Meeting of Japanese Society for Neurochemistry

2. Representative
   Akio Wanaka (Department of Anatomy and Neuroscience, Nara Medical University Faculty of Medicine)

3. Opening period and Place
   September 30th to October 1st, 2021
   The meeting was held on-line (The headquarter of the web broadcasting was placed at Nara Medical University).

4. Numbers of participants / Numbers of participating countries and areas
   Total 452 participants / 1 from Australia, 1 from US (New York)

5. Total cost
   13,028,650 JPY

6. Main use of subsidy
   Meeting website maintenance: 200,000 JPY
   Fee for technical staff (Web broadcasting): 100,000 JPY
   Rental fee for internet access: 100,000 JPY
   Total: 400,000 JPY

7. Result and Impression

   We had originally planned to hold the conference face-to-face (at the Nara Convention Center), but as of the end of July 2021 (two months before the conference), the infection situation of the new coronavirus was not under control, so we decided to hold the conference completely online. The deadline for registration was extended and the number of registrations increased significantly with the switch to an online format, and we were able to receive over 450 registrations, which was close to our original plan (500). This was our first experience with the online format, but thanks to the efforts of the administration office, we were able to successfully register abstracts and participants, and abstracts were prepared in the form of online downloads. The program was rather tight, with three symposia and two oral presentations running simultaneously. Since I was at the web-broadcasting center, I was in a position to have a bird's eye view of the progress and discussions at each venue in real time. There are advantages and disadvantages to online conferences but considering that we could not have expected such a large number of participants in a face-to-face format, I am very glad that we decided to hold the conference online. Each day started with a special lecture, followed by symposia (a total of 12 symposia over the two days), the Young Scientists' Dojo (one of the features of this conference, where young researchers are given ample time to present and discuss their research), general oral presentations, and poster presentations. All the presentations

were very lively. All of them were full of lively exchanges, and I am very pleased as the conference chair that the conference was made possible by the grant from your foundation and that the members of our society were greatly stimulated by it. I would like to express my gratitude once again to the Novartis Foundation for their generosity in making such an opportunity possible.

8. Additional description
None

Report on Research Meeting

November 26, 2021

1. Name of Research Meeting / Conference
The 39th Japan Endocrine Society (JES) Summer Seminar on Endocrinology and Metabolism

2. Representative
Tomoaki Tanaka

3. Opening period and Place
From July 8th 2021 to July 10th 2021.
At Kamogawa Grand Hotel
Address: 〒296−0044　820 Ichiba, Kamogawa, Chiba, Japan

4. Number of participants / Number of participating countries and areas
476 person / 2 countries and 6 areas (JAPAN and USA; Chiba, Tokyo, Kyusyu, Kyoto, Kanazawa, State of Indiana in USA, and so on)

5. Total cost
14,433,078 yen

6. Main use of subsidy
Additional description・Orijinarukuokādo insatsubutsu・Internet line usage fee

7. Result and Impression

I am pleased to report that we have safely we have successfully conducted “the 39th JES Summer Seminar on Endocrinology and Metabolism” with great success even under COVID-19 situation. It was my great pleasure to have been appointed as the chairman of the 39th Endocrinology and Metabolism Summer Seminar of the Japanese Endocrine Society, and it has been held on Thursday July 8th, Friday 9th and Saturday 10th, 2021 at the Kamogawa Grand Hotel (Kamogawa, Chiba Prefecture). Historically speaking, the Japanese Society of Endocrinology, founded in April 1927, has a history and tradition of 93 years and has contributed greatly to the development of clinical practice and research in endocrinology in Japan. In this context, the Endocrinology and Metabolism Summer Seminar is an academic meeting that integrates not only the clinical field but also the research field in the field of endocrinology and metabolism. This year, we experienced a new type of infectious viral disease, bona-fide “COVID-19”, that we human beings had never expected before. Even in the midst of new social risks in the with/after-corona era, we physicians and medical researchers should continue to walk with hope toward the future in order to protect our health and livelihoods and build a safe and secure healthy society for our children in the future. Under these circumstances, I believe that this seminar achieved an important mission to help young doctors and young medical researchers, who will lead the next generation, to lead Japan's endocrinology

and metabolism with their dreams and contribute to the health and welfare of many patients. Therefore, in addition to providing a safe and secure environment for discussion at the local venue, we have prepared and then organized to hold the seminar in a new hybrid style that is future-oriented by preparing both on-site meeting and live webcasting with on-demand transmission to respond to the new social style of the post-corona era.

The theme of this year's seminar was "Endocrinology from a Molecular Perspective: New Diagnosis and Treatment with New Technology." On the clinical side, we discussed topics such as new therapeutic agents and new diagnostic methods for endocrine disorders and lifestyle-related diseases such as diabetes. In terms of research, we provided the latest topics such as diagnostic methods and disease prediction using artificial intelligence (AI) and machine learning. Actually, we also provided a wide range of information that would be useful to many clinicians and researchers, and discussed novel diagnostic and therapeutic possibilities. Importantly, there was insightful and constructive discussion and scientific debating between those who attended with on-site conference and those who participated in the live-streaming webcasting. In particular, the topics of basic research using state-of-the-art technology and educational lectures were very successful, with meaningful and active interactions and discussions.

8. Additional description
None

35th Grant Report (FY2021)

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 2021 are as follows.

35th Novartis Research Grant:	40 Researchers (JPY 1 mil.),	Subtotal JPY 40.0mil.
Research Meeting Grant:	5 Meetings (JPY 0.4 mil.),	Subtotal JPY 2.0mil.
		Total JPY 42.0mil.

35th Novartis Research Grant (FY2021)

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	Shuhei Nakamura	Graduate School of Frontier Biosciences, Osaka University	Associate Professor	Molecular mechanism of lysosomal damage repair by TFEB
2	Kenichi Kimura	Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance, University of Tsukuba	Assistant Professor	Investigation of cellular dynamics of CD73-expressing mesenchymal stem cells in the bone marrow microenvironment
3	Michio Sato	Institute of Resource Development and Analysis , Kumamoto University	Assistant Professor	A new challenge to HFpEF focusing on the cardioprotective effects of a novel lncRNA
4	Asuka Higo	Center for Gene Research, Nagoya University	Designated Assistant Professor	Revealing the mechanism to inhibit early senescence initiation in plants by switching transcription start sites.
5	Fumio Motegi	Institute for Genetic Medicine, Div. of Developmental Physiology, Hokkaido University	Professor	Analysis of cellular self-organizing mechanics by novel opto-thermogenetic approach
6	Kazuhiro Abe	Graduate school of Pharmaceutical Sciences, Nagoya University	Associate Proffessor	Structural and functional analysis of primary cation transporters and elucidation of the substrate specificity by gain-of-function mutant
7	Takayoshi Matsumura	Division of Inflammation Research, Center for Molecular Medicine. Division of Cardiovascular Medicine. Jichi Medical University	Lecturer	Hematopoietic stem cell aging and mitochondria aging
8	Hayato Nakagawa	Department of Gastroenterology and Hepatology, Mie University	Professor	Significance and spatial profiling of phospholipid metabolism in NASH
9	Takafumi Toyohara	Division of Medical Science, Tohoku University	Specially appointed assistant Professor	Investigating the mechanism of vascular aging using iPSC-derived 3D vascular model

#	Name	Institution	Title	Research Project
10	Shiki Takamura	Department of Immunology, Kindai University Faculty of Medicine	Associate Professor	Mechanisms of differentiation of highly infiltrating CD8 T cells in the tumor
11	Tomokatsu Ikawa	Research Institute for Biomedical Sciences, Tokyo University of Science	Professor	Dissection of epigenetic reprogramming underlying tumorigenesis using acute lymphoblastic leukemia inducing model
12	Kensuke Okuda	Laboratory of Bioorganic & Natural Products Chemistry, Kobe Pharmaceutical University	Professor	Exploratory study for the discovery of zinc homeostasis modulators utilizing a highly sensitive fluorescence imaging probe
13	Syota Matsumoto	Institute for Quantitative Biosciences, The university of Tokyo	Research Assistant Professor	Cryo-electron microscopy studies for disease pathogenesis of Xeroderma Pigmentosum
14	Kenichiro Kuwako	School of Medicine, Department of Neural and Muscular Physiology, Shimane University	Associate Professor	Analysis of brain aging caused by the disruption of "nuclear-axon crosstalk" machinery
15	Satoshi Inoue	Division of Immune Response, Aichi Cancer Center Research Institute	Section Head	Establishment of precision medicine for subsequent solid tumors after hematopoietic cell transplantation based on comprehensive genomic analyses
16	Taichi Kano	Graduate School of Engineering, Tokyo University of Agriculture and Technology	Professor	Development of asymmetric synthesis of sulfoximine analogs as bioisosteres
17	Keiichiro Mine	Division of Metabolism and Endocrinology, Department of Internal Medicine, Faculty of Medicine, Saga University	Project Assistant Professor	Achieve a comprehensive understanding of the pathogenesis of type 1 diabetes mellitus, leading to development of the prevention measures
18	Hiroyuki Konno	Graduate School of Science and Engineering, Yamagata University	Professor	Synthesis of amino acid derivatives and its application for the inhibitors against SARS-CoV2 protease
19	Mikiko Ohno	Department of Pharmacology, Shiga University of Medical Science	Associate Professor	Potential implications of enzymatic activity of the multifunctional protease NRDC as a new therapeutic target for cardiovascular disease
20	Aiko Sada	International Research Center for Medical Sciences (IRCMS), Kumamoto University	Associate Professor	Elucidating the mechanism of skin stem cell aging by targeting anti-aging matrix Fibulin-7
21	Kenshi Hayashi	School of Health Sciences, College of Medical, Pharmaceutical and Health Sciences, Kanazawa University	Associate professor	Studies of genetic basis of early onset cardiac conduction system disease and disease mechanism-based personalized medicine
22	Shigeki Arawaka	Department of Internal Medicine IV, Division of Neurology, Faculty of Medicine, Osaka Medical and Pharmaceutical University	professor	Analysis of the mechanism underlying extracellular release of tau for treatment of Alzheimer's disease
23	Keiro Shirotani	The Laboratory of Genome-based Drug Discovery, Nagasaki University	Associate Professor	Study of functional deficits of microglia and accelerated onset of neurodegenerative disorders

#	Name	Institution	Title	Research Project
24	Hiromitsu Hara	Graduate School of Medical and Dental Sciences, Department of Immunology, Kagoshima University	Professor	Modulation of itch by neuro-immune interaction
25	Kouji Hirota	Department of Chemistry, Graduate School of Science, Metropolitan University	Professor	Analysis of ncRNA transcription mediated chromatin modulation mechanism
26	Naofumi Uesaka	Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University	Professor	Communication between tumor cells and neurons regulating brain tumor
27	Mio Nakanishi	Graduate School of Medicine, Chiba University	Lecturer	Establishment of tissue maturaiton induction method to obtain adult tissues from pluripotent stem cells
28	Hideaki Nishihara	Department of Neurotherapeutics, Yamaguchi University	Assistant Professor	Intrinsic barrier dysfunction in multiple sclerosis patient
29	Yoshio Fujitani	Laboratory for Developmental Biology and Metabolism, Institute for Molecular and Cellular Regulation, Gunma University	Professor	Identification of PP cells as an origin of pancreatic ductal adenocarcinoma
30	Kazuma Ogawa	Institute for Frontier Science Initiative, Kanazawa University	Professor	Development of an innovative diagnostic and therapeutic method that combines radionuclide therapy and novel boron neutron capture therapy
31	Rie Wakabayashi	Goto-Kamiya laboratory, Department of Applied Chemistry, Faculty of Engineering, Kyushu University	Assistant Professor	Investigation on adjuvant effect of peptide-stabilized emulsions
32	Shintaro Kinugawa	Department of Cardiovascular Medicine, Faculty of Medical Sciences, Kyushu University	Associate Professor	Development of novel therapy for sarcopenia based on the regulation of O-linked N-acetylglcosamine motif (O-GlcNacylation)
33	Hiroo Takahashi	Department of Molecular Neurobiology, Kagawa University	Assistant Professor	Molecular mechanism of neuroprotection after ischemic stroke
34	Bisei Ohkawara	Division of Neurogenetics, Nagoya University Graduate School of Medicine	Associate Professor	Involvement of fusion proteins on morphogenesis of neuromuscular junction
35	Megumu Tanaka	Department of Cardiovascular Research, Shinshu University School of Medicine	Postdoctoral Researcher	Inhibition of cancer metastasis by selective regulation of AM-RAMP2 and AM-RAMP3 systems
36	Etsuko Toda	Department of Analytic Human Pathology, Nippon Medical School	Assistant Professor	Molecular mechanism for the mutual relationship between macrophage chemotaxis and activation
37	Akiko Ogawa	Institute of Development, Aging, and Cancer. Dept of Modomics medicine, Tohoku University	Assistant Professor	Metabolic signaling regulation by RNA modification

#	Name	Institution	Title	Research Project
38	Keisuke Asano	Laboratory of Organic Reaction Chemistry, Department of Material Chemistry, Graduate School of Engineering, Kyoto University	Assistant Professor	Catalytic Asymmetric Indole Halocyclization for Construction of Bioactive Structures
39	Noriko Inada	Graduate School of Life and Environmental Sciences, Osaka Prefecture University	Professor	Analysis of structures and functions of plant nuclear actin microfilaments
40	Katsuyoshi Takaoka	Laboratory for Embryology, Institute of Advanced Medical Sciences, Tokushima university	Associate professor	Establishment of embryonic diapause induction technique for in vitro culture of animal embryos

FY2021 Research Meeting Grant

(JPY 400 thousand x5 = 2.0 million)

#	Meeting	Place / Date	Institution / Title	Name
1	20th International Symposium on Spontaneously Hypertensive Rats	Kyoto/ 2022.10.16-17	Department of Gene Diagnostics and Therapeutics,Research Institute, National Center for Global Health and Medicine/ Director	Norihiro Kato
2	10th Asian Biological Inorganic Chemistry Conference	Kobe/ 2022.11.28-12.3	Graduate School of Engineering, Division of Applied Chemistry, Department of Molecular Chemistry, Biofunctional Chemistry Lab.,Osaka University/ Professor	Shinobu Itoh
3	Symposium on Molecular and Cellular Cognition	Tokyo/ 2022.6.27-28	Department of Applied Biological Chemistry, Graduate School of Agriculture and Life Sciences,The University of Tokyo/ Professor	Satoshi Kida
4	11th International Tunicate Meeting	Kobe/ 2022.7.10-15	Department of Biology, Konan University/ Professor	Takehiro Kusakabe
5	JSICR/MMCB 2022 Joint Symposium	Tokyo/ 2022.6.9-10	Laboratory of Biomedical Science, Graduate School of Agricultural and Life Sciences,The University of Tokyo/ Associate Professor	Shigeru Kakuta

第35期（2021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。2021年度は、下記の総額4,200万円の助成事業を実施しました。

第35回ノバルティス研究奨励金	40件（1件100万円）	4,000万円
研究集会助成	5件（1件 40万円）	200万円
		総額 4,200万円

第35回ノバルティス研究奨励金（2021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	中村　修平	大阪大学 大学院生命機能研究科	准教授	TFEB による損傷リソソーム修復機構の解明
2	木村　健一	筑波大学 生存ダイナミクス研究センター	助教	CD73 発現細胞による骨髄微小環境の細胞ダイナミクスの解明
3	佐藤　迪夫	熊本大学 生命資源研究・支援センター	特任助教	新規 lncRNA の心臓保護作用に着目した心不全（HFpEF）への挑戦
4	肥後あすか	名古屋大学　遺伝子実験施設	特任助教	転写開始点のスイッチングによる植物の早期老化開始抑止機構の解明
5	茂木　文夫	北海道大学　遺伝子病制御研究所 発生生理学分野	教授	新規の光温度遺伝学技術で探求する生体自律化メカニクス
6	阿部　一啓	国立大学法人東海国立大学機構 名古屋大学　創薬科学研究科	准教授	カチオン能動輸送体の構造機能解析と機能獲得変異体による基質特異性の解明
7	松村　貴由	自治医科大学 分子病態治療研究センター 炎症・免疫研究部　兼任 循環器内科	講師	ミトコンドリアに着目した老化造血幹細胞多様性の解析
8	中川　勇人	三重大学 大学院医学系研究科消化器内科学	教授	非アルコール性脂肪肝炎におけるリン脂質代謝異常の意義とその空間的理解
9	豊原　敬文	東北大学　分子病態医工学分野	特任助教	iPS 細胞由来 3D 血管を用いた血管老化メカニズムの解明
10	高村　史記	近畿大学医学部　免疫学教室	講師	腫瘍内高浸潤型 CD8T 細胞分化機構の解明
11	伊川　友活	東京理科大学　生命医科学研究所	教授	急性リンパ性白血病誘導モデルを用いた初期リプログラミング機構の解明
12	奥田　健介	神戸薬科大学　薬化学研究室	教授	高感度化亜鉛蛍光イメージングプローブを活用する細胞内亜鉛ホメオスタシスに干渉する化合物の探索
13	松本　翔太	東京大学 定量生命科学研究所 胡桃坂研究室	助教	クライオ電子顕微鏡を用いた色素性乾皮症発症メカニズムの解明
14	桑子賢一郎	島根大学 医学部 神経・筋肉生理学	准教授	“核－軸索クロストーク制御システム”の破綻による脳機能老化

No.	氏　名	所　属	職位	研究課題
15	井上　聡	愛知県がんセンター研究所 腫瘍免疫応答研究分野	ユニット長	網羅的ゲノム解析に基づく造血細胞移植後 2 次固形がんのゲノム医療の実現
16	加納　太一	東京農工大学　大学院工学研究府	教授	生物学的等価体としてのスルホキシイミン類縁体の不斉合成法の開発
17	三根敬一朗	佐賀大学 肝臓・糖尿病・内分泌内科	特任助教	1 型糖尿病発症機序の包括的な理解とその予防法の開発
18	今野　博行	山形大学　大学院理工学研究科	教授	アミノ類の網羅的合成と SARS-CoV2 Pro 阻害剤の開発
19	大野美紀子	国立大学法人 滋賀医科大学 薬理学講座	准教授	新たな心血管病治療標的としての多機能プロテアーゼ NRDC 酵素活性の意義
20	佐田亜衣子	熊本大学　国際先端医学研究機構	特任 准教授	抗老化マトリクス Fibulin-7 に着眼した皮膚幹細胞老化メカニズムの解明
21	林　研至	金沢大学 医薬保健研究域保健学系	准教授	若年発症心臓刺激伝導障害の遺伝子基盤解明とそれに基づく個別化医療の実践
22	荒若　繁樹	大阪医科薬科大学 医学部内科学 IV 教室脳神経内科	教授	アルツハイマー病治療を目指したタウ分子の細胞外放出機構の解明
23	城谷　圭朗	長崎大学 ゲノム創薬学研究室	准教授	ミクログリアの機能障害と神経変性疾患の発症機序の研究
24	原　博満	鹿児島大学 大学院医歯学総合研究科 免疫学分野	教授	神経ー免疫連関による痒み抑制機構の解明
25	廣田　耕志	東京都立大学　理学部化学科	教授	転写物の配列に依存しない非コード RNA 転写と共役した普遍的染色体機能調節機構の解明
26	上阪　直史	東京医科歯科大学 大学院医歯学総合研究科 認知神経生物学研究室	教授	脳腫瘍を悪性化させる腫瘍細胞―神経細胞間コミュニケーション
27	中西　未央	千葉大学　大学院医学研究院	講師	組織成熟化誘導法樹立による多能性幹細胞から成体組織形成の実現
28	西原　秀昭	山口大学 神経・筋難病治療学講座	助教	多発性硬化症患者に存在する血液脳関門破綻の遺伝的素因の解明
29	藤谷与士夫	群馬大学　生体調節研究所 分子糖代謝制御分野	教授	膵癌の新たな origin の同定　～膵ランゲルハンス島を由来とする膵癌発生新仮説の提唱～
30	小川　数馬	金沢大学　新学術創成研究機構	教授	核医学治療と新規ホウ素中性子捕捉療法を融合した革新的診断治療法の開発
31	若林　里衣	九州大学 工学研究院応用化学部門 後藤・神谷研究室	助教	ペプチドエマルションのアジュバント効果の検証
32	絹川真太郎	九州大学　大学院医学研究院 循環器内科学分野	准教授	O- 結合グリコシル化制御に基づくサルコペニアの新規治療法の開発
33	高橋　弘雄	香川大学 分子神経生物学	助教	脳梗塞から神経細胞を守る分子メカニズムの解析
34	大河原美静	名古屋大学大学院　医学系研究科 神経遺伝情報学	准教授	神経筋接合部の形態形成における細胞膜融合蛋白質群の役割
35	田中　愛	信州大学　医学部 循環病態学教室	博士 研究員	AM-RAMP2 系と AM-RAMP3 系の選択的制御による癌転移抑制法の開発
36	遠田　悦子	日本医科大学 解析人体病理学	助教	マクロファージの「動き」と「活性化」の相互連関メカニズム
37	小川亜希子	東北大学　加齢医学研究所 モドミクス医学分野	助教	RNA 修飾代謝による生体シグナル応答の機序解明にむけて
38	浅野　圭佑	京都大学　大学院　工学研究科 材料化学専攻 有機反応化学分野	助教	インドール類の触媒的不斉ハロ環化反応による生物活性骨格構築
39	稲田のりこ	大阪府立大学 大学院生命環境科学研究科	教授	植物細胞核内アクチン繊維の構造および機能の解明
40	高岡　勝吉	徳島大学 先端酵素学研究所発生生物学分野	准教授	胎生動物胚の体外培養を目指した発生休止誘導技術の開発

2021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。2021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	加藤　規弘	国立研究開発法人 国立国際医療研究センター研究所 遺伝子診断治療開発研究部	部　長	京都／ 2022.10.16-17	第20回 国際SHRシンポジウム
3	伊東　　忍	大阪大学 工学研究科応用化学専攻分子創成化学コース生命機能化学研究室	教　授	神戸市／ 2022.11.28-12.3	第10回 アジア生物無機化学国際会議
4	喜田　　聡	東京大学 大学院農学生命科学研究科応用生命化学専攻	教　授	東京／ 2022.6.27-28	分子細胞認知学シンポジウム
5	日下部岳広	甲南大学 大学院自然科学研究科生物学専攻	教　授	神戸／ 2022.7.10-15	第11回 国際被嚢動物学会議
6	角田　　茂	東京大学 農学生命科学研究科実験動物学研究室	准教授	東京／ 2022.6.9-10	JSICR/MMCB 2022 合同シンポジウム

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
Total	1,463	174,660	255	11,720	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
Total	13	2,600	14	582	1,899	217,813

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
Total	1,463	174,660	255	11,720	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
Total	13	2,600	14	582	1,899	217,813

35th Financial Report

Balance Sheet

As of March 31, 2022

(Unit：JP Yen)

Account	Amount
Ⅰ Assets	
1. Current Assets	
Current Assets Total	24,163,100
2. Fixed Assets	
(1) Basic Fund	
Basic Fund Total	1,100,000,000
(2) Specific Assets	
Specific Assets Total	10,594
(3) Other Long - term Assets	
Other Long - term Assets Total	80,056,790
Fixed Assets Total	1,180,067,384
Assets Total	1,204,230,484
Ⅱ Liabilities	
1. Current Liabilities	
Current Liabilities Total	42,085,176
Liabilities Total	42,085,176
Ⅲ Equity（Net Assets）	
1. Designated Net Assets	
Designated Net Assets Total	1,000,010,594
（Amount Appropriating to basic Fund）	(1,000,000,000)
(Amount Appropriating to specific assets)	10,594
2.General Net Assets	162,134,714
（Amount Appropriating to）	(100,000,000)
Equity Total（Net Assets）	1,162,145,308
Liabilities & Equity Total	1,204,230,484

Statement of Net Assets

From April 1 st, 2021 to March 31, 2022

(Unit：JP Yen)

Account	Amount
Ⅰ General Net Assets Changes	
1. Ordinary income & Expenditure	
(1) Ordinary income	
Interest from basic fund	14,876,376
Donation	40,110,000
Other Income	562,040
Ordinary Income Total	55,548,416
(2) Ordinaty Expenditure	
Project Expense	50,731,211
Grant Expense	42,000,000
Novartis Research Grant	40,000,000
Research Meeting Grant	2,000,000
Administrative Expense	2,963,306
Ordinary Expenditure Total	53,694,517
Ordinary Balance without Appraisal Profit or Loss	1,853,899
2. Nonrecurring Profit & Loss	
Nonrecurring Balance of Current Period	0
General Net Assets Ending Balance	162,134,714
Ⅱ Designated Net Assets Changes	
Designated Net Assets Change	(110,000)
Designated Net Assets Ending Balance	1,000,010,594
Ⅲ Net Assets Balance Ending Balance	1,162,145,308

第35期（2021年度）財務報告

貸借対照表

2022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	24,163,100
2．固定資産	
⑴ 基本財産	
基本財産合計	1,100,000,000
⑵ 特定資産	
特定資産合計	10,594
⑶ その他固定資産	
その他固定資産合計	80,056,790
固定資産合計	1,180,067,384
資産合計	1,204,230,484
Ⅱ負債の部	
1．流動負債	
流動負債合計	42,085,176
負債合計	42,085,176
Ⅲ正味財産の部	
1．指定正味財産	
指定正味財産合計	1,000,010,594
（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）	(10,594)
2．一般正味財産	162,134,714
（うち基本財産への充当額）	(100,000,000)
正味財産合計	1,162,145,308
負債及び正味財産合計	1,204,230,484

正味財産増減計算書

2021年4月1日から2022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	14,876,376
受取寄付金	40,110,000
雑収益	562,040
経常収益　計	55,548,416
⑵ 経常費用	
事業費	50,731,211
支払助成金	42,000,00
ノバルティス研究奨励金	40,000,000
研究集会助成金	2,000,000
管理費	2,963,306
経常費用　計	53,694,517
当期経常増減額	1,853,899
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	162,134,714
Ⅱ指定正味財産増減の部	
当期指定正味財産増減額	(110,000)
指定正味財産期末残高	1,000,010,594
Ⅲ正味財産期末残高	1,162,145,308

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

[Board of Trustees] 5 trustees, 2 auditors As of Sept 15, 2022

Post	Name	Title
Chairman	Kuniaki Takata, Ph.D.	President, Gunma Prefectural Public University Corporation
Trustee	Sadayoshi Ito, M.D., Ph.D.	Special Administrator, Katta General Hospital Professor Emeritus, Tohoku University
	Akimichi Kaneko, M.D., Ph.D.	Professor Emeritus, Keio University
	Fujio Murakami, Ph.D.	Professor Emeritus, Osaka University
	Leo Lee	President, Novartis Pharma K.K.
Auditor	Tokuzo Nakajima, CPA	Representative, Tokuzo Nakajima CPA Firm
	Masanori Fuse	Financial Advisor, Novartis Pharma K.K.

[Board of Councilors] 10 councilors As of Sept 15, 2022

Post	Name	Title
Chairman	Takao Shimizu, M.D., Ph.D.	Director, Institute of Microbial Chemistry, Microbial Chemistry Research Foundation Project Leader, National Center for Global Health and Medicine Professor Emeritus, University of Tokyo
Councilor	Masamitsu Iino, M.D., Ph.D.	Special Advisor, International Research Center for Neurointelligence, University of Tokyo Professor Emeritus, University of Tokyo
	Hiroyuki Kawashima, Ph.D	Former Professor, Niigata University
	Tsuneyoshi Kuroiwa, Ph.D.	Member of the Japan Academy; Professor Emeritus, University of Tokyo
	Masakatsu Shibasaki, Ph.D.	President, Microbial Chemistry Research Foundation Professor Emeritus, University of Tokyo
	Akihiko Nakano, Ph.D.	Senior Advisor/Deputy Director, RIKEN Center for Advanced Photonics Professor 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
	Miwako Mori, Ph.D.	Professor Emeritus, Hokkaido University
	Tohru Hirose, Ph.D.	Managing Director, Novartis Pharma K.K. Head, Global Drug Development, Novartis Pharma K.K.

[Grantee Selection Committee] 20 members As of Sept 15, 2022

Post	Name	Title
Chairman	Toshiaki Ohteki, D.D.S., Ph.D.	Professor, Medical Research Institute, Tokyo Medical and Dental University
Member	Yasuyoshi Sakai, Ph.D.	Professor, Kyoto University Graduate School of Agriculture
	Taisuke Tomita, Ph.D.	Professor, University of Tokyo Graduate School of Pharmaceutical Sciences
	Fumiko Toyoshima, Ph.D.	Professor, Institute for Life and Medical Sciences, Kyoto University.
	Tohru Minamino, M.D., Ph.D	Professor, Juntendo University Graduate School of Medicine
	Yasuhiro Yamada, M.D., Ph.D.	Professor, The Institute of Medical Science, University of Tokyo
	Masaki Ieda, M.D., Ph.D.	Professor, University of Tsukuba Faculty of Medicine
	Yasuteru Urano, Ph.D.	Professor, University of Tokyo Graduate School of Pharmaceutical Sciences
	Erina Kuranaga, Ph.D.	Professor, Tohoku University Graduate School of Life Sciences
	Mitinori Saito, M.D., Ph.D.	Professor, Kyoto University Institute for Advanced Study
	Fumitoshi Kakiuchi, Ph.D.	Professor, Keio University School of Fundamental Science and Technology
	Hiroshi Kawasaki, M.D., Ph.D.	Professor, Kanazawa University Faculty of Medicine
	Shoen Kume, Ph.D.	Professor, Tokyo Institute of Technology School of Life Science and Technology
	Kiyoshi Takeda, M.D., Ph.D.	Professor, Osaka University Graduate School of Medicine
	Kazuhiro Nakamura, Ph.D.	Professor, Nagoya University Graduate School of Medicine
	Tetsuya Higashiyama, Ph.D.	Professor, University of Tokyo Graduate School of Science
	Sachiko Miyake, M.D., Ph.D.	Professor, Juntendo University Graduate School of Medicine
	Hozumi Motohashi, M.D., Ph.D.	Professor, Tohoku University Institute of Development, Aging and Cancer
	Yoshihiro Sato, Ph.D	Professor, Hokkaido University Graduate School of Pharmaceutical Sciences
	Yutaka Takahashi M.D., Ph.D.	Professor, Nara Medical University

公益財団法人ノバルティス科学振興財団

［理事・監事］

任期　2022年6月15日～ 2024年6月　　2022年9月15日現在（敬称略）

職 名	氏 名	現　　職
代表理事	高田　邦昭	群馬県公立大学法人 理事長
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
	金子　章道	慶應義塾大学名誉教授
	村上富士夫	大阪大学名誉教授
	レオ リー	ノバルティス ファーマ株式会社 代表取締役社長

任期　2020年6月17日～ 2024年6月

監 事	中嶋　德三	公認会計士 中嶋德三事務所
	布施　正則	ノバルティス ファーマ株式会社 企画管理本部ファイナンシャルアドバイザー

［評議員］

任期　2020年6月17日～ 2024年6月　　2022年9月15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 微生物化学研究所　所長 国立国際医療研究センター プロジェクト長 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
	川島　博行	元新潟大学大学院医歯学総合研究科 教授
	黒岩　常祥	日本学士院会員 東京大学名誉教授
	柴崎　正勝	公益財団法人 微生物化学研究会 理事長 東京大学名誉教授
	中野　明彦	理化学研究所 光量子工学研究センター 特別顧問・副センター長 東京大学名誉教授
	鍋島　陽一	京都大学 大学院医学研究科　特任教授 京都大学名誉教授
	藤本　豊士	順天堂大学 大学院医学研究科 特任教授 名古屋大学名誉教授
	森　美和子	北海道大学名誉教授
	廣瀬　　徹	ノバルティス ファーマ株式会社 常務取締役グローバル医薬品開発本部長

［選考委員］

2022年9月15日現在（敬称略）

職 名	氏 名	現　　職
選考委員長	樗木　俊聡	東京医科歯科大学 難治疾患研究所 教授
選考委員	阪井　康能	京都大学 大学院農学研究科 教授
	富田　泰輔	東京大学 大学院薬学系研究科 教授
	豊島　文子	京都大学 医生物学研究所 教授
	南野　　徹	順天堂大学 大学院医学研究科 教授
	山田　泰広	東京大学 医科学研究所 教授
	家田　真樹	筑波大学 医学医療系 教授
	浦野　泰照	東京大学 大学院薬学系研究科 教授
	倉永英里奈	東北大学 大学院生命科学研究科 教授
	斎藤　通紀	京都大学 高等研究院 教授
	垣内　史敏	慶應義塾大学 理工学部 教授
	河崎　洋志	金沢大学 医学系 教授
	粂　　昭苑	東京工業大学 生命理工学院 教授
	竹田　　潔	大阪大学 大学院医学系研究科 教授
	中村　和弘	名古屋大学 大学院医学系研究科 教授
	東山　哲也	東京大学 大学院理学系研究科 教授
	三宅　幸子	順天堂大学 大学院医学研究科 教授
	本橋ほづみ	東北大学 加齢医学研究所 教授
	佐藤　美洋	北海道大学　大学院薬学研究院　教授
	高橋　　裕	奈良県立医科大学　教授

事 務 局 便 り

ご寄付のお願い

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

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

当財団へのご寄付には、下記の税法上の優遇措置が適用されます。

優遇措置の概略

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

法人：支出した寄付金は、通常一般の寄付金の損金算入限度額とは別枠で、下記の範囲内で損金の算入できます。

（資本金等の額×当期の月数/12×0.375%＋所得の金額×6.25%）÷2

ご寄付は、随時受付けております。詳しくは、財団事務局までお問合せください。

（E-メール：foundation.japan@novartis.com）

事務局より

2022年度も お陰様で 財団年報を発行する運びとなりました。2020年の初めからの新型コロナウイルス感染症によって、いろんな常識が常識でなくなり、それは、日々の生活や仕事に大きく影響しました。財団の仕事も会社の仕事も在宅が常識となり、科学技術の進歩の恩恵によって、大きな障害とならずに日々の仕事が進んでいます。これも改めて考えてみると驚くべきことで、ワクチンやデジタルによる恩恵にとどまらず、日々の基礎研究の成果なのだと感じています。

このような新しい日常の中で、基礎研究を中心としている当財団の事業である研究奨励金助成や研究集会助成事業に対して 今年も多くの応募があり、その中から選ばれた研究に助成が行われ、それぞれに成果を上げられていることを嬉しく思います。

当財団は1987年9月の財団設立以来、助成件数は**総数で1899件、総額で 約21.8億円**となりました。当財団は、自然科学の創造的研究への助成によって、日本の学術発展に寄与することを目指しており、助成を受けられた研究成果がすぐに応用につながらなくとも、将来、新分野につながることを夢見て、この事業を継続して参ります。

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

事務局長　原　健記
2022年10月13日

公益財団法人 ノバルティス科学振興財団
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