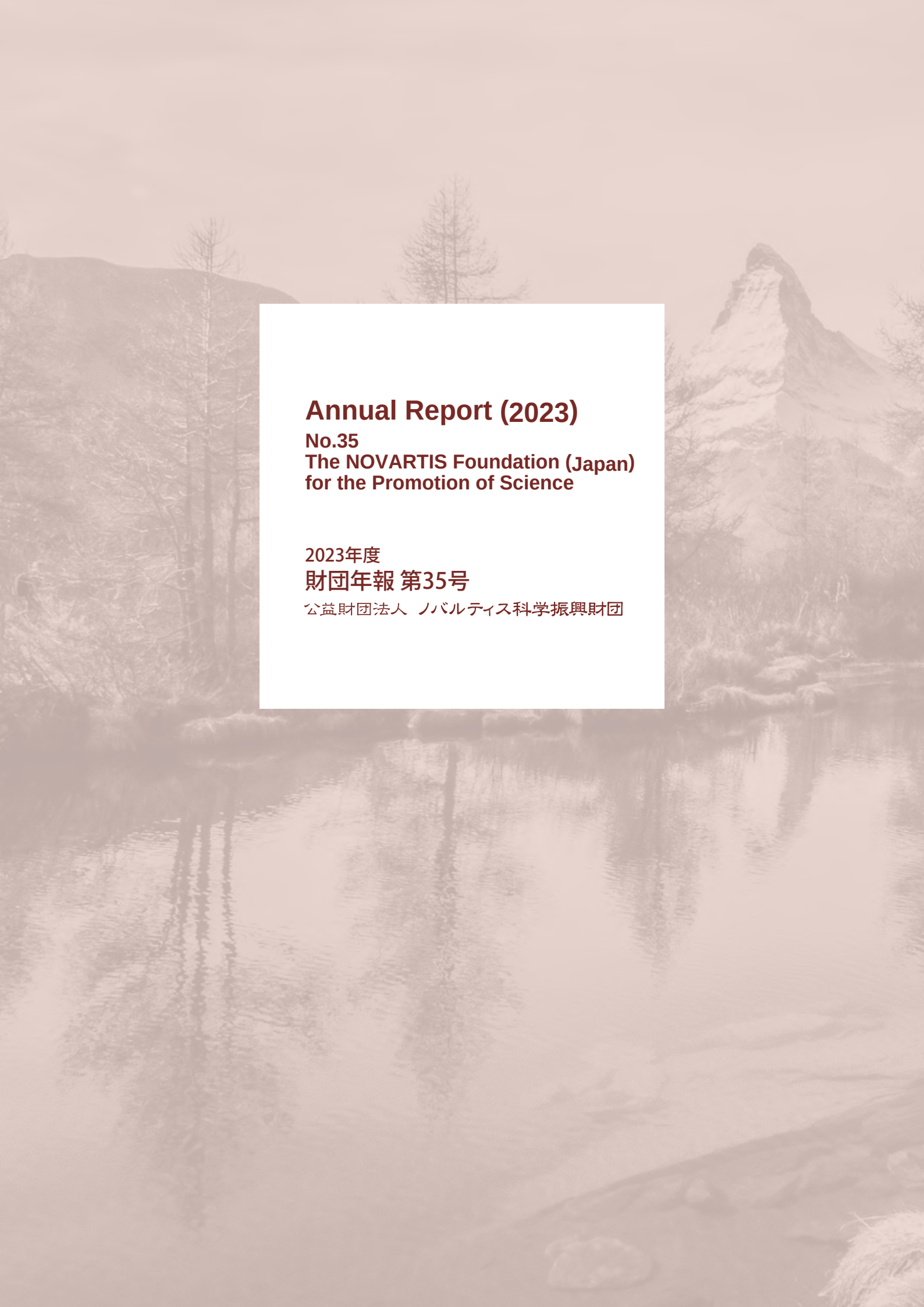
Annual Report (2023)

No.35
The NOVARTIS Foundation (Japan) for the Promotion of Science

2023年度

財団年報 第35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 35th Novartis Research Grant recipients (research from April 2022 to March 2023: 40 research grants and 5 research meetings).

The coronavirus (COVID-19) infection that began in 2019 raged around the world. In 2023 the situation has stabilized and society has regained its composure. During the pandemic period, while human traffic was greatly restricted, remote conferencing and information exchange using digital technology became commonplace, causing a major tectonic shift in the traffic of information. Through these experiences, we have learned that in many cases, things can be done efficiently and effectively through Internet-based communication. On the other hand, we have also recognized the importance of direct person-to-person contact. Furthermore, it was a great surprise to see the practical application of generative AI technology such as ChatGPT, which allows people to easily communicate with AI through natural languages. Witnessing the rapid development of AI and other information science technologies, we are reminded once again what unique creativity of human beings can bring about.

The Novartis Foundation (Japan) for the Promotion of Science was established in 1987 with a donation of 1 billion yen from Ciba-Geigy AG (now Novartis AG) of Switzerland. The purpose of the Foundation is clearly stated in its Articles of Incorporation as "to promote science by encouraging creative research in the natural sciences, thereby contributing to the improvement of the health and welfare of the people". In addition, a document titled "Prospectus for Establishment" written in the year of the Foundation's establishment states that the Foundation "seeks to promote and support creative research in the natural sciences, which will be the axis of science in the coming 21st century, thereby contributing to the welfare of mankind" and that the Foundation "provides financial support for research and offers opportunities for cross-border exchange". The policy of the Foundation is to "promote creative research in the natural sciences and thereby contribute to the welfare of humanity". Based on this policy, the Foundation has provided a total of 1,943 grants over the past 36 years, amounting to approximately 2.22 billion yen. In these times of rapid change, the Foundation is determined to return to this starting point and support the excellent research that will open the way to the next era.

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

はじめに

代表理事　高田　邦昭

本年報には、第35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（2022年4月～2023年3月の研究：研究奨励金40件、研究集会5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ましたが、2023年になり状況が安定するとともに、社会も落ち着きを取り戻してきました。この間、人の往き来が大きく制限された中で、デジタル技術を活用したリモートでの会議や情報発信が一般化し、情報流通に大きな地殻変動が起きました。この様な経験を通じて、私達はインターネットによるコミュニケーションで効率良く効果的に事を運ぶことができる場合が多いことを知る一方で、人と人とが直接向き合うことの重要性も認識しました。さらには、ChatGPTに代表される生成AI技術が実用化され、自然言語により人がAIと簡単にコミュニケーションできるようになったのは大いなる驚きでした。この様なAIを始めとする情報科学技術の急速な発展を目の当たりにするにつけ、改めて人にしかできない創造性とは何だろうかと考えさせられたりもします。

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、36年間で総計1,943件、金額にして約22.2億円の助成を行ってきました。日々目まぐるしい変化の起こる現在においてこそ、財団は改めてこの原点に立ち返り、独自のアイデアを膨らませている方、新たなプロジェクトを考えている方などの、次の時代を拓く優れた研究を支援して行きたいと考えています。

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

Ⅱ.

Reports from the Recipients of Novartis Research Grants

Molecular mechanism of lysosomal damage repair by TFEB

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Summary Abstract

Damaged lysosomes can be detrimental for cell, but their repair mechanism is largely unclear. We have previously found that TFEB, a master transcription factor for autophagy and lysosome biogenesis, is activated upon lysosomal damage and is essential to repair of damaged lysosomes (Nakamura et al., *Nat Cell Biol*, 2020). In the current study, to further reveal the regulatory mechanism of TFEB, we identified key molecules which work both upstream and downstream of TFEB. On-going functional analysis of these key molecules will contribute to the understanding of molecular mechanism to repair damaged lysosomes.
Key Words : TFEB, lysosome, autophagy

Introduction

Lysosomes are acidic organelles that contain a variety of digestive enzymes and are responsible for the degradation of intracellular and extracellular components. Various irritant particles such as silica, calcium oxalate crystals, and toxins are delivered to lysosomes by endocytosis pathway, and these are known to often damage lysosomes. Damaged lysosomes are extremely harmful to cells because they induce inflammation, oxidative stress, and apoptosis by releasing their acidic contents into the cytoplasm, leading to several prominent disease including neurodegenerative disease and kidney disease. In this study, we aim to reveal how cells repair damaged lysosomes by focusing on TFEB function.

Results

We have recently found that TFEB, a master regulator of autophagy and lysosomal biogenesis, is activated upon lysosomal damage and plays an essential role in the repair of damaged lysosomes (Nakamura et al., Nat Cell Biol, 2020). Autophagy is known as an intracellular bulk degradation system and is strongly induced by starvation and various stresses. When autophagy is induced, a double-membrane structure called an autophagosome is formed, which envelopes various unwanted intracellular materials and then fuses with lysosomes for degradation. Interestingly, this activation of TFEB requires non-autophagic functions of LC3 protein, which normally plays an essential role in autophagy. We newly found that when lysosomes are damaged, LC3 localizes not only to autophagosomes but also to lysosomes and activates TFEB through the function of calcium channel TRPML1. However, fundamental and important questions remain unanswered, such as (1) how LC3 localizes to lysosomes, (2) why LC3 ultimately leads to TFEB activation, and (3) how TFEB repairs damaged lysosomes. In order to answer these questions, we carried out the research with the following three aims.

Aim 1: Molecular mechanism of lysosomal localization of LC3 during lysosomal damage

It has been shown that treatment of cells with LLOMe, a lysosome-specific damaging agent, causes internal calcium leakage when lysosomes are damaged. Therefore, we investigated the possibility that this calcium may be involved in the lysosomal localization of LC3. We found that LC3 localizes to lysosomes when calcium leakage from lysosomes is artificially induced by administration of ML-SA1, an agonist of TRPML1, a calcium channel in lysosomes. On the other hand, LLOMe-treated damaged lysosomal localization of LC3 was inhibited by administration of BAPTA-AM, a calcium chelator. These results indicate that local calcium efflux from lysosomes is necessary and sufficient for lysosomal localization of LC3. In addition, analysis of various mutations in ATG16L1, which is responsible for lipid modification and membrane localization of LC3, revealed that a specific region is essential for the lysosomal localization of LC3. We are currently searching for factors that interact with LC3 and ATG16L1 during lysosomal damage and will use this information to elucidate the detailed localization mechanism.

Aim 2: Elucidation of the mechanism of TFEB activation by lysosomal LC3

The activity of TFEB is known to be regulated by its phosphorylation status. During lysosomal damage, lysosomal-localized LC3 interacts with TRPML1, which promotes calcium efflux, resulting in dephosphorylation, nuclear translocation and activation of TFEB. These findings suggest that there is a phosphatase that is activated by LC3 and calcium, but the entity of this enzyme is unknown. Therefore, we screened 298 phosphatases fusing a RNAi library and identified a candidate phosphatase responsible for nuclear translocation and activation of TFEB upon lysosomal damage. Importantly, this phosphatase played a role in lysosome damage-specific manner but not in other TFEB activation conditions such as starvation. Interestingly, this phosphatase was normally located in the nucleus, but only upon lysosomal damage it localized on damaged lysosomes. Furthermore, we found that this localization is dependent on the lipidation of LC3. We plan to further characterize the role of this phosphatase to understand how LC3 activates TFEB in our future study.

Aim 3: Elucidation of the mechanism to repair damaged lysosomes by TFEB

Targets of TFEB during lysosomal damage and their function in lysosomal repair are not well understood. To reveal how TFEB repairs damaged lysosomes, we performed transcriptome analysis using RNAseq to search for novel targets of TFEB during lysosomal damage. The results revealed that there are many TFEB targets that are completely different from those induced by starvation. Screening of these factors for their ability to repair damaged lysosomes, followed by ChIP-qPCR analysis, led to the identification of an enzyme working in one metabolic pathway as a novel direct TFEB target. Interestingly, this enzyme is mainly localized to mitochondria and plays an essential role both in mitochondrial and lysosomal homeostasis independently of its enzymatic activity. We are currently identifying factors that interact with this metabolic enzyme, and have discovered a new regulatory mechanism of mitochondrial and lysosomal homeostasis through these factors. Although both lysosomes and mitochondria are known to become dysfunctional in aging and various important diseases, the mechanism by which homeostasis of these two organelles is simultaneously regulated is not well understood. We expect that the function of this metabolic enzyme downstream of TFEB identified in this study will be the key to uncover this mystery.

Discussion & Conclusion

Damaged lysosomes have been found in many diseases such as neurodegenerative diseases, crystalline nephropathy, silicosis, and infectious diseases, as well as in individual aging and cellular aging processes, suggesting that the failure of damaged lysosome repair mechanisms leads to the onset and development of these diseases and aging. We believe that further functional analysis of the obtained key factors and TFEB using pathological models and aging models lead to the establishment of new therapeutic methods for lysosomal damage-associated diseases and inhibitors of aging itself.

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一般の皆様へ

リソソームは細胞内の分解を担うのみならず様々なシグナル伝達の司令塔として働き、細胞や個体の恒常性維持に必須の役割を果たす。リソソーム機能の破綻は神経変性疾患や感染症など多くの疾患に加えて老化の要因になることが示唆されているが、リソソーム恒常性がどの様に維持されているかはあまりよくわかっていない。本研究ではリソソーム恒常性維持に必須の働きを持つ TFEB の制御機構について解析を行い、いくつかこの制御の鍵を握る因子を同定することができた。今後、これら因子の病態や老化モデルを用いた機能解析を進めることで、リソソーム機能不全を伴う疾患の新たな治療法や老化そのものの抑制法確立のシーズ創出につながる可能性があると考えている。

Investigation of cellular dynamics of CD73-expressing mesenchymal stem cells in the bone marrow microenvironment

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Summary Abstract

Mesenchymal stem cells (MSCs) are a multipotent and considered to be of great potential for regenerative medicine. We have currently identified a cell surface enzyme CD73 as a novel marker for MSCs. However, since a part of MSCs lost CD73 expression after differentiation into progenitor cells, their final fate remain unclear. In this study, we generated CD73-Cre driver lineage tracing model and tried to analyze their fate during bone development and fracture repair.

Key Words : Mesenchymal stem cells, bone, cartilage, CD73

Introduction

MSCs have attracted attention as a tool for regenerative medicine that utilizes their multilineage differentiation and tissue regeneration potential, and clinical trials have begun for many diseases, including bone disease treatment. However, in vivo cellular dynamics and differentiation potential of MSCs remain unclear, and in many cases, clinical application of MSCs has been preceded by clinical trials with unclear therapeutic mechanism of action. Therefore, it is desirable to elucidate the properties of MSCs in vivo in order to establish safe and effective therapeutic approaches. We have currently identified CD73 which labels MSCs efficiently in vivo. Therefore, we aim to elucidate the dynamics of CD73-expressing cells in niche formation and remodeling using cell lineage analysis mouse.

Results

MSCs are maintained in vivo by special microenvironments. The bone marrow niche, known as one of these niches, is formed by sinusoidal endothelial cells, hematopoietic cells, and pericytes which tightly regulated by various humoral factors, cytokines, and intercellular adhesion. To clarify the localization and properties of MSCs, we performed screening of gene expression profiles and identified CD73 (5'-Nucleotidase Ecto: *Nt5e*) as a promising molecule. We generated CD73-EGFP reporter mouse, in which EGFP is expressed under control of a CD73 bacterial artificial chromosome (BAC) promoter including distal regulatory elements (Breitbach*, Kimura*, Cell Stem Cell, 2018). CD73-EGFP reporter mouse labeled a subgroup of MSCs as well as sinusoidal endothelial cells in the bone marrow. CD73-EGFP positive MSCs displayed in vitro high proliferation potential and differentiated into osteoblast and chondrocyte compared to EGFP negative MSCs. To investigate whether EGFP positive MSCs contribute to bone repairing, we generated femoral fracture model. At day 4 post surgery, endogenous CD73-EGFP positive MSCs migrated to bone repair sites and differentiated into bone and chondrocytes. Furthermore, some CD73-positive MSCs contributed to neovascularization, suggesting that they are involved in niche remodeling.

To chase their lineage in detail, we generated tamoxifen inducible Cre knock-in mouse, CD73-CreERT2 mouse, by CRISPR/Cas9 system. At first, we confirmed whether CD73-CreERT2 mouse could label MSCs and sinusoidal endothelial cells in the bone marrow, we administrated 2 mg tamoxifen for three consecutive days in the CD73-CreERT2 crossed with tdTomato reporter mouse at 8 weeks of age. tdTomato positive cells were appeared at the periosteum, endosteum, articular cartilage and metaphysis, but sinusoidal endothelial cells in the bone marrow were not labeled. Next, we administrated tamoxifen to pregnant mother to investigate when CD73 positive MSCs emerge in the bone. At postnatal day 14 after tamoxifen administrated at embryonic day 14.5, tdTomato positive cells appeared at the metaphysis and a part of articular cartilage, and the number of tdTomato positive cells rather increased in the cancellous bone, articular cartilage and synovium at embryonic day 16.5 tamoxifen administration (Figure 1). The tdTomato positive cells in articular cartilage expressed Sox9, suggesting that they are chondrogenic progenitors. The tdTomato positive cells localize perivascular region in the metaphysis, where osteoblastic progenitors are abundant and undergo active bone differentiation and maturation. These tdTomato positive cells expressed osteo progenitor marker Osterix. The tdTomato positive cells were also observed in the endosteum in the diaphysis, and they differentiated into osteocytes with aged.

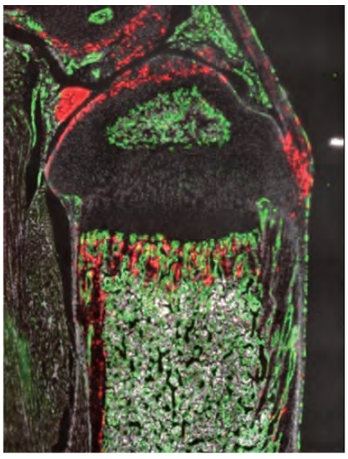

Figure 1. An example of lineage tracing analysis of CD73 positive cells in the bone. Green: CD31 positive vessels, Red: tdTomato positive cells.

Bone repair is accompanied by disruption of the bone and vascular network, resulting in massive remodeling of the niche. We examined how MSCs differentiate and migrate during this process. After labeling CD73 positive cells with tamoxifen administration, we created femur bone fracture model. At day 14 post surgery, tdTomato positive cells formed cluster within the callus. These tdTomato positive cells positive for chondrocyte marker, indicating that they differentiated into chondrocytes and contributed to cartilage formation. tdTomato positive cells were also found differentiated osteoblast within the hard callus. Thus, these data revealed that CD73 is suitable marker to enrich for MSCs that give rise to chondrocyte and osteocytes during fracture repair.

Discussion & Conclusion

These results indicate that CD73 positive MSCs are a population with high proliferative and osteo-chondrogenic differentiation potential. We found that CD73 positive cells contribute to the regenerative process of fracture repair by promoting callus formation, suggesting that CD73 marks osteo-chondroprogenitor cells. Since CD73 serve AMP to adenosine in which adenosine signal regulate osteo and chondrogenic differentiation, CD73 may play role in not only as a marker for MSCs and also involving in homeostasis in bone and cartilage. Their final fate upon bone development and fracture repair and the involved mechanisms of intercellular signaling need to be addressed in future studies.

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一般の皆様へ

間葉系幹細胞（MSCs）は、再生医療のツールとして骨疾患治療への実用化に向けた研究が進んでいます。しかし、治療の有効性や作用機序についてはまだ明らかとなっていないことが多く残されています。MSCs がどのような性質を持っているのかが分かれば、MSCs を利用した新たな治療法の開発に繋がります。この研究では、MSCs の局在とその性質を制御する微小環境について明らかにすることを目指しています。これによって、MSCs の生体内の機能解明につながり、安全で効果的な治療法確立へ貢献できると考えています。

A new challenge to HFpEF focusing on the cardioprotective effects of a novel lncRNA

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Summary Abstract

Recently, using a comprehensive Gene Trap approach, the applicants identified a novel long non-coding RNA Caren (lncRNA cardiomyocyte-enriched non-coding transcript), which is highly expressed specifically in cardiomyocytes and localized to the cytoplasm. The applicants have shown that Caren acts on multiple targets, attenuating the "DNA damage response" and exerting cardioprotective effects by "enhancing mitochondrial biosynthesis and function. While ncRNAs are generally not conserved among species, we succeeded in identifying a human CAREN candidate and confirmed that its Tg mice exhibit cardioprotective effects.

Key Words : Heart Failure (HF), Mitochondria, Aging, non-coding RNA

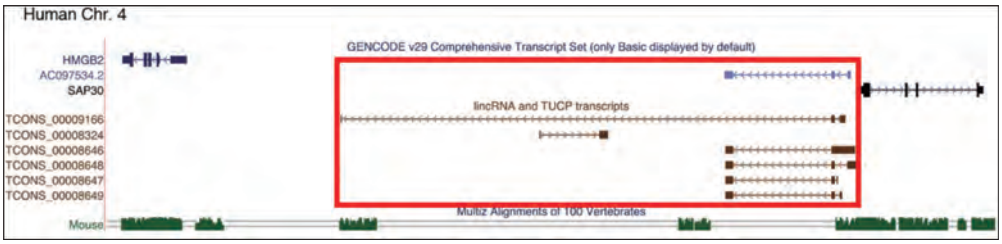
Introduction

The number of patients with heart failure (HF) is increasing in world-wide, especially in HF preserved Ejection Fraction (HFpEF), which accounts for about half of all HF and is associated with a mortality rate no less than that of HF Ejection Fraction (HFrEF). HFpEF accounts for about half of all HF, and its mortality rate is no less than that of HFrEF. However, HFpEF, including its molecular basis, is still largely unknown, and effective treatment has not yet been established. Moreover, patients with HFpEF are predominantly the elderly. We have reported that identified lncRNA Caren (cardiomyocyte-enriched non-coding transcript) has cardioprotective effects against pressure-load-induced HF, and we investigated whether this Caren could ameliorate age-related decline in cardiac function including diastolic dysfunction.

Results

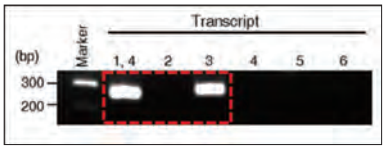
Analysis of a human genomic DNA database revealed that the intergenic region between human HMGB2 and SAP30 gives rise to 6 lincRNA transcripts (Figure1).

Figure1



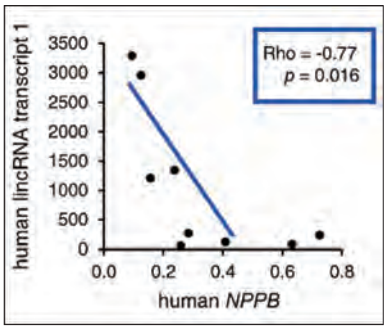
This region is the same where mouse Caren exists. Two of them were expressed in human inducible pluripotent stem cell-derived cardiomyocytes (Figure2).

Figure2



Next, the expression of lincRNA transcript 1 was inversely correlated with that of NPPB, a marker of left ventricular dysfunction (ρ = –0.77, p = 0.016), although we observed no correlation between the expression of lincRNA transcript 3 and NPPB (Figure3).

Figure3



Moreover, we generated transcript1 or 3 Tg mice and evaluated these mice by TAC (transverse aortic constriction) model.

As a result, transcript1 Tg mice showed cardiac protective effects like mice *Caren* Tg mice. Based on these results, we determined that the identified transcript 1 is *Caren* human homologue. Finally, we expect that the elucidation of the mechanism of human *CAREN* lead to the development of novel HF therapies in the future.

Discussion & Conclusion

The majority of lncRNAs reported so far are localized in the nucleus, target a single adjacent gene, and exert their biological effects via some form of regulation under "Cis-manner". Furthermore, among lncRNAs, lncRNAs that have been shown to exert physiological or pathophysiological effects in vivo. In addition, only a few lncRNAs have been confirmed to have physiological or pathophysiological5 effects in vivo. On the other hand, the newly identified "Caren" is characterized by the fact that (1) it is localized in the cytoplasm and (2) multiple actions have been confirmed in vivo. Therefore, the detailed elucidation of the mechanism of action of Caren in this study is expected to lead to the development of a new RNA world by understanding the regulatory mechanism of "multiple" target molecules by lncRNAs localized in the cytoplasm, which has not been elucidated. Moreover, the mechanism of cardioprotective action of Caren differs from existing cardiac failure drugs in that it (1) reduces the DNA damage response and (2) maintains and enhances the energy supply to the heart by maintaining and improving mitochondrial function.

Our research is expected to elucidate the molecular basis of HF (especially HFpEF) and lead to the development of innovative novel therapies.

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一般の皆様へ

日本を含めた先進各国において、心不全患者は増加の一途を辿っています。心不全は、様々な要因・疾患により、心臓の機能が低下した状態です。心不全の死亡率は、主要な悪性腫瘍に劣りません。しかし、心不全の根本的な治療法は確立していません。そのような状況の中で、私はミトコンドリアに着目した研究を行っています。加齢や心負荷により、ミトコンドリア機能が低下すると、心不全の進展に繋がります。私は、タンパクを作らないRNA（non-coding RNA）を用いて、心臓ミトコンドリアを増やすことにより、心機能を改善できるのではないかと期待して、研究を進めています。

Revealing the mechanism to inhibit early senescence initiation in plants by switching transcription start sites.

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Summary Abstract

For plants, controlling the timing and extent of senescence may be important to properly respond to stress, but such molecular mechanisms are unknown. The genes encoding a novel family of receptor kinases that inhibit the onset of leaf senescence have multiple transcription start sites and are interestingly characterized by their ability to switch ligand-accepting extracellular domains (ECDs) by changing the transcription start sites. In this study, comprehensive analysis of transcription start sites by CAGE-seq revealed that transcription is initiated upstream of all ECDs and that the active transcription start sites vary depending on the leaf age. Detailed expression analysis showed that the spatio-temporal expression patterns varied among ECDs. Furthermore, this receptor-kinase family is involved in senescence induced by nitrogen deprivation stress in addition to age-related senescence.
Key Words : Arabidopsis thaliana, senescence, receptor-like-kinase

Introduction

For land plants, it is necessary to sense and respond to changes in the surrounding environment and their own growth stage, and to adjust the growth of each organ and the entire organism. Plants store nutrients obtained through photosynthesis in their leaves and distribute them to all organs of the body as needed to regulate the growth of the entire organism. When plants sense stress, they choose whether to initiate senescence in only some organs or in the entire individual as a survival strategy. On the other hand, early senescence would result in a lack of leaves for photosynthesis, making it impossible to maintain the individual. Thus, controlling the timing and extent of senescence initiation may be important to properly respond to stress, but such molecular mechanisms are unknown.

Results

The genes encoding a novel receptor-kinases family that repress the onset of leaf senescence have multiple transcription start sites and have the interesting feature of switching the Extracellular domain (ECD), the ligand-receptive part, by changing the transcription start sites. The purpose of this study is to find clues to elucidate the molecular mechanism that suppresses early senescence initiation by analyzing the usage of each transcript in detail by exploring the growth conditions and stress stimuli that change the transcription start sites of thisfamily. To comprehensively identify the transcription start sites of each of the four genes encoding the novel receptor-kinase family and to detect changes in transcription start sites, CAGE-seq analysis was performed on young leaves in the middle of wild-type maturation and old leaves that have started senescence. The results showed that transcription is initiated upstream of all ECD-encoding exons. Furthermore, we found that the actively transcribed ECDs were altered between young leaves and those that had initiated senescence. Next, we compared the changes in transcription of each ECD along developmental stages under normal growth conditions. Using qRT-PCR with total RNA extracted from the third and fourth leaves of various day-old plants, we analyzed changes in transcript levels over time in the entire leaf. The results showed that each ECD showed a different pattern of expression level changes along the developmental stages. Some ECDs showed a pattern of continually increasing expression levels along their age, while others showed a pattern of increasing up to a certain days of age and then decreasing. The degree of up-regulation also differed among the ECDs, with some ECDs showing only a 1.5-fold up-regulation, while others showed up to a 28-fold up-regulation. Furthermore, a reporter line expressing the GUS (β-glucuronidase) gene under the control of the promoter region of each ECD was created, and a detailed analysis of the spatial expression pattern of each ECD sequence revealed that the tissues expressing each ECD were different. All ECD promoters showed strong activity in vascular tissue, but some showed activity throughout the vascular bundle, while others showed activity only around the xylem. Some promoters were also active in leaf fleshy tissue. To examine the differences in function of each ECD in senescence induced in response to stress in addition to aging under normal growing conditions, we first examined the stresses in which the receptor kinases we were focusing on were involved. Using a medium deficient in essential nutrients for growth such as sugar, nitrogen, phosphorus, and potassium, we compared the time of senescence induction in the wild type and mutants of the receptor kinase. The results showed that the focused receptor kinase was involved in senescence induced by nitrogen in particular.

Discussion&. Conclusion

This study showed that the spatio-temporal expression pattern of ECDs differs among ECDs during aging under normal growth conditions. We would like to further verify this in terms of changes in the ratio of ECDs along the growth stages by performing absolute quantification of ECDs in the future. We are currently preparing mutants in which only a single ECD is non-functional by introducing mutations in each ECD with CRISPR-Cas9, and are observing the aging phase. Preliminary experiments suggest that in aging-induced senescence, different ECDs lacking function may have different onset times of senescence, i.e., the contribution of each ECD in suppressing senescence may be different. In addition, we would like to examine the use of different ECDs in stress-induced senescence by observing the different expression patterns of each ECD and the onset time of senescence of mutants of each ECD in the induction system of senescence by nitrogen deprivation established this time.

一般の皆様へ

動くことのできない植物は、周囲の環境変化や自身の成長段階を感知して応答し、それぞれの器官および個体全体の成長を調整する必要があります。そのような応答の一つに、老化開始時期の制御がありますが、植物が、様々な情報を統合して老化の開始時期を調節するための分子機構はわかっていません。本研究で機能解析を進めた受容体キナーゼは、その遺伝子が欠失すると老化開始時期が早まることから、老化開始の調節に関わっていることが示唆されます。本研究での成果を足がかりに、今後さらに機能解明を進めることで、植物が老化開始時期を調節する仕組みを明らかにし、将来的には、人工的に操作する技術の開発にもつながることが期待されます。

Analysis of cellular self-organizing mechanics by novel opto-thermo-genetic approach

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Summary Abstract

An embryo undergoes the generation of cells with specific fates, forms, and functions through collective interactions of biological components, referred to as self-organization. This project aims to develop new paradigms for morphogenesis by developing novel techniques for the measurement and manipulation of mechanical forces in the multi-cellular organism, *C. elegans*. We employed tools and techniques needed to interrogate the mechanical processes to analyze how embryonic patterning and oogenesis are regulated through the coupling of chemical reactions and mechanical forces.
Key Words : Mechanics, Embryogenesis, Force, Cell polarity, *C. elegans*

Introduction

The establishment of global order within an organism generally emerges from local interactions among molecules and cells. These collective interactions, commonly referred to as self-organization, give rise to the emergent properties encompassing the fate, form, and function of cells, ultimately leading to morphogenesis of tissues and organs. Mechanical forces play a crucial role in driving these transformative changes, and recent investigations have shed light on their potential to influence cellular fate and function. These findings suggest the existence of a complex feedback loop between forces and cellular physiology. To elucidate the underlying design principles governing such mechano-chemical feedbacks, it is imperative to develop advanced technologies capable of quantifying and manipulating forces within biological systems.

Results

This study aimed to develop analytical techniques to investigate the physiological significance of mechanical forces in multi-cellular systems. We attempted to establish a "thermo-genetics" method by which the functions of target proteins involved in force generation can be quickly inhibited in multi-cellular stage animal, C. elegans. Using a combination of microfluidic device and infrared light device, temperature in early embryos and oocytes were globally maintained at 15ºC and locally increased it to 20-25ºC. This local increase in cellular temperature was sensed by genetically modified cytoskeletons, which could be promptly inactivated when exposed to a restrictive (high) temperature. By utilizing this technology, we were able to elucidate the effects of force generation attributed to microtubules and the actin cytoskeleton on the patterning of cells and tissues. Our investigation specifically focused on asymmetric cell division during the early-stage embryo development and oogenesis in adult hermaphrodite animals.

We first screened temperature-sensitive mutations in various cytoskeleton genes and cell polarity genes that exhibited a rapid response to a temperature shift from 15ºC to 25ºC. We confirmed that missense mutations in the type II myosin heavy chain NMY-2 (ne3409), the actin nucleator formin CYK-1 (or596), and tubulin TBA-1 (or594) led to the inhibition of cytoskeletal structures within 10 seconds upon the rapid temperature shift. Similarly, temperature-sensitive mutations of the centriole component SPD-2 (or293), the midbody protein CYK-4 (or749), the PAR complex proteins PAR-2 (or539) and PKC-3 (ne4246) caused inhibition of their respective structures within approximately 30 seconds following the temperature shift. Based on these observations, we successfully demonstrated that local temperature changes can effectively manipulate mechanical forces within a confined region of multicellular organisms.

We next employed the thermos-genetics technique to investigate the impact of force generation derived from the actin skeleton on the patterning of fertilized zygotes. Shortly after fertilization, the zygote initiates polarization along the antero-posterior axis and segregates the dedicated polarity regulators, PAR proteins, on the cell cortex during mitosis. Previous studies have established the significance of the actin cytoskeleton in the initiation and establishment of PAR polarity. By using the thermo-genetics approach, we aimed to examine whether the actin cytoskeleton also contributes to the maintenance of the established pattern of PAR proteins during late mitosis. We found that inactivation of NMY-2 and CYK-1 during anaphase, but not from prometaphase to metaphase, led to the mis-segregation of PARs during cytokinesis. This finding confirms the efficacy of the thermo-genetics technique in exerting temporal and spatial control over actin cytoskeleton-mediated forces. The thermo-genetics technique offers valuable tool to explore the temporal and spatial control of the actin cytoskeleton-mediated forces.

We are employing this technique to analyze the impact of both microtubule- and actin skeleton-derived forces on the spatial pattern of multicellular-stage embryos. Previous studies have highlighted distinct mechanisms in the initiation and pattern formation of PAR proteins between zygotes and two-cell stage embryos. In the formation of germ cell precursors during the two-cell-stage, polarity formation relies not only on the actin cytoskeleton but also on microtubules. To investigate this phenomenon further, we compromised the architecture of microtubules by inhibiting the function of tubulin and centrosomes. As a result, we observed that although PAR proteins initially established an asymmetric pattern, they eventually depolarized during mitosis. This finding a crosstalk between microtubule- and actin-dependent forces in the maintenance of the polarity pattern during early embryonic development. By combining our thermo-genetics technique with these mutant strains, we aim to gain a deeper understanding of the intricate interplay between mechanics and developmental processes.

Discussion & Conclusion

Through the utilization of a hybrid approach involving a microfluidic device and an infrared light device, we successfully demonstrated that local manipulation of temperature can be used for acute inactivation of cellular forces exerted by the cytoskeletons within multi-cellular stage embryos. By employing this method, we can delve deeper into understanding the intricate interplay between mechanics and tissue patterning during animal development.

一般の皆様へ

生物は細胞集団の空間パターンを組織・器官スケールで秩序化することで、生体の形と機能を創生する。この細胞集団の秩序化では、細胞が機械的力の発生を感知・応答することで化学シグナル伝達を調節する「力学化学カップリング」を必要とすることが示された。しかしながら、力作用は直接可視化することができないので、この仕組みと役割には未だに不明な点が多い。本研究では、力発生の役割を解明するための技術開発を目的とし、細胞内温度変化を利用して、力発生に関わる標的因子の機能を素早く阻害する「温度遺伝学法」を開発し、線虫多細胞期胚における非対称分裂を司るメカニクスの機能を解析した。この技術を活用して、細胞自律的な自己組織化に対して、細胞非自律的な力学的作用が及ぼす影響を調べることで、マクロスケールの生体秩序化を司る基盤原理が解明できる。

Structural and functional analysis of primary cation transporters and elucidation of the substrate specificity by gain-of-function mutant

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Summary Abstract

We study primary-active transporter via P-type ATPases using functional and structural analyses to demonstrate that four mutations transform the non-gastric proton pump, a strict H^+-selective electroneutral ATPase, into a bona fide Na^+-dependent electrogenic sodium pump, providing insight on how Na^+ binding drives a concerted mechanism leading to Na^+/K^+ pump phosphorylation.

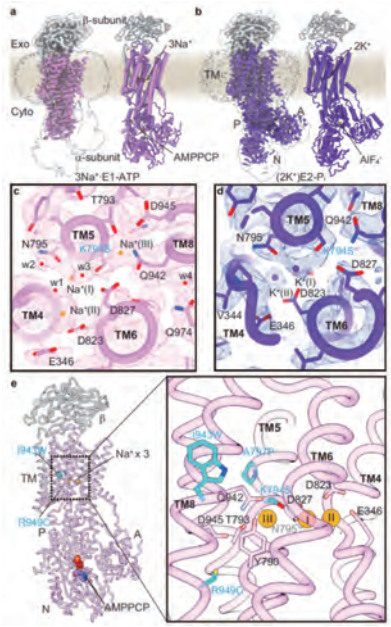
Key Words : P-type ATPase, proton pump, sodium pump, cryo-EM, membrane proteins

Introduction

Despite high sequence conservation between proton and sodium pump (more than 65%), their transport substrate (H^+ or Na^+) and their stoichiometry ($1H^+/1K^+$ for proton pump, $3Na^+/2K^+$ for sodium pump) is strictly discriminated. In order to gain the molecular insight into their substrate specificity, we attempted to identify Na^+-transporting gain-of-function mutant of proton pump, and determine its structure by cryo-EM.

Results

1. We determined a crystal structure of non-gastric proton pump (ngHKA), and structurally defines its transport stoichiometry of $1H^+/1K^+$ per ATP-hydrolysis.
2. Staring from WT ngHKA, we design tailored mutants based on the structural data, previous results and sequence comparison. The substrate specificity of these mutants is evaluated utilizing kinetics, electrophysiology, and cryo-EM analyses.
3. Finally, we success to generate quadruple mutant of ngHKA (SPWC mutant, see figure) that mediate Na^+/K^+-specific, electrogenic transport like genuine sodium pump. Cryo-EM analysis determined three Na^+ and two K^+ are bound to the TM cation site in E1 and E2 state, respectively. We further identified sequential conformational change induced by the Na^+-binding leading autophosphorylation.



Discussion & Conclusion

We presented the first structure of the ngHKA, defined its transport stoichiometry and proton extrusion mechanism, and revealed the requirements to transform this strict electroneutral $1H^+/1K^+$ pump into a strict electrogenic $3Na^+/2K^+$ pump. This transformation reveals how evolution exploited the same mechanism to build electrochemical gradient of different magnitude for dissimilar ions.

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一般の皆様へ

細胞膜を隔てたイオンの不均衡分布は、例えば神経の情報伝達や栄養素の細胞への取り込み等、生命現象と深くかかわっています。これは ATP のエネルギーを利用してイオンを輸送する非常によく似たタンパク質、能動輸送体によって形成されています。しかしながら、輸送されるイオンの種類やその個数は厳密に決まっていて、決して間違えたりはしません。我々はプロトンポンプをナトリウムポンプに変換することに挑戦し、たった4つのアミノ酸を置換するだけで、Na+を輸送するポンプを創る事に成功しました。構造解析によって、3つの Na+ と2つの K+ を結合していることが確認できました。この研究によって、プロトンポンプとナトリウムポンプがどのように輸送するイオンの種類を認識しているかが詳しく理解できました。

Hematopoietic stem cell aging and mitochondria aging

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Summary Abstract

In this study, given the diversity and heterogeneity of aging hematopoietic stem cells (HSC), comprehensive gene expression analysis and epigenomic analysis at the single cell level (single-cell RNA sequencing and single-cell ATAC sequencing) were performed. Among HSCs obtained from aged mice, we found some cell populations with gene expression and epigenomic changes similar to those of HSCs obtained from young mice. In the future, we would like to establish a method to select such HSC populations from elderly individuals and use them in the treatment of various age-related diseases.
Key Words : Hematopoietic stem cells, aging, mitochondria

Introduction

Hematopoietic stem cells (HSCs) reside at the apex of the hierarchy of hematopoietic cells, uniquely capable of dividing without differentiating (self-renewal) and also differentiating to all the different cellular components within the blood. Thus the precise regulation of HSC self-renewal and differentiation without malignant transformation is critical. However, HSC functions alter with age: aged HSCs exhibit lower repopulation potential, loss of homing potential, and a higher risk of malignant transformation. In this project, we have extended our current research on HSC aging by using single cell RNA-sequencing (scRNA-seq) and single cell assay for transposase-accessible chromatin using sequencing (scATAC-seq).

Results

Dendra2 mice (*Gt(ROSA)26Sortm1.1(CAG-COX8A/Dendra2)Dcc/J* mice), whose intracellular mitochondria emit green fluorescence, were used in this study. Slam HSCs (CD150+CD48-Lin-Sca1+Kit+) were sorted from bone marrow of young (2-4 months old) and old (18 months old) Dendra2 mice using FACS, and further sub-divided into Dendra2-high HSCs (HSCs with high mitochondrial mass) and Dendra2-low HSCs (HSCs with low mitochondrial mass). Comprehensive gene expression analysis (scRNA-seq) and epigenomic analysis (scATAC-seq to identify open chromatin regions in the whole genome) were performed for each single cell from 4 groups (young mitochondria-low, young mitochondria-high, old mitochondria-low, and old mitochondria-high HSCs).

Researchers from all over the world have proposed various methods to analyze scRNA-seq, and it is not yet clear which one is superior. Furthermore, simultaneous analysis of scRNA-seq and scATAC-seq is still relatively new, and no established methods are published or publicly available on how to integrate multiple scRNA-seq and scATAC-seq data considering their batch effects. Hence we tried and compared several bioinformatic analyses, and

decided to: 1) merge scRNA-seq data from each sample after individual quality check, 2) merge scATAC-seq data from each sample after individual quality check, 3) further merge two pre-merged data using the weighted neighborhood (WNN) analysis and a feature dimension reduction method called Uniform Manifold Approximation Projection (UMAP), 4) conduct clustering using information both from scRNA-seq and scATAC-seq, 5) perform scRNA-seq analysis both on each cluster and on the whole dataset, and 6) perform scATAC-seq analysis both on each cluster and on the whole dataset. The know-how established during this project was applicable also to scRNA-seq data on normal hematopoietic differentiation (reference 1).

HSCs were divided into four groups in order of cell numbers: 1) average HSCs, 2) HSCs with a high protein synthesis capacity, 3) HSCs with cell cycling, and 4) HSCs with increased inflammatory signals. The most striking feature of HSCs fromaged mice was that cells with cell cycling reduced, especially in aged HSCs with high mitochondrial mass. This may reflect cell cycle arrest or senescence in these cells. Secondly, we noticed that a specific subset of HSCs increased in HSCs from aged mice, suggesting that this subset represents a cell population with particularly reduced HSC function. Third, another cell population located on the opposite side in the UMAP showed a higher protein synthesis capacity than other populations, and was considered to be a population of cells in which stem cell function was maintained. This population was well preserved even in aged mice, demonstrating gene expression and epigenomic changes very similar to HSCs from young mice. This cell population can be a potential candidate for 'young-like' HSCs in old mice. We are currently investigating which surface antigens (cell surface proteins that can be used for FACS sorting) are suitable for sorting this cell population. In addition, by analyzing the above-mentioned HSCs with particularly reduced function in detail, we identified a surface protein X was specifically expressed on more aged HSCs among aged mice. We successfully established the method to sort these cells by FACS using antibodies against protein X. To sort protein X-negative HSCs can be another strategy to enrich ‘young-like’ HSCs.

Discussion & Conclusion

We have found that a cell population among HSCs obtained from aged mice shows gene expression and epigenomic changes very similar to HSCs obtained from young mice and that protein synthesis is enhanced in this cell population compared to other cell populations. In the future, we would like to establish a method to select such HSC populations from elderly individuals. When a method to identify and enrich 'young-like' HSCs is established in the near future, it will be possible to raise the age of bone marrow transplantation donors. In the longer term, knowledge gained in the process of elucidating HSC aging can be applied to research on stem cell aging in various tissues other than bone marrow. It could also be applied to personalized medicine to identify individuals likely to develop abnormalities associated with aging, not only in blood diseases but also in diseases of various organs.

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一般の皆様へ

造血幹細胞は全ての血液細胞になる（分化する）ことが可能な特別な細胞です。悪性腫瘍化することなく生涯にわたる血液の産生を維持するために、造血幹細胞の機能は厳密に制御されなければいけません。しかし、残念ながら、造血幹細胞の機能は老化とともに低下し、悪性腫瘍化の危険性は上昇します。今回の研究で、年老いたネズミの造血幹細胞を詳細に解析したところ、年老いたネズミの中にも比較的その機能が保たれた、いわば、“まだ若い”造血幹細胞がいる可能性があります。今後は、このような“若い”細胞をヒトからも精製する方法を確立したいと考えております。

Significance and spatial profiling of phospholipid metabolism in NASH

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Summary Abstract

Enhanced de novo lipogenesis mediated by SREBP is thought to be involved in nonalcoholic steatohepatitis (NASH) pathogenesis. In this study, we assessed the impact of SREBP inhibition on NASH and liver cancer development in murine models. Unexpectedly, SREBP inhibition via deletion of SCAP in the liver exacerbated liver injury, fibrosis, and carcinogenesis, despite markedly reduced hepatic steatosis. SCAP–SREBP pathway inhibition altered the fatty acid (FA) composition of phosphatidylcholines due to both impaired FA synthesis and disorganized FA incorporation into phosphatidylcholine via LPCAT3 downregulation, which led to endoplasmic reticulum stress and hepatocyte injury. Thus, excessively strong and broad lipogenesis inhibition was counterproductive for NASH therapy, which will have important clinical implications in NASH treatment.

Key Words : Nonalcoholic fatty liver disease, Lysophosphatidylcholine acyltransferase, hepatocellular carcinoma, autophagy, lipidomics

Introduction

Enhanced de novo lipogenesis mediated by SREBP is thought to be involved in NASH pathogenesis. At present, several drugs targeting enzymes involved in hepatic de novo lipogenesis such as ACC, FASN, and SCD1, which are all regulated by SREBP, are under development (1). However, to the best of our knowledge, there is no conclusive experimental evidence demonstrating that SREBP inhibition can prevent NASH or NASH-related hepatocellular carcinoma (HCC) development. Thus, we assessed the impact of SREBP inhibition on NASH and HCC development in murine models.

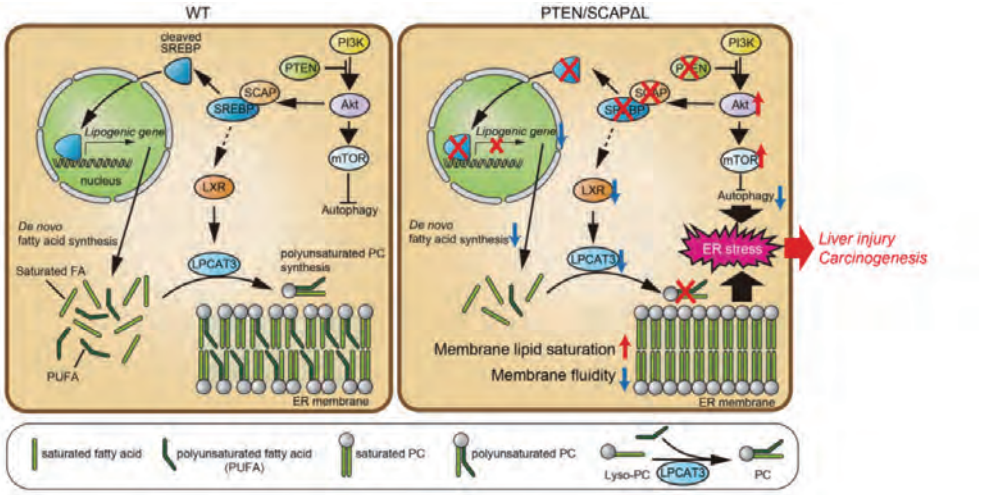
Results

We used a well-known NASH-HCC mouse model: a liver-specific phosphatase and tensin homolog (PTEN) knockout mouse (PTENΔL) generated by crossing a Ptenflox/flox mouse and an albumin-Cre mouse (Alb-Cre). PTENΔL mice show constitutive upregulation of SREBP due to PI3K–Akt pathway activation, which leads to spontaneous fatty liver and subsequent HCC development. To examine the effects of SCAP–SREBP pathway inhibition in PTENΔL mice, we generated liver-specific PTEN/SCAP double-knockout mice by crossing PTENΔL mice with Scapflox/flox mice (PTEN/SCAPΔL). Unexpectedly, SREBP inhibition via deletion of SCAP in the liver exacerbated liver injury, fibrosis, and carcinogenesis, despite markedly reduced hepatic steatosis. These phenotypes were ameliorated by restoring SREBP function through the transgenic or AAV-mediated approach. Moreover, enhanced hepatocarcinogenesis through SCAP deletion was observed in another NASH model, the choline-deficient, L-amino acid-defined, high-fat-diet model.

Transcriptome and lipidome analyses revealed that SCAP–SREBP pathway inhibition altered the fatty acid (FA) composition of phosphatidylcholines due to both impaired FA synthesis and disorganized FA incorporation into phosphatidylcholine via lysophosphatidylcholine acyltransferase 3 (LPCAT3) downregulation, which led to endoplasmic reticulum (ER) stress and hepatocyte injury. Supplementation of phosphatidylcholines significantly improved liver injury and ER stress induced by SCAP deletion. Spatial profiling of phospholipids revealed that zonal distribution of phosphatidylcholines was observed in wild type mice, while it was markedly disorganized in PTEN/SCAPΔL mice.

Hepatic steatosis is a hallmark of NAFLD. However, hepatic fat deposition is often significantly decreased in advanced NASH, referred to as “burned-out NASH.” We performed RNA-seq analysis using liver biopsies obtained from NAFLD patients and found that the SREBP-mediated lipogenesis pathway was significantly downregulated in burned-out NASH. Furthermore, the activity of SCAP-SREBP-LPCAT3 axis was found inversely associated with liver fibrosis severity in human NASH, suggesting that the downregulation of this axis may be involved in the pathogenesis of advanced burned-out NASH.

Although phospholipid composition was also disturbed in SCAP single knockout mice (SCAPΔL), SCAPΔL mice did not exhibit spontaneous liver injury, suggesting that an additional factor induced by PTEN deletion is required for liver injury. We identified that SREBP inhibition cooperated with impaired autophagy to trigger liver injury. In fact, additional deletion of the autophagy-essential gene ATG5 in SCAPΔL mice significantly aggravated liver injury and HCC development accompanied by enhanced ER stress .



Discussion & Conclusion

We found that the strong inhibition of SCAP/SREBP-mediated lipogenesis unexpectedly exacerbated liver injury, fibrosis, and carcinogenesis in murine NASH via the disturbance of phospholipid metabolism. These findings are critical to developing NASH treatment strategies because they indicate that excessively broad and strong inhibition of the lipogenic pathway may be counterproductive in NASH therapy. Importantly, the SREBP-mediated lipogenesis pathway was significantly downregulated in patients with advanced burned-out NASH, which was consistent with a previous report (2). Although the significance of downregulation of SREBP pathway in advanced NASH has been unknown, our present study arises the possibility that downregulation of SREBP pathway may be a disease-promoting factor in advanced stage NASH. In fact, less steatosis in patients with NASH-related cirrhosis is associated with higher risk for HCC development and mortality, even among patients with the same Child–Turcotte–Pugh score (3). Thus, both excessive and insufficient SREBP activation may result in NASH disease progression, and appropriate SREBP activity may be essential for maintaining liver homeostasis. Furthermore, phospholipid supplementation or correction of abnormal FA incorporation into phospholipids may be treatment options for advanced stage NASH (4).

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一般の皆様へ

非アルコール性脂肪肝炎（NASH）は、肝臓への脂肪沈着（脂肪肝）をきっかけに、炎症・線維化がおこり、肝硬変や肝癌に至る進行性の疾患です。今回の研究から、進行したNASHでは肝細胞内の脂質、特にリン脂質のバランスが崩れ、細胞が障害されやすくなっていることが明らかとなりました。リン脂質の補充やリン脂質バランスの改善が、進行NASHの治療法の一つとなると期待されます。

Investigating the mechanism of vascular aging using iPSC-derived 3D vascular model

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Summary Abstract

Lifestyle diseases such as hypertension, diabetes, and dyslipidemia are ultimately 'vascular diseases' which causes vascular aging. However, no effective way has been developed to accurately predict and treat vascular aging. In this research, 3D blood vessel was created from patient-derived iPS cells. We are investigating the physical effects of blood flow on vascular aging using the 3D vasculature.

Key Words : Vascular aging, iPS cells, Vascular smooth muscle cells, Endothelial cells

Introduction

As average life expectancy is increasing and the population aged 100 and over is expected to reach 300,000 by 2040 in Japan, it is necessary that elderly people can stay healthy and play an active role in society. Lifestyle diseases such as hypertension, diabetes, and dyslipidemia are ultimately 'vascular diseases' which cause vascular aging. Despite significant progress in the field, curative treatments have yet to be developed directly targeting the vascular aging.

Results

Since models using mouse vascular tissue is unable to fully recapitulate human pathophysiology observed in the clinic, due to the differences in gene expression from that of humans, human iPSC-derived tissue-engineered blood vessels (TEBV) will serve as a more robust model to elucidate the mechanisms leading to vascular aging. Although some TEBVs with vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) have already been established in the field, these models have caveats in their ability to mimic the physiology of the human artery. For example, the contraction of these vessels remains weak due to the lack of circumferential alignment of VSMCs and the lack of flexibility in the scaffolds. These two characteristics are important to address because it has been well established that contraction and dilation play a crucial role in vascular diseases.

In this research, we established a novel rolling-up molding method to form a physiologically deformable TEBV from human iPSCs. First, a VSMC fiber was formed with a 3D-printed microchannel mold and metallic anchors. The iPSCs-derived VSMCs suspended in a collagen hydrogel spontaneously contracted about 95% in volume and aligned axially in 24 hours. This fiber is rolled up by a 3D-printed inclined mold to spontaneously form a spiral shape. Finally, rolled-up VSMCs were embedded into collagen hydrogel with perfusion connectors, and ECs were seeded inside to establish the TEBV. As a proof of concept, our TEBV was also challenged to undergo other physiologically-relevant conditions including, maintenance under

pulsatile perfusion, treatment with U46619, a thromboxane A2 agonist, and normalized with contraction by KCl. The relative contraction ratio was reported to be 78.0 ± 5.1%, similar to previously reported ex vivo experiments.

We prepared TEBV using iPS cells derived from patients with Werner syndrome, a type of progeria, to create a model of vascular aging, and confirm differences in vascular contractility and compliance between Werner syndrome and healthy control. The TEBV derived from a patient with Werner syndrome tended to show increased contraction, similar to the result observed in previous studies. We are currently performing computational fluid dynamics using patient clinical data and the TEBV to clarify what types of blood flow affect vascular aging. Furthermore, RNA-seq, metabolomics, and lipidomics are planned to elucidate the mechanism of vascular aging.

Discussion & Conclusion

Our TEBV can mimic the phenotype of vascular aging, which can be utilized to examine the effect of blood flow on vascular aging. Once we clarify the type of flow affecting vascular aging, prediction of the vascular site where aging is likely to occur will be possible. It will also have the potential to create a method for monitoring vascular aging. This research also has the potential developing new therapies that can mitigate vascular aging. Taken together, our model will serve as a more physiologically-relevant model to investigate the mechanisms behind vascular aging and thus will lead to therapeutic discoveries.

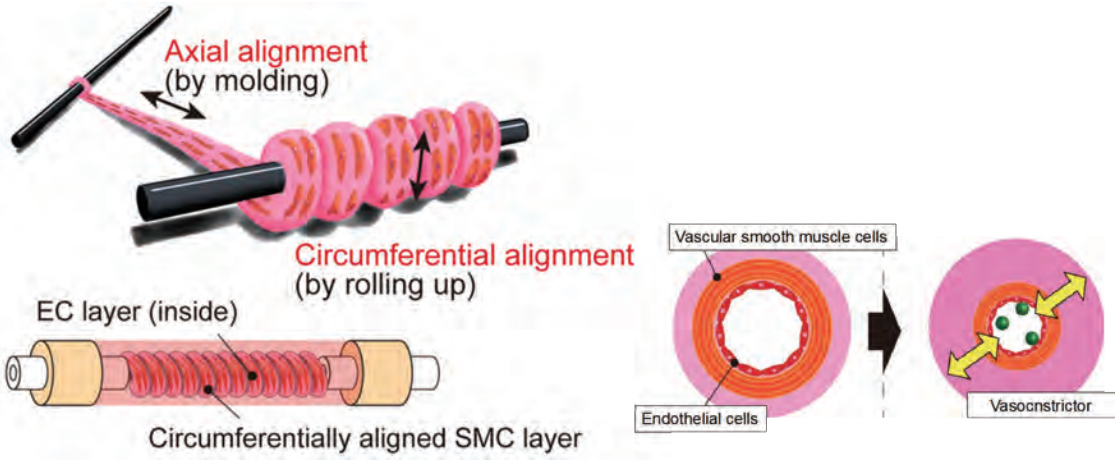

Figure 1.
A novel rolling-up molding method to form a physiologically deformable TEBV from human iPSCs. The TEBV is contracted with vasoconstrictors such as thromboxane A2 agonist.

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一般の皆様へ

血管は年齢とともに老化し動脈硬化のリスクとなりますが、その原因は解明されておらず、治療法もありません。我々はiPS細胞を用いて3D血管を作製し、血流が血管老化に及ぼす影響を解析しています。本研究によって血管老化を起こす要因を明らかにすることで、血管の老化を予防、治療する方法を開発していきたいと思っております。

Mechanisms of differentiation of highly infiltrating CD8 T cells in the tumor

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Summary Abstract

$CD8^+$ T cells with tissue-resident memory (TRM-like TIL) characteristics are found in the tumor, and their presence is critically associated with preferable clinical outcomes. However, the mechanisms by which T_{RM}-like TIL are generated in the tumor are poorly understood. Through the analysis of tissue section of human cancer patients, we identified the actual sites where T_{RM}-like TIL are generated in the tumor, and local antigen signaling are likely to be a factor that promotes the differentiation of T_{RM}-like TIL. In the future, we will investigate the possibility that macrophages located in the differentiation sites provide the necessary signaling for the differentiation of T_{RM}-like TIL.

Key Words : Tumor, CD8 T cell, TRM-like TIL,

Introduction

Tissue-resident memory T cells (T_{RM}) that permanently reside in epithelial tissues play a predominant role in protective immunity[1]. It has recently been shown in human patients that some tumors harbor $CD8^+$ T cells with T_{RM} characteristics (T_{RM}-like TIL) and high frequencies of these are associated with an improved response to tumor immunotherapy[2]. However, the mechanisms by which T_{RM}-like TIL are generated in the tumor are poorly understood. In this study, by using human tissue samples and the mouse model, we attempt to understand how T_{RM}-like TIL are generated in the tumor.

Results

Our histological analysis of tumor specimens from patients with head and neck cancer (squamous cell carcinoma) revealed that the generation of T_{RM}-like TIL (double-positive for CD103 and CD8) is unique to some patients and that T_{RM}-like TIL, but not $CD103^-$ CD8 T cells predominantly infiltrate the tumor, suggesting that CD8 T cells acquire T_{RM} characteristics before infiltrating the tumor (in the stroma). We classified patients into three groups based on the infiltration of CD8 T cells and their differentiation to T_{RM} -like TIL: 1) differentiation of T_{RM}-like TILs in the stroma and infiltration of these cells in the tumor, 2) infiltration of $CD8^+$ TILs ($CD103^-$) in the stroma without T_{RM} differentiation, and 3) poor infiltration of $CD8^+$ TILs in the stroma. In group 1, T_{RM} -like TIL have a superior ability to infiltrate deep into the tumor, reflecting their reputational advantage for antitumor immunity. Although T_{RM}-like TIL in the tumor highly express checkpoint inhibitory molecules PD-1 and Tim-3, most cells are still positive for IFNγ and perforin, indicating that these cells are not fully exhausted. It is well known that TCF^+ progenitor exhausted CD8 T cells, which is capable of effectively responding to checkpoint blockade immunotherapy, are preferentially generated in the tertiary lymphoid structures

(TLS)[3]. On the other hand, T_{RM} -like TIL are rarely found in TLS, indicating that TCF^+ progenitor exhausted CD8 T cells and T_{RM} -like TIL are generated by distinct mechanisms. We identified at least two distinct sites where T_{RM}-like TIL are generated in the stroma: one is at the tumor border where anti-tumor immunity is actively induced (differentiation site: DS), and the other is surrounding the areas where tumor cells undergo necrosis (remodeling site: RS). Given the morphological differences of these sites, we hypothesize that T_{RM}-like TIL in DS and RS are distinct subsets, effector, and memory, respectively, and that signals necessary for their differentiation likely differ. In support of this, we found that DS mainly consisted of fibroblasts, whereas RS consisted of αSMA expressing myofibroblasts, a cellular hallmark of tissue regeneration. Utilizing a spatial transcriptomic approach (Nanostring, GeoMx), we found that T_{RM}-like TIL in both DS and RS highly express genes related to activation as compared to $CD8^+$ $CD103^-$ TILs in the stroma, suggesting that local antigen signaling plays a role in the differentiation of T_{RM}-like TIL. However, we did not see large differences between T_{RM}-like TIL in DS and RS. In this analysis, we realized that CD8 T cells isolated from DS and RS contain significant amounts of genes related to plasma cells, indicating that the neighbor cells' genes are also involved in. Thus, another spatial transcriptome approach with single-cell resolution, such as CosMx, would be preferable.

The lack of a mouse model is a major obstacle to the study of T_{RM}-like TIL as conventional tumor-cell injection models result in low, if any, generation of T_{RM} -like TIL. To address this problem, we have analyzed a genetically engineered mouse model (Cre-mediated tissue-specific genetic alteration) that develops ovarian cancer with a typical clinical course, including progressive accumulation of hemorrhagic ascites and development of peritoneal metastasis[4]. We have found that T_{RM}-like TIL are effectively generated in tumor tissues that have malignantly developed around the peritoneal surfaces. In order to investigate the gene signatures of T_{RM}-like TIL and their ontogeny in the circulation, we isolate $CD8^+$ T cells from the peritoneal metastasis (tumor sites) and spleen (circulation) and performed single-cell TCR/RNA sequence. We just received the data and analysis is ongoing.

Discussion & Conclusion

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

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一般の皆様へ

組織滞在型メモリー CD8T 細胞（TRM）は粘膜組織に定着し、感染防御免疫の第一線を担う。これに極めて類似した特徴もつ CD8T 細胞（TRM-like TIL）は強力な抗腫瘍活性を持つことが知られているが、この細胞が腫瘍内にてどのように分化するのかは不明である。我々はヒト腫瘍組織にてその分化部位を特定すると共に、TRM-like TIL 局所抗原刺激が重要であること、また、分化部位に存在するマクロファージが抗原刺激の供給源となっている可能性を示唆する結果を得た。今後はこのマクロファージが TRM-like TIL 分化誘導に果たす役割を追及する。

Dissection of epigenetic reprogramming underlying tumorigenesis using acute lymphoblastic leukemia inducing model

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Summary Abstract

B-precursor acute lymphoblastic leukemia (B-ALL) is the most common childhood tumor and the leading cause of cancer-related death in children. TCF-PBX1 fusion protein which is produced from the t(1;19)(q23;p13) translocation is present in about 6% of childhood B-ALL cases. Although previous studies have shown the pathogenesis of the patient, the mechanisms of TCF3-PBX1 B-ALL disease onset still remain unclear. We have recently established a mouse model of TCF3-PBX1 B-ALL. Using this, we dissected the molecular mechanisms underlying the B-ALL development.

Key Words : B-ALL, leukemia, hematopoietic stem cell, differentiation, B cell

Introduction

TCF3-PBX1 B-ALL has a poor prognosis, especially in children. Although various mouse models have been developed to elucidate the pathogenesis of B-ALL, the efficient models were not established yet. We have previously developed a method that allows unlimited expansion of mouse and human hematopoietic stem / progenitor cells by inhibiting the function of E2A, a transcription factor essential for B cell differentiation. These cells were named induced Leukocyte Stem (iLS) cells because they have the ability to differentiate into T, B lymphocytes and myeloid cells. By transducing the TCF3-PBX1 to the iLS cells, we have established TCF3-PBX1-positive iLS cells.

Results

The TCF3-PBX1-positive iLS cells can be maintained in a same culture system and have similar morphology and proliferation potential to normal iLS cells.

To determine the differentiation potential of the TCF3-PBX1-positve iLS cells to B lineage cells, the cells were cultured on OP9 stromal cells in the presence of IL-7 and FLT3-ligand. After 6 days of the culture, the cells were harvested and analyzed by flow cytometer. The number of B cells were considerably fewer than control, suggesting that the TCF3-PBX1 inhibited the B cell differentiation. To examine the potential for B-ALL development, the TCF3-PBX1-positve iLS cells were transferred to sublethally-irradiated mice. All the mice died at around 3-4months of injection. Pro-B-ALL like cells were expanded and infiltrated in bone marrow, spleen and lymph nodes. RT-qPCR analysis demonstrated the downregulation of B-lineage genes, *Pax5*, *Vpreb1* and *Ikzf1*, while the expression of metabolism-related genes, *Glut1* and *Insr* was upregulated. RNA-seq analysis also demonstrated that the global expression pattern of the pro-B ALL derived from TCF3-PBX1 iLS cells was quite similar to the one of patient samples reported before. Moreover, the genomic mutation in the *Pax5, Ikzf1, Nras, Kras* and

Cdkn2a loci, which is also frequently observed in the TCF3-PBX1 B-ALL patients was found in the TCF3-PBX1 pro-B ALL cells, indicating that the mouse model faithfully reflected the pathogenesis of the ALL patient.

Next, in order to elucidate the onset mechanism, we analyzed the gene expression pattern using preleukemic cells (TCF3-PBX1-expressing pro-B cells), and found that the expression of *Hoxa9*, *Lmo2*, and *Igf1* was markedly elevated. All of these genes have been implicated in association with hematopoietic malignancies such as acute myeloid leukemia and T-cell ALL (T-ALL). Interestingly, *Hoxa9* is a direct target gene of the transcription factor PBX1 (Bijl et al. 2005), and the expression of *Lmo2* and *Igf1* is regulated by HOXA9 (Calero-Nieto et al. 2013; Steger et al. 2015), suggesting that the cascade of TCF3-PBX1 → HOXA9 → LMO2, IGF1 is important in the early stage of B-ALL onset.

To determine the role of HOXA9 in early stages of B cell differentiation, HOXA9 inhibitor (DB818) was added to the culture of the TCF3-PBX1-positive iLS cells differentiating into B cells. The inhibitor did not affect the generation of B cells, suggesting that the HOXA9 is not essential for B cell differentiation of the TCF3-PBX1-positive iLS cells.

To determine if the HOXA9 is involved in the proliferation of multipotent progenitors, the *Hoxa9* of TCF3-PBX1-positive / negative iLS cells was deleted using CRISPR-Cas9 system. Interestingly, the proliferation potential of both iLS cells was affected, while the growth of Hoxa9$^{-/-}$TCF3-PBX1-positive iLS cells was more severely suppressed than that of Hoxa9$^{+/+}$TCF3-PBX1-positive iLS cells, indicating that growth of TCF3-PBX1-positive iLS cells was dependent on the HOXA9 activity.

Discussion & Conclusion

In this study, we succeeded in establishing a mouse model of TCF3-PBX1 B-ALL using iLS cells. The model faithfully reflected the patient pathogenesis, based on the gene expression profile or single nucleotide polymorphism (SNP). Using this model, we found that the expression of *Hoxa9*, *Lmo2*, and *Igf1* was markedly elevated in TCF3-PBX1-positive pro-B cells. The knockdown of *Hoxa9* significantly suppressed the growth of TCF3-PBX1 iLS cells. These results indicated that the HOXA9 is involved in the early stages of B-ALL development. The mouse model will be useful for examining the molecular mechanisms of the B-ALL development and the screening of novel drugs. This method, which uses B-ALL-inducing system of iLS cells to study the mechanism of leukemia onset and to develop a new treatment method, can be applied to the research of not only TCF3-PBX1 but also leukemias caused by other fusion proteins and genetic mutation. We hope the study will lead to create a new trend in hematopoietic malignancy research.

一般の皆様へ

近年、急性リンパ性白血病（ALL）の治療成績は飛躍的に向上したものの、未だに予後不良な転座型も存在します。我々は、難治性 ALL の一つである1;19転座型（*TCF3-PBX 型*）B 細胞性 ALL（B-ALL）に着目して研究を行いました。独自に開発した動物モデルを用いて、発症初期の分子メカニズムの一端を解明しつつあります。今後は本研究で見出した分子機構を B-ALL の新規治療法の開発に結びつけたいと考えています。

Exploratory study for the discovery of zinc homeostasis modulators utilizing a highly sensitive fluorescence imaging probe

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Summary Abstract

As part of our ongoing research to improve a highly sensitive intracellular zinc fluorescent probe Dpa-LBC, we have successfully developed a MRI probe candidate Dpa-SoxLdiF. Contrary to fluorescence imaging modality with poor permeability, MRI modality is suitable for *in vivo* imaging based on its high permeability. We expect that Dpa-SoxLdiF is a lead compound to image zinc dynamics *in vivo*.

Key Words : zinc, imaging, probe, fluorescence, MRI

Introduction

Lately, mobile zinc has been proposed to function as a mediator in signal transduction pathways. It has also become clear that disruption of zinc homeostasis is associated with various diseases such as Alzheimer's disease, diabetes mellitus, and cancer. Therefore, targeting zinc metabolism is now attracting attention as a new therapeutic strategy for intractable diseases related to zinc homeostasis disruption. In this context, an assay system that can efficiently visualize the intracellular status of zinc is needed to accelerate the discovery of therapeutic agents that can be used to maintain or intervene in the zinc homeostasis of the organism.

Results

Recently, we have successfully developed a small molecule fluorescent probe, Dpa-LBC, which greatly exceeds the sensitivity of existing fluorescent probes by incorporating a signal enhancement system into the probe design with respect to fluorescent imaging of intracellular zinc.[1)] Therefore, we expected that Dpa-LBC could be applicable to a high-content screening system (HCS) to explore for compounds that would regulate mobile zinc in living cells. However, unlike the high-sensitivity system used in the development of Dpa-LBC, which uses a confocal fluorescence microscope with an objective lens collecting more light and a glass-bottom dish, the usual HCS system uses a widefield fluorescence microscope with a conventional objective lens and a plastic-bottom well plate from the aspect of cost. Preliminary results using the HCS system indicate that it is difficult to capture zinc perturbations by Dpa-LBC. The objectives of this study were 1) to modify this novel highly sensitive zinc fluorescent probe to be applicable to a HCS system to detect mobile zinc in living cells, and 2) to establish a high-throughput screening (HTS) system to explore intracellular zinc modulators.

To improve the sensitivity of Dpa-LBC, accelerating the zinc-catalyzed reaction of the probe and increasing the catalytic turnover rate are necessary. Dpa-LBC consists of three substructures: a zinc-recognizing ligand moiety (Dpa: dipicolylamine), a zinc-responsive linker, and a quenched fluorophore. Of these three elements, the ligand moiety and the fluorophore can be freely converted. Therefore, we aimed to improve the reactivity of the probe by optimizing the ligand moiety for zinc responsiveness. In parallel, we also attempted to convert the fluorophore for fluorescence imaging to magnetic resonance responsive moiety for magnetic resonance imaging (MRI).

First, in terms of optimizing the Dpa moiety, it is possible to increase the affinity between the probe ligand and zinc with lower affinity of the decomposed zinc complex after release reaction of the fluorophore to quickly dissociate the zinc and increase the catalytic turnover. In this study, we designed and synthesized Dpaderivatives with 4- and/or 6-substituted pyridine(s) aiming to accelerate the release of fluorophore by adjusting the affinity to zinc and improving the nucleophilicity of the coordinating water on the zinc complex to trigger the fluorescent turn on reaction. Then we designed a simplified compounds with a straightforward linker between the modified Dpa ligands and the fluorophore to achieve a prompt evaluation of the zinc-catalyzed reaction, but the synthesis was unexpectedly difficult. Further studies are currently underway to establish an evaluation system.

On the other hand, some plausible results were obtained on efforts to expand the fluorescent probe to MRI probe. The details are as follows. As a first-generation zinc MRI probe, Dpa-SoxLdiF was designed and synthesized by replacing the quenched fluorophore, a substructure of Dpa-LBC described above, with 2,6-difluorophenol as a ^{19}F-MRI signal-responsive moiety and converting the sulfide to a sulfoxide and eliminating *p*-acetoxybenzyl moiety for synthetic convenience. Dpa-SoxLdiF was expected to release 2,6-difluorophenol by zinc-catalyzed reaction like Dpa-LBC. First, ^{19}F-NMR characterization was performed on Dpa-SoxLdiF. Difference in chemical shifts of 2,6-difluorophenol, which is expected to be released by the reaction of zinc with Dpa-SoxLdiF, and Dpa-SoxLdiF itself measured by ^{19}F-NMR, was 6.8 ppm. This difference was sufficiently distinguishable by ^{19}F-MRI. The reaction with catalytic amount of zinc with Dpa-SoxLdiF was subsequently examined by ^{19}F-NMR, which revealed that Dpa-SoxLdiF reacted completely and was quantitatively converted to 2,6-difluorophenol, indicating that zinc amplified MR signal by catalyzing the release reaction of 2,6-difluorophenol..

Discussion & Conclusion

The results which I have mentioned above indicate that Dpa-SoxLdiF is a promising ^{19}F-MRI probe molecule for amplifying zinc signal in buffer solution. Glutathione, which exists in high concentration under intracellular environment, coordinates with zinc and competes with the zinc ligand moiety of the probe. Therefore, it may inhibit the probe response to zinc. We then evaluated the response of Dpa-SoxLdiF in the presence of glutathione (10 mM), which mimicked the intracellular environment, and found that the response of Dpa-SoxLdiF to zinc was maintained, although a slight inhibition was observed. Nevertheless, zinc catalyzed the probe reaction to enhance MR signal even in the presence of high level glutathione. In conclusion, Dpa-SoxLdiF is useful as a lead compound for ^{19}F-MRI probes that can detect intracellular zinc. Evaluating the sensitivity to other divalent metals is currently underway to assess zinc selectivity.

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一般の皆様へ

亜鉛ホメオスタシスの崩壊とアルツハイマー病、糖尿病、がんなどの多様な疾患との関連性が明らかとなっており、亜鉛ホメオスタシスを維持あるいは介入するために使用可能な治療薬の発見を加速するためには、亜鉛の細胞内の状態を効率的に可視化できるアッセイ系が必要である。本研究では私たちが開発した高感度細胞内亜鉛蛍光プローブの改良を行って本アッセイ系の構築を試みるとともに、個体レベルでの生体イメージングに適した MRI プローブへの展開を図ったところ、第一世代の亜鉛 MRI プローブの開発に成功した。

Cryo-electron microscopy studies for disease pathogenesis of Xeroderma Pigmentosum

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Summary Abstract

Xeroderma pigmentosum (XP) is a genetic disease caused by the defect of nucleotide excision repair (NER). UV-DDB is a specific recognition protein for NER, and efficiently binds to UV lesions in chromatin, in which DNA is tightly packed. However, it is unclear how UV-DDB recognize DNA lesions in chromatin. Our result indicated by cryo-electron microscopy how UV-DDB binds to DNA lesions in chromatin. This achievement may contribute to the development of treatments for XP patients.
Key Words : DNA repair, NER, chromatin, cryo-EM, nucleosome

Introduction

Xeroderma Pigmentosum (XP) is a representative disease caused by the defect of nucleotide excision repair (NER) [Ref. 1]. NER is one of the important pathway to eliminate DNA lesions generated by ultraviolet (UV) from genome. In NER, UV-damaged DNA binding protein (UV-DDB) recognizes DNA lesions on genome, and then, recruits other NER factors to damaged sites, which is important to proceed NER [Ref. 2]. On the other hand, DNA is wrapped around histone proteins in cells, and forms high order structures, called chromatin. It is still unclear how NER efficiently works in chromatin, which could be a barrier for UV-DDB to recognize lesions on DNA.

Results

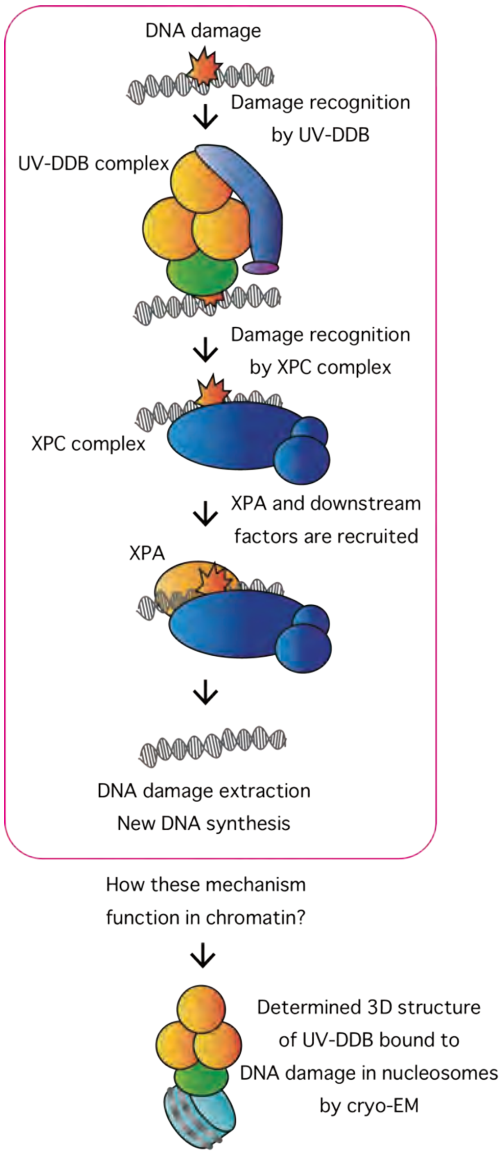
To understand the detail of NER mechanism in chromatin, we tried to directly observe UV-DDB bound to DNA lesion in chromatin by cryo-electron microscopy (cryo-EM). We focused on *in vitro* reconstitution of UV-DDB bound to nucleosome, a minimum unit of chromatin, containing UV lesion [Figure]. Nucleosomes consist of four types of histone protein (each two molecules of H2A, H2B, H3, H4) and about 145 bp DNA. To reconstitute damaged nucleosomes *in vitro*, we purified all histones from E. coli. We chemically synthesized DNA lesion on 145 bp DNA. After mixing purified histones with damaged DNA, we reconstituted damaged nucleosomes *in vitro*. We expressed UV-DDB complexes using baculo virus in insect cells, and purified UV-DDB complex. To assess the binding activity of UV-DDB to damaged nucleosomes, we performed gelshift and fluorescent polarization (FP) assay. These results suggested that UV-DDB has the capability to bind to DNA lesion even in nucleosomes. As a next, to reveal how UV-DDB binds to DNA lesion in nucleosomes, we reconstituted UV-DDB-NCP complex, and applied for cryo-EM analysis. By cryo-EM analysis, we successfully revealed the structure of UV-DDB-NCP complex at high resolution. This structure indicated that UV-

DDB directly binds to UV lesion in nucleosomes and UV-DDB doesn't affect the core structure of NCP by its binding.

In addition to UV-DDB, we also investigated the downstream factors, Xeroderma pigmentosum complementation group C (XPC) and group A (XPA). We purified XPC and XPA in the same method as we described above. Gelshift assay with damaged nucleosomes and these factors indicated that XPC and XPA potentially binds to damaged nucleosomes.

Now we are working on revealing UV-DDB-XPC-XPA assembled on damaged nucleosomes. We hope further research could investigate how XPC and XPA are recruited in damaged nucleosomes.

Recently, the structure of XPC, XPA and its downstream factor, transcription factor IIH (TFIIH), bound to damaged DNA was solved by cryo-EM [Ref. 3]. Following these structure, XPC binds to the DNA lesion from the opposite direction of UV-DDB. Our result raises the question that how XPC recognizes DNA lesion after UV-DDB binding in chromatin because the binding structure of XPC seems to crash into the histone core in nucleosome. This is also interesting mechanism and further studies are needed to figure them out.



Discussion & Conclusion

Our result indicated how UV-DDB recognizes UV lesions in nucleosomes, and it may help us to understand the basic mechanism of the damage detection in chromatin in NER. Our cryo-EM structure of UV-DDB-NCP might be also important for screening seeds of potential drugs affecting the DNA repair mechanism including NER. The defect of DNA repair pathway is related to various diseases, and XP is one of them, which derived from the defect of NER. Therefore, in the near future, our achievement of this research could contribute to develop the treatments for XP patients. Not only these patients, our achievements also might propose the developments of the methods of UV protection from sunlight, which risks are increasing because of the global warming and ozone layer depletion in the world.

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一般の皆様へ

私たちの遺伝情報はゲノムと呼ばれるDNA配列に保存されています。遺伝子の設計図とも言われるDNAは、ほぼ全ての細胞に等しく保存されており、それは安定に子孫へと引き継がれていきます。ところが紫外線など外から強力なエネルギーを受けると、DNAが損傷を受け、遺伝子が変異を起してがんが発生するリスクが高まります。この損傷を修復するメカニズムの一つが、ヌクレオチド除去修復であり、絶えず私たちに生じた損傷を取り除き続けています。

ヌクレオチド除去修復に異常が起こると、紫外線による損傷を修復できなくなり、さまざまな疾患が引き起こされます。色素性乾皮症もその一つであり、特定難病に指定された治療法がない遺伝病です。私たちはクライオ電子顕微鏡という最先端の顕微鏡により、この修復メカニズムを可視化して理解することを目指しています。この研究が進めば、紫外線によるがんの発生を抑制できると期待され、将来的に色素性乾皮症の治療法の確立へと繋がることも夢ではありません。

Analysis of brain aging caused by the disruption of "nuclear-axon crosstalk" machinery

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Summary Abstract

The linker of nucleoskeleton and cytoskeleton (LINC) complex, a nuclear membrane protein complex, plays an important role in the regulation of cytoskeletal network. In this study, we show that the LINC complex is essential for the regulation of neural activity via axon initial segment (AIS) which is required for the generation of action potential. Furthermore, we found that the expression levels of the LINC complex components on the nuclear envelope were markedly reduced with aging, leading to structural abnormality in the AIS of aged neurons. These findings indicate that the LINC complex-mediated AIS regulation may be involved in physiological brain aging.
Key Words : Brain aging, Neural activity, Axon initial segment, LINC complex

Introduction

As our country enters the era of super-aging, extending the healthy life span of the brain has become an extremely important issue. Physiological brain aging is thought to be caused by a gradual decline in the activity and plasticity of individual neurons, leading to a decrease in neurological function (Burke and Barnes, *Nat Rev Neurosci* 2006). However, the mechanism for physiological brain aging is still largely unknown, and effective strategies to prevent it based on solid molecular mechanisms have yet to be established.

Results

In this study, we analyzed the mechanism of brain aging by focusing on the nucleus-mediated regulation of neural activity. The LINC complex that penetrates the nuclear envelope is a nuclear membrane complex composed of Sun proteins on the inner membrane and Nesprin proteins on the outer membrane. By binding of Nesprin to the cytoskeleton, including microtubules and actin fibers, the LINC complex functions as the center of the cytoskeletal network and is involved in various cellular events such as cell polarity formation and migration (Horn, *Curr Top Dev Biol,* 2014). AIS is located at the proximal site of axon in neurons and is a physical and physiological border between the somatodendritic and axonal domains. AIS contains specific cytoskeletal structures and voltage-gated sodium and potassium channels, and plays a pivotal role in regulating neuronal excitability via action potential generation (Leterrier, *J Neurosci* 2018). Recent studies demonstrate that AIS alters its length, position and molecular composition in response to changes in neural activity level, indicating that AIS is one of the important factors subserving neuronal plasticity.

To clarify the importance of the LINC complex in AIS regulation, we first inhibited LINC complex function by forced expression of the dominant negative Nesprin1 mutant (Nesprin1 DN) in mouse primary neurons. We found that the AIS length was significantly shortened in Nesprin1 DN-expressing neurons including cortical neurons, hippocampal neurons and cerebellar Purkinje cells, compared to GFP-expressing control neurons. The expression of Nesprin1 DN in cortical pyramidal neurons in vivo by in utero electroporation also significantly shortened their AIS length. We next examined the structural plasticity of AIS

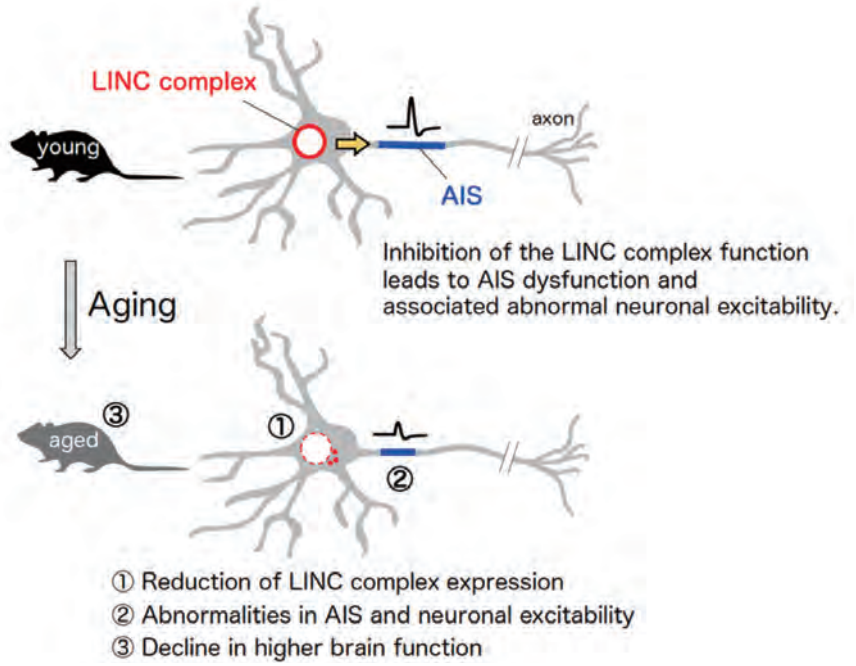

Figure 1. LINC complex-mediated AIS regulation and neuronal aging

Discussion & Conclusion

In this study, we show for the first time that the LINC complex-mediated AIS regulation is important for the regulation of neuronal excitability. We also show that the disruption of this system affects brain functions such as locomotion and emotion in mice. Furthermore, we show that aging causes a reduction in the expression of the LINC complex and the structural abnormality of AIS, which may lead to the decline of brain functions.

In the future, it will be necessary to elucidate the detailed molecular mechanisms of AIS regulation via the LINC complex in order to fully understand the system of the nucleus-mediated neural activity control. Furthermore, it is very important to verify whether preventing the reduction of the LINC complex expression with aging can deter the decline in brain function in old age. The success of these future studies is expected to lead to the implementation of innovative preventive medicine aimed at extending the healthy life span of the brain.

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一般の皆様へ

私たちの脳は、痴呆症などの神経疾患に罹らなくても、加齢に伴って徐々にその機能が衰えていきます。しかし、どのようにしてその生理的変化が起こるのかはよくわかっておらず、有効な予防法もありません。今回の研究では、これまで知られていなかった神経細胞の活動レベルを制御する新たなしくみを発見し、さらに老化によってその機構が破綻することで脳機能の衰退が起こる可能性を見出しました。今後は、今回発見した機構を老齢期でも正常に維持することで加齢による脳機能の低下を抑止できるかどうかを検証していきます。そして、将来的には、これらの知見を脳老化の予防法の開発につなげたいと思います。

Establishment of precision medicine for subsequent solid tumors after hematopoietic cell transplantation based on comprehensive genomic analyses

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Summary Abstract

Long-term survivors of hematopoietic cell transplantation represent significant risk of diagnosis of subsequent malignant neoplasms (SMNs). The genomic features of SMNs are unknown. The genomic features of SMNs are unknown. Here, we used next-generation sequencing of SMNs, to detect distinct mutational signatures (BRCAness and MSI) compared to primary malignant neoplasms without transplantation, raising a possibility that distinct molecular pathogenesis caused by a series of therapy of hematopoietic diseases such as chemotherapy, radiation and graft versus host disease.

Key Words : Hematopoietic cell transplantation, Subsequent malignant neoplasms, Genomic features, Graft versus host disease

Introduction

The population of long-term survivors of hematopoietic cell transplantation (HCT) is growing because of improvement of HCT-based therapy. Long-term survivors of HCT have an increased risk of diagnosis of subsequent malignant neoplasms (SMNs). Although HCT therapy is effective to cure primary hematopoietic cancer, it also produces harmful genetic damages within normal cells. Several factors are thought to be contributed to the development of SMNs, including irradiation, genotoxic agents, immunosuppressive therapy, and chronic graft-versus-host disease (GHVD). The genomic features of SMNs are unknown.

Results

To perform comprehensive genomic analyses of SMN, we collected tumor samples from 86 individuals with a mean age of 59 years. In 20 individuals, chronological multi-sampling of tumor from SMNs were collected. We also collected multi-regional sampling of tumor and adjacent normal tissues from 12 SMN individuals.

High coverage whole exome sequencing (WES) was performed on these FFPE or frozen samples with a mean coverage of 100-150x in tumor tissues and adjacent normal tissues; and ~50x in both donor- and recipient-derived germline control samples. Both somatic synonymous and nonsynonymous mutations were called using three publicly available mutation callers. We developed effective pipelines to eliminate mutations were artificially caused by FFPE, so called FFPE artefact.

We successfully detected reliable somatic mutations from 86 SMNs and 77 primary malignant neoplasms (PMNs) without transplantation (non-SSC). No obvious differences of

recurrent driver mutations were observed between PMNs and SMNs (eg: *APC* and *TP53* in colorectal cancer; *TP53* in esophagus cancer; *TP53* and *CDKN2A* in oral cancer. However, tumor mutation burden (TMB) of SMNs is higher than PMNs in oral and colorectal cancers. Indeed, we detected genomic alterations of TMB-H-related genes and mutational signatures in some TMB-H tumors. As TMB-H has been proposed as a potential biomarker for immunotherapy such as anti-PD1 Ab, raising a possibility that immunotherapy is more efficacious than chemotherapy in SMNs of oral and colorectal cancers.

Chronological multi-sample sequencing revealed tumors with same individual are primarily clonally heterogenous in 75% (15/20) of SMNs, suggesting these tumors developed independently rather than relapsed from primary tumors (Figure 1).

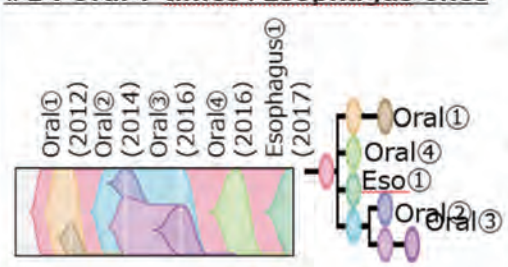

Figure 1 Chronological multi-sample sequencing. Individual #1 with SMNs developed oral cancer four times and esophagus cancer once. Clonal analyses revealed that these tumors developed independently rather than relapsed from primary tumors.

Sequencing of non-tumor regions in individuals with SMNs show that marked genomic alterations are observed compared to individuals with PMNs. Sequencing of multi-regional sampling of semi-microdissected regions unveiled extremely heterogenous, multiple/widespread expanded clones with pathogenic mutations such as *TP53* are developed all over the normal tissues (oral and esophagus) (Figure 2), suggesting extensive heterogeneity and distinct mutagenesis across clones in normal epithelial tissues a potential explanation of different clinical and pathological features of SMNs caused by exposure of a series of mutagens and/or GVHD during the therapy. In contrast, there is no obvious “field cancerization” were observed in colorectal cancer of SMNs, explaining that lack of recurrent colorectal cancers developed in SMNs. Taken together, these insights into the biology of these distinct conditions may enable future efforts to optimize the treatment and surveillance of individuals with more of this diagnosis.

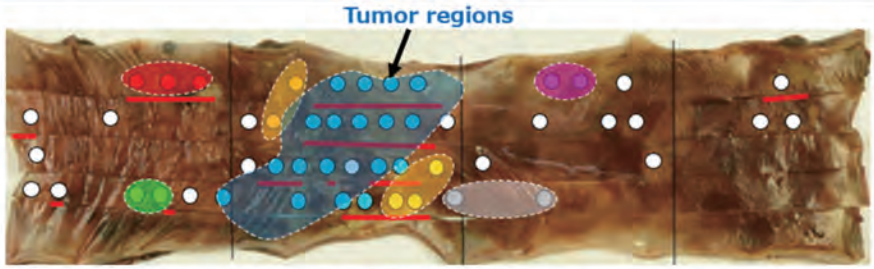

Figure 2 Multi-reginal sampling clonal analyses. Heterogenous clones within esophagus cancer lesion (blue and yellow), and multiple/widespread expanded clones with pathogenic mutations such as *TP53* are developed all over the normal tissues.

Discussion & Conclusion

Our novel NGS-based study provides fresh insights into the pathogenesis of SMNs. We have shown that: i) TMB of SMN is higher than that of PMN; ii) SMNs tumors developed independently rather than relapsed from primary tumors (Figures 1); and iii) extensive heterogeneity and distinct mutagenesis across clones in normal epithelial tissues (Figure 2). Obviously, further investigations are required to establish the validity of this approach, our findings urge requirement of genetically-guided therapy and/or relapse risk assessment for SMNs.

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一般の皆様へ

同種造血細胞移植は、白血病などの難治性血液疾患の根治を可能とする治療法です。移植技術の向上に伴い、長期生存者数が増加しています。しかしながら移植治療による副作用として正常細胞がダメージを受けてしまい、口腔や食道などの2次固形がんの発症に至ってしまいます。しかしその原因や病態は不明であるため、1次がんに準じた治療法が選択せざる得ないのが現状です。そこで大規模2次がん検体バンクと空間・経時的マルチサンプリングゲノム解析を組み合わせることで、移植症例に対するゲノム医療による個別化至適治療の実現に向けた分子基盤を構築することを目的としました。その結果、2次がんに特徴的なゲノム異常が認められ、免疫チェックポイント阻害剤などの個別化至適治療法の適用対象になり得る可能性が示唆されました。

Development of asymmetric synthesis of sulfoximine analogs as bioisosteres

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Summary Abstract

Key Words : Sulfoximine, Sulfinamidine

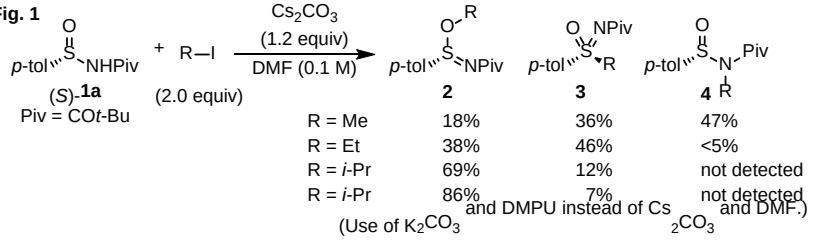
Introduction

Sulfoximines with a chiral center on the sulfur atom have attracted attention in recent years as new substructures for pharmaceuticals and agrochemicals. Asymmetric synthesis methods for such sulfoximines have been established to date.[1,2] On the other hand, asymmetric synthesis of nitrogen analogues of sulfoximines, such as sulfonediimines and sulfonimidoamides, is quite difficult, although they are expected to be new transition state analogs. In this study, we established a simple asymmetric synthesis method for sulfinamidines which are the precursors of these compounds.

Results

First, we began our investigation on an *O*-selective alkylation of *N*-pivaloyl-protected sulfinamide **1a** (Fig. 1). The reaction using methyl iodide as an alkylating agent and Cs_2CO_3 as a base in DMF at 50 °C gave a low yield of the desired product, sulfinimidate ester **2** (R = Me) along with a substantial amount of *S*-alkylation product **3** and *N*-alkylation product **4**. Use of ethylating agent led to suppression of the *N*-alkylation while providing a significant amount of the undesired *S*-alkylation product **3** (R = Et). The bulky *tert*-butyl group of the pivaloyl group may shield the nitrogen atom and prevent the approach of the ethylating agent. Use of sterically more hindered isopropyl iodide dramatically improved the oxygen-selectivity, giving 69% yield of **2a** (R = *i*-Pr). We then investigated the influence of base and solvent on the product ratio in the reaction of **1a** with isopropyl iodide at 70 °C. The choice of base also significantly affected the reaction, and the less expensive K_2CO_3 outperformed the other bases tested. Among the solvents screened, DMPU (*N*,*N*'-dimethylpropyleneurea) afforded optimal yield. With the optimized conditions in hand, we examined the substrate scope in the *O*-selective alkylation of sulfinamides, and various aryl and alkyl sulfinamides were found to be applicable to the present method. Chiral HPLC analysis confirmed that most of the sulfinimidate esters were obtained without racemization.

Fig. 1

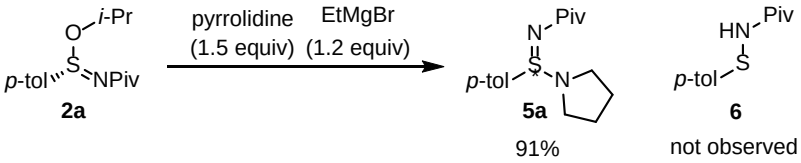


	2	3	4
R = Me	18%	36%	47%
R = Et	38%	46%	<5%
R = *i*-Pr	69%	12%	not detected
R = *i*-Pr	86%	7%	not detected

(Use of K_2CO_3 and DMPU instead of Cs_2CO_3 and DMF.)

Next, we tested chiral sulfinamidine synthesis by the reaction of sulfinimidate ester **2a** with a magnesium amide (Fig. 2). The reaction of sulfinimidate ester **2a** with magnesium amide that was generated from pyrrolidine (1.5 equiv) and ethylmagnesium bromide (1.2 equiv) gave sulfinamidine **5a** in good yield without racemization. In this reaction, the reduction product **6** was not observed, while a substantial amount of **6** was formed as byproduct in the reaction using excess amounts of ethylmagnesium bromide (2 equiv).

Fig. 2



We then investigated the substrate scope of magnesium amides in the sulfinamidine synthesis. The reactions of sulfinimidate ester **2a** with various magnesium amides were performed under the optimized reaction conditions. In the case of the magnesium *N*,*N*-diethylamide that was generated from ethylmagnesium bromine and diethylamine, the corresponding sulfinamidine was obtained in good yield (86%) without racemization (99% ee). These results suggested that both cyclic and acyclic aliphatic amides can be introduced stereospecifically. On the other hand, when *N*-ethylaniline was used as an amide source, the reaction gave the corresponding sulfinamidine in moderate yield (54%) with decreased optical purity (60% ee). In this reaction, a less nucleophilic magnesium amide was generated from the aromatic amine, which may decrease the reaction rate of the desired reaction and increase the undesired racemization. Additionally, the reaction using a primary amine (e.g. cyclopropylmethylamine) afforded the desired sulfinamidine in good yield (70%). However, the optical purity of the obtained sulfinamidine was found to be drastically decreased (3% ee). We are currently investigating the appropriate conditions to suppress optical purity degradation of sulfinamidines when using aromatic amines and primary amines. In addition, since the stereochemistry of the obtained sulfinamidine is not clear, we are investigating a method for determining the stereochemistry.

Discussion & Conclusion

In summary, we have developed an efficient method for the synthesis of chiral sulfinamidines from easily available chiral sulfinamides. The key step of this method is stereospecific *O*-selective alkylation of chiral sulfinamides, which is accomplished by using the appropriate base, solvent, and sterically hindered isopropyl iodide as the alkylating agent. Various chiral sulfinamidines are obtained in high yield without racemization by the nucleophilic addition of the magnesium amides derived from secondary amines to chiral sulfinimidate esters. However, the reaction of magnesium amides derived from aromatic amines and primary amines was found to give the racemized sulfinamidines, and it is necessary to find the optimum conditions to suppress the racemization. The further transformation of the obtained chiral sulfinamidines to other compounds containing an S=N bond is underway in our laboratory.

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一般の皆様へ

医薬品や農薬などの生物活性物質には、硫黄原子を含むスルホキシイミンと呼ばれるものがあります。スルホキシイミンにはその鏡像となるものが存在するため、どちらか一方だけを合成する不斉合成法の開発が必要となります。本研究では、スルホキシイミンの酸素原子を窒素原子で置換したスルフィンアミジンの不斉合成法を開発し、新しい生物活性物質の合成が可能となりました。今後は医薬品開発への応用が期待されます。

Achieve a comprehensive understanding of the pathogenesis of type 1 diabetes mellitus, leading to development of the prevention measures

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Summary Abstract

Type 1 diabetes (T1D) is caused by insulin-producing pancreatic β-cell destruction, usually leading to absolute insulin deficiency. Genetic and environmental factors have been widely reported as risk or protection factors for T1D. We have reported that Tyrosine kinase 2 (Tyk2) is a susceptibility gene for virus-induced T1D in mice and humans. Tyk2, a member of Janus kinase (Jak) family, is involved in cytokine signaling. Since the association between Tyk2 and autoimmune T1D has not been reported, we examined the role of Tyk2 in autoimmune T1D to understand the pathogenesis of T1D from various viewpoints. In this study, we found that Tyk2 plays an important role in the development of autoimmune T1D.
Key Words : Type 1 diabetes, NOD mouse, Autoimmunity

Introduction

Type 1 diabetes (T1D) is caused by destruction of insulin producing pancreatic β-cells. The pathogenesis of T1D is related to genetic and environmental factors. Viruses are one of the important environmental factors in T1D (1). We have reported that Tyrosine kinase 2 (Tyk2) is a susceptibility gene for virus-induced diabetes in mice and humans (2, 3). Tyk2, a member of Janus kinase (Jak) family (Jak1, Jak2, Jak3, and Tyk2), is involved in cytokine signalings including type 1 interferons (IFNs), interleukin (IL)-6, and IL-10. After binding of the cytokine(s) to the cell surface receptor(s), Tyk2 gets activated, phosphorylate intracellular tail of the receptor(s), and recruits Signal transducers and activators of transcriptions (Stats). Recruited Stats become phosphorylated themselves, dimerize, and translocate to the nucleus to regulate gene expression by direct binding to DNA regulatory elements (4). The Tyk2 mediated signaling plays a role in anti-microorganism responses; however, aberrant activation of the signaling has been implicated in the pathogenesis of inflammatory diseases such as psoriasis (5, 6). Since the association between Tyk2 and autoimmune T1D has not been reported, we examined the role of Tyk2 in autoimmune T1D to understand the pathogenesis of T1D from various viewpoints and develop the safety prevention measures for T1D.

Results

Tyk2 knockout NOD mice showed reduced incidence of autoimmune T1D compared with Tyk2 sufficient NOD mice (Figure 1, p<0.001).

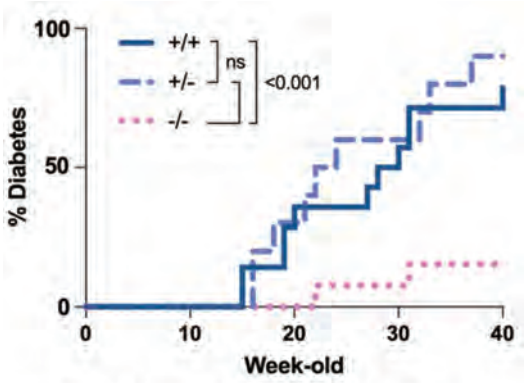

Figure 1. The incidence of autoimmune T1D

The transfer experiments of naïve NY8.3 CD8 T cells into NOD mice suggested that Tyk2 expression in antigen-presenting cells (APCs) is important for the proliferation of CD8 T cells in pancreatic lymph node. We found that MHC class 1 expression in CD8-positive tissue-resident dendritic cells (CD8+ rDC) was reduced by Tyk2 deficiency. To test the T cell priming capacity of CD8+ rDC, sorted CD8+ rDC was cultured with naïve NY8.3 CD8 T cells and IGRP peptide. The proliferation of NY8.3 CD8 T cell was reduced when it cultured with Tyk2 KO CD8+ rDC compared with Tyk2 WT CD8+ rDC, indicating that Tyk2 KO CD8+ rDC has a reduced ability to proliferate of islet autoreactive CD8 T cells.

RNA-sequencing analysis revealed that Tyk2 KO CD8 T cells in the pancreas showed decreased gene expression of interferon (IFN)- γ and granzyme.

These results suggest that Tyk2 is a potential target for the prevention of T1D onset. Four weeks of Tyk2 inhibitor treatment starting at 6 weeks of age prevented the onset of diabetes; this inhibition was not observed when treatment was started at 12 weeks of age.

Discussion & Conclusion

Our results indicate that Tyk2 plays an important role in the development of autoimmune T1D. Reduced expression of Tyk2 in CD8+ rDC and CD8 T cells suppresses its function in the acquired immune response. Although the risk of infection must be considered, Tyk2 inhibition is a potential methods to prevent the onset of autoimmune T1D.

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一般の皆様へ

本研究は血糖値を下げるホルモンであるインスリンを作る細胞が破壊され糖尿病（1型糖尿病）になる機序を解析することで、有効で安全な1型糖尿病の発症予防法を確立することを目的としています。1型糖尿病は生涯にわたってインスリンを補充する以外に治療法がなく、現在のところ不治の病です。我々の研究によって1型糖尿病の発症を防ぐ方法を開発し、患者数の減少と人々のQOL の向上へ貢献したいと考えています。

ある遺伝子とその遺伝子から作られるタンパク質の働きを阻害することで、1型糖尿病の発症が抑制されることが明らかになりました。その発症抑制機序を詳細に解析し、標的として最適だと考えられる細胞の種類を特定することができました。

この研究成果は、副作用を限りなく抑えた1型糖尿病発症予防法の開発へ繋がると考えられます。

Synthesis of amino acid derivatives and its application for the inhibitors against SARS-CoV2 protease

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Summary Abstract

Synthesis of amino acid derivatives using chiral Ni(II) Schiff base are described. For the first time, dynamic kinetic resolution of unprotected amino acids with active side chains was attempted to afford chiral Ni(II) Schiff base derivatives in good yield and diastereoselectivity. After the conversion of free side chain, protected amino acids were isolated by removal of chiral ligand.

Key Words : Chiral Ni(II) Schiff base, dynamic kinetic resolution, tailor-made amino acids, SARS-CoV2 protease inhibitor

Introduction

Tailor-made amino acids are important structural units in the development of modern pharmaceuticals, biomaterials, and so on. Synthesis of tailor-made amino acids via Ni(II) complex intermediates is one of the effective preparation method. We have reported that synthesis of unusual amino acids for the total synthesis of peptidyl natural products using chiral Ni(II) glycine Schiff base [1].

On the other hand, SARS-CoV2 protease is responsible for the processing of two virus precursors polypeptides produced during virus replication. Therefore, SARS-CoV2 protease is essential for life cycle and reasonable target protein for the drug discovery [2][3].

Results

In the present study, we attempted dynamic kinetic resolutions or (S)/(R) interconversions of unprotected amino acids with active side chains. The amino acids derivatives we synthesized are used for the creation of SARS-CoV2 protease inhibitors.

All dynamic kinetic resolutions of amino acids were conducted under the following conditions as a synthetic protocol. The (*R*)-chiral ligands (0.1 mmol) with (*rac or L*)-amino acids (0.11 mmol) and $Ni(OAc)_2.4H_2O$ (0.11 mmol) in the presence of K_2CO_3 (0.6 mmol) in MeOH (1.6 mL) were treated at 50 ^oC in N_2 atmosphere. Each reaction was monitored by TLC, NMR and/or HPLC. After the completion of the reactions, the mixtures were quenched by 10% AcOH (3.2 mL) and concentration in vacuo. Crude compounds were protected with active side chains and purified by column chromatography over silica gel ($CHCl_3$-MeOH) to afford the Ni(II) complexs (*R,R*)-form. Each diastereomeric excess of the Ni(II) complexs was determined by ^1H-NMR analysis. Using above a synthetic protocol, dynamic kinetic resolution of various (*rac or L*)-amino acids with active functional groups on the side chains was performed using (*R*)-chiral ligand and $Ni(OAc)_2$. Resultantly, dynamic kinetic resolutions of amino acids with active side chains (Gln, Orn, Lys, Glu, Tyr) without protecting groups even for linear (Nle, Met), branched

chain (Val, Ile, Leu) proceeded to give (*R,R*)-forms in 68-99% yields and high diastereoselectivity (>93%de). Surprisingly, Orn and Lys with two free amino groups and Glu with two free carboxylic acids constructed with chiral ligand to afford selectively the Ni(II) complexs (*R,R*)-forms in excellent yield. However, the dynamic kinetic resolution of Asp and Cys did not work and those of Asn showed low stereoselectivity (~10%de). The use of Ser and Thr gave undesired diastereoisomer (*R,S*)-forms in high yield compared with the desired isomers (*R,R*)-forms. On the other hand, homoCys, homoSer, Asp(OtBu), His(Trt), Ser(tBu), and Thr(tBu) were successful to obtain the corresponding products in good yields. It is noted that amino acids with hetero atoms on β-carbon were unsuccessful. It guess that the formation of amino acids and Ni(II) directly proceeded and functionalities of side chains of amino acids react to Ni(II) complex (*R,R*)-forms. Cheap (*rac or L*)-amino acids are converted easily to expensive (*D*)-amino acids by the methodology. Additionally, cheap (*rac*)-amino acids can be converted to (*L*)-amino acids. Moreover, the synthesized Ni(II) complex Lys type (*R,R*)-form was attempted to modify the side chain by Cbz, Ac, Boc, Fmoc, Alloc, phthalimide, and urea derivatives in satisfactory chemical yields. The homoSer derivative was converted to TBS and Ac forms in good yields. By these reactions, the side chains were protected and the corresponding compounds can be used as protected amino acids for the peptide synthesis. The synthesized Ni(II) complexs could be disassembled with HCl to give amino acids and chiral ligand in moderate yields [4][5][6].

Next, we attempted the preparation of α,α-disubstituted amino acids using the methodology. As use of Ni(II) complex Phe type (*R,R*)-form, α-substituted reaction was smoothly proceeded to give Ni(II) complex Phe type (*R,R*)-form with methyl group in the α position in moderate yield. However, benzyl group was not introduced in the α position to give dibenzyl derivatives. DOPA and its derivatives did not react with chiral ligand with $Ni(OAc)_2.4H_2O$. To produce SARS-CoV2 3CL protease inhibitors from the docking simulation, preparation of several amino acid derivatives was also hard work.

Discussion & Conclusion

By the use of Ni(II) chiral Schiff base, dynamic kinetic resolution and (S)/(R) interconversions of amino acids and alkylation with Gly Schiff base can be performed to give tailor made amino acids. In this study, we found the reaction using unprotected amino acids with active side chains proceeds to afford desired Ni(II) complexs. These results mean that this chemistry has great potential for the amino acids synthesis and creates new chemical space for the drug discovery and material science. There are several tasks to be solved, molecular design, optimized conditions. For the production of new SARS-CoV2 3CL protease inhibitors, we will attempt new amino acids synthesis and evaluate the inhibitory activities.

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一般の皆様へ

アミノ酸は最も重要な部分構造の一つであり、医薬品、農薬、食品添加物などに利用されている。アミノ酸は多彩な側鎖構造を持つことから需要が高く、その効率的な製造技術の開発は重要課題となっている。それらの製造プロセスにおいて、一般性があり、環境にやさしく、なおかつ安全、安価な方法論の確立が求められていた。筆者は光学活性なニッケルリガンドを用いて安価なL体を高価なD体に変換しつつ、保護基を効率的に導入しうる方法の開発に成功した。この結果は従来法と比較して様々な利点があり、社会実装への展開が期待される。

Potential implications of enzymatic activity of the multifunctional protease NRDC as a new therapeutic target for cardiovascular disease

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Summary Abstract

Nardilysin, a multifunctional protease abbreviated as NRDC, is a potential therapeutic target for cardiovascular diseases. We analyzed mutant NRDC mice lacking enzyme activity to clarify the pathophysiological significance of enzyme activity.

Key Words：metalloprotease, enzymatic activity

Introduction

We reported that nardilysin (NRDC), an M16 family metalloprotease, is a multifunctional protein dependent on the cellular localization. NRDC acts as an enhancer of ectodomain shedding making a complex with ADAM17 and enhances ectodomain shedding of membrane anchored proteins on the cell surface (ref.1-4), and acts as a transcriptional co-regulator making complexes with other transcriptional factors such as PGC1α, Islt1, p53, and HDACs in the nucleus (ref. 5-9, 14-15). Systemic NRDC knockout mice (Nrdc-/-) show the cardiovascular phenotypes such as bradycardia and hypotension despite increased sympathetic nerve activity (ref. 10). These findings suggest that NRDC may be a key player of the regulation of vascular tone. We also reported that serum NRDC concentration was elevated in ACS patients compared with healthy volunteer and that NRDC is a novel biomarker for the early detection of ACS (ref.11-13). However, the pathophysiological significance of enzymatic activity of NRDC in cardiovascular disease is still unclear. In this study, we generated Nrdc E>A knock-in mice (Nrdc E>A KI/KI) lacking enzymatic activity of NRDC by changing the glutamic acid in the center of enzyme activity to alanine and analyzed.

Results

This research explored the following major themes.

1) Cardiac phenotypic analysis of adult Nrdc E>A KI/KI.

To clarify the effect of enzymatic activity of NRDC regulating heart rate, Nrdc E>A KI/KI were analyzed using an electrocardiographic telemetry system. The average heart rate after the stimulation of atropine and metoprolol to remove the autonomic nerve effect was significantly lower in Nrdc E>A KI/KI compared with control mice littermates indicating that heart rate is regulated by NRDC enzymatic activity.

Furthermore, we confirmed decreased mRNA expression of ion channels essential for sinus node automaticity formation in Nrdc E>A KI/KI hearts using RT-PCR. In addition, we performed RNA-seq using Nrdc E>A KI/KI hearts and found that several novel mRNA expressions essential for the cardiac conduction system were altered compared to control mice.

2) Significance of NRDC in a model of cardiac disease (takotsubo cardiomyopathy model)
We have previously shown that serum NRDC levels were significantly elevated in human acute coronary syndrome (ACS) and takotsubo cardiomyopathy (TSS) compared to healthy controls and patients with stable angina pectoris (ref. 8 and unpublished). We generated a model of takotsubo cardiomyopathy (isoproterenol overdose injection) in wild-type and cardiovascular-specific Nrdc-deficient mice (Sm22α-Nrdc-KO) as well as in Nrdc E>A KI/KI, which is a model of stress-induced cardiomyopathy believed to be caused by overexcitation of the sympathetic nervous system. We focused on how the enzymatic activity of NRDC is involved in catecholamine-induced myocardial damage, focusing on changes in downstream signaling of β-receptors and reactive oxygen species.
3) Screening of compounds which reduce the enzymatic activity of NRDC
MEF cells derived from wild-type, Nrdc E>A KI/KI, and Nrdc-KO mice were treated with small molecule compounds from a library that have been previously shown to inhibit the enzymatic activity of NRDC, and we examined how the expression of the target molecules is altered in these cells.

Discussion & Conclusion

Cardiac phenotyping of mutant NRDC knock-in mice lacking enzyme activity revealed that these mice, like Nrdc-KO mice, exhibit bradycardia and reduced expression of ion channels responsible for sinus node automaticity. Previously, we reported that NRDC forms complexes with other transcription factors, suggesting that the enzymatic activity of NRDC may regulate ion channel expression in coordination with other transcription factors. However, the detailed mechanism by which transcription factors NRDC forms complexes in the heart and how NRDC modulates these transcription factors enzymatically remain to be elucidated and require further investigation.

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一般の皆様へ

我々は、プロテアーゼの一つであるナルディライジンについて、生体内でどのような役割を持つのか長年研究しています。遺伝子改変マウスの解析系やヒト血清ナルディライジン値を測定できるシステムを立ち上げ、様々な病態での役割を明らかにしてきました。今回の研究ではナルディライジンによる心臓の拍動（心拍数）調節のしくみを明らかにしました。また、その酵素活性による意義を明らかにするために、ナルディライジンの酵素活性を持たない変異型ナルディライジンを持つマウスや、ナルディライジンの酵素活性を抑制する小分子化合物を解析し、心筋梗塞やタコツボ型心筋症などの心臓病において、ナルディライジンが新しい治療薬開発の標的となり得るかどうかを検討しました。今後、ナルディライジン酵素活性を抑制することによって、実際に心臓病の改善が見込めるかどうか、また、さらに詳細なメカニズムを解析し、将来の治療薬候補を探索していきます。

Elucidating the mechanism of skin stem cell aging by targeting anti-aging matrix Fibulin-7

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Summary Abstract

Tissue stem cells divide infrequently as a protective mechanism against internal and external stresses associated with aging. Here, we demonstrated that mice lacking fibulin-7, an extracellular matrix (ECM), show early impairments resembling epidermal stem cell aging, such as the loss of fast-cycling clones, delayed wound healing, and increased expression of inflammation- and differentiation-related genes. Fibulin-7 interacts with ECM proteins, and the overexpression of fibulin-7 in primary keratinocytes results in slower proliferation. Our results suggest that fibulin-7 maintains epidermal stem cell heterogeneity, thereby protecting skin from the detrimental effects of aging and maintaining long-term tissue resilience.
Key Words : skin aging, epidermal stem cells, extracellular matrix, microenvironment, Fibulin-7

Introduction

The extracellular matrix (ECM) provides an essential microenvironment for the tissue stem cells, and its alterations can induce aging-associated skin dysfunction during aging. Among the genes upregulated in epidermal stem cells in aged skin with the unknown function was fibulin-7, a secreted glycoprotein belonging to the short fibulin family of ECM proteins[1]. Fibulin-7 regulates cell differentiation and migration through interaction with other ECM proteins, heparin, and cell surface receptors such as β1 integrins to mediate binding to odontoblasts, monocytes, and endothelial cells[2,3,4]. However, the role of fibulin-7 in the skin remains largely unknown.

Results

1. Loss of fibulin-7 leads to age-dependent depletion of fast-cycling stem cell clones and increased inflammatory response

The fibulin-7 protein was localized to the basement membrane, and the expression was significantly lower in the aged skin. Mice lacking fibulin-7 showed an aging skin-like phenotype, with an accelerated decrease in the number of fast-cycling epidermal stem cell clones and a delay in wound healing. To understand how fibulin-7 maintains epidermal stem cells at the molecular level, we performed RNA sequencing using epidermal stem cells isolated from the skin of fibulin-7-deficient mice. The results showed that the expression of a group of inflammation-related genes, including antigen presentation and cytokine production, was elevated in fibulin-7-deficient mice. In a previous report, abnormal expression of a group of genes that define the properties of epidermal stem cells was reported as part of the inflammatory response of the skin. Analysis of these marker genes revealed that the characteristic

molecular changes seen in aged mice, such as enhanced epidermal differentiation markers, were already present in fibulin-7-deficient mice at the age of 1 year. This suggests that the aging phenotype of epidermal stem cells may be accelerated in fibulin-7 deficient mice.

2. Fibulin-7 binds to extracellular matrix proteins and regulates the microenvironment of epidermal stem cells

To address the biochemical function of Fibulin-7, proteins that bind to fibulin-7 were identified by affinity chromatography and mass spectrometry, and included basement membrane structural proteins, growth regulators, matricellular proteins, and matrix proteases. Fibulin-7 interacted with basement membrane structural proteins such as Collagen IV, and fibulin-7-deficient mice showed irregular basement membrane thickening patterns and abnormal expression. This aligns with a previous report describing the upregulation of col IV in thickened BM, which led to niche stiffness in aged skin[5]. Moreover, collagen XVII proteolysis promotes skin aging[6], and we confirmed that collagen XVII staining was significantly decreased in the aged WT mice as well as in the 1-y-old fibulin-7 KO mice. These results indicate that fibulin-7 maintains the microenvironment of epidermal stem cells through physical interaction with extracellular matrix proteins.

3. Fibulin-7 regulates the undifferentiated state and proliferative response to growth factors in epidermal stem cells

Finally, we investigated the possibility that overexpression of fibulin-7 could exert a protective effect on epidermal stem cells using cultured epidermal stem cells. Epidermal stem cells overexpressing fibulin-7 are maintained in an undifferentiated and slow-cycling state. The regulation of proliferation and differentiation was shown to be dependent on different domains of the fibulin-7 protein.

Fibulin-7 overexpression was able to inhibit differentiation markers not only in the basal state but also during calcium induction in the presence or absence of col IV coating, albeit a more significant suppression was observed with col IV coating. Binding with col IV may enhance fibulin 7 function through their protein–protein interactions.

To mimic the microenvironment of aging skin, we examined the effects of low serum or inflammation-like stress conditions and found that fibulin-7 maintains low proliferation of epidermal stem cells under both conditions. These results suggest that fibulin-7 may act as an "anti-aging matrix" that maintains epidermal stem cell capacity over the long-term.

Discussion & Conclusion

Young skin is supported by heterogeneous stem cell populations having the high capacity for injury recovery and resilience to stress. We demonstrated that the deletion of fibulin-7 accelerated the age-dependent loss of fast-cycling stem cell clones, accompanied by defective skin regeneration after tissue damage[7]. Fibulin-7 maintains epidermal stem cells in part by regulating inflammatory stress responses and the fate balance of stem cells through direct binding to ECM proteins. Our results suggest that regulation of the extracellular environment by fibulin-7 maintains heterogeneous epidermal stem cell populations over the long-term, which may be an essential mechanism in controlling skin aging.

The slower cycling rate induced by fibulin-7 overexpression was only partially enhanced by IL-6, a positive regulator of keratinocyte proliferation[8]. This indicates that fibulin-7 may dampen keratinocyte response to excess inflammatory cytokine signaling. Fibulin-7 could represent a potential molecule for protecting epidermal stem cells against the detrimental effects of aging-associated inflammation, lineage misspecifications, and a premature differentiation-like state. Taken together, fibulin-7 could be a valuable candidate for the intervention of chronic wounds observed more frequently in older patients.

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一般の皆様へ

皮膚は、外的・内的ストレスを常に受けながらも、柔軟に応答し、組織を回復する力を持つレジリエンスの高い臓器の一つですが、加齢とともに徐々にその能力を喪失していきます。本研究では、細胞外ではたらくタンパク質である fibulin-7が、表皮幹細胞周囲の微小環境を構築することで、皮膚の老化を防ぐ鍵になる因子の一つであることを解明しました。本研究成果は、老化した表皮幹細胞や環境因子を標的とした皮膚の老化予防・制御法の創出へとつながることが期待されます。

Studies of genetic basis of early onset cardiac conduction system disease and disease mechanism-based personalized medicine

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Summary Abstract

High-throughput sequencing targeting 180 arrhythmia and cardiomyopathy-related genes with functional studies identified 21 pathogenic or likely pathogenic variants in 20 of 51 early-onset (<65 years) cardiac conduction system disease (CCSD) probands (39%), who showed an AV block and/or a sick sinus syndrome (SSS) with pacemaker implantation (PMI) or a family history of PMI. Functional analyses of a cellular electrophysiological study and in vivo zebrafish cardiac assay might be useful for determining the pathogenicity of rare variants in patients with CCSD. LMNA and SCN10A may be the major development factors in CCSD.
Key Words : Cardiac conduction system disease, 2015 ACMG standards and guidelines, CRISPR/Cas9-mediated gene knock-out in zebrafish, Cellular electrophysiological study

Introduction

The genetic cause of cardiac conduction system disease (CCSD) has not been fully elucidated. One of the main challenges facing genetic testing is that interpretation of the testing is complicated by the high prevalence variants of uncertain significance (VUS), which cannot be applied in clinical practice. We aimed to identify pathogenic or likely pathogenic variants in CCSD patients by using the targeted next-generation sequencing of candidate genes and 2015 American College of Medical Genetics and Genomics (ACMG) standards and guidelines as well as evaluating the usefulness of functional studies for determining them.

Results

We performed the targeted next-generation sequencing of 180 candidate genes linked to arrhythmogenic diseases or cardiomyopathies of 51 probands diagnosed with early-onset (<65 years) CCSD, who showed an AV block and/or a sick sinus syndrome (SSS) with pacemaker implantation (PMI) or a family history of PMI.

The mean age of the study participants was 45±19 years at

the diagnosis of CCSD. Of 51 subjects, 27 (53%) were women, 28 (55%) had SSS, 30 (59%) had AV block, and 39 (76%) underwent PMI. Seventeen (33%) had a first-degree family history of PMI. Echocardiographic data revealed a normal mean ejection fraction. Atrial fibrillation (AF) and muscular disease were complicated in 17 (33%) and 2 (4%) subjects, respectively.

Gene analysis showed that 15 variants with pathogenic or likely pathogenic were identified in *LMNA* (n=5), *EMD* (n=2), *MYH7* (n=2), *SCN5A* (n=1), *SCN10A* (n=1), *KCNA5* (n=1),

TNNT2 (n=1), *OBSCN* (n=1) and *LAMP2* (n=1) in 15 probands (29%). In addition to these 15 variants, 25 variants with VUS were identified in 21 genes in 15 probands (29%).

To evaluate the functional changes brought about by these variants, we generated a knockout zebrafish with CRISPR-mediated insertions or deletions of the *EMD* or *LMNA* homologs in zebrafish. At 48 hpf after the microinjection of sgRNA and Cas9 protein, the mean heart rate and conduction velocities in the CRISPR/Cas9-injected embryos were significantly decreased. We also evaluated F2 zebrafish embryos. The mean heart rate and conduction velocities of $lmna^{del/del}$ embryos were significantly decreased. We next conducted functional study of one MYH6 variant using zebrafish. We performed targeted myh6 knockdown with ATG-blocking morpholino antisense oligonucleotide (ATG-MO). Myh6 morphants showed a significantly slower HR and conduction velocities of ventricle compared with non-injected embryos. Co-injection of MYH6 WT cRNA partially rescued the reduced mean conduction velocities, while co-injection of MYH6 variant cRNA did not.

We also conducted cellular electrophysiological study to evaluate 2 variants in *KCNH2* and *SCN5A*, 3 variants in *SCN10A*. Electrophysiological study demonstrated that 4 variants in *KCNH2*, *SCN5A*, *SCN10A* showed loss-of-function effect, and one variant in *SCN10A* showed gain-of-function effect.

Six variants in *MYH6*, *KCNH2*, *SCN5A* and *SCN10A* changed their clinical significance from 'uncertain significance' to 'likely pathogenic' when added the functional study results.

Discussion & Conclusion

High-throughput sequencing targeting 180 arrhythmia and cardiomyopathy-related genes with functional studies identified 21 pathogenic or likely pathogenic variants in 20 of 51 CCSD probands (39%). Functional analyses of a cellular electrophysiological study and in vivo zebrafish cardiac assay might be useful for determining the pathogenicity of rare variants in patients with CCSD. *LMNA* and *SCN10A* may be the major development factors in CCSD.

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一般の皆様へ

若年発症徐脈性不整脈の原因遺伝子の詳細は明らかにされていない。また、遺伝子解析によって見いだされるバリアントの中にはその病的意義が不明なものが数多く含まれる。我々は次世代シーケンサーを用いて65才未満で発症した徐脈性不整脈51症例に対して拡大候補遺伝子解析を行った。遺伝子解析の結果、15症例に15個の病的バリアントを見出し、15症例に25個の病的意義不明のバリアントを見出した。病的意義不明のバリアントに対してゼブラフィッシュあるいは動物培養細胞を用いて機能解析を行ったところ、6個のバリアントの機能異常を確認した。最終的に若年発症徐脈性不整脈51症例のうち、20症例に21個の病的バリアントを見出し、陽性率は39% であった。その中には LMNA 遺伝子と SCN10A 遺伝子のバリアントが多く含まれ、若年発症徐脈性不整脈の主要原因遺伝子であることが示唆された。

Analysis of the mechanism underlying extracellular release of tau for treatment of Alzheimer's disease

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Summary Abstract

Cell-to-cell propagation of tau pathology is thought to cause spread of neuronal degeneration in Alzheimer's disease. Extracellular release of tau proteins is a key step for this propagation. However, the mechanism underlying extracellular release of tau is not fully understood. In this study, we found that treatment with autophagy inducer rapamycin facilitated physiological release of tau in mouse primary cortical neurons. Additionally, treatment with lysosomal inhibitor cocktail facilitated release of tau. These findings suggest that tau was physiologically released via an autophagy-based mechanism, and this release is facilitated during inhibition of lysosome function as a compensatory phenomenon.

Key Words : Alzheimer's disease, tau, secretion, autophagy

Introduction

Cell-to-cell propagation of tau pathology is thought to cause spread of neuronal degeneration in Alzheimer's disease. Extracellular release of tau proteins is a key step for this propagation. However, the mechanism underlying extracellular release of tau is not fully understood. To elucidate the pathway that regulates release of tau, we tested whether tau was physiologically released via an autophagy-based mechanism in mouse primary cortical neurons.

Results

In this study, to obtain cell lysates, mouse primary cortical neurons were suspended in ice cold lysis buffer, sonicated at 30W for 3 sec 5 times, and kept on ice for 30 min. Samples were centrifugated at 12,000×*g* for 30 min at 4°C. The resultant supernatants were collected as cell lysates and stored at −80°C until use. Protein concentration was measured by BCA Protein Assay Kit. To assess extracellular tau, collected conditioned media (CM) were centrifuged at 6,000×*g* for 5 min at 4°C to remove cells and cell debris, and then 1/4 volume of 100% trichloroacetic acid was added to CM. CM were kept on ice for 30 min and centrifuged at 20,000×*g* for 30 min at 4°C. The resultant pellets were washed 3 times with 1 ml of cold acetone, air dried, and dissolved in 100 µl of Laemmli's sample buffer containing 2.5% 2-mercaptoethanol with brief sonication. For assessment of intracellular levels and extracellular release of target proteins, we performed SDS-PAGE and western blotting. For quantitative analysis, the signals of b-actin were used as a loading control. To obtain detergent-insoluble tau, neurons were sonicated on ice in buffer containing 1% Triton X-100 and centrifuged at 100,000 × g for 30 min at 4°C. The supernatant was collected as 1% Triton X-100 soluble fraction. After washing pellets with buffer, resultant pellets were dissolved in the equal aliquot

of the solution containing 8 M urea and 2% SDS and centrifuged at 100,000 × g for 30 min. The supernatant was collected as 1% Triton X-100 insoluble fraction.

To explore a mechanism underlying extracellular release of tau, we investigated the effects of autophagy on release of tau in mouse primary cortical neurons. Treatment with autophagy inducer rapamycin facilitated release of tau and decreased intracellular levels of soluble tau. Treatment with rapamycin increased intracellular generation of LC3-II with reducing intracellular levels of p62, indicating that rapamycin stimulated autophagy flux under the present condition. Also, treatment with rapamycin did not increase LDH release, showing that the elevation of tau release was not caused by drug-induced cell death. These findings suggested that tau was physiologically released via an autophagy-based mechanism.

Then, we investigated whether degradative function of autophagy affected release of tau. To address this issue, we treated primary neurons with cocktail of protease inhibitors, E-64 and leupeptin, which inhibit lysosomal degradation. Degradative function of autophagy is exerted by fusion of lysosomes with autophagic vacuoles. Treatment with these inhibitors facilitated extracellular release of tau without altering LDH release, whereas it increased intracellular levels of soluble and detergent-insoluble tau. Protease inhibitor cocktail increased intracellular levels of LC3-II and p62, showing that it blocked autophagy flux. These findings suggested that autophagic release of tau was facilitated in a fashion compensatory to dysfunction of autophagic degradation.

To see the relationship between extracellular release and degradation in autophagy, we investigated the effects of rapamycin on tau metabolism under inhibition of lysosomal degradation. When we co-treated primary neurons with rapamycin and cocktail of proteasome inhibitors E-64 and leupeptin, extracellular release of tau was increased more significantly than that in treatment with either rapamycin or cocktail of proteasome inhibitors. However, proteasome inhibitor-induced accumulation of soluble and insoluble tau was not reduced by addition of rapamycin. Increased levels of LC3-II and p62 were also sustained. These findings suggested that rapamycin further facilitated extracellular release of tau under inhibition of autophagic degradation, but this effect did not lead to reduction in intracellular accumulation of soluble and insoluble tau.

Discussion & Conclusion

Autophagy plays a pivotal role in maintenance of proteostasis by remove unnecessary organelles and misfolded proteins under basal and stress conditions. In addition to this degradative function, accumulating lines of evidence have indicated the role of extracellular release of “leaderless proteins”, which lack an endoplasmic reticulum-translating sequence. We found that treatment with autophagy inducer rapamycin facilitated physiological release of tau in mouse primary cortical neurons. This finding suggests that tau was physiologically released via an autophagy-based mechanism as one of leaderless proteins. Additionally, treatment with lysosome inhibitor cocktail facilitated release of tau. Co-treatment with rapamycin and lysosome inhibitor cocktail further increased extracellular release of tau, whereas rapamycin failed to reduce lysosome inhibitor-induced accumulation of soluble and insoluble tau. These findings suggest that autophagic release of tau is facilitated during inhibition of autophagic degradation as a compensatory phenomenon, but this effect was insufficient to normalize intracellular accumulation of soluble and insoluble tau. Proteostasis of tau protein may be maintained by cooperation of degradative and releasing functions of autophagy. Dysregulation of autophagic functions may lead to cause the formation of tau aggregates and cell-to-cell propagation of tau pathology in Alzheimer’s disease.

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In preparation for submission.

一般の皆様へ

アルツハイマー病の発症には、タウタンパク質の細胞間伝播が深く関与している。本研究では、細胞間伝播に必須のステップであるタウタンパク質の細胞外放出機構に焦点をあてて解析を行った。その結果、マウス大脳皮質初代神経細胞において、タウタンパク質は生理的にオートファジーを介して細胞外に放出されること、この放出はオートファジーの分解機能を阻害すると代償的に亢進することを見出した。これらの所見は、オートファジーの機能異常は、タウのタンパク質恒常性維持を乱し、アルツハイマー病の病勢進展に関与する可能性を示唆していた。

Study of functional deficits of microglia and accelerated onset of neurodegenerative disorders

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Summary Abstract

Knock-in mice whose exon 2 was replaced with human exon 2 with Alzheimer's disease risk allele R47H (hE2R47H), were generated and its mRNA levels were determined to be at similar levels as knock-in mice with wild-type human exon 2 (hE2WT) or wild-type mouse, suggesting the successful generation without unwanted splicing as described before. The $Trem2^{hE2WT/hE2WT}$ and $Trem2^{hE2R47H/hE2R47H}$ homo knock-in mice were crossed with $App^{NLF/NLF}$ Alzheimer's disease model mice and are being aged and analyzed. In phagocytosis assay using primary macrophages, the phagocytic activities were not different between wild-type and Trem2 knock-out mice. However, phagocytic activities of wild-type macrophages stimulated by lipopolysaccharide (LPS) were higher than those of Trem2 knock-out. Finally, phagocytic activities of $Trem2^{hE2WT/hE2WT}$ and $Trem2^{hE2R47H/hE2R47H}$ homo knock-in mice were compared but no difference was observed even when stimulated by LPS.
Key Words : Alzheimer's disease, microglia, TREM2

Introduction

Genetic studies identified triggering receptor expressed on myeloid cells 2 (TREM2) as an Alzheimer's disease (AD)-associated risk factor and so far researchers have tried to establish knocked-in mouse models with TREM2 risk allele (R47H). However, the knock-in mice showed unwanted splicing and did not express Trem2 mRNA or proteins. Our research group have succeeded in generation of the Trem2 knock-in mice model by exchanging mouse exon2 with human exon2 including R47H mutation. In this study we characterize the expression of Trem2 mRNA in the mouse brain and investigated the function of Trem2 R47H allele hE2R47H compared with Trem2 wild type hE2WT.

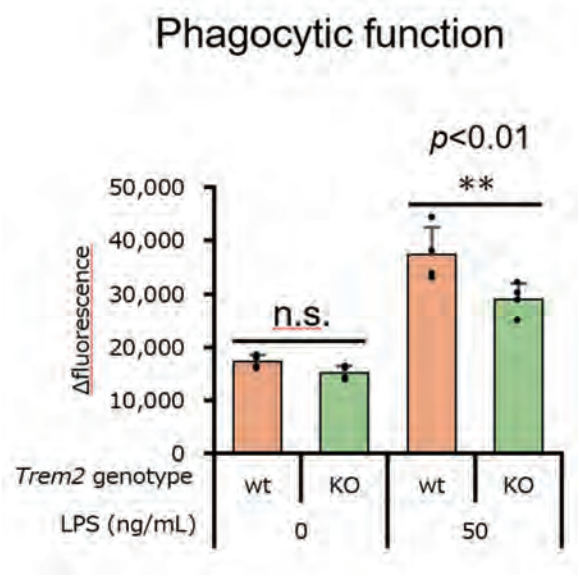
Results

Trem2 mRNA levels of the generated homozygous knock-in mice whose exon2 were replaced with human exon2 wild-type (hE2WT) or human exon2 R47H risk allele (hE2R47H) were quantified by qPCR in adult mouse cerebral cortex. I have found that Trem2 mRNA levels of both knocked-in mice were expressed at similar levels as wild-type mice, suggesting the successful generation of the knock-in mice without unwanted splicing.

The $Trem2^{hE2WT/hE2WT}$ and $Trem2^{hE2R47H/hE2R47H}$ homo knock-in mice were crossed with $App^{NLF/NLF}$ Alzheimer's disease model mice and about thirty mice of $Trem2^{hE2WT/hE2WT}/App^{NLF/NLF}$ and $Trem2^{hE2R47H/hE2R47H}/App^{NLF/NLF}$ male or female were obtained. Amyloid accumulation was investigated in cerebral cortex and hippocampus of $App^{NLF/NLF}$ mice and I have found that few amyloid plaques were observed in 12 months old mice. Therefore chronology of amyloid ac-

cumulation study was determined as 12, 18, 24 months. Currently Trem2$^{hE2WT/hE2WT}$/App$^{NLF/NLF}$ and Trem2$^{hE2R47H/hE2R47H}$/App$^{NLF/NLF}$ mice are being aged, and their brains are removed and analyzed at these time points.

Phagocytosis assay, which measures the microglial important function for onset and progression of Alzheimer's disease, was established. In brief, pHrodo-labelled E.coli, which is non-fluorescent outside the cell at neutral pH but fluorescent in intracellular acidic pH environments, were incubated with primary microglia prepared from wild-type mice. However, because microglial cell numbers obtained from these mice were not enough, primary bone marrow-derived macrophages (BMDM) which are naïve immune cells as microglia and have overlapped functions, were used. First, to demonstrate that Trem2 is involved in the phagocytosis, phagocytic activities of wild-type and Trem2 knock-out (KO) BMDM were compared. Unexpectedly, phagocytic activities were not different between those BMDM. However, phagocytic activities of wild-type BMDM stimulated by lipopolysaccharide (LPS) were higher than those of Trem2 knock-out BMDM (Figure). Finally, phagocytic activities of hE2WT and hE2R47H BMDM homo knock-in mice (Trem2$^{hE2WT/hE2WT}$ and Trem2$^{hE2R47H/hE2R47H}$) were compared but no difference was observed.



Discussion & Conclusion

So far Trem2 R47H knock-in mice displayed unexpected splicing and lost Trem2 expression. In this study the Trem2 hE2R47H knock-in mice generated by our group expressed Trem2 at similar levels as that in Trem2 hE2WT control mice and wild-type mice. These new mouse models should help understanding how TREM2 increases the incidence of Alzheimer's disease. At the moment aged model mice are under investigation how Trem2 affects amyloid accumulation, microglial activation, and tau phosphorylation.

We established phagocytosis assay which are related to Trem2 function because wild-type macrophages stimulated by LPS enhanced phagocytic activity much more than Trem2 knock-out macrophages. In this system, however, Trem2^{hE2R47H} homozygous macrophages showed similar phagocytic activity as Trem2^{hE2WT} homozygous macrophages even when stimulated by LPS. This suggests that Trem2 activates phagocytic activity induced by LPS but this function may not be related to pathology of Alzheimer's disease. That may suggest that functional deficits of Trem2^{hE2R47H} should be analyzed in vivo. We are currently investigating the pathological aspects in aged mice.

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一般の皆様へ

アルツハイマー病の根本治療薬はアミロイドの抗体医療薬のみが米国で上市されている。しかし認知機能改善効果は弱いので他の作用点の治療薬開発が必要である。脳の免疫細胞ミクログリアが発症や進行に関わっているという最近の知見から本研究ではミクログリアが持つ TREM2というタンパク質を標的とした創薬を行っている。これまでの研究上の問題点はアルツハイマー病の発症頻度を高める TREM2R47H 変異を導入したモデルマウスでは TREM2 自身が発現しないことであった。本研究ではこの問題点を克服しモデルマウスの作製に成功し、実際に TREM2が発現していることを確認した。また初代培養細胞の実験では TREM2の変異があっても貪食機能に大きな影響を与えないことがわかった。これよりモデルマウスでの機能解析研究が非常に重要であることが示唆され、今後は本研究で作製したマウスでアルツハイマー病の発症機序あるいは治療薬開発研究を進めていく。

Modulation of itch by neuro-immune interaction

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Summary Abstract

The type 2 cytokines IL-4, IL-13 and IL-31 act as itch mediators associated with allergic dermatitis directly through their receptors expressed on pruritic neurons in dorsal root ganglia. IL-27 is an immunoregulatory cytokine that signals via its heterodimeric receptor composed of WSX-1 and gp130. Although IL-27 is known to suppress Th2 and ILC2 responses, the effect of IL-27 on the neural system is unknown. Here we show that IL-27 acts directly on pruritic sensory neurons and suppresses itch.
Key Words : itch, cytokine, IL-27

Introduction

Physiological itch is essential for host defense as scratching behavior leads to the removal of potentially harmful organisms from the skin. However, in chronic diseases such as atopic dermatitis, itch is associated with unpleasant effects. Recent reports have shown that the type 2 cytokines IL-4, IL-13 and IL-31 control itch in allergic dermatitis by directly activating and/or sensitizing pruriceptors in the dorsal root ganglion (Ref.1). IL-27 is an immunoregulatory cytokine that signals via its heterodimeric receptor composed of WSX-1 and gp130. Although IL-27 suppresses Th2 and ILC2 responses, the role of IL-27 in the neural system is unknown.

Results

To investigate whether IL-27 is involved in itch sensation, we intradermally injected several pruritogens into WSX-1-deficient (WSX-1-/-) and wild-type control mice and monitored itch behavior. We found that WSX-1-/- mice showed significantly enhanced scratching behavior to several pruritogens, suggesting that IL-27 controls itch sensation in the physiological state. Conversely, we found that intradermal administration of recombinant IL-27 could suppress pruritogen-induced scratching behavior in wild-type mice. Since IL-27 receptor engages Jak1/2-STAT1/3 pathway as a downstream signaling, we next investigated a contribution of JAKs in the suppressive effect of IL-27 on itch by using a JAK(1/2) inhibitor, ruxolitinib. Although administration of recombinant IL-27 suppressed histamine-induced scratching behavior, treatment with the JAK inhibitor partially but significantly reduced the suppressive effect of rIL-27, suggesting that the effect of IL-27 on itch sensation is dependent on JAK activation. Next, to investigate whether IL-27 acts directly or indirectly on pruriceptive neurons, we generated conditional knockout mice, Nav1.8-Cre/WSX-1flox/flox, which lack WSX-1 expression specifically in nociceptive neurons including pruriceptors. We found that the suppressive effect of IL-27 on itch sensation was abolished in Nav1.8-Cre/WSX-1flox/flox mice, suggesting that IL-27 acts directly on sensory neurons to suppress itch sensation. To identify a possible source of IL-27 in normal skin, we used immunohistochemistry of skin from p28-Venus report-

er mice, which express the fluorescent protein Venus in synchrony with the expression of p28, a component of IL-27. We found that IL-27 was produced mainly by CD11c+ dermal dendritic cells (DCs). Flow cytometric analysis of skin DC subsets revealed that CD207-CD11b+ dermal DCs most abundantly produced IL-27.

Discussion & Conclusion

Our data suggest that dermal DCs constitutively produce IL-27 in the skin under steady-state conditions and suppress excessive itch sensation by directly acting on the pruriceptive DRG neuron. The significance of IL-27 regulation of itch in pathogenic conditions such as allergic dermatitis is still unknown and requires further investigation. This study provides important new insights into the regulation of peripheral sensation by immune and nervous interactions. Further studies would open up the IL-27-Jak pathway as a potential target for the treatment of pruritus.

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一般の皆様へ

アトピー性皮膚炎に代表される慢性の痒みは患者の QOL を著しく低下させる。今回の我々の研究により、これまでアトピー性皮膚炎などの炎症を抑制するサイトカインとして知られていたIL-27が、痒みを伝達する感覚神経にも直接作用して痒みを抑制することが明らかとなった。これは、免疫細胞が産生するサイトカインが痒み感覚の抑制に働くことを示した初めて発見であり、この研究をさらに進めることで、新しい掻痒治療薬の開発につながるものと期待される。

Analysis of ncRNA transcription mediated chromatin modulation mechanism

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Summary Abstract

In eukaryotic cells, transcription from genomic regions that do not contain protein-coding information has been found, and such transcripts are called non-coding RNAs. Among them, long non-coding RNAs that are expressed at promoters and involved in the regulation of gene expression are called promoter non-coding RNAs, but such RNA's functions remain unknown. The non-promoter-coding "mlonRNA" originally discovered by the applicant, which is expressed on the fission yeast *fbp1* promoter, is expressed during glucose starvation stress and contributes to transcriptional activation by promoting the binding of transcription factors by opening the chromatin structure of the passed region of transcription polymerase. In this study, we elucidate whether the pioneer polymerase transcribing mlonRNAs and opening chromatin, is involved in DNA associated reaction other than transcription such as recombination in fission yeast genome.

Key Words : Chromatin, non-coding RNA, transcription, *fbp1*, fission yeast

Introduction

Many forms of life, including humans, engage in sexual reproduction, which involves the exchange of the genome between two individuals, a male and a female. This process produces offspring with a divergent genotype that differs from that of the parent, which is advantageous in responding to crises such as environmental change. In sexual reproduction, this genetic diversity is brought about by an exchange reaction between the father and mother through homologous recombination of the genome between the father and mother, which is accompanied by the halving of the genome through meiosis. Meiotic homologous recombination is initiated by the introduction of a DNA double-strand break (DSB) in the genome. DSBs are not randomly distributed; some regions of the genome frequently introduce DNA dsDNA breaks while others do not, but the regulatory mechanism of DSB distribution remains unresolved. In addition, since many of the meiotic recombination sites in fission yeast localize similarly to non-coding RNA transcription sites, the possibility of DSB regulation by non-coding RNA has been suggested, but its molecular mechanism remains completely unknown.

Results

To investigate the possible roles of ncRNA expression in the determination of meiotic DSB sites, we compared the frequency and distribution of meiotic DSBs in fbp1 upstream region in the presence and absence of glucose. In the presence of glucose, we found faint DSB signal around UAS1 in the fbp1 upstream region at 4 and 6 hours after induction of meiosis (Fig. 1).

By contrast, we found at least two prominent meiotic DSB signals around UAS1 and UAS2 in *fbp1* upstream region (Fig. 1). These results indicate redistributions and upregulations of meiotic DSB sites are induced in *fbp1* upstream region in response to glucose starvation. To address the involvement of ncRNA transcriptions in the redistribution of meiotic DSBs, we examined the expression profile of mlonRNAs and mRNA upon glucose starvation stress in meiosis. In contrast to previous study (1) , leaky expressions of mlonRNAs and mRNA was observed before glucose starvation in meiosis. This might be caused by the difference of culture conditions. To synchronously induce meiosis, we cultured cells in the medium lacking nitrogen source to arrest cell cycle in G1 phase once and released in meiotic condition at time 0. Thus, the observed leaky expressions may be caused by nitrogen starvation condition. Nevertheless, this slight difference of *fbp1* induction upon glucose starvation in mitosis and meiosis, each mlonRNA (a, b, and c) and mRNA of *fbp1* were induced in stepwise manner during glucose starvation in meiosis, while such inductions were not seen in glucose-added condition. We analyzed chromatin configuration in *fbp1* upstream region by investigating the distribution of micrococcal nuclease (MNase) sensitive sites. The distribution patterns of MNase sensitive sites in mitotic cells, G1-arested cells and meiotic cells in glucose-added condition were comparable, whereas meiotic cells in glucose starvation condition exhibited drastic changes of the distribution patterns; A couple of MNase sensitive sites appeared at UAS1 and intense MNase sensitive bands appeared around UAS2–TATA box region at 60–180 min after glucose starvation, which were coincident with the robust *fbp1* mRNA transcription. Collectively, these results indicate that transcriptions of mlonRNA/mRNA of *fbp1* in response to glucose starvation stress are associated with chromatin conversions and this transcription-associated chromatin changing induces redistribution and upregulation of meiotic DSB sites in *fbp1* upstream region.

We next wish to study which transcription, mlonRNA or mRNA, is involved in the introduction of meiotic DSBs in *fbp1* upstream region. To this end, we mutated several critical *cis* elements required for these transcriptions. We analyzed mutant cells carrying a point mutation in UAS1 or UAS2 (33), the critical binding sites for transcription factor Atf1-Pcr1 or Rst2, respectively. Both elements are required for *fbp1*-mRNA expression, while UAS1 but not UAS2 is required for the inductions of mlonRNA. In addition to these two *cis* elements, we analyzed mlonRNA initiation element (mlon-IE), which is involved in the mlonRNA-c transcription. Moreover, we also analyzed *fbp1*-TATA-box, which is required for *fbp1*-mRNA expression but not

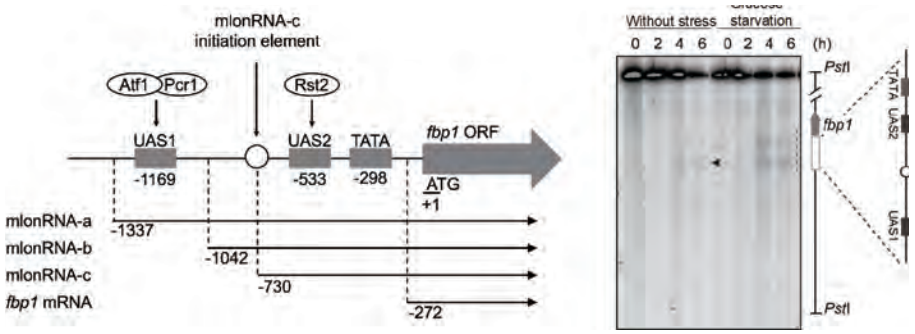

Fig. 1 ncRNA initiations induce new meiotic DSBs in fbp1 upstream region
(left) Schematic drawing of fbp1 locus. Arrows represent expressed ncRNA and mRNA of fbp1. Gray boxes represent cis element required for fbp1 expression. White circle represents mlonBox required for the initiation of mlonRNA-c. (right) DSBs introduced in fbp1 upstream region are induced by glucose starvation stress.

for the inductions of mlonRNA. The cells carrying a mutation in UAS2 and cells lacking *fbp1*-TATA-box showed very similar DSB distribution compared to wild-type cells in glucose starvation condition, indicating that expression of *fbp1*-mRNA is not important for the creation of DSB sites in *fbp1* upstream region upon glucose starvation stress. The cells with a mutation in UAS1 showed critical reduction of DSBs around UAS 1 and UAS2 compared to wild-type cells. Moreover, mutant cells in which mlon-IE is replaced with a part of *act1* sequence also showed reduction of DSB around UAS2 compared to wild-type cells. These results indicate that inductions of mlonRNAs rather than *fbp1*-mRNA are required for meiotic DSB formation in *fbp1* in response to glucose starvation stress.

Discussion & Conclusion

Evolution is a continuous process of genetic variation and phenotypic selection (2). At a molecular level, mutations in genes make diversity of the functionality of genes, and it is believed that genes possessing advantages to fit environmental condition are selected from such diverse genes. Meanwhile, a study using bacteria model showed that whole genome-shuffling *via* homologous recombination between homologous DNA sequences has great advantage over the simple mutation-mediated improvement of gene function. In the current study, we showed that transcription events induced by environmental changes trigger meiotic recombination events. These events might be induced around gene-promoter region, since transcription factor binding elements are usually located in gene promoter region, and thus DNA sequences not only within gene body but also in promoter region should be efficiently diversified. This view is consistent with the recent study showing a pivotal role of mutations in non-coding regulatory DNA sequences such as promoter sequences. Moreover, frequent recombination-mediated genome-shuffling between homologous DNAs at the actively used genes in response to various stresses might be reasonable for the rapid molecular evolution upon the environmental clue.

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一般の皆様へ

本研究では、子孫に遺伝子を繋ぐ機構、減数分裂における、配偶子の多様性の創出機構を新規に解明しました。子孫の多様性は、環境変動への生物種としての対応や進化の原動力として重要であることは皆さんご存知のことと思います。例えば、接木で全国にクローン増殖した「桜」の木は、一度病原菌や虫におかされると全滅してしまいますね。本研究をさらに発展させ、減数分裂期の「組換え」による子孫の多様性創出の秘密をさらに解明し、効率的な品種改良技術など応用研究にも繋げたいと思います。

Communication between tumor cells and neurons regulating brain tumor

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Summary Abstract

Key Words : Brain tumor, synapse, Neural activity, glia, cell-cell communication

Introduction

It is now known that not only the characteristics of the tumor cells, but also the surrounding microenvironment is important in the malignant transformation of brain tumors. However, it remains unclear how the interaction between brain tumor cells and the microenvironment causes brain tumors to become malignant or benign. In this study, we aim to test the hypothesis that "tumor cells form networks with normal cells in the microenvironment and exploit bioactive substances from normal cells to promote tumor cell growth.

Results

We first investigated the onset of tumor cell growth and glial cell changes in the brains of glioma model mice. It is known that tumors (gliomas) are generated in the mouse brain by knocking out or knocking down three tumor suppressor genes, Trp53, Pten, and Nf1, which have been found mutated in patients with human brain tumors, by the CRISPR/Cas9 system. Using this model mouse, we analyzed the growing tumor cells and glial cells. The results showed that glioma increased cell division from around 4 weeks of age, and the number of neurons and oligodendrocytes decreased markedly from 8 weeks of age due to tumor cell progression. In contrast, microglia increased within the tumor from 6 weeks of age, and astrocytes increased in the peritumoral area. These results indicate that tumor cell-neuron and tumor cell-glial cell interactions can be analyzed using this model system.

Next, to analyze the mode of interaction of tumor cells with normal cells and tumor progression, we aimed to develop an in vivo experimental system to detect activity dynamics and transitions in the neuron-tumor cell network. Calcium imaging has been used in neuroscience field to measure cell activity and analyze intercellular networks, and the applicant applied this method to tumor cells. The applicant used adeno-associated virus and electroporation to express calcium sensor genes (genetically encoded calcium indicator) in neurons and tumor cells. One week later or late, the craniums of tumor model mice were opened and the tumor cells and neurons expressing the calcium sensor gene were observed under an objective lens. We found that brain tumor cells exhibit calcium activity, albeit at low frequency (approximately once every 3 minutes), and that specific cell populations are synchronously active. In addition, neurons surrounding tumor cells showed calcium transients. We also screened for the best calcium-directed genes for detecting calcium activity in tumor cells and found that jG-CamP8m best detected calcium activity in tumor cells.

We used the calcium imaging method to analyze the transition of tumor cell activity during tumor progression, and found that only a few tumor cells showed activity at 4-5 weeks of age, soon after tumor initiation. However, at 7-8 weeks of age, when tumor progression is more pronounced, more than half of the tumor cells showed calcium activity. These results suggest that the number of tumor cells receiving transmission and sensory input from neurons increases with tumor progression, supporting the hypothesis of this study that tumor cells form networks with neurons and glial cells during tumor progression.

To investigate whether neuronal activity and tumor cell activity affect tumor progression, we used adeno-associated viruses to express artificial synthetic receptors (DREADD) on neurons and tumor cells, which, when expressed in cells, respond to their ligand CNO and induce calcium activity in these cells. We analyzed tumor model mice expressing DREADD in neurons and intraperitoneally injected with CNO to increase neuronal activity. Compared to tumor model mice without elevated neuronal activity, tumor model mice with elevated neuronal activity had an increased number of Ki67-positive cells, a marker of mitotic cells, in brain tumor tissue. Elevated neural activity also increased the number of microglia and oligodendrocytes in the tumors. Moreover, elevated tumor cell activity resulted in the increased number of Ki67-positive cells. These results indicate that neuronal activity promotes tumor progression and recruits peritumoral brain endogenous cells into the tumor.

Discussion & Conclusion

This research has significance in establishing “brain tumor physiology” by combining cutting-edge technologies in neuroscience and basic medical research, ultimately leading to the cure of brain tumors. By elucidating the contribution of neurotransmission and gliotransmission to tumor progression, this research will provide a foothold for developing a new therapeutic approach that "targets factors that support tumor progression," rather than the conventional approach of targeting the tumor cells themselves. If we can identify specific neurotransmission and gliotransmission, we will be able to predict which neuronal network will become abnormal at an early stage of development and establish a new diagnostic method for brain tumors to detect the disease in the preliminary stage when the tumor grows and the patient recognizes the disease through pain. This could lead to new diagnostic methods for detecting brain tumors before the tumor has grown and the patient is aware of the disease in pain. The strategies in this study can be applied to other types of brain tumors and tumors arising in organs where nerves are present (e.g., the intestine where the vagus nerve is located).

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一般の皆様へ

脳が正常に働くには脳内の環境が安定していることが必要不可欠です。脳疾患の原因や病態として、脳内の局所環境が乱れていることが考えられています。脳腫瘍は脳内の局所環境が乱れている疾患の1つです。脳腫瘍は、腫瘍細胞自体が持つ性質だけでなく、周りに存在する正常細胞が進展や悪化に影響することが分かっています。しかし、腫瘍細胞と周囲の脳内環境にある正常細胞とがどのように相互作用して脳腫瘍を進行させるのかはほとんど分かっていません。この研究では、正常な神経細胞やグリア細胞と腫瘍細胞との相互作用に注目しています。腫瘍細胞は、正常な神経細胞やグリア細胞と相互作用して、腫瘍細胞にとって必要な脳内環境を形成していると考えられています。この研究では、腫瘍細胞が神経細胞やグリア細胞からの物質を悪用して腫瘍を進行させる可能性を調べました。今後、それらの物質を標的とする脳腫瘍の新規治療法の開発につなげることを目指しています。

Establishment of tissue maturation induction method to obtain adult tissues from pluripotent stem cells

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Summary Abstract

In this research, we aimed to develop a new method to induce cellular maturation from pluripotent stem cells to functional hematopoietic stem cells. We identified fetal-liver derived niche factors via in silico and in vitro screening using human induced pluripotent stem cells (hiPSCs) derived pre-hematopoietic stem cells (pre-HSCs). Using these newly identified niche factors, we successfully established a method to induce maturation of the hiPSCs-derived pre-HSCs into HSCs expressing CD201, an essential marker of transplantable fetal-liver HSCs.

Key Words : pluripotent stem cells, hematopoietic stem and progenitor cells, fetal liver, maturation,

Introduction

Recently, enormous number of studies have been performed to induce directed differentiation of human pluripotent stem cells (hPSCs) into various cells and tissues, attempting to apply them to future regenerative medicine and drug development. However, these hPSC-derived cells and tissues are still immature in terms of gene-expression, epigenetic states, structures, and functions and are most similar to the counterparts at fetal stages. This 'roadblock to maturation' in hPSC differentiation is thought to be a most serious obstacle to hPSC application to regenerative medicine and drug development. In this research project, I aim to (1) understand a common mechanism underlying functional maturation, and (2) remove the 'roadblock to maturation' by manipulating the maturation-machinery and obtain adult-like cells/tissues from hPSCs in a shorter time.

Results

First step of human hematopoietic stem cell (HSC) development takes place in a specific region of mesoderm (AGM region). Approximately 1 month after fertilization, epithelial cells at the AGM region differentiate into primitive blood cells called pre-HSCs Two months after fertilization, the pre-HSCs translocate to fetal liver and undergo proliferation and maturation into fetal-liver HSCs. As early as 3 months after fertilization, CD201(+) fetal-liver HSCs obtain transplantability and bone-marrow reconstitution ability. Among such multistep paths of maturation into functional HSCs, the maturity of human pluripotent stem cell (hPSC) derived hematopoietic cells remained at the primitive CD45(-) pre-HSCs (Nat Biotechnol 34 1168).

By using recently-develped monolayer-culture method, we first optimized conditions to induce differentiation of hPSCs into pre-HSCs and successfully obtained culture containing >75% CD34(+) CD43(+) CD45(+) pre-HSCs, that have not been obtained with >5% efficiency

through the previous methods. Flow cytometric and transcriptome analyses revealed that these triple-positive cells were most similar to pre-HSCs when translocating to fetal-liver 2 months after fertilization in the human development.

To induce further maturation of the pre-HSCs to fetal-liver harboring transplantibility and bone-marrow reconstitution ability, we sought a way to find unknown fetal-liver-derived niche factors that presumably enhance maturation of the pre-HSCs into HSCs. To this end, we performed in silico analysis of single-cell transcriptome data for fetal-liver cells containing both hematopoietic and non-hematopoietic niche cells (Nature 574 365). Based on the transcriptome data, we predicted fetal-liver niche factors that were supposed to be secreted by fetal hepatocytes, fibroblasts, epithelial cells, or macrophages and responded by pre-HSCs. Then we tested >20 predicted niche factors by in-vitro screening system using the hPSC-derived pre-HSCs. As a result, we could successfully identified several potential niche factors that enhanced maturation of pre-HSCs into feta-liver-HSC-like cells expressing CD201. Of note, CD201 was recently identified as a specific marker for transplantable fetal-liver HSCs (Nat Comms 13 1103).

Our ongoing analyses on the CD201(+) HSC-like cells may allow us to confirm their bone-marrow reconstitution ability and transcriptional- and epigenetic similarities between the hPSC-derived HSCs and bona fide fetal-liver HSCs.

Discussion & Conclusion

Overall, despite a slight change from the approach described in the proposal, we successfully established a novel method to induce fetal HSC-like cells from pluripotent stem cells. Further experiments are ongoing to develop additional method to induce further maturation from the fetal-liver HSCs to neonatal- (e.g. cord blood) and adult HSCs that takes longer time in human development. Strategy of our research should be applicable to other studies aiming to identify unknown maturation factors for other cells and tissues.

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Nat Biotechnol 34 1168
Nature 574 365
Nature Communications 13 1103

一般の皆様へ

本研究ではヒト多能性幹細胞由来の未熟な細胞を試験管内で短期間に成人の細胞へと成熟させることを目的に、造血幹細胞をモデルとして成熟化誘導法の開発をおこなった。従来の方法でヒト多能性幹細胞から得られるのは、CD45陰性の primitive な pre-HSC に留まっていたが、研究代表者は造血細胞の更なる成熟の場である胎児肝臓内のニッチ因子同定をおこない、同定された胎児肝臓ニッチ因子を使って造血再構築能をもった細胞に特異的な CD201陽性の胎児肝臓造血幹細胞様の細胞を得た。現在、これらの細胞の機能を詳細に解析中であるほか、胎児型幹細胞から成人幹細胞へと成熟化技術をより進化させるため、さらなる研究を進めている。

Intrinsic barrier dysfunction in multiple sclerosis patients

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Summary Abstract

Recently, disease susceptibility genes for multiple sclerosis (MS) have been localized to endothelial cells[1], making blood-brain barrier (BBB)-forming endothelial cells as an interesting candidate for novel therapeutic targets. However, research on the BBB has been limited due to difficulties in accessing patient-derived BBB samples. We generated BBB models from MS patients-derived induced pluripotent stem cells (iPSCs) to overcome this issue. We have demonstrated that our *in vitro* BBB model can replicate BBB abnormalities observed in post-mortem brains of MS patients. We identified over 400 differentially expressed genes in MS compare to healthy individual. We further demonstrated that inhibition of candidate gene X in BBB-forming endothelial cells derived from healthy individuals led to downregulation of claudin-5 and increased permeability of small molecules, thus replicating BBB dysfunction similar to MS patients.

Key Words : Multiple Sclerosis (MS), induced pluripotent stem cells (iPSCs), blood-brain barrier (BBB)

Introduction

The detailed pathogenesis of MS is not yet fully understood, and there is currently no cure for the disease. It is widely accepted that the infiltration of immune cells into the central nervous system and the breakdown of the blood-brain barrier (BBB) are early pathological hallmarks in MS lesions[2]. However, due to the limited access to brain samples, how BBB changes in MS contribute disease development and progress is poorly understood. Here, we employed induced pluripotent stem cells (iPSCs) to establish BBB model from MS-patient and explored active contribution of BBB-forming endothelial cells for MS pathogenesis.

Results

1. Transcriptome analysis MS patients-derived BBB-forming endothelial cells contain more than 400 genes with altered expression compared to healthy controls.

We confirmed that our method (extended endothelial cell culture method-derived brain microvascular endothelial cell-like cell: EECM-BMEC-like cell) recapitulates tight junction disruption and enhanced adhesion molecule expression observed in autopsy brain samples of MS patients as previously published[3]. To identify candidate genes involved in BBB disruption in MS patients, transcriptome analysis was conducted on EECM-BMEC-like cells. There are over 400 differential expression genes (DEGs) in EECM-BMEC-like cells derived from MS patients compared to healthy individuals. Reactome pathway analysis based on DEGs identified several pathways. Among these pathways, two pathways have been previously reported to be associated with BBB development and maintenance. Furthermore, we identified that a

candidate X, which has been reported as a susceptibility gene for MS (literature omitted), is significantly decreased in MS patients.

2. Candidate X induced tight junction disruption in healthy individual-derived EECM-BMEC-like cells as observed in MS patients.

To analyze the function of candidate X in BBB-forming endothelial cells, multiple small molecules (compound A-D) known to inhibit the function of candidate X were applied to healthy individual-derived EECM-BMEC-like. None of these compounds altered the expression of VE-cadherin, which is an adherence junction protein, and cell-cell junctions were maintained (Figure 1). Interestingly, compound A and B disrupted claudin-5, which is a tight junction protein in EECM-BMEC-like cells, while Compound C and D, known to inhibit Candidate X, did not cause claudin-5 disruption (Figure 1).

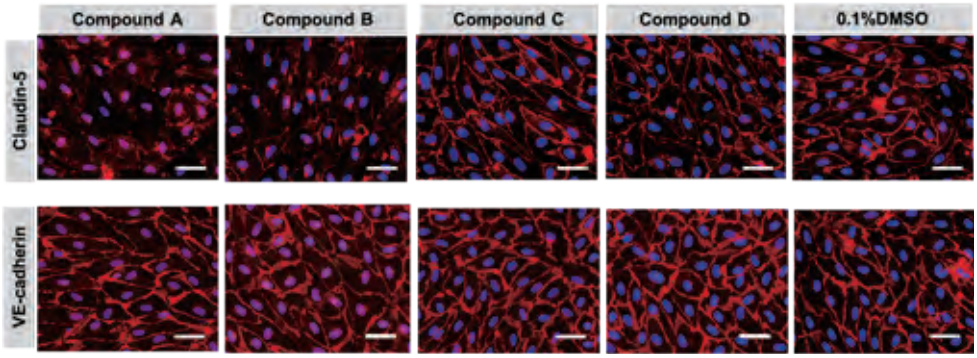

Figure 1. BBB disruption caused by candidate X in healthy individual-derived EECM-BMEC-like cells
The healthy individual-derived EECM-BMEC-like cells were cultured on chamber slides and treated with multiple small molecules (Compound A-D) known to inhibit candidate X, as well as a control (DMSO). Immunostaining was performed for claudin-5, a tight junction protein, and VE-cadherin, an adherence junction protein, shown in red. Nuclei were stained with DAPI (blue). (Scale bar: 50 μm).

Next, we examined whether compound A, which was found to disrupt tight junctions, affected the permeability of small molecules, an important function of BBB. EECM-BMEC-like cells were cultured on cell culture inserts with 0.4 μm pores in the presence of compound A or DMSO as a control. After 6 days of culture to establish a confluent monolayer, the permeability of a small molecule, sodium fluorescein (NaFl; molecular weight 376 Da), was evaluated. In healthy individual-derived EECM-BMEC-like cells treated with compound A, the permeability of the small molecule was significantly increased (Figure 2).

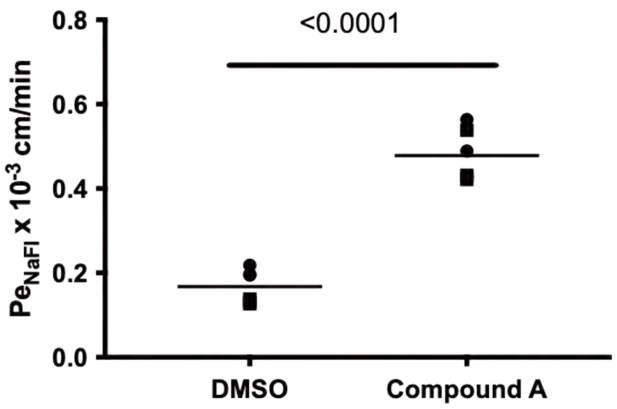

Figure 2. The effect of compound A on small molecule permeability
Healthy individual-derived EECM-BMEC-like cells were cultured on cell culture inserts with 0.4 μm pores and treated with small molecules (compound A) known to inhibit candidate X, as well as a control (DMSO). After 6 days of culture, permeability to sodium fluorescein (NaFl) was measured as previously described4, 5. Statistical analysis using t-test showed a significant change in permeability (P=<0.0001).

Discussion & Conclusion

These results indicate that BBB disruption observed in MS patients can be reproduced in artificially manipulated healthy individual-derived EECM-BMEC-like cells. It is speculated that candidate X has multiple functions and the mode of inhibition differs for each small molecule, resulting in differential downstream signaling and varying degrees of claudin-5 disruption. Further investigation of differences among the compounds will help to identify the detailed molecular mechanism and downstream signaling of candidate X in BBB function. This leads to the establishment of 1) strategies to strengthen BBB integrity and 2) potential applications in drug delivery to the central nervous system by transiently disrupting BBB..

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一般の皆様へ

血液脳関門という脳を守るバリアシステムに着目して多発性硬化症の病態解明、創薬研究を行いました。本研究では患者さん由来の血液脳関門モデルを iPS 細胞の技術を用いて実験室に再現しました。また、健常人由来の血液脳関門モデルを人為的に改変することで多発性硬化症患者にみられる変化を再現することができました。これらの発見は、今後血液脳関門を標的とした、血液脳関門を強固にする治療法、もしくは脳内に薬物を届ける薬物輸送研究に発展できると期待しています。

Identification of PP cells as an origin of pancreatic ductal adenocarcinoma

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Summary Abstract

Unlike previously known pancreatic adenocarcinomas arising from pancreatic acinar or ductal cells, we have revealed the existence of a new type of pancreatic adenocarcinoma development mechanism that arises from PP cells (*Ppy*-expressing cells). This study is expected to provide new insights into the diagnosis and treatment of pancreatic cancer in the future.
Key Words : PP cells, *Ppy* gene, Pancreatic islet, Pancreatic cancer, PDAC,

Introduction

The applicants focused on PP cells, one of the endocrine cells of the pancreas whose function has not been well analyzed, and reported the details of their gene profile by single cell analysis (1). To expand this work, we attempted to generate a cell line of PP cells by constructing a mouse model in which PP cells express the oncogene SV40 Large T. Initially, the mice were expected to develop PPoma, an endocrine tumor, but surprisingly, tumors that appeared to be pancreatic ductal adenocarcinoma (PDAC) developed very early, at 3 to 4 weeks of age, and all died by 7 weeks of age.

Results

By crossing *Ppy-Cre* mice with *Rosa26-Lox-Stop-Lox-Large T* (*Rosa26*^{LargeT}) mice, we generated a mouse model *Ppy-Cre;Rosa26*^{LargeT} that expresses an oncogene by targeting *Ppy*-expressing cells. The mice were expected to develop PPoma, an endocrine tumor, in the pancreas, but contrary to expectations, pancreatic adenocarcinoma (PDAC) development was observed in all cases as early as 3 to 4 weeks of age.

The average survival time of *Ppy-Cre;Rosa26*^{LargeT} mice was 4.7 weeks, and none of the analyzed mice survived longer than 7 weeks of age. Body weight and blood glucose levels were significantly lower than those of control mice, and at 4 weeks, most of the pancreas was occupied by abnormal duct structures, tumors and stromal tissue, and the amount of differentiated islets and acinar cells was markedly reduced. The most characteristic feature is that the abnormal duct-like structures appear to extend directly from the islets from about 2 weeks of age. It is considered that SV40 Large T antigen is expressed in the periphery of the islets where PP cells are originally located, and these Large T-expressing cells protrude and extend from the islets while proliferating, forming abnormal pancreatic duct structures. It was suggested that *Ppy-Cre;Rosa26*$^{LargeT/tdTomato}$ mice develop pancreatic adenocarcinoma (PDAC) within 3 weeks after birth. As one of the methods to verify that the lesions are cancerous, transplantation experiments into immunodeficient mice were conducted. Islets were isolated at 7 days of age and transplanted subcutaneously into athymic mice, and tumors developed

within one month. *Ppy-Cre;Rosa26*$^{LargeT/tdTomato}$ mouse islets were shown to develop typical CK-19 positive PDAC with glandular duct structures. The PDAC was also considered to be derived from *Ppy*-expressing cells since they were Tomato-positive.

To elucidate what kind of changes in gene expression profiles were induced by the activation of Large T in *Ppy*-expressing cells, we sorted tomato-positive cells from *Ppy-Cre;Rosa26*^{tdTomato} mice and *Ppy-Cre;Rosa26*$^{LargeT/tdTomato}$ mice, respectively, using FACS. Then, cDNA libraries were prepared from the RNA, and RNA-sequence was performed using a next-generation sequencer to identify the variable genes. As a result, 1362 genes were observed as variable expression genes. Among them, we observed decreased expression of pancreatic endocrine cell marker genes such as *Ppy*, *Gcg*, *Pyy*, etc., increased expression of duct cell markers such as *Krt19* (CK-19), increased expression of *DNMT1*, which is known to induce hypermethylation of tumor suppressor gene promoters (2), increased expression of *mycL1,* a known reprogramming gene. In addition, increased expression of *mycL1* and *sox2*, which are known as reprogramming genes, were observed. In addition, a number of genes that have been reported to be associated with PDAC in the literature were listed. Pathway analysis revealed that pathways related to secretion such as peptide secretion and peptide transport were down-regulated, while those related to cell proliferation such as DNA replication and DNA repair were enriched as up-regulated pathways. These results strongly suggest that the differentiation of pancreatic endocrine cells into cancer cells (PDAC) is induced from the viewpoint of gene signature.

Discussion & Conclusion

(1) It has been believed that pancreatic acinar cells and duct cells are the cells of origin of pancreatic adenocarcinoma. One of the reasons for this is that lesions diagnosed as pancreatic cancer morphologically show an adenoductal structure. In addition, animal models have been developed in which pancreatic carcinogenesis occurs when oncogenes such as KRAS are activated and tumor suppressor genes are inactivated in pancreatic acinar cells or duct cells, but no animal model has been reported in which carcinogenesis occurs from endocrine cells (3).

(2) RNA sequencing analysis in this study revealed a number of genes with altered expression levels associated with the development of pancreatic cancer from *Ppy*-expressing cells. By comparing the results of RNA sequencing analysis with those of mouse models of pancreatic cancer (*Ptf1a-Cre;LSL-KRAS*^{G12D}*;Trp53f/+*) and human pancreatic cancer that have been deposited so far, we plan identify several unique marker gene groups for pancreatic cancer arising from *Ppy*-expressing endocrine cells created in this study. We will also compare the results with those of RNA sequencing of human pancreatic cancers. Using these marker genes, we plan to test whether similar pathways exist in human pancreatic cancer.

This study reveals a previously undescribed pathway for the development of pancreatic adenocarcinoma from *Ppy*-expressing cells, which are pancreatic endocrine cells.

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一般の皆様へ

膵がんは早期発見が困難な癌種であり，本邦における5年生存率は7%と他の癌に比べて極端に低い．従って、膵がんの発生進展様式を理解することは喫緊の課題である．

膵臓は腺房細胞，膵管細胞そして膵ランゲルハンス島（内分泌細胞）という3つのパートから成るが，膵がんは腺房細胞あるいは導管細胞を源として発生すると現在は考えられている．我々は，膵ランゲルハンス島に存在する内分泌細胞の一つであるPP細胞を起源として再現性よく膵がんを発生するユニークな動物モデルを開発した．このモデル動物を詳細に分子レベルで調べることによって，これまでには報告されていない，ユニークな膵癌の発症様式が存在することが明らかになった．我々の発見は，あらたな膵がんの診断や治療法の開発につながる可能性がある．

Development of an innovative diagnostic and therapeutic method that combines radionuclide therapy and novel boron neutron capture therapy

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Summary Abstract

This study aimed to synthesize and evaluate a probe closododecaborate-(Ga-DOTA)-c(RGDfK) (**1**) containing closo-dodecaborate ($[B_{12}H_{12}]^{2-}$) as a boron cluster, a [^{67}Ga]Ga-DOTA derivative for nuclear medicine imaging, and an RGD peptide for tumor targeting. Moreover, we prepared a radioiodinated probe [^{125}I]**2** in which I-125 is introduced into a closo-dodecaborate moiety of **1**. [^{67}Ga]**1** and [^{125}I]**2** showed high stability and high uptake in cancer cells in vitro. Biodistribution experiments in tumor-bearing mice revealed similar biodistribution patterns between [^{67}Ga]**1** and [^{125}I]**2**. Meanwhile, [^{125}I]**2** exhibited higher accumulation in most tissues, including the tumor, than [^{67}Ga]**1**, probably because of higher albumin binding.
Key Words : BNCT, $\alpha_v\beta_3$ integrin, RGD peptide

Introduction

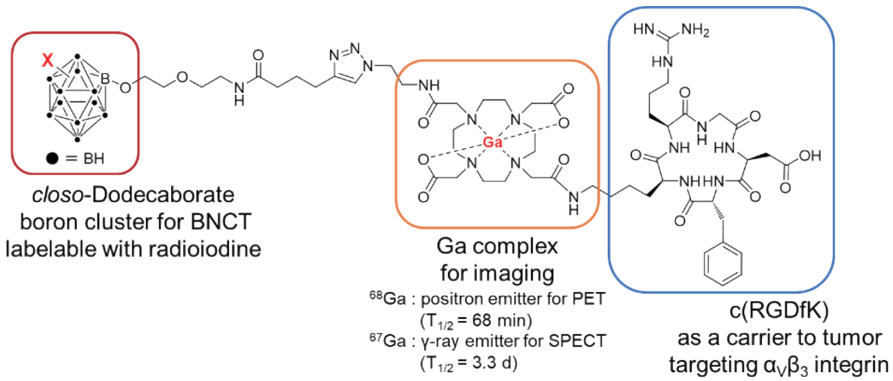
A boron neutron capture therapy (BNCT) system, using boron-10-introduced agents coupled with companion diagnostics, is anticipated as a promising cancer theranostic. To develop a novel BNCT system using drugs with the same chemical structure between a compound containing ^{10}B for BNCT and a PET probe for companion diagnostics, I designed, synthesized, and evaluated a probe closo-dodecaborate-(Ga-DOTA)-c(RGDfK) **1** containing closo-dodecaborate ($[B_{12}H_{12}]^{2-}$) as a boron cluster to introduce multiple ^{10}B molecules into a probe, a [^{68}Ga]Ga-DOTA derivative as a stable ^{68}Ga complex for PET imaging, and an arginine–glycine–aspartic acid (RGD) peptide for tumor-targeting.

Results

^{67}Ga was used as an alternative radionuclide instead of ^{68}Ga because of its relatively long half-life [1]. [^{67}Ga]**1** was prepared via conjugation of a [^{67}Ga]Ga-DOTA-c(RGDfK) derivative with a dodecaborate derivative by alkyne-azide cycloaddition. Radioiodinated compound [^{125}I]**2** was prepared from **1** using chloramine T as an oxidant. [^{67}Ga]**1** and [^{125}I]**2** were synthesized with a radiochemical purity of over 95%.

The affinity of **1** for $\alpha_v\beta_3$ integrin was determined via a competitive binding assay using U-87 MG human glioblastoma cells with high $\alpha_v\beta_3$ integrin expression. Figure The half maximal inhibitory concentration (IC_{50}) value (nM) for **1** was 10.9 ± 1.4, which was not significantly different from that of c(RGDfK) (10.9 ± 4.2 nM) in our previous study [2]. Therefore, introducing a relatively large molecule, a Ga-DOTA complex conjugated dodecaborate derivative, into c(RGDfK) via the ε-amino group of the lysine residue did not significantly impede the affinity of c(RGDfK) for $\alpha_v\beta_3$ integrin. Cellular uptake experiments with a human glioma cell line,

U-87 MG, and biodistribution experiments of [^{67}Ga]**1** and [^{125}I]**2** were performed. The uptakes of [^{67}Ga]**1** and [^{125}I]**2** in U-87 MG cells were similar and increased in a time-dependent manner. These results indicate that the introduction of iodine into a dodecaborate structure hardly alters their affinity for $\alpha_v\beta_3$ integrin. The biodistribution experiments of [^{67}Ga]**1** and [^{125}I]**2** in tumor-bearing mice were similar, such as high tumor uptake and relatively low uptake in other non-target tissues, except the kidney, liver, and intestine. Therefore, it indicates that [^{67}Ga]**1** and [^{125}I]**2** could be distributed to these tissues in intact forms. The result of urine analysis reconfirmed their high metabolic stabilities of [^{67}Ga]**1** and [^{125}I]**2** since most radioactivity, 89.3% for [^{67}Ga]**1** and 84.5% for [^{125}I]**2**, was observed as intact forms in urine at 1 h postinjection. Meanwhile, unexpectedly, the radioactivity of [^{125}I]**2** in most tissues, including the tumor, was higher than that of [^{67}Ga]**1**. We supposed that the higher [^{125}I]**2** radioactivity should be derived from delayed blood clearance due to its higher protein binding in plasma. Thus, to confirm the difference in blood clearance and protein binding between [^{67}Ga]**1** and [^{125}I]**2** in detail, we performed the biodistribution experiments of them in many time points of normal mice and the in vitro protein binding experiment. As the results the halflives of the distribution phase (α phase) of [^{67}Ga]**1** and [^{125}I]**2** from the blood were calculated as 7.7 and 5.7 min, respectively. And then, we revealed the percentages of the protein bindings of [^{67}Ga]**1** and [^{125}I]**2** in murine plasma as 56.2 ± 4.3 and 80.2 ± 1.9%, respectively ($p < 0.001$), and the binding percentages in the purified human serum albumin (HSA) as 82.1 ± 0.8 and 94.9 ± 0.3%, respectively ($p < 0.001$). The results indicate that introducing an iodine atom into closo-dodecaborate significantly increased the protein binding ratio in the plasma, supporting the difference in the blood clearance and biodistribution between [^{67}Ga]**1** and [^{125}I]**2**.



closo-Dodecaborate-(Ga-DOTA)-c(RGDfK) **1** (X = H)
Iodo-*closo*-dodecaborate-(Ga-DOTA)-c(RGDfK) **2** (X = I)

Discussion & Conclusion

We synthesized a ^{67}Ga-labeled compound ([^{67}Ga]**1**) and a corresponding nonradioactive compound containing RGD peptide, Ga-DOTA, and closo-dodecaborate for BNCT and its companion diagnostics. Moreover, we synthesized an I-125-introduced compound ([^{125}I]**2**) into a closo-dodecaborate of **1**. Biodistribution experiments in tumor-bearing mice revealed that [^{67}Ga]**1** and [^{125}I]**2** showed high tumor uptake and similar biodistribution patterns. Meanwhile, [^{125}I]**2** showed higher accumulation in most tissues, including the tumor, derived from higher albumin binding than [^{67}Ga]**1**. The higher accumulation in the tumor is desirable for BNCT, and the iodo-closo-dodecaborate site may work as an albumin binder. These results provide useful information for developing novel drugs for BNCT and its companion diagnostic imaging. However, determining the boron element in the biodistribution experiments and the therapeutic study by neutron irradiation is necessary for the future because this report is a preliminary study.

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一般の皆様へ

BNCTは、2020年に世界に先駆けて日本で承認された最先端のがん治療法です。ホウ素（^{10}B）をがんに集積させて中性子線を照射し、ホウ素の分裂により放出される放射線によりがんを殺傷する治療法であるため、ホウ素をがんに運ぶ薬剤が非常に重要です。本研究コンセプトのような同一構造化合物によるPET診断情報を利用した精密な治療計画を行うことにより、それぞれの患者により最適なBNCT治療の供給へつながると考えられます。現在、構造を最適化した更なる薬剤開発など、実用化を目指した研究を進めています。

Investigation on adjuvant effect of peptide-stabilized emulsions

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Summary Abstract

This study aimed to develop adjuvants that exhibit immunostimulant effects against protein antigens. We developed oil-in-water emulsions with interface stabilized with self-assembling peptides that can directly carry protein antigens by enzymatic reaction. The enhanced antigen delivery to immune cells was confirmed depending on the design of the self-assembling peptides.

Key Words : vaccine, adjuvant, oil-in-water emulsion, self-assembly peptide

Introduction

Vaccines are effective tools to prevent various infectious diseases or to prevent the severity of symptoms. The use of adjuvants, which are immunostimulants, is important to induce effective immune responses by vaccines. Therefore, the development of effective adjuvants with identified mechanisms of action is one of the most important issues in vaccine development. In this study, we focused on an oil-in-water (O/W) emulsion as the adjuvant formulation and aimed to develop self-assembled peptide-based O/W emulsions that show adjuvant effects for vaccines carrying protein antigens.

Results

We have prepared self-assembled peptide-based O/W emulsions by using peptide amphiphiles (PAs) (Fig. 1A). PAs spontaneously self-assemble in aqueous solutions by multiple intermolecular interactions, such as hydrophobic interaction and hydrogen bond formation. We have previously designed PAs with microbial transglutaminase (MTG) enzyme recognition sequences and demonstrated that proteins can be integrated on their self-assembled structures via the MTG reaction.[1,2] In this study, we synthesized several PAs with aromatic Pyrenyl and Fmoc groups in the hydrophobic part and LLQG sequence as an MTG recognition sequence. Additional amino acid was introduced between the aromatic groups and the LLQG sequence to investigate the influence of PA design on self-assembly, MTG reactivity, emulsion formation, and interaction with immune system. The obtained PAs were subjected to self-assemble in several aqueous solutions (water, citrate and phosphate buffers). Spectroscopic analyzes (fluorescence and Fourier transform infrared spectra) revealed that these PAs self-assembled by π-π stacking interactions between aromatic groups and hydrogen bond formation between peptides. The stacking interactions were influenced by the PA design or solution conditions, while hydrogen bond formation remained similar among PAs used in this study. The formation of fibrous structures was confirmed for all PAs by transmission electron microscopy and confocal laser scanning microscopy (CLSM) images.

A green fluorescent protein containing an MTG-reactive Lys-containing peptide sequence

(Ktag-EGFP) was used as a model antigen protein. Ktag-EGFP was reacted with pre-formed PA fibrous assemblies by the addition of MTG at 37 °C for 2 h. More than 80% of the Ktag-EGFP proteins reacted with PAs, which was confirmed by high performance liquid chromatography. The accumulation of Ktag-EGFP on the PA fibers was directly observed by CLSM. The antigen protein-loaded fibers were used to make O/W emulsions with squalene as the oil phase. Emulsion particles with diameters ranging from several hundred nanometers to several micrometers were produced, depending on the type of PA used. CLSM showed that most of the antigenic proteins and PA fibers located at the interface between the oil and aqueous phases, suggesting that these fibrous structures could stabilize the O/W emulsions (Fig. 1B).

Murine dendritic cells, DC2.4 cells, were used as immune cells for in vitro antigenic protein delivery study. The cells were treated with the O/W emulsions prepared above. As controls, fibers loaded with Ktag-EGFP protein, and Ktag-EGFP protein alone were also applied. The delivery rate of Ktag-EGFP into the DC2.4 cells was quantified by flow cytometry. The amount of proteins delivered to the cells was significantly higher when emulsions or fibers with direct conjugation of Ktag-EGFP were applied than when Ktag-EGFP alone was applied. The protein delivery efficiency showed the dependance on the design of the PAs, and tended to be higher when PAs containing basic or hydrophobic amino acids in the peptide sequence were used. The observation by CLSM indicated that the PA assemblies with higher protein delivery nature have tendency to accumulate on/near the cell membranes. The higher protein delivery efficiency by O/W emulsions or PA fibers was not observed when proteins were not directly bound to PAs by MTG reaction but were physically mixed with emulsions or fibers. Moreover, the difference in the cellular internalization pathway was suggested for emulsions and fibrous states.

Discussion & Conclusion

The loading of antigenic proteins directly onto the fiber-like structures formed by PAs using enzymatic reactions was shown to improve the protein delivery ratio to immune cells in a PA design-dependent manner. The accumulation of a large number of proteins on PA structures to form larger objects and the moderate hydrophobicity of the PA aggregates may have enhanced their affinity to cells. Using this PA fiber, a new form of O/W emulsion-type vaccine with antigenic proteins integrated at the O/W emulsion interface was successfully created. Although the immunizing effect could not be verified using experimental animals, the newly developed formulation in this study is expected to show high vaccine efficacy, since the delivery ability to immune cells was improved in vitro.

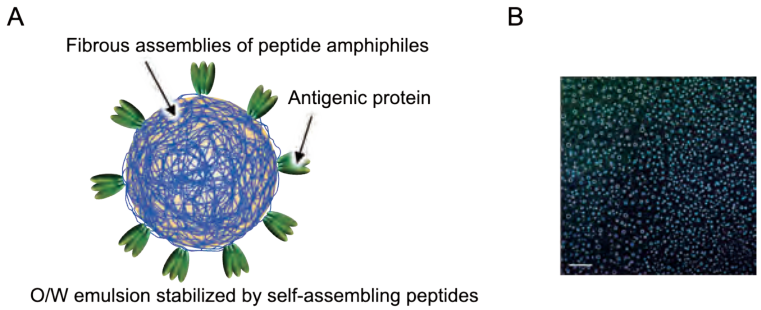

Fig. 1 (A) Schematic representation and (B) confocal microscopic image of O/W emulsion adjuvant developed in this study. Bar: 10 μm.

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一般の皆様へ

ワクチンは、感染症の感染予防あるいは感染による症状の重篤化を抑えるために有効です。ワクチン効果を高めるために、アジュバントと呼ばれる添加剤が使われます。本研究は、水の中に油滴が分散したエマルションという形状が示すアジュバント効果に着目しました。特に、水中で繊維状の構造体を形成する自己組織化ペプチドを用いて、水と油の界面に抗原となるタンパク質を集めることが可能な、新しいワクチンアジュバントを開発し、免疫細胞へ効率的に抗原タンパク質を運ぶことに成功しました。

Development of novel therapy for sarcopenia based on the regulation of O-linked N-acetylglcosamine motif

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Summary Abstract

In this study, we clarified whether O-GlcNAcylation of skeletal muscle proteins is involved in the progression of age-related skeletal muscle atrophy. Skeletal muscle in mice atrophied with age. This age-related skeletal muscle atrophy is achieved by an imbalance between protein synthesis and degradation. At the same time, O-GlcNAcylation of skeletal muscle proteins increased and GFAT1 protein expression increased. It was suggested that O-GlcNAcylation might be involved in the progression of skeletal muscle atrophy.
Key Words : Sarcopenia, skeletal muscle atrophy, O-GlcNAcylation

Introduction

The development of preventive and therapeutic methods for age-related sarcopenia is an extremely important research topic. Skeletal muscle atrophy is caused by an imbalance between protein synthesis and degradation [1]. O-GlcNAcylation is one of the post-translational modifications of proteins, which alters the functions of proteins in response to cellular stresses [2]. However, the role of O-GlcNAcylation in skeletal muscle atrophy has not been elucidated so far. The purpose of this study was to clarify whether O-GlcNAcylation of various proteins involved in protein synthesis and degradation plays an important role in the progression of age-related skeletal muscle atrophy.

Results

2-month-old, 5-month-old, and 11-month-old C57BL/6J mice were purchased (Japan SLC Co., Ltd.). Each mouse was housed under a 12-hour light/dark cycle for one month in an environment with free access to standard diet and water. All animal experiments, breeding and storage of mice used in experiments, and experimental plans were approved by the Kyushu University Animal Care and Use Committee, and were carried out in accordance with the Kyushu University Animal Experiment Practice Manual. At 3 months, 6 months, and 12 months of age, lower extremity skeletal muscle and epididymal fat were excised from each mouse under deep anesthesia (intraperitoneal administration of 200 mg/kg of pentobarbital). After weighing, skeletal muscle tissue was used for histological evaluation (hematoxylin-eosin staining), and Western blotting. Protein extraction, electrophoresis, and immunoblotting from skeletal muscle tissue samples were performed by standard methods. As primary antibodies, Akt, phospho-Akt, mTOR, phospho-mTOR, atrogin-1, O-GlcNAc, MuRF1, glutamine-fructose-6-phosphate amidotransferase (GFAT) 1, GFAT2, O-GlcNAc transferase (OGT), An antibody against O-GlcNAcase (OGA) was used.

The body weight of mice was increased significantly with age (3 months old: 28±1 g, 6 months old: 28±2 g, 12 months old: 32±3 g, $p<0.05$). Consistent with the increase in body weight, the fat weight-to-body weight ratio of 12-month-old mice was increased by 93% compared to 3-month-old mice. On the other hand, the lower extremity skeletal muscle weight-body weight ratio was decreased with aging (3 months old: 14.3±0.3 mg/g, 6 months old: 13.4±0.6 mg/g, 12 months old: 12.3±0.5 mg/g, $p <0.05$). Histological evaluation of skeletal muscle showed a 10% reduction in cell cross-sectional area in skeletal muscle of 12-month-old mice compared with 3-month-old mice. Skeletal muscle atrophy was found to occur with aging. Therefore, we evaluated molecules related to protein synthesis and degradation systems. Akt and mTOR protein expression did not differ at each age. Akt phosphorylation levels were reduced by 22% in skeletal muscle of 12-month-old mice compared to 3-month-old mice, and mTOR phosphorylation levels were reduced by 19%. Furthermore, atrogin-1 and MuRF1 protein expression were increased by 43% and 71% in skeletal muscle of 12-month-old mice compared with 3-month-old mice. Therefore, the balance of the protein synthesis/degradation system was broken. O-GlcNAcylation modification of skeletal muscle proteins was significantly increased in skeletal muscle of 12-month-old mice compared with 3-month-old mice ($p<0.05$). Among molecular proteins regulating O-GlcNAcylation, GFAT1 was significantly increased. On the other hand, GFAT2, OGT and OGA did not change significantly.

Discussion & Conclusion

In this study, skeletal muscle atrophy in aged (12 months old) mice was observed. This was achieved by decreasing the protein synthesis and increasing the protein degradation. At this time, it became clear that the proteins subjected to O-GlcNAc modification were increased. Increased O-GLcNAcylation was thought to be regulated by an increase in GFAT1. O-GlcNAcylation modification of skeletal muscle extract protein increased with age-related skeletal muscle atrophy. In this study, we could not clarify the causal relationship between increased O-GlcNAcylation and skeletal muscle atrophy. Treatment of cultured C2C12 skeletal muscle cells with dexamethasone has been shown to cause cell atrophy [3]. In this experiment, O-GlcNAcylation was increased, decreased Akt phosphorylation and increased Atrogin-1, MuRF1 expression [3]. This cultured cell result supports our current findings. In addition, O-GlcNAcylation in the myocardium was increased in a pressure-overload hypertrophic heart model, which was closely related to the activation of Akt phosphorylation, a key molecule for cardiac hypertrophy signaling [4]. Since the sites of O-GlcNAcylation and phosphorylation occur at or near the same site, it is thought to play an important role in regulating protein phosphorylation [5]. Therefore, it is suggested that age-related skeletal muscle atrophy, decreased skeletal muscle Akt phosphorylation, and O-GlcNAcylation modification are closely related. It is necessary to examine the causal relationship of this point in detail in the future.

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一般の皆様へ

本研究において、加齢に伴う骨格筋萎縮の進展に骨格筋タンパクの O-GlcNAc 化が関係していることを明らかにした。骨格筋萎縮の新たな分子機序を明らかにした点が極めて意義が高く、今後 O-GlcNAc 化を制御する薬剤の開発により、現代の社会的な問題である老化によるサルコペニアの克服に貢献できると考える。

Molecular mechanism of neuroprotection after ischemic stroke

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Summary Abstract

Ischemic stroke is the frequent cause of death and disability in adults worldwide. We investigated endogenous neuroprotective mechanisms for ischemic stroke, by using animal models. Our findings reveal that the condition of the brain just before stroke has a great impact on ischemic cell death. Transcription factor *Npas4*-dependent and *Npas4*-independent neuroprotective mechanisms function to promote cell survival in ischemic neurons.
Key Words : Ischemic stroke, neuroprotection, Npas4

Introduction

Ischemic stroke is the second leading cause of death and the most frequent cause of disability in adults worldwide. Stroke initiates complex pathological events in the brain, resulting in cell damage and loss of neurological function. It is important to know the factors that influence neuronal survival or death after stroke. In this study, we focused on endogenous neuroprotective mechanisms for ischemic stroke.

ResultsT

When the brain is pre-exposed to a brief period of ischemia, it can withstand subsequent lethal ischemia. This phenomenon is called ischemic tolerance. Intriguingly, non-noxious stimuli could also cause ischemic tolerance. For example, exercise for several weeks helps develop ischemic tolerance. However, it was unknown whether short-term exposure to non-noxious stimuli can induce ischemic tolerance. Recently, we found that mice pre-exposed to an enriched cage (provided with tubes, hiding place, glove etc) for a short period (40 min) acquired resistance to ischemic cell death (**1**). Activation of cortical neurons using AAV vectors carrying the DREADD (Designer Receptors Exclusively Activated by Designer Drugs) system showed that transient neuronal activity is sufficient to activate endogenous neuroprotective mechanisms. We named this phenomenon as “activity-dependent ischemic tolerance” (**2**). The activity-dependent transcription factor Npas4 (neuron PAS domain protein) is required for this activity-dependent ischemia tolerance (**1, 2**). The condition of the brain just before stroke has a great impact on ischemic cell death.

Previous study has reported that stroke onset time influences the effect of drugs on ischemic stroke (**3**). It suggests that brain conditions altered by circadian rhythms may affect ischemic resistance of the brain. Therefore, we investigated whether stroke onset time has a significant effect on cell death by stroke. We performed permanent MCAO (middle cerebral artery occlusion) surgery for wild-type mice (male, 6 weeks) during the day (zeitgeber time (ZT) 6) and nighttime (ZT6). Twenty-four hours after the surgery, live cells were stained with TTC (2,3,5-triphenyl tetrazolium chloride) and the volume of the dead cell area was measured.

Interestingly, infract volume in the cerebral cortex was remarkably reduced, when a stroke occurred at ZT15 compared to when a stroke occurred at ZT 6. It suggests that endogenous neuroprotective mechanisms function prominently at night. Since mice are nocturnal animals, their activity increases significantly at night. We speculated that the expression of the activity-dependent neuroprotective gene *Npas4* may remarkably increase in mice brain at night and facilitate cell survival after stroke. To reveal this point, we analyzed *Npas4* expression pattern in the cerebral cortex due to circadian rhythm. As expected, Npas4 was strongly expressed in the cerebral cortex during the night compared to the day. Next, we studied the contribution of increased Npas4 expression to neuroprotective mechanisms activated at night, by using Npas4-KO mice (male, 6 weeks).

Unexpectedly, *Npas4*-KO mice also showed the reduced cell death after stroke, when a stroke occurred at ZT 15 compared with at ZT 6. This result suggests that this time-dependent reduction in cell death is *Npas4*-independent. To reveal the molecular mechanisms underlying this *Npas4*-independent neuroprotection, we searched for genes that were remarkably increased (or decreased) in the cerebral cortex at night, and affected the cell survival after stroke. We found that some clock genes are more strongly expressed in cortical neurons at night than during the day. To reveal the neuroprotective function of these genes, we prepared dissociated cultured neurons from cerebral cortex at embryonic day 15. After the electroporation of candidate genes, cultured neurons were treated oxygen-glucose deprivation conditions (1% oxygen, no glucose). By using *in vitro* ischemic culture conditions, we are currently analyzing the neuroprotective functions of these candidate genes.

Discussion & Conclusion

Our findings suggest that several neuroprotective mechanisms function in ischemic neurons. One is activated by neuronal activity. This mechanism is induced by *Npas4* expression. We found that Npas4 induces the expression of the small Ras-like GTPase *Gem*, and Gem prevents the abnormal activation of L-type voltage gated calcium channels and promotes cell survival in ischemic neurons (**1**, **2**). On the other hand, *Npas4*-independent neuroprotective mechanism is strongly activated in mice brain during nighttime. We found several candidate molecules that are strongly induced in the cerebral cortex at night. We are currently analyzing the neuroprotective functions of these candidate genes.

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一般の皆様へ

脳梗塞により、脳では大規模な神経細胞死が起こります。一度失われた神経細胞は再生しないため、神経細胞を守ることが予後の改善に極めて重要です。私達は、脳梗塞の直後に、脳が自身を守るためのメカニズムを活性化することを見出しました。これらのメカニズムを人為的に強く活性化することができれば、新たな脳梗塞の治療法に繋がると考え、その基盤となる基礎研究を進めています。

Involvement of fusion proteins on morphogenesis of neuromuscular junction

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Summary Abstract

Skeletal muscle fiber is a large syncytium with multiple and evenly distributed nuclei. We performed single-nucleus RNA sequencing (snRNA-seq) analysis of embryonic mouse diaphragms, and found that some mRNA for cell fusion were specifically detected in neuromuscular junction (NMJ) nuclei in the transcriptome analysis. With further experiments, we revealed that proteins, which are encoded by the mRNA for cell fusion, were not specifically localized in NMJ of the skeletal muscles and acetylcholine receptors-accumulated regions of in vitro myotubes. We here revealed that some scaffold proteins for NMJ formation was involved in NMJ formation.

Key Words : Neuromuscular junction, snRNA-seq

Introduction

The neuromuscular junctions exploit a coordinated interaction between a presynaptic region comprised of the spinal motor neurons (SMNs) and a postsynaptic region comprised of multinucleated myofibers. The formation of postsynaptic compartmentalization in myofibers involves multiple mechanisms to create a sharp delineation between the synaptic and extrasynaptic domains of the myofibers. This has been most extensively studied by analyzing the expression patterns of acetylcholine receptor (AChR) subunits and signaling molecules to make AChR clusters. We already know that these proteins are specifically expressed in the subsynaptic myonuclei.

Results

We performed single-nucleus RNA sequencing (snRNA-seq) analysis of diaphragms in mouse embryos at embryonic day (E)12.5, 15.5, 18.5, and postnatal day (P)1.5. Furthermore, we investigated the transcriptional profiles of myonuclei in the embryonic myofibers by Seurat software. To our knowledge, this is the first report of snRNA-seq of embryonic skeletal muscles at different developmental stages. However, our analysis had a following limitation; snRNA-seq analysis at four developmental stages of E12.5, E15.5, E18.5 and P1.5 was likely to be insufficient to clearly delineate the lineage of nuclei of our interest.

Anyway, we found that each myonucleus had a distinct transcriptome pattern during the NMJ formation in embryogenesis (unpublished data). By the analyses, we found some mRNA for cell fusion including *Cav3*, which were specifically detected in neuromuscular junction (NMJ) myonuclei. Using transfection of fusion proteins, Cav3-GFP and clathrin heavy chain-GFP in myofibers, we revealed that Cav3 proteins, which are encoded by the *Cav3*, were not specifically localized in the subsynaptic NMJ region of the skeletal muscles, stained with

AChR clustering (Figure 1). Surprisingly, we also found that protein for clathrin heavy chain were specifically colocalized in the regions with AChR clustering. It suggested that membrane characters for subsynaptic region for NMJ were different to the other regions.

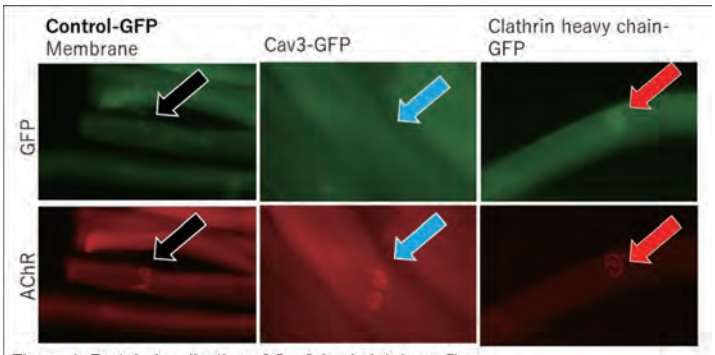

Figure 1. Protein localization of Cav3 in skeletal myofibers
Although clathrin heavy chain was colozalised with acetylcholine receptor (AChR), Cav3 protein was not

We thus focused on the genes, which encode proteins for clathrin-dependent structures with certain scaffold proteins and are expressed together with the genes for AChR subunits and signaling molecules, *Dok7*. We found that some genes for the scaffold protein complex beneath the plasma membrane were also expressed specifically, significantly, and correlatedly in the subsynaptic myonuclei together with genes for NMJ formation by the transcriptomic analysis. Using quantitative real-time PCR (qRT-PCR), we also confirmed that the genes for scaffold proteins were significantly expressed (1) in the center of E18.5 mouse diaphragms, and (2) in the *in vitro* C2C12 myotubes when the cells were differentiated and fused for 2 days. Using small hairpin RNA (shRNA) against some genes for the scaffold proteins, we revealed that the scaffold proteins were only involved in size and number of AChR clustering in C2C12 myotube, but did not affect gene expressions for the signaling pathway X in the myotubes.

By analyzing snRNA-seq, we counted number of myonuclei for total, NMJ, and MTJ as well as number of nuclei for the other cells such as; tendon cells, Schwann cells, perichondrium, proximal mesenchymal cells, endothelium cells, and chondrocytes in diaphragms during embryogenesis. These data newly revealed the characters of myogenic cells and other cells in embryonic diaphragms. In addition to subsynaptic myonuclei for NMJ, we also found expression profiles of myonuclei, which were likely localized closely to the myotendinous junction (MTJ) in the embryonic myofibers. We revealed that MTJ myonuclei expressed genes not only for myofibers but also for extracellular matrix. Interestingly, we found that both NMJ and MTJ myonuclei had gene profiles which showed activated signaling pathway of “X”. Previous reports suggested SMNs and cells in cartilage tissues expresses ligands which activate the signaling pathway X during embryogenesis to develop skeletal myofibers. In total, our further analysis resulted in novel coordinated gene expressions in embryonic NMJ and MTJ myonuclei.

Discussion & Conclusion

Taken together with all results, we have identified gene expression profiles of embryonic subsynaptic nuclei at the NMJ and MTJ in myofibers of the skeletal muscles. Genes which are associated with signaling pathway X and other NMJ-specific genes are attractive candidates to dissect the formation of the embryonic NMJ at the molecular level. Similarly, MTJ myonuclei has activated signaling pathway X and is likely to be stimulated by the ligands released from perichondrium and cartilage. Dissection of genes for the compartments of skeletal myofibers will be able to unveil finely tuned communications among myofibers and other cells in the skeletal muscle tissues during embryogenesis.

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Currently, a manuscript with the data of snRNA-seq is submitted to J. Neurochemistry.

一般の皆様へ

本研究の目的は、身体の中で比較的大きな細胞で、多核である筋肉細胞が胎生期にどのように発達し、領域を決定しているのかを、最新の技術であるシングル核 RNA-seq という手法で明らかにしたものです。本研究の結果は各発生段階の筋細胞の特徴を明らかにしただけでなく、今後筋肉の病態研究を行う際の基盤となる知識を含んでいます。

Inhibition of cancer metastasis by selective regulation of AM-RAMP2 and AM-RAMP3 systems

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Summary Abstract

Adrenomedullin (AM) is a multifunctional peptide regulated by receptor activity-modifying proteins (RAMPs). We investigated the actions of the AM-RAMP2 and 3 systems in the tumor microenvironment and their impact on metastasis. PAN02 pancreatic cancer cells were injected into the spleens of mice, leading to spontaneous liver metastasis. Tumor metastasis was enhanced in vascular endothelial cell-specific RAMP2 knockout mice (DI-E-RAMP2-/-). By contrast, metastasis was suppressed in RAMP3-/- mice, where the number of podoplanin (PDPN)-positive cancer-associated fibroblasts (CAFs) was reduced. Because PDPN-positive CAFs are a hallmark of tumor malignancy, we assessed the regulation of PDPN and found that Src/Cas/PDPN signaling is mediated by RAMP3. Activation of RAMP2 in RAMP3-/- mice suppressed both tumor growth and metastasis.

We suggest that upregulation of PDPN in DI-E-RAMP2-/- mice increases malignancy, while downregulation of PDPN in RAMP3-/- mice reduces it.

Key Words : Metastasis, Adrenomedullin, RAMP

Introduction

Adrenomedullin (AM) is a peptide that is widely produced throughout the body and has a wide variety of biological activities. AM activity is mainly regulated by the receptor activity modifying proteins RAMP2 and RAMP3. We have previously shown that AM-/- mice and RAMP2-/- mice are embryonic lethal due to abnormal vascular development. We have established mice that can induce the loss of RAMP2 in vascular endothelial cells (DI-E-RAMP2-/-) in adults and performed subcutaneous transplantation of melanoma cells. We found that metastasis to the lungs was enhanced and reported that vascular homeostasis by the AM-RAMP2 system suppressed cancer metastasis.

On the other hand, an important problem is that pancreatic cancer has a poor prognosis and is prone to postoperative liver metastasis. It has also been reported that RAMP3 expression is elevated in human pancreatic cancer samples.

In this study, we investigated the significance of the AM-RAMP2 and 3 system in organ-to-organ metastasis using DI-E-RAMP2-/- and RAMP3-/- mice.

Results

PAN02 pancreatic cancer cells were transplanted into the spleen and examined for metastasis to the liver. In DI-E-RAMP2-/-, primary tumor size was reduced, whereas metastasis to the liver was enhanced, confirming the reproducibility of the previous experimental results. Observing liver metastases in DI-E-RAMP2-/-, we found increased fibrosis around the cancer and an increase in αSMA-positive cancer-associated fibroblasts (CAFs), a marker of myofibroblasts, and these CAFs were podoplanin (PDPN)-positive. Clinically, cancers with PDPN-positive CAFs have been reported to have a poor prognosis. In contrast, DI-E-RAMP2-/- had a compensatory increase in RAMP3 expression with a decrease in RAMP2 expression. Therefore, we next investigated a similar organ-to-organ transition model in RAMP3-/- mice.

Cancer metastasis experiments using RAMP3-/- showed that, contrary to DI-E-RAMP2-/-, metastasis to the liver was suppressed. Furthermore, contrary to DI-E-RAMP2-/-, PDPN-positive CAFs around the tumor were reduced. Therefore, we proceeded to investigate the relationship between RAMP3 and PDPN in fibroblasts. Knockdown of RAMP3 in fetal-derived fibroblasts (MEFs) also reduced PDPN expression. Furthermore, in RAMP3-/- MEFs stimulated with PAN02 cell supernatants, the activities of Src and Cas, which are involved in regulating PDPN expression, were also reduced, indicating the existence of a Src-Cas-PDPN system downstream of RAMP3.

Next, we proceeded to examine primary cultures of CAFs from around metastases, and found reduced expression of αSMA in RAMP3-/- derived CAFs. In RAMP3-/- CAFs, intracytoplasmic stress fiber formation was suppressed, while actin formation just below the plasma membrane was enhanced, suggesting that mesenchymal epithelial transition (MET) occurred. Furthermore, RAMP3-/- CAFs suppressed cell migration and proliferation in a co-culture system with PAN02 cells. In array analysis, RAMP3-/- CAFs showed decreased expression of cancer-promoting factors and increased expression of inhibitory factors. Therefore, when cell supernatants of RAMP3-/- CAFs were added to PAN02 cells, the expression of the mesenchymal marker Vimentin was reduced and the proliferation and migration of PAN02 cells were also inhibited. In fact, when RAMP3-/- CAFs were mixed with PAN02 cells and transplanted into mice, tumour size was suppressed and liver metastasis was reduced, suggesting that RAMP3-/- CAFs are so to speak good CAFs that suppress cancer growth and metastasis.

Finally, when AM was continuously administered to RAMP3-/- to selectively activate the AM-RAMP2 system while the AM-RAMP3 system was blocked, cancer metastasis was further suppressed than in RAMP3-/-.

Discussion & Conclusion

These results suggest that in DI-E-RAMP2-/-, compensatory enhancement of the AM-RAMP3 system resulted in increased PDPN expression and enhanced cancer malignancy. In contrast, in RAMP3-/-, the reduction of malignant PDPN-positive CAFs was considered to be a consequence of reduced cancer metastasis. Selective RAMP2 activation and RAMP3 inhibition are expected to be a new therapeutic approach to suppress cancer metastasis.

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The endothelial adrenomedullin-RAMP2 system regulates vascular integrity and suppresses tumor metastasis.

Cardiovasc Res. 111(4):398-409. 2016

一般の皆様へ

この研究から、AM-RAMP2系が血管の恒常性維持作用を介して癌の転移を抑制するのに対して、AM-RAMP3系が癌関連線維芽細胞の悪性度を高め、癌転移を促進させることが分かりました。選択的なAM-RAMP系の制御が、癌転移を抑制する治療法に繋がることが期待されます。

Molecular mechanism for the mutual relationship between macrophage chemotaxis and activation

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Summary Abstract

The activation state of macrophages and their precursor cells, monocytes, is closely linked to the pathogenesis of inflammatory diseases and presents a potential therapeutic target. In this study, we explored the role of FROUNT, a molecule regulating the migration of monocytes/macrophages, in their activation. We discovered that inhibiting FROUNT suppressed not only the accumulation of monocytes and macrophages at inflammatory sites but also the expression of inflammatory cytokines, resulting in the amelioration of nephritis. These findings suggest that FROUNT may be a promising therapeutic target capable of regulating both the excessive accumulation and activation of monocytes/macrophages in inflammatory diseases.
Key Words : chemokine, macrophage, FROUNT, chemotaxis

Introduction

Activated monocytes/macrophages play a crucial role in promoting immune and inflammatory responses in various inflammatory diseases. Chemokines are known to mediate the migration and activation of immune cells at sites of inflammation (*1*). However, the molecular mechanisms of chemokine-mediated cellular activation are not yet fully understood. We previously reported that FROUNT mediates monocyte/macrophage chemotaxis by promoting the signaling of chemokine receptors CCR2 and CCR5 (*2, 3*). More recently, we demonstrated that disulfiram (DSF), an established drug used for treating alcoholism, potently inhibits FROUNT function (*4*). In the current study, we investigate the molecular mechanism of FROUNT-mediated monocyte-macrophage activation, as well as the inhibitory effects of DSF as a potential immune response modulator.

Results

To clarify the involvement of the chemokine signaling regulator FROUNT in the regulation of macrophage activation, morphological and gene expression analyses of inflammatory tissue from a nephritis model and bone marrow-derived macrophages were performed as follows:

1. Involvement of FROUNT in macrophage activation in response to various stimuli.

To assess the involvement of FROUNT in macrophage activation, cultured macrophages prepared from the bone marrow of FROUNT-deficient mice and macrophages treated with the FROUNT inhibitor disulfiram (DSF) were subjected to various stimuli, including LPS and Fc receptor ligation. Total RNA prepared from the stimulated macrophages were examined for cytokine expression by reverse transcription-quantitative PCR (RT-qPCR). Results show that FROUNT deficiency or DSF treatment reduces the expression of inflammatory cytokines and chemokines in stimulated macrophages. To investigate the signaling pathways affected in the FROUNT-deficient state, Western blot analysis was performed. The results showed that some kinases showed re-

duced phosphorylation, while others showed up-regulated phosphorylation in FROUNT-deficient macrophages. Morphological observations revealed that control macrophages showed lamellipodia-like pseudopodia upon stimulation. In contrast, FROUNT-deficient macrophages showed filopodia-rich pseudopodia before stimulation and showed little stimulation-dependent morphological changes.

2. Evaluation of FROUNT-targeting therapy in a model of macrophage-associated inflammation

The efficacy of targeting FROUNT on monocyte/macrophage activation and migration was investigated in a WKY rat model of anti-glomerular basement membrane (GBM) glomerulonephritis. In the control group, urinary albumin excretion, which reflects glomerular damage, gradually increased from day5, and a large amount of albuminuria was observed on day7. In contrast, no increase in albuminuria was observed in the DSF-treated group during the observation period. In accordance with them, crescentic formation and necrotizing glomeruli, the pathological feature of this disease, is significantly suppressed in DSF-treated group. DSF markedly reduced monocyte/macrophage numbers in glomeruli on day3, before glomerular damage occurred. In controls, monocytes accumulating in the glomeruli developed pseudopodia in the glomerular capillaries, whereas pseudopodia formation was inhibited in the DSF-treated group. This is consistent with in vitro observations that DSF blocks chemokine-dependent pseudopodia formation and chemotaxis of bone marrow-derived monocytes/macrophages. As for CD163-positive M2-like macrophage, they appear on day 7 in the control group, but are hardly found in DSF group. The RT-qPCR analysis showed that glomerular mRNA of inflammatory cytokines and chemokines such as TNFα, CCL2, and CXCL9 were significantly downregulated in the DSF-treated group compared with the control group. In addition, podocyte loss, which is reported to be induced by inflammatory cytokines such as TNFα, was reduced in the DSF group, suggesting that DSF inhibits the activation of macrophages accumulated in glomeruli and prevents glomerular damage with further monocyte recruitment and macrophage activation.

3. Crosstalk analysis of migration and activation signals.

To investigate whether there is crosstalk between chemokine receptor signaling and activation signaling, and to clarify the interconnection between cell motility and activation signaling, we tested the cytokine response of activated macrophages in CCR2-deficient condition or in the presence of the actin polymerization inhibitors, which alter cell motility and morphology. Macrophages from each condition were examined for morphological changes and cytokine expression. Although the results were not entirely consistent with FROUNT deficiency, they did provide new insights into the relationship between changes in cell movement and morphology and cytokine expression.

Discussion & Conclusion

These results suggest that both FROUNT deficiency and its inhibition via DSF treatment suppress the chemotactic response of monocytes/macrophages and their subsequent activation, leading to a reduction in the production of cytokines and chemokines. This results in a significant decrease in monocyte/macrophage numbers at inflammation sites. In cases of anti-GBM nephritis, this dampened inflammatory cytokine response leads to an attenuation of podocyte loss. Therefore, targeting FROUNT with disulfiram could serve as a highly effective and safe strategy in the treatment of glomerulonephritis, blocking both the monocyte/macrophage chemotactic response and their activation within the glomerulus (*5*). This study revealed the dual regulation

of monocytes/macrophage migration and activation via FROUNT, offering a potential new way to control harmful and excessive immune and inflammatory responses with fewer side effects. In conclusion, the strategy of targeting FROUNT appears promising in controlling macrophage-related responses. We plan to investigate further the underlying molecular mechanism in future studies.

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一般の皆様へ

私たちはこれまで、炎症・免疫反応において重要な働きを担う単球・マクロファージの遊走を促進する分子、FROUNT の機能について解析してきました。新たに、FROUNT が活性化に伴う炎症性サイトカインの発現にも関与することを明らかにしました。ラットの腎炎モデルにおいては、FROUNT の阻害活性をもつ既存の嫌酒薬ジスルフィラムが腎炎を強力に抑制することを見出しました。単球・マクロファージの遊走と活性化は相互に関連しており、これらが過剰に働きすぎると組織傷害や病的な線維化の原因ともなることから、FROUNT はこれら双方を制御可能な標的分子として期待されます。

Metabolic signaling regulation by RNA modification

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Summary Abstract

Key Words : RNA, RNA modification, metabolism, epitranscriptome, GPCR

Introduction

Among the soluble factors in vivo, factors of nucleic acid-type is limited to adenosine and ATP, and their diversity is extremely poor compared to other soluble factors. Recently, it has been discovered that RNA has as many as 150 different chemical modifications, which are responsible for post-transcriptional regulation of gene expression, and that abnormal RNA modifications can cause disease onset. In this study, we aimed to clarify the metabolism and physiological significance of soluble factors (modified nucleosides) derived from RNA modifications.

Results

1. Establishment of a comprehensive detection method for RNA modification metabolism

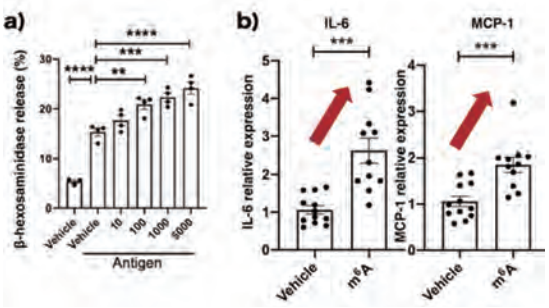
Biological samples were stored at -80°C after collection, thawed on the day of measurement, deproteinized by adding organic solvent, and then water was added to separate the liquid layer into two layers. The aqueous layer was collected after ultrafiltration. The collected aqueous layer was centrifugally concentrated and redissolved in ultrapure water, and the RNA-modified metabolites were detected by mass spectrometry.

2. Examination of bioactivity of modified RNA-derived catabolites

Using the above detection method, the distribution of RNA catabolites in extracellular fluid of various species including human was determined. Since these metabolites are modified nucleosides, we screened them for their activities as GPCR ligands. As a result, we identified N6-methyladenosine (m6A) as having specific activity against the adenosine A3 receptor. The activity was more than 10-fold stronger than that of unmodified adenosine, and m6A had activity against the adenosine A3 receptor even at only 1 nM.

3. Elucidation of the mechanism of activity of RNA-modified metabolites and identification of their physiological actions

To elucidate the mechanism of the potent receptor activity of m6A, we investigated the predicted structure by homology modeling and further supported by mutant assays to identify hydrophobic amino acid residues specific for m6A binding.



Next, we examined the regulatory mechanism of m6A by applying various external stimuli, and found that extracellular m6A increases by lysosomal degradation during cytotoxic stimulation such as cell injury, as demonstrated by the generation of a knockout cell line of the modifying enzyme. Furthermore, downstream signaling of the m6A-adenosine A3 receptor increases the production of type I allergy or local inflammatory cytokines in vivo(fig a & b).

Discussion & Conclusion

Receptor screening of modified nucleosides present in the human body using the shedding method revealed that m6A (N6-methyladenosine) binds specifically to the adenosine A3 receptor. The binding potency was about 10 times stronger than that of unmodified adenosine. Structural analysis identified amino acid residues involved in the specificity of m6A. m6A caused phosphorylation of ERK and elevation of intracellular calcium concentration, and these signaling pathways were confirmed to be adenosine A3 receptor dependent by inhibitor administration. In both the mast cell degranulation test and the passive cutaneous anaphylaxis (PCA) model in mice, m6A elicited allergic reactions. Furthermore, m6A administration to mice increased inflammatory cytokines and chemokines such as IL6 and MCP-1.

Recently, it has become clear that not only DNA and proteins but also RNA undergoes chemical modifications such as methylation and acetylation, and RNA modification is becoming a new research field next to epigenetics. In this study, we focused on RNA modification metabolism, optimized its comprehensive detection, and analyzed various samples using this method. Furthermore, a new metabolic pathway of m6A is currently being analyzed and is in preparation for submission (unpublished data not shown in this report). We were also able to clarify the RNA modification kinetics of the global pandemic COVID-19 vaccine (Ref2) or the receptor activity of the adverse effects of nucleic acid therapeutics (Ref1).

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2022 年 46 巻 2 号 p. 105-113
* 直接の成果となっている論文は投稿前です。

一般の皆様へ

近年、DNA やタンパク質だけでなく、RNA もメチル化やアセチル化といった化学修飾を受けることが明らかになり、RNA 修飾がエピジェネティクスに次ぐ新たな研究分野として定着しつつある。本研究では RNA 修飾代謝に着目し、その網羅的検出の最適化を行い、更にこの手法を用いて様々な検体を解析することにより成果を得た。更に m6A の新たな代謝経路についても現在解析を進め、投稿準備中である。また、世界的パンデミック COVID-19ワクチンの RNA 修飾動態あるいは核酸治療薬の副作用の受容体活性についても明らかにすることができた。

Catalytic Asymmetric Indole Halocyclization for Construction of Bioactive Structures

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Summary Abstract

Cooperative catalysis which consists of a *trans*-cyclooctene derivative, which retards background reactions, and an asymmetric catalyst, which provides enantioselectivity, enabled catalytic asymmetric induction in rapid halogenation reactions which have been difficult to conduct due to their background reactions. The concept of the catalyst system was also applied to catalytic asymmetric indole halocyclization.

Key Words : *trans*-cyclooctene, asymmetric catalyst, background reaction, bromination, indole

Introduction

Bromination is indispensable synthetic reactions because bromo-organic compounds are useful as functional molecules and synthetic intermediates. However, fast non-catalyzed background reactions disturb the catalytic control of enantioselectivity even when using *N*-bromoamide reagents, the reactivity of which is milder than that of bromine (Br_2). Bromocyclization of Indole derivatives also suffered from the problem. In this study, we developed catalyst systems to solve the problem and enable catalytic asymmetric indole bromocyclization.

Results

We developed a *trans*-cyclooctene (TCO) derivative to retard background reaction pathways in several bromocyclization reactions. In addition, mechanistic studies including NMR and Raman analyses of the reaction mixtures and control experiments revealed that a small amount of Br_2 generated in situ from *N*-bromosuccinimide (NBS) causes the background reactions. This is the first study to clarify the mechanism of the rapid background reactions which take place even when NBS is employed as a mild brominating reagent. Moreover, we found that the TCO reacts with Br_2 to deactivate it, thereby retarding the background reactions. It is notable that the use of less than stoichiometric amount of the TCO was sufficient to shut out the non-catalyzed reactions based on the Br_2-involved chain mechanism. Furthermore, the key species which was formed through the reaction of the TCO and Br_2 was isolated by gel permeation chromatography (GPC) purification, which allowed for investigations of its reactivity and characterization of its structure.

Next, based on these results, we developed cooperative catalysis which consists of the TCO and a chiral bromination catalyst. First, proof of concept was obtained in asymmetric bromocyclization of allylic amides: the cooperative catalysis improved the enantioselectivity and alleviated the reaction rate. Catalytic asymmetric bromocyclization of indole derivatives was also investigated, and some chiral catalysts provided enantioselectivity. However, this

reaction was halted in the presence of the TCO even with the chiral bromination catalyst. It indicates that Br_2 was also involved in the reaction mechanism of the chiral bromination catalyst cycle, and this mechanism efficiently inhibited the background reaction pathway. Thus, in this reaction, the chiral bromination catalyst proved to play two roles as the catalyst decelerating the background reaction and as the chiral bromination catalyst. These results provided another methodology controlling the background reactions involving Br_2.In addition, through the above-mentioned research, we focused on the ability of the TCO to inhibit not only non-catalytic background reaction pathways but also catalytic reaction pathways involving Br_2. Thus, we consider that the TCO serves as an indicator to investigate whether Br_2 is involved in the mechanism of unknown bromination reactions. Thus, we added the TCO to a catalytic asymmetric reaction which was reported to involve Br_2 in the catalytic reaction pathway,[1] and the TCO completely inhibited the catalytic reaction. Thus, we developed a function of the TCO as an indicator useful for mechanistic studies on bromination reactions like 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), which has been often used in mechanistic studies on radical reactions.

Discussion & Conclusion

In summary, we revealed that in situ generated Br_2 causes the non-catalyzed reaction pathways in several bromocyclization, and the TCO derivative was developed to efficiently retard the background reactions. Notably, the use of less than stoichiometric amount of the TCO was sufficient to shut out the non-catalyzed reactions based on the Br_2-involved chain mechanism. Furthermore, the TCO was useful as an additive for improving the enantioselectivity in the catalytic asymmetric reaction. The cooperative catalytic system using the TCO with chiral catalysts offers alternative methods for catalytic asymmetric induction in bromination that suffers from non-selective background reactions. In addition, the extended methodology using the asymmetric catalysts playing the two roles was useful for catalytic asymmetric indole bromocyclization. Moreover, the TCO also served as the indicator of Br_2 in the catalytic reaction pathway, and thus it was demonstrated to be useful for mechanistic studies to know whether Br_2 is involved in bromination reactions of interest.

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一般の皆様へ

医薬品などの機能性分子を効率よく合成することは、その安価・安定供給や新物質創成につながる基盤技術になり、人々の生活を豊かにします。不斉触媒はそれを実現する武器になりますが、速すぎる反応にはこれが利用できませんでした。この問題を解決するために、反応をあえて遅くする触媒を開発し、ハロゲン化反応による生物活性分子の合成を短工程化する反応原理を創り出しました。これをきっかけに、不斉触媒による医薬品合成がこれまで以上に発展すると考えています。

Analysis of structures and functions of plant nuclear actin microfilaments

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Summary Abstract

Recent studies using animal cells showed that actin microfilaments (AFs), one of cytoskeletons that functions in intracellular transports, are transiently formed in the nucleus and function in regulation of gene expression. However, the presence of nuclear AFs in plants was unknown. This study strongly suggested that nuclear AFs do exist and function in regulation of various plant physiologies.

Key Words : Actin microfilaments, *Arabidopsis thaliana*, gene expression, nucleus

Introduction

We previously found that *Arabidopsis thaliana* mutants of ACTIN DEPOLYMERIZING FACTOR (ADF), actin binding protein that regulates organization and dynamics of actin microfilaments (AFs), functions in the nucleus to regulate responses against pathogen. In addition, our preliminary data showed that *adf* mutants showed altered nuclear morphology and gene expression. Together with the recent animal research regarding nuclear AFs that transiently formed to regulate gene expression, we hypothesized that *A. thaliana* ADF regulates gene expression through regulation of nuclear AFs.

Results

A. thaliana genome possesses 11 *ADF* genes, which are divided into 4 subclasses. Subclass I *ADF*, containing *ADF1*, *-2*, *-3* and *-4*, express throughout the plant (Inada, 2017). We previously showed that *adf4* knockout mutant and *ADF1-4* knockdown plants (*ADF1-4Ri*) showed an increased resistance against powdery mildew fungus (Inada et al., 2016). In addition, both *adf4* and *ADF1-4Ri* exhibited an increase of plant size and an enhancement of endoreplication (Inada et al., 2021). Both pathogen resistance and increased plant size phenotypes were more significantly observed in *ADF1-4Ri* compared to *adf4*. Furthermore, we showed that nuclear localization of ADF4 is important for response against powdery mildew fungus (Inada et al., 2016).

While the main function of plant AFs is in regulation of intracellular transport at the cell periphery, recent research using animal cells showed that transiently formed nuclear AFs have various functions including regulation of gene expression. Gene expression is also affected by nuclear morphology (Pontvianne&Grob 2020). Based on those results, we hypothesized that *A. thaliana* ADFs regulate expression of genes for pathogen responses and plant growth through regulation of organization and dynamics of nuclear AFs.

To test this hypothesis, we first analyzed nuclear morphology in epidermal cells of fixed mature leaves of wild type (WT) Col-0, *adf4* and *ADF1-4Ri*. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) and observed with confocal laser scanning microscope (CLSM). Obtained fluorescent images were quantitatively analyzed using ImageJ. We found that *adf4* and *ADF1-4Ri* showed a significant decrease in size of heterochromatin, which is a nuclear domain in which chromatins are highly condensed and gene expression is suppressed. Neither heterochromatin number per nucleus nor nuclear size were altered in *adf4* and *ADF1-4Ri* (Matsumoto et al, 2023).

If alteration in heterochromatin size in *adf4* and *ADF1-4Ri* was caused through ADF's function in regulation of AFs, loss of actin could cause similar alteration in heterochromatin. *A. thaliana* genome encode 8 *ACTIN* genes, of which *ACT2*, *ACT7*, and *ACT8* are expressed in vegetative tissues, where subclass I ADFs express (Kandasamy et al.,2010). While *act2/8* double mutant did not show alteration in heterochromatin size, we found that the size of heterochromatin in *act7* increased compared to WT (Matsumoto et al, 2023).

Then we performed microarray analysis using Col-0, *adf4* and *ADF1-4Ri* to see if loss of *ADF*s causes alteration in gene expression. We found that expression of more than 1800 genes was altered in *adf4* and *ADF1-4Ri*. Particularly, genes related to pathogen responses showed altered expression. Those results clearly showed that ADF functions in regulation of nuclear organization and gene expression (Matsumoto et al., 2023).

To examine the possibility that regulation of nuclear AFs by ADF functions in regulation of nuclear organization and gene expression, we established *A. thaliana* lines for nuclear AF visualization. Actin-binding peptide Lifeact conjugated with GFP and nuclear localization signal (NLS) was expressed in Col-0, *adf4* and *ADF1-4Ri*. We found filamentous structures in nuclei in roots of Col-0, *adf4*, and *ADF1-4Ri*. The frequency that nuclei with filamentous structures was higher in *ADF1-4Ri* compared to Col-0 and *adf4*. The length of the filamentous structures was longer in *ADF1-4Ri* compared to Col-0 and *adf4*. Those filamentous structures labeled with Lifeact-GFP-NLS were more often observed in root tips, where cells were undifferentiated.

Discussion & Conclusion

By a microscopic observation of the nuclei, we showed that *A. thaliana* ADFs and ACTs function in regulation of nuclear organization. Furthermore, large-scale expression analysis revealed that a number of genes showed altered expression in *adf* mutants, indicating that ADFs function in regulation of gene expression. We hypothesized that ADFs regulate organization and dynamics of nuclear AFs, which regulate gene expression. We established plants that express actin filament-binding peptide conjugated with fluorescent protein and nuclear localization signal, and found that filamentous structures were observed in root cells. The frequency that filamentous structures were observed was higher, and length of nuclear filamentous structures was longer in *adf* mutants. Those results strongly suggest that ADF function in regulation of nuclear AFs, which are involved in regulation of gene expression.

References

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Pontvianne, F., and Grob, S. (2020) Three-dimensional nuclear organization in *Arabidopsis thaliana*. *Journal of Plant Research* 133, 479-488.

一般の皆様へ

細胞骨格の一種であるアクチン繊維は、人や植物などの生物を構成する細胞内において、主に物質の輸送を制御する器官である。最近の動物における研究で、アクチン繊維が細胞核の中でも遺伝子発現制御に働くことが明らかになっている。私たちは本研究で、これまで一部の動物細胞でしか解析されていなかった細胞核内アクチン繊維が、植物細胞でも存在し、遺伝子発現制御を通して植物の病害応答や成長に寄与していることを強く示唆した。今後は、植物生理機能の制御における植物細胞核内アクチン繊維の役割を更に明らかにしていきたい。

Establishment of embryonic diapause induction technique for in vitro culture of animal embryos.

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Summary Abstract

Many conventional studies on developmental regeneration have predominantly focused on "activity" and have achieved a certain degree of success. However, human and mouse embryos implanted in vitro or reconstructed from undifferentiated cultured cells bypass the "developmental quiescence" observed in vivo and proceed to the implantation process at a developmental stage unsuitable for successful implantation, giving rise to various functional and efficiency issues. From this study, we found that apparently developmentally diapause embryos are a mixture of embryos that can and cannot reactivate.
Key Words : Embryonic diapause, Implantation

Introduction

Embryonic diapause is a phenomenon exhibited by mammalian embryos, wherein the maternal intrauterine environment undergoes alterations in response to environmental cues, resulting in the temporary arrest of the cell cycle and cell differentiation in preimplantation embryos. The induction of developmental pauses acts as a regulatory mechanism for embryonic self-organization and facilitates a stable pregnancy by temporarily halting the embryo at a developmental stage conducive to implantation. Therefore, embryonic diapause represents a critically significant embryonic event; however, the molecular mechanisms underlying this process remain largely unknown.

Results

While developmental processes encompass both "active" and "quiescent" aspects, conventional research has predominantly focused on the former and achieved a certain level of success. Presently, worldwide efforts are underway to develop organoid technologies for regenerative medicine, aiming to generate diverse tissues and organs from embryonic stem (ES) or induced pluripotent stem (iPS) cells. However, significant challenges persist regarding functionality, size, and quality of these organoids. One contributing factor is the prevailing bias towards techniques that promote the release and activation of the quiescent state during embryonic development. Notably, human and mouse embryos implanted in vitro or reconstructed using undifferentiated cultured cells bypass the "Embryonic diapause" that would normally occur in vivo. Consequently, they proceed to the implantation stage at a developmental phase unsuitable for successful implantation, resulting in numerous functional and efficiency-related issues.

The objective of this study is to establish the groundwork for the realization of complete in vitro culture of embryos from embryonic animals through fundamental research on developmental arrest. By investigating the mechanisms underlying developmental pauses, this research endeavors to lay the foundation for achieving comprehensive in vitro embryonic culture.

In conventional studies on embryonic diapause, there has been a general assumption that all mouse embryos possess the ability to undergo embryonic diapause due to the observation of a significant number of reactivating mouse embryos during this developmental stage. However, through this study conducted by the principal investigator, which specifically aimed to explore individual variations, it was discovered that only approximately 70% of mouse embryos are capable of successfully reemerging, even when they exhibit apparent dormancy. In essence, the population of diapausing mouse embryos consists of a combination of genuinely hibernating embryos and those falsely categorized as diapause.

The transcriptome analysis using RNA-seq on individual embryos revealed the presence of two distinct groups. Furthermore, the identification of 35 differentially expressed genes (DEGs) associated with principal components (PC) provided insight into their potential involvement in determining the regenerative capacity of the embryos. Notably, some of these candidate factors underwent phenotypic screening, wherein knockout (KO) embryos for four of them exhibited disrupted developmental pauses. Based on this evidence, we posit a strong likelihood of an association between the PC component and the presence or absence of regenerative potential.

Moving forward, our future investigations will aim to ascertain whether a correlation exists between the individual variations observed in dormant embryos, as mentioned above, and the occurrence of regenerative processes. And we aim for in vitro culture of future embryonic embryos of embryonic animals.

The academic results obtained will lead to a breakthrough in the essential understanding of the phenomenon of developmental arrest, which will lead to the discovery of developmental arrest in primates, including human embryos, and its applied research. For example, they will lead to the development of technologies that have been considered an unattainable dream, such as artificial birth timing control and complete in vitro culture technology for embryos of embryonic animals, and their impact will be felt in many fields, including animal husbandry and human clinical practice. In this way, this research has the potential to produce innovations of great significance in both the academic and technological fields.

Discussion & Conclusion

Reflecting upon the trajectory of scientific progress, the domains of apoptosis, a form of cell death that garnered the Nobel Prize in Physiology or Medicine, and autophagy, an intracellular degradation mechanism, have evolved as academic disciplines through the identification and systematic understanding of fundamental principles underlying diverse biological phenomena. By elucidating a segment of the foundational model concerning inactivation phenomena through the proposed research, it becomes feasible to detect and analyze such inactivation phenomena in various contexts, including latent cancer. Moreover, through the discovery of fundamental principles governing inactivity across a broad spectrum of biological events and the elucidation of their biological significance, we will establish a novel research paradigm of programmed inactivity, akin to apoptosis (programmed cell death) and autophagy (programmed self-degradation).

一般の皆様へ

本研究では、発生休止というこれまで分子学的に研究されてこなかった現象の分子メカニズムを明らかにします。得られた知見は、将来の胎生動物胚の完全試験管培養といった夢のような技術の開発につながります。

Publication - FY2021 Grant Recipients

Title of the research project	Molecular mechanism of lysosomal damage repair by TFEB
Recipient (Institution)	Shuhei Nakamura (Graduate School of Frontier Biosciences, Osaka University)
Journal article / other material	PLOS Genetics doi.org/10.1371/journal.pgen.1010264
Title of the paper	PACSIN1 is indispensable for amphisomelysosome fusion during basal autophagy and subsets of selective autophagy

Title of the research project	Structural and functional analysis of primary cation transporters and elucidation of the substrate specificity by gain-of-function mutant
Recipient (Institution)	Kazuhiro Abe (Graduate school of Pharmaceutical Sciences, Nagoya University)
Journal article / other material	Nature communications 09 Sept. 2022 doi.org/10.1038/s41467-022-32793-0
Title of the paper	Structure and function of H+/K+ pump mutants reveal Na+/K+ pump mechanisms

Title of the research project	Hematopoietic stem cell aging and mitochondria aging
Recipient (Institution)	Takayoshi Matsumura (Division of Inflammation Research, Center for Molecular Medicine. Division of Cardiovascular Medicine., Jichi Medical University)
Journal article / other material	Nature Communications | (2022) 13:7064 doi.org/10.1038/s41467-022-34906-1
Title of the paper	A Myb enhancer-guided analysis of basophil and mast cell differentiation

Title of the research project	Significance and spatial profiling of phospholipid metabolism in NASH
Recipient (Institution)	Hayato Nakagawa (Department of Gastroenterology and Hepatology, Mie University)
Journal article / other material	J Clin Invest. 2022;132(11):e151895. doi.org/10.1172/JCI151895.
Title of the paper	Inhibiting SCAP/SREBP exacerbates liver injury and carcinogenesis in murine nonalcoholic steatohepatitis

Title of the research project	Cryo-electron microscopy studies for disease pathogenesis of Xeroderma Pigmentosum
Recipient (Institution)	Syota Matsumoto (Institute for Quantitative Biosciences, The university of Tokyo)
Journal article / other material	Cancer Science.23-May-2023, doi.org/10.1111/cas.15850
Title of the paper	Chromatin structure related to oncogenesis

Title of the research project	Analysis of brain aging caused by the disruption of "nuclear-axon crosstalk" machinery
Recipient (Institution)	Kenichiro Kuwako (School of Medicine, Department of Neural and Muscular Physiology , Shimane University)
Journal article / other material	Development (2022) 149, dev201214. doi:10.1242/dev.201214
Title of the paper	N-WASP-Arp2/3 signaling controls multiple steps of dendrite maturation in Purkinje cells in vivo

Title of the research project	Elucidating the mechanism of skin stem cell aging by targeting anti-aging matrix Fibulin-7
Recipient (Institution)	Aiko Sada (International Research Center for Medical Sciences (IRCMS), Kumamoto University)
Journal article / other material	EMBO Rep . 2022 Dec 6;23(12):e55478. doi: 10.15252/embr.202255478. Epub 2022 Oct 24.
Title of the paper	The extracellular matrix fibulin 7 maintains epidermal stem cell heterogeneity during skin aging

Title of the research project	Studies of genetic basis of early onset cardiac conduction system disease and disease mechanism-based personalized medicine
Recipient (Institution)	Kenshi Hayashi (School of Health Sciences, College of Medical, Pharmaceutical and Health Sciences, Kanazawa University)
Journal article / other material	Journal of Molecular and Cellular Cardiology doi.org/10.1016/ j.yjmcc.2023.03.001
Title of the paper	The utility of zebrafish cardiac arrhythmia model to predict the pathogenicity of KCNQ1 variants

Title of the research project	Study of functional deficits of microglia and accelerated onset of neurodegenerative disorders
Recipient (Institution)	Keiro Shirotani (The Laboratory of Genome-based Drug Discovery, Nagasaki University)
Journal article / other material	J. Biochem 2022;00(00):1–7 doi.org/10.1093/jb/mvac073
Title of the paper	The role of TREM2 N-glycans in trafficking to the cell surface and signal transduction of TREM2

Title of the research project	Intrinsic barrier dysfunction in multiple sclerosis patient
Recipient (Institution)	Hideaki Nishihara (Department of Neurotherapeutics, Yamaguchi University)
Journal article / other material	Adv. Healthcare Mater. 2022, 11, 2200804 doi: 10.1002/adhm.202200804
Title of the paper	The Modular μSiM: A Mass Produced, Rapidly Assembled,and Reconfigurable Platform for the Study of Barrier Tissue Models In Vitro

Title of the research project	Molecular mechanism of neuroprotection after ischemic stroke
Recipient (Institution)	Hiroo Takahashi (Department of Molecular Neurobiology, Kagawa University)
Journal article / other material	Neuroscience Research 186 (2023) 3.9 doi: 10.1016/j.neures.2022.10.005. Epub 2022
Title of the paper	Molecular mechanisms underlying activity-dependent ischemic tolerance in the brain

Title of the research project	Molecular mechanism for the mutual relationship between macrophage chemotaxis and activation
Recipient (Institution)	Etsuko Toda (Department of Analytic Human Pathology, Nippon Medical School)
Journal article / other material	Kidney Int.2022 Dec. doi.org/10.1016/j.kint.2022.07.031
Title of the paper	nhibition of the chemokine signal regulator FROUNT by disulfiram ameliorates crescentic glomerulonephritis

Title of the research project	Catalytic Asymmetric Indole Halocyclization for Construction of Bioactive Structures
Recipient (Institution)	Keisuke Asano (Institute for Catalysis, Hokkaido University)
Journal article / other material	Chem. Rec. 2022, e202200200 doi.org/10.1002/tcr.202200200 tcr.wiley-vch.de
Title of the paper	Organocatalytic Access to Tetrasubstituted Chiral Carbons Integrating Functional Groups

＜論文掲載＞ 2021 年度受賞者

助成タイトル	TFEB による損傷リソソーム修復機構の解明
受賞者	中村 修平 (大阪大学 大学院生命機能研究科)
論文掲載誌・書誌事項	PLOS Genetics doi.org/10.1371/journal.pgen.1010264
論文タイトル	PACSIN1 is indispensable for amphisomelysosome fusion during basal autophagy and subsets of selective autophagy

助成タイトル	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
受賞者	阿部 一啓 (名古屋大学 創薬科学研究科)
論文掲載誌・書誌事項	Nature communications 09 Sept. 2022 doi.org/10.1038/s41467-022-32793-0
論文タイトル	Structure and function of H+/K+ pump mutants reveal Na+/K+ pump mechanisms

助成タイトル	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
受賞者	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)
論文掲載誌・書誌事項	Nature Communications	(2022) 13:7064 doi.org/10.1038/s41467-022-34906-1
論文タイトル	A Myb enhancer–guided analysis of basophil and mast cell differentiation

助成タイトル	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
受賞者	中川 勇人 (三重大学 大学院医学系研究科消化器内科学)
論文掲載誌・書誌事項	J Clin Invest. 2022;132(11):e151895. doi.org/10.1172/JCI151895.
論文タイトル	Inhibiting SCAP/SREBP exacerbates liver injury and carcinogenesis in murine nonalcoholic steatohepatitis

助成タイトル	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
受賞者	松本 翔太（東京大学 定量生命科学研究所　胡桃坂研究室）
論文掲載誌・書誌事項	Cancer Science.23-May-2023, doi.org/10.1111/cas.15850
論文タイトル	Chromatin structure related to oncogenesis

助成タイトル	“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
受賞者	桑子 賢一郎（島根大学 医学部 神経・筋肉生理学）
論文掲載誌・書誌事項	Development (2022) 149, dev201214. doi:10.1242/dev.201214
論文タイトル	N-WASP-Arp2/3 signaling controls multiple steps of dendrite maturation in Purkinje cells in vivo

助成タイトル	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
受賞者	佐田 亜衣子（熊本大学 国際先端医学研究機構）
論文掲載誌・書誌事項	EMBO Rep . 2022 Dec 6;23(12):e55478. doi: 10.15252/embr.202255478. Epub 2022 Oct 24.
論文タイトル	The extracellular matrix fibulin 7 maintains epidermal stem cell heterogeneity during skin aging

助成タイトル	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
受賞者	林 研至（金沢大学 医薬保健研究域保健学系）
論文掲載誌・書誌事項	Journal of Molecular and Cellular Cardiology doi.org/10.1016/j.yjmcc.2023.03.001
論文タイトル	The utility of zebrafish cardiac arrhythmia model to predict the pathogenicity of KCNQ1 variants

助成タイトル	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
受賞者	城谷 圭朗 (長崎大学 ゲノム創薬学研究室)
論文掲載誌・書誌事項	J. Biochem 2022;00(00):1-7 doi.org/10.1093/jb/mvac073
論文タイトル	The role of TREM2 N-glycans in trafficking to the cell surface and signal transduction of TREM2

助成タイトル	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
受賞者	西原 秀昭 (山口大学 神経・筋難病治療学講座)
論文掲載誌・書誌事項	Adv. Healthcare Mater. 2022, 11, 2200804 doi: 10.1002/adhm.202200804
論文タイトル	The Modular μ SiM: A Mass Produced, Rapidly Assembled,and Reconfigurable Platform for the Study of Barrier Tissue Models In Vitro

助成タイトル	脳梗塞から神経細胞を守る分子メカニズムの解析
受賞者	高橋 弘雄 (香川大学 分子神経生物学)
論文掲載誌・書誌事項	Neuroscience Research 186 (2023) 3.9 doi: 10.1016/j.neures.2022.10.005. Epub 2022
論文タイトル	Molecular mechanisms underlying activity-dependent ischemic tolerance in the brain

助成タイトル	マクロファージの「動き」と「活性化」の相互連関メカニズム
受賞者	遠田 悦子 (日本医科大学 解析人体病理学)
論文掲載誌・書誌事項	Kidney Int.2022 Dec. doi.org/10.1016/j.kint.2022.07.031
論文タイトル	nhibition of the chemokine signal regulator FROUNT by disulfiram ameliorates crescentic glomerulonephritis

助成タイトル	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
受賞者	浅野 圭佑（北海道大学 触媒科学研究所）
論文掲載誌・書誌事項	Chem. Rec. 2022, e202200200 doi.org/10.1002/tcr.202200200 tcr.wiley-vch.de
論文タイトル	Organocatalytic Access to Tetrasubstituted Chiral Carbons Integrating Functional Groups

Ⅲ.

Reports from the Recipients of Grants for International Meetings

Report on Research Meeting

1. Name of Research Meeting / Conference
   The 20th International Symposium on Spontaneously Hypertensive Rats

2. Representative
   Norihiro Kato (chair of the meeting)

3. Opening period and Place
   October 16-17, 2022
   Shiraikaikan Annex, 11-1 Yoshida-Konoe-cho, Sakyo-ku, Kyoto, JAPAN

4. Number of participants / Number of participating countries and areas
   53 participants / 9 countries in Asia, Europe, North America and Oceania

5. Total cost
   JPY 1,419,618

6. Main use of subsidy
   Equipment operating expenses

7. Result and Impression

   The International SHR (Spontaneously Hypertensive Rats) Symposium was first held in Kyoto in 1971 with the aim of contributing to the development of preventive and therapeutic methods for hypertension and stroke by bringing together researchers from around the world who are engaged in various research projects related to hypertension using disease models such as SHR to present and discuss their research results and to raise awareness of research on hypertension and stroke and other organ complications.

   Since then, the symposium has continued to be held about once every two years, and a total of nine countries have hosted the symposium so far. Since the International Society of Hypertension (ISH) held in Fukuoka in 2006, the symposium has been recognized as a satellite symposium of the ISH.

   In 2022, the ISH would be held in Japan (Kyoto), and this meeting was planned as a satellite symposium to discuss (1) achievements and prospects of disease models and (2) prevention strategies based on precision medicine.

   The meeting was initially planned to be held in a hybrid format, with a significant number of local (i.e., on-site) participants and viewers expected. However, due to the COVID-19 pandemic, we decided to hold the two-day conference in a semi-hybrid format, which reduced the number of on-site participants as much as possible and increased the weight of online participation.

   On the afternoon of the first day (October 16), there were oral presentations at two sessions and poster presentations. On the morning of the second day (October 17), oral presentations were given at two more sessions. This conference (all English session) featured presentations and Q&A sessions by a total of 21 leading researchers from Japan and abroad. The titles of the sessions and symposiums are as follows

Session 1: System-oriented study on hypertension and cardiovascular complications.
① Symposium 1 (Yamori et al., 4 speakers): Hypertension model research in the past, present and future.
② Symposium 2 (presented by Kato et al.): Mechanistic understanding of hypertension.
Session 2: Precision medicine and prevention of hypertension-related disease
③ Symposium 3 (presented by Charchar et al.): Recent progress in the pathophysiology of hypertension
④ Symposium 4 (presented by Munroe et al.): Bridging the gap between bench and bedside

Thus, the participants were able to discuss and exchange new knowledge with researchers from Japan and abroad regarding research using disease models for lifestyle-related diseases.

8. Additional description

Due to the time difference between Japan and the local area, the order of the presentations was slightly changed. However, many researchers participated in real time from overseas despite the late night and early morning, and we had a lively exchange of opinions.

Lastly, we would like to express our sincere gratitude to the NOVARTIS Foundation (Japan) for the Promotion of Science for their support.

Report on Research Meeting

2022/12/19

1. Name of Research Meeting / Conference
   10th Asian Biological Inorganic Chemistry Conference (AsBIC10)

2. Representative
   Shinobu Itoh, Professor
   Osaka University, Graduate School of Engineering, Division of Applied Chemistry
   2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

3. Opening period and Place
   November 28 – December 3, 2022
   Kobe International Conference Center, Ariston Hotel Kobe, Kobe Portopia Hotel

4. Number of participants / Number of participating countries and areas
   354 participants / 26 countries / Japan, Korea, India, Hong Kong, China, Australia, Singapore, Taiwan, New Zealand, Philippine, Israel, Malaysia, Cyprus, USA, UK, France, Germany, Switzerland, Poland, Canada, Belgium, Spain, Italy, Iceland, Hungary, South Africa

5. Total cost
   ¥33,950,000

6. Main use of subsidy
   Support for the accommodations of plenary and award lectures

7. Result and Impression
   AsBIC10 will bring together researchers, educators, postdoctoral fellows, and students working on 'bioinorganic chemistry' to share the latest information and promote the development of this field and international mutual understanding as well as to foster young researchers. To this end, 10 plenary lectures, 5 AsBIC award lectures, 32 keynote lectures, 99 invited talks, 25 oral presentations, and 127 poster presentations were presented related on the following themes: (1) Metal Homeostasis, (2) Metallodrugs, (3) Metal-based Mechanism in Diseases, (4) Metal Sensing, (5) Natural and Artificial Photosynthesis, (6) Engineering Metalloproteins, (7) Metalloproteins and Functional Models, (8) Models and Mechanism, (9) Bioinspired Catalysis, (10) Nano/Tech Material, (11) Integrated Biometal Sciences, (12) Molecular Movies in Structural Metallo-biology.

8. Additional description
   This is an unprecedented large-scale, full face-to-face international conference carried out a difficult condition due to the Corona Disaster. We hope that the success of this conference will serve as a foundation for future international conferences.

ReportonResearchMeeting

1. Name of Research Meeting / Conference
Symposium on Molecular and Cellular Cognition

2. Representative
Satoshi Kida; President of MCCS-Asia, Professor, the University of Tokyo

3. Opening period and Place
Date: June 27-28, 2022
AM9-PM7, June 27
AM9-AM5:30, June 28
Venue: Ito Hall, The Ito International Research Center, The University of Tokyo 7-3-1, Hongo, Bunkyou-ku, Tokyo ZIP 113-0033
https://www.u-tokyo.ac.jp/adm/iirc/en/hall.html

4. Number of participants / Number of participating countries and areas
The number of participants; about 200 (Approximately 70 people participated in the event locally)
Number of participating countries and areas;9 countries including Japan (Australia, Canada, China, France, Israel, South Korea, UK, USA)

5. Total cost
400,000yen

6. Main use of subsidy
Travelexpensesforspeakers

7. Result and Impression
MCCS-Asia is the Asian branch of MCCS (headquartered in the United States), which holds an international symposium in Asian countries every two years. The symposium was originally scheduled to be held in 2021, but due to the difficulty of holding the symposium because of the COVID-19 pandemic, it was postponed for one year and held on June 27-28, 2022, inviting top-class researchers from Japan and abroad. The theme of the symposium focused on research to elucidate the mechanisms of brain function and psychiatric disorders. Since the impact of the COVID-19 pandemic is still strong and we have travel restrictions to Japan, we decided to have the meeting in a hybrid manner. Researchers who were unable to come to Japan were asked to make online presentations. Registration was required for those who wished to participate, but there was no charge for participation. We also took thorough measures to prevent infection at COVID-19, such as installing acrylic partitions on the podium and requiring the wearing of masks.
On both June 27 and 28, from 9:00 a.m. to 5:00 p.m., a total of 30 speakers gave 20-minute talks followed by a 5-minute Q&A. Alicia Che (Yale University, USA), Timothy

W. Bready (University of Queensland, Australia), Anatol Kreitzer (University of California, San Francisco, USA), Ted Abel (University of Iowa, USA), Mazen Kheirbek (University of California, San Francisco, USA), and Juan Song (University of North Carolina, USA), Matthew Girgenti (Yale University, USA), Paul Frankland (University of Toronto, Canada), Takashi Kitamura (University of Texas, USA), Gisella Vetere (ESPCI Paris, France), and Yong-Seok Lee (Seoul National University, Korea) visited Japan and gave lectures there. On the other hand, Jelena Radulovic (Albert Einstein College of Medicine, USA), Gustavo Turecki (McGill University, Canada), Weidong Li (Shanghai Jiao Tong University, China), Zhihua Gao (Zhejiang University, China), Hyungju Park (Korea Brain Res Inst, Korea), Kobi Rosenblum (University of Haifa, Israel), Gemma Modinos (King's College London, UK), and Justin Lee (Inst Basic Sci, Korea) gave their lectures online. 11 researchers from Japan gave them in the Ito hall: Yasunori Hayashi (Kyoto University), Satoshi Kida (University of Tokyo), Tadafumi Kato (Juntendo University), Kanzo Suzuki (Tokyo University of Science), Takanobu Nakazawa (Tokyo University of Agriculture), Akiko Hayashi (RIKEN), Takeshi Imai (Kyushu University), Shusaku Uchida (Kyoto University), Tomoyuki Furuyashiki (Kobe University), Atsushi Kasai (Osaka University), and Takuya Sasaki (Tohoku University). The presentations were at the cutting edge of research results. The Q&A session was so active that many questions were asked both onsite and online, so much so that the break time was shortened.

In addition, 19 poster presentations were given on June 27 from 5:00 p.m. to 7:00 p.m. The speakers who attended the conference room scored the poster presentations, and four of them received the Poster Best Presentation Award.

Although there were significant restrictions due to the COVID-19 pandemic, lectures in the Ito Hall and online performances from overseas were also conducted without delay. In addition, online delivery and question-and-answer sessions online as well as locally were conducted without problems. In addition, poster presentations were also successful, although only locally. As described above, the international symposium was conducted the same way as before the COVID-19 pandemic, and it was more convenient to conduct the symposium because it allowed online presentations and participation. I was grateful that researchers from overseas also understood and cooperated with the mask-wearing requirement in Japan, although they are not required to wear masks in their own countries. I would also like to thank almost all the participants from my laboratory who took part in this symposium and played a behind-the- scenes role. I have no information that any participants were infected with the COVID-19 after participating in the symposium.

8. Additional description

I would like to express my sincere gratitude to the NOVARTIS Foundation (Japan) for the Promotion of Science for their cooperation in organizing such an excellent symposium.

The pictures of the conference.

Report on Research Meeting

Date of Report: September 13, 2022

1. Name of Research Meeting / Conference
   11th International Tunicate Meeting (11th ITM)

2. Representative
   TAKEHIRO KUSAKABE
   Professor
   Graduate School of Natural Science, Konan University

3. Opening period and Place
   July 11, 2022 - July 15, 2022 (5 days)
   Konan University - Okamoto Campus 8-9-1 Okamoto, Higashinada-ku Kobe 658-8501, Japan

4. Number of participants / Number of participating countries and areas
   151 participants / 18 countries (Japan and 17 others)

5. Total cost
   2,091,500yen

6. Main use of subsidy

   Labor costs: Graduate students and undergraduate students of Department of Biology and Department of Nanobiochemistry of Konan University were hired as meeting staffs.

   Travel fellowships: Travel fellowships (called ITM-NOVARTIS Travel Fellowship) were awarded to four graduate students (three from Japanese Universities and one from abroad) who presented a high quality paper at the meeting and whose travel expenses were not fully supported by their own institutes and other fellowships or grants.

7. Result and Impression

   Under the Covid-19 pandemic situation, we decided to organize the meeting in a hybrid style, combination of on-site and on-line presentations/participation. Every paper was given by sharing the presentation slides in zoom meeting, irrespective of whether speakers are on site or not. When talks were given in Zoom by online speakers, the talks were also projected on the screen in the meeting room. Zoom movies of talks were recorded in real-time during the meeting days and were uploaded for on-demand viewing shortly in the same day. Preparation and running of the hybrid system was very hard due to both technical reasons and time differences among countries and areas, but the self-sacrificing works of the meeting staffs, most of them were graduate students hired by the grant from the NOVARTIS Foundation for the Promotion of Science, made it possible.

   As we reported above, we had 151 participants, including 8 oversea on-site attendees (from USA, Belgium, France, Italy, and Israel). Among them, 67 are residents in Japan

and 84 are from other countries. Ratio of principal investigators/senior researchers : post-docs/students are 81:70. Thus, nearly half of the participants were post-docs and students and we could support their attendance by the travel fellowship from the grant from the NOVARTIS Foundation for the Promotion of Science. We had 74 regular talks as well as a technical session and round table discussion. The round table discussion was scheduled at 24:00-25:00 on 14th of Japanese time, because this would be a time slot at which most of people on the globe was able to attend the session.

The following are some of the messages from the meeting participants that we received after the meeting:

"Dear organizers, What a wonderful conference! You all did such a terrific job in organizing and putting everything together in a marvelous way. Please also deliver my deepest thanks to the students that worked so hard behind the scenes."

"Thanks to the organizers and participants for a very successful 11th International Tunicate Meeting in Kobe (and around the world!). Despite all the challenges presented by the uncertainties of the past few years, and the hybrid format of the meeting, the organizers were able to create a fully functional international meeting and meet a critical need for the tunicate research community. The science was as strong as at any prior meeting, showing the great resilience of the community during the coronavirus pandemic. Congratulations to all who contributed to the organization, and to all who presented their work. All very much appreciated."

As we can tell from the above messages from participants, the meeting was frank and enthusiastic atmosphere and we had very active discussion throughout the meeting. As a whole, the meeting was really successful. On behalf of the Organizing Committee, I would like to express our sincere gratitude to the NOVARTIS Foundation for the Promotion of Science.

Report on Research Meeting

1. Date
   Nov. 29, 2022

2. Name of Research Meeting / Conference
   JSICR/MMCB 2022 Joint Symposium

3. Representative
   Shigeru Kakuta, PhD., DVM.
   Chair, The 86th Annual Meeting of the Japanese Society of Interferon & Cytokine Research (JSICR2022)
   Laboratory of Biomedical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo

4. Opening period and Place June 9-10, 2022
   Yayoi Auditorium, The University of Tokyo

5. Number of participants / Number of participating countries and areas
   Number of participants: 185
   Number of participating countries: 5

6. Total cost
   JPY 9,252,000-

7. Main use of subsidy
   Labor cost for stuffs (¥150,000)
   Others (website creation and maintenance ¥50,000; Video device and streming ¥200,000)

8. Result and Impression

   The main theme of JSICR/MMCB 2022 Joint Symposium was “New horizons in Immune- mediated homeostasis and diseases.” 4 symposiums by each 4-5 invited speakers and 2 special talks by Dr. Matsushima and Dr. Yoshimura were held by on-site (Yayoi Auditorium)/on-line (Zoom) hybrid style. Among invited speakers from foreign countries, Glen Barber (Miami University), Helen Goodridge (Cedars-Sinai Medical Center), Simon Yona (Hebrew University) and Dmitry Gabrilovich (AstraZeneca) came to Japan and talked about their research on-site, and the others joined online. We could directly discuss with speakers using excellent online tools. In addition, 56 regular presentations were held by on-demand video presentation style, but interactive Q&A was possible using ONLINE CONF system. Among these presentations, JSICR/MMCB 2022 Young Investigator Award was given to 6 young researchers, Kohei Soga, Ryosuke Hiranuma (The University of Tokyo), Tanakorn Srirat (Keio University), Haruka Shimizu (Tokyo University of Science), Ka Man Tse (Kyoto University) and Takashi Kato (Wakayama Medical University). Although an official party could not be held on during this symposium, participants enjoyed and got the latest scientific information.

As special plans, Symposium 1 (June 9) “Immune Regulation against viral infection and type 2 immunity” was cosponsored by AMED iD3 catalyst unit and held as “The 7th Translational and Regulatory Sciences Symposium”. 120 non-members attended to this symposium by Zoom via AMED iD3 catalyst unit. Symposium 1 (June 10) “Immune Regulation by Innate Immune Sensor Molecules” was held as “The 2nd ARS seminar, 2022” in Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo. 49 graduate students of The University of Tokyo attended to this symposium by Zoom to earn a credit. As a result, not only our society members, but also many participants form various community joined to this symposium.

36th Grant Report (FY2022)

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 2022 are as follows.

36th Novartis Research Grant:	39 Researchers (JPY 1.0 mil.),	Subtotal JPY 39.0mil.
Research Meeting Grant:	5 Meetings (JPY 0.4 mil.),	Subtotal JPY 2.0mil.
		Total JPY 41.0mil.

36th Novartis Research Grant (FY2022)

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	Soichi Sano	Department of Cardiology, Graduate School of Medicine, Osaka Metropolitan University	Junior Associate Professor / Lecturer	Y Chromosomal Genes Protective against Heart Failure
2	Susumu Goyama	Division of Molecular Oncology, Graduate School of Frontier Sciences, The Tokyo University	Professor	Development of in vivo gene therapy using HSC-targeting lipid nanoparticles
3	Mika Nomoto	Center for Gene Research, Nagoya University	Assistant professor	Elucidation of the molecular mechanism of plant immune response activated by mechanosensory trichomes
4	Juro Sakai	Division of Metabolic Medicine, The University of Tokyo, Research Center for Advanced Science and Technology	Professor	Elucidation of anti-obesity effect by signal sensing epigenetic modifier-mediated mitochondrial biogenesis
5	Kojiro Mukai	Laboratory of Organelle Pathophysiology, Graduate School of Life Sciences, Tohoku University	Assistant Professor	The molecular mechanism governing STING activation
6	Tomoaki Tanaka	Department of Molecular Diagnosis, Chiba University	Professor	Elucidation of Novel Regulatory Mechanism of Glucose Metabolism in Liver Type 2 Innate Lymphocytes by Single Cell Analysis and Spatial Transcriptome Analysis
7	Haruko Takano	Department of Molecular Pathophysiology, Institute of Advanced Medical Science,, Nippon Medical School	Assistant professor	Elucidation of the role of alveolar capillary in alveolar formation for clinical application
8	Kazuya Kanemoto	Graduate School of Pharmaceutical Sciences, Tohoku University	Assistant Professor	Development of three component reaction for site-selective peptide modification
9	Takayuki Nojima	Medical institute of Bioregulation, Kyushu University	Associate Professor	Study about a mechanism of cancer-specific transcription termination that produces noncoding RNA

#	Name	Institution	Title	Research Project
10	Junki Miyamoto	Institute of Global Innovation Research, Tokyo University of Agriculture and Technology	Associate Professor	Diet intervention and chronic obstructive pulmonary disease
11	Masahito Kawazu	Research Institute, Division of Cell Therapy, Chiba Cancer Center	Division head	Immunogenomic analysis to elucidate the mechanism of immune evasion in endometrial cancer with high microsatellite instability
12	Hisaaki Hirose	Institute for Chemical Research, Kyoto University	Program-Specific Associate Professor	Molecular basis of calcium ion-regulated cellular uptake of nutrients into cancer cells
13	Yoshiaki Abe	Department of Hematology, Faculty of Medicine, University of Tsukuba	Post graduate student	Exploring follicular lymphoma-specific immunity
14	Hiroki Kobayashi	Laboratory of Oncology, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences	Assistant Professor	Development of novel PFK1 inhibitors for the therapy of mitochondrial diseases
15	Koji Kikuchi	Department of Chromosome Biology, Kumamoto University	Lecturer	Elucidating the mechanism of Sertoli cell polarity that plays a crucial role in the differentiation of male germ cell lineage.
16	Osamu Tskamoto	Medical Biochemistry, Osaka University Graduate School of Frontier Biosciences	Associate Professor	Clarification of the transcriptional regulatory mechanism of natriuretic peptide genes by the heart-specific super-enhancer CR9 that senses hemodynamic loads
17	Yoji Kawano	Institute of Plant Science and Resources, Okayama University	Professor	Evolution and activation mechanisms of NLR immune receptors
18	Kyota Aoyagi	Department of Cellular Biochemistry, Kyorin University School of Medicine	Associate Professor	Defective mitophagy in pancreatic beta-cells leads to diabetes
19	Yosuke Tanaka	IRCMS, Laboratory of Stem Cell Regulation, Kumamoto University	Lecturer	Differential Regulation of Stem Cell Ability by Calcium Signaling in Normal and Abnormal Hematopoietic Stem Cells and Therapeutic Applications
20	Satoru Okamoto	Department of Agriculture, Niigata University	Assistant professor	A study about a molecular mechanism of regulation of flowering from roots
21	Soichiro Yamanaka	Department of Biological Sciences, Graduate School of Science, The University of Tokyo	Associate Professor	The analysis on the re-establishment of epigenome in mouse gonocyte
22	Di Wu	Animal Cell Function Laboratory, Nagoya University	Assistant Professor	Research on the vertebrate CMP-sialic acid synthetase as a novel regulatory protein of neurogenesis by interacting with particular proteins
23	Jun Shirakawa	Institute for Molecular and Cellular Regulation (IMCR),Gunma University	Professor	Interorgan networks of islets with liver, fat, or macrophages to regulate β-cell mass
24	Takumi Oti	Faculty of Science, Department of Biological Science, Kanagawa University	Assistant Professor	Copulatory experience and neural mechanisms controlling male sexual function: focusing on the spinal gastrin-releasing peptide receptor neurons
25	Hirosuke Shiura	Graduate Faculty of Interdisciplinary Research, University of Yamanashi	Assistant Professor	The mechanism of early placental development regulated by a virus-derived gene

#	Name	Institution	Title	Research Project
26	Hiroki Ohara	Department of Functional Pathology, Shimane University	Lecture	Development of novel preventive and therapeutic approaches for hypertensive disease based on elucidation of the immunogenetic mechanisms
27	Jun Hamazaki	Laboratory of Protein Metabolism, The University of Tokyo	Assistant Professor	Elucidation of the proteasome maintenance mechanism and its application to drug discovery
28	Yasuhiro Horibata	Department of Biochemistry, Dokkyo Medical University School of Medicine	Associate Professor	Elucidation of the disease development caused by disruption of a novel phospholipid transport factor required for mitochondrial homeostasis
29	Mineko Kengaku	Institute for Advanced Study, Institute for Integrated Cell-Material Sciences, Kyoto University	Professor	Molecular mechanism of dendrite remodeling during the critical period of brain development
30	Akira Takai	Dept. of Cell Biol., Grad. Sch. of Med.,The University of Tokyo	Assistant Professor	Phase separation dynamics of pathogenic RNA granules uncovered with novel visualization and manipulation method of endogenous RNAs
31	Shunsuke Kitajima	Cancer Institute, Cell Biology, Japanese Foundation for Cancer Research	Staff Scientist	Molecular mechanism of intrinsic resistance to KRAS G12C inhibitor associated with LKB1/KEAP1 mutation
32	Tsuyoshi Udagawa	Graduate School of Pharmaceutical Sciences, Laboratory of Biological Chemistry, Nagoya City University	Associate Professor	Optimization of protein amino (N)-terminal regions of artificial mRNAs by an evolutionary engineering approach
33	Minako Hirano	Faculty of Interdisciplinary Science and Engineering in Health Systems, Okayama University	Associate Professor	Search and creation of photo-activated adenylyl cyclases for optogenetics tools
34	Ryuichi Ohgaki	Department of Bio-system Pharmacology, Graduate School of Medicine, Osaka University	Associate Professor	Novel lipid metabolism essential for the ceramide homeostasis in stratum corneum
35	Takeshi Fuchigami	Laboratory of Clinical Analytical Sciences, Graduate School of Medical Sciences, Kanazawa University	Associate Professor	Development of multifunctional molecular probes with high sensitivity for detecting precancerous lesions in pancreatic cancer
36	Tatsuo Mano	Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry	Section Chief	Stratification of Alzheimer's Disease by Integrative Multi-Omics Analysis
37	Haruki Ochi	Institute for Promotion of Medical Science Research, Yamagata University	Associate Professor	Research for the assembly of PML nuclear bodies regulated by Neddylation and the promotion of kidney regeneration
38	Kazuhiko Matsuo	Division of Chemotherapy, Faculty of Pharmacy, Kindai University	Lecturer	Pathological roles of a novel receptor for chemokine ELC/CCL19 in psoriasis
39	Tetsuya Tsujihara	Department of Medicinal and Organic Chemistry, School of Pharmacy,Iwate Medical University	Associate Professor	Development and application of stereoselective synthesis of cyclopropane-fused heteropolycyclic compounds

FY2022 Research Meeting Grant

(JPY 400 thousand x5 = 2.0 million)

#	Meeting	Place / Date	Institution	Name
1	DNA29 - 29th International Conference on DNA Computing and Molecular Programming	Sendai/ 2023.9.11-15	Department of Robotics, Graduate School of Engineering, Tohoku University/ Professor	Satoshi Murata
2	2nd Asian Palaeontological Congress	Tokyo/ 2023.8.3-7	Department of Earth and Planetary Science, The University of Tokyo/ Professor	Kazuyoshi Endo
3	“Molecular Movies” International Symposium 2023: Molecular Movies; to be continued	Awaji/ 2023.11.30-12.1	Department of Life Science, Graduate School of Science, University of Hyogo/ Professor	Minoru Kubo
4	10th International MDM2 workshop	Tokyo/ 2023.10.15-18	Laboratory of Fundamental Oncology, National Cancer Center Research Institute/ Laboratory Head	Rieko Ohki
5	International symposium on skin stem cell dynamics	Tokyo/ 2023.5.14-15	Center for Biosystems Dynamics Research, Laboratory for Tissue Microenvironment, RIKEN/ Team Leader	Hironobu Fujiwara

第36期（2022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。2022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36回ノバルティス研究奨励金	39件（1件100万円）	3,900万円
研究集会助成	5件（1件 40万円）	200万円
		総額 4,100万円

第36回ノバルティス研究奨励金（2022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	佐野　宗一	大阪公立大学 大学院医学研究科循環器内科学	特任講師	心不全の悪化に関連する Y 染色体遺伝子の特定とそのメカニズムの解明
2	合山　　進	東京大学 新領域創成科学研究科先進分子腫瘍学分野	教　授	造血幹細胞指向型脂質ナノ粒子を活用した生体内遺伝子治療法の開発
3	野元　美佳	名古屋大学 遺伝子実験施設	助　教	植物葉面の毛状突起トライコームが活性化する免疫応答の分子機構解明
4	酒井　寿郎	東京大学先端科学技術研究センター 代謝医学分野	教　授	シグナル感知型エピゲノム修飾因子によるミトコンドリア生合成を標的とした抗肥満効果の解明
5	向井康治朗	東北大学 大学院生命科学研究科細胞小器官疾患学分野	助　教	自然免疫シグナル STING 経路の活性化機構の解明
6	田中　知明	千葉大学 分子病態解析学講座	教　授	シングルセル解析と空間的トランスクリプトーム解析から捉える肝臓 2 型自然リンパ球の新たな糖代謝調節機構の解明
7	高野　晴子	日本医科大学 先端医学研究所病態解析学部門	助　教	肺胞形成機構における血管内皮細胞の役割の解明と肺胞再生治療への応用
8	金本　和也	東北大学大学院 薬学研究科	助　教	ペプチドのピンポイント修飾を可能にする三成分連結反応の開発
9	野島　孝之	九州大学 生体防御医学研究所	准教授	非コード RNA を創出するがん特異的な転写終結機構の解明
10	宮本　潤基	東京農工大学 大学院グローバルイノベーション研究院	テニュアトラック准教授	食事介入による慢性閉塞性肺疾患モデルマウスに対する治療法の確立
11	河津　正人	千葉県がんセンター 研究所細胞治療開発研究部	部　長	マイクロサテライト高度不安定性子宮体がんの免疫回避のメカニズム解明
12	広瀬　久昭	京都大学 化学研究所	特定准教授	Ca イオンを基軸とした、がん細胞の栄養取り込み機構の分子基盤
13	安部　佳亮	筑波大学 血液内科研究室	日本学術振興会特別研究員	濾胞性リンパ腫特異的免疫環境の探索

No.	氏　名	所　属	職位	研究課題
14	小林　大貴	東京薬科大学 生命科学部 腫瘍医科学研究室	助　教	ミトコンドリア病を標的とした新規ホスホフルクトキナーゼ（PFK1）阻害剤開発
15	菊池　浩二	熊本大学 染色体制御分野	講　師	生殖細胞系列の分化を支えるセルトリ細胞の極性化メカニズムの解明
16	塚本　　蔵	大阪大学大学院生命機能研究科 医化学講座	准教授	力学的負荷を感知する心臓特異的スーパーエンハンサー CR9 によるナトリウム利尿ペプチド遺伝子発現制御機構の解明
17	河野　洋治	岡山大学 資源植物科学研究所	教　授	NLR 免疫受容体の進化と活性化機構の解明
18	青柳　共太	杏林大学 医学部 細胞生化学	准教授	膵β細胞におけるマイトファジー不全による糖尿病発症機構
19	田中　洋介	熊本大学　国際先端医学研究機構 幹細胞制御研究室	特任講師	正常・異常造血幹細胞におけるカルシウムシグナルによる幹細胞性制御機構の相違点の解明と治療応用
20	岡本　　暁	新潟大学 農学部	助　教	根から花成を制御する分子機構の研究
21	山中総一郎	東京大学 理学系研究科 生物科学専攻	准教授	マウス胎仔期雄性生殖細胞におけるエピゲノム再構築原理の解明
22	呉　　迪	名古屋大学 動物細胞機能研究室	助　教	CMP- シアル酸合成酵素が神経形成を制御する新奇相互作用分子であることの研究
23	白川　　純	群馬大学 生体調節研究所 代謝疾患医科学分野	教　授	膵島と肝臓、脂肪、マクロファージとの相互作用による膵β細胞量調節機構
24	越智　拓海	神奈川大学 理学部生物科学科	特別助教	交尾経験に着目した脊髄 GRP 受容体ニューロンによる性機能制御メカニズムの解明
25	志浦　寛相	山梨大学 大学院総合研究部	助　教	ウイルス由来遺伝子がもたらした哺乳類胎盤初期分化メカニズムの解明
26	大原　浩貴	島根大学 医学部病理学講座 病態病理学	講　師	免疫遺伝学的メカニズムの解明に基づく高血圧性疾患の新たな予防・治療法の開発
27	濱崎　　純	東京大学　大学院　薬学系研究科 蛋白質代謝学教室	助　教	プロテアソーム維持機構の解明と創薬への応用
28	堀端　康博	獨協医科大学 医学部生化学講座	准教授	ミトコンドリア恒常性に必要な新規リン脂質輸送因子の破綻による疾患発症機構の解明
29	見學美根子	京都大学 高等研究院 物質 - 細胞統合システム拠点	教　授	臨界期脳の樹状突起リモデリングを制御する分子シグナルの解明
30	高井　　啓	東京大学大学院 医学系研究科 細胞生物学分野	助　教	内在性 RNA の可視化・制御法で解き明かす病原性 RNA 顆粒の相分離動態
31	北嶋　俊輔	公益財団法人がん研究会 がん研究所 細胞生物部	研究員	LKB1/KEAP1 遺伝子変異に伴う KRAS G12C 阻害剤治療抵抗性の誘導とその分子機序解明
32	宇田川　剛	名古屋市立大学 大学院薬学 研究科遺伝情報学分野	准教授	進化工学的アプローチによる人工 mRNA のタンパク質アミノ（N）末端領域の最適化
33	平野美奈子	岡山大学 学術研究院 ヘルスシステム統合科学学域	准教授	cAMP の高時間分解能計測による光活性化 cAMP 産生酵素の探索と創製
34	大垣　隆一	大阪大学 大学院医学系研究科 生体システム薬理学	准教授	角質層セラミドの恒常性に不可欠な新たな脂質代謝機構の解明

No.	氏　名	所　属	職位	研 究 課 題
35	淵上　剛志	金沢大学 医薬保健研究域薬学系　臨床分析科学研究室	准教授	膵癌の前癌病変を高感度に検出できる多機能性分子プローブ開発
36	間野　達雄	国立精神・神経医療研究センター 神経研究所 疾病研究第四部	室　長	統合的マルチオミクス解析によるアルツハイマー病の層別化
37	越智　陽城	山形大学 医学部 メディカルサイエンス推進研究所 生化学解析センター	准教授	ネジル化修飾を介した PML ボディの凝集と腎組織再生促進の研究
38	松尾　一彦	近畿大学 薬学部 化学療法学研究室	講　師	乾癬病態形成におけるケモカイン ELC/CCL19 の新規受容体の病理的役割の解明
39	辻原　哲也	岩手医科大学 薬学部 薬科学講座 創薬有機化学分野	准教授	シクロプロパンが縮環した複素多環式化合物の立体選択的合成法の開発と応用

2022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。2022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	村田　智	東北大学 大学院工学研究科ロボティクス専攻	教　授	仙台／2023.9.11–15	DNA29 - 第29回DNAコンピューティングと分子プログラミングに関する国際会議
2	遠藤　一佳	東京大学 理学系研究科地球惑星科学専攻	教　授	東京／2023.8.3–7	第2回アジア古生物学会議
3	久保　稔	兵庫県立大学大学院理学研究科生命科学専攻	教　授	淡路／2023.11.30–12.1	「高速分子動画」国際シンポジウム2023: 高分子動画；今後の展開に繋げて
4	大木理恵子	国立がん研究センター研究所基礎腫瘍学ユニット	独立ユニット長	東京／2023.10.15–18	第10回　国際MDM2ワークショップ
5	藤原　裕展	理化学研究所 生命機能科学研究センター細胞外環境研究チーム	チームリーダー	東京／2023.5.14–15	国際シンポジウム「皮膚幹細胞ダイナミクス」

Promotion Results according to the PROGRAM

(Unit : mil yen)

Year	Research Grants		Meeting Grants		Japan-Europe Research Exchange		Oversea Reseach Trip	
	# of people	Amount	# of people	Amount	# of people	Amount	# of people	Amount
1987	18	1,800	8	400	0	0	0	0
1988	39	3,900	8	400	7	1,740	0	0
1989	42	4,200	8	400	9	2,260	0	0
1990	51	7,650	10	500	8	2,440	0	0
1991	55	11,000	11	550	9	2,710	9	250
1992	50	10,000	10	500	10	3,315	8	265
1993	50	10,000	10	500	11	3,511	9	300
1994	50	10,000	10	500	8	2,530	6	155
1995	50	6,500	10	500	7	2,020	6	170
1996	45	5,850	10	500	6	1,600	4	120
1997	41	4,920	10	500	6	1,610	2	55
1998	41	4,920	10	500	4	1,070	8	160
1999	41	4,920	10	500	4	710	8	160
2000	41	4,100	8	400	3	660	0	0
2001	41	4,100	7	350	2	440	0	0
2002	40	4,000	8	400	0	0	0	0
2003	40	4,000	4	200	0	0	0	0
2004	45	4,500	5	200	0	0	0	0
2005	45	4,500	5	200	0	0	0	0
2006	46	4,600	6	240	0	0	0	0
2007	50	5,000	6	240	0	0	0	0
2008	45	4,500	7	280	0	0	0	0
2009	30	3,000	6	240	0	0	0	0
2010	38	3,800	5	200	0	0	0	0
2011	41	4,100	6	240	0	0	0	0
2012	40	4,000	6	240	0	0	0	0
2013	42	4,200	5	200	0	0	0	0
2014	42	4,200	6	240	0	0	0	0
2015	35	3,500	6	240	0	0	0	0
2016	35	3,500	5	200	0	0	0	0
2017	41	4,100	5	200	0	0	0	0
2018	37	3,700	5	200	0	0	0	0
2019	37	3,700	5	200	0	0	0	0
2020	39	3,900	9	360	0	0	0	0
2021	40	4,000	5	200	0	0	0	0
2022	39	3,900	5	200	0	0	0	0
Total	1,502	178,560	260	11,920	94	26,616	60	1,635

Promotion Results according to the PROGRAM

(Unit : mil yen)

Year	Travel Expense to Japan		Special Grant		Total # of people	Total Amount
	# of people	Amount	# of people	Amount		
1987	0	0	0	0	26	2,200
1988	0	0	0	0	54	6,040
1989	0	0	0	0	59	6,860
1990	0	0	0	0	69	10,590
1991	0	0	0	0	84	14,510
1992	0	0	0	0	78	14,080
1993	0	0	0	0	80	14,311
1994	0	0	2	110	76	13,295
1995	0	0	1	50	74	9,240
1996	0	0	0	0	65	8,070
1997	0	0	1	30	60	7,115
1998	0	0	0	0	63	6,650
1999	0	0	4	130	67	6,420
2000	0	0	3	142	55	5,302
2001	0	0	3	120	53	5,010
2002	0	0	0	0	48	4,400
2003	0	0	0	0	44	4,200
2004	0	0	0	0	50	4,700
2005	0	0	0	0	50	4,700
2006	0	0	0	0	52	4,840
2007	5	1,000	0	0	61	6,240
2008	5	1,000	0	0	57	5,780
2009	3	600	0	0	39	3,840
2010	0	0	0	0	43	4,000
2011	0	0	0	0	47	4,340
2012	0	0	0	0	46	4,240
2013	0	0	0	0	47	4,400
2014	0	0	0	0	48	4,440
2015	0	0	0	0	41	3,740
2016	0	0	0	0	40	3,700
2017	0	0	0	0	46	4,300
2018	0	0	0	0	42	3,900
2019	0	0	0	0	42	3,900
2020	0	0	0	0	48	4,260
2021	0	0	0	0	45	4,200
2022	0	0	0	0	44	4,100
Total	13	2,600	14	582	1,943	221,913

助成金実績一覧表

（単位：万円）

年号	研究奨励金		研究集会		日欧研究交流		海外出張助成	
	人数	助成額	人数	助成額	人数	助成金額	人数	助成金額
1987	18	1,800	8	400	0	0	0	0
1988	39	3,900	8	400	7	1,740	0	0
1989	42	4,200	8	400	9	2,260	0	0
1990	51	7,650	10	500	8	2,440	0	0
1991	55	11,000	11	550	9	2,710	9	250
1992	50	10,000	10	500	10	3,315	8	265
1993	50	10,000	10	500	11	3,511	9	300
1994	50	10,000	10	500	8	2,530	6	155
1995	50	6,500	10	500	7	2,020	6	170
1996	45	5,850	10	500	6	1,600	4	120
1997	41	4,920	10	500	6	1,610	2	55
1998	41	4,920	10	500	4	1,070	8	160
1999	41	4,920	10	500	4	710	8	160
2000	41	4,100	8	400	3	660	0	0
2001	41	4,100	7	350	2	440	0	0
2002	40	4,000	8	400	0	0	0	0
2003	40	4,000	4	200	0	0	0	0
2004	45	4,500	5	200	0	0	0	0
2005	45	4,500	5	200	0	0	0	0
2006	46	4,600	6	240	0	0	0	0
2007	50	5,000	6	240	0	0	0	0
2008	45	4,500	7	280	0	0	0	0
2009	30	3,000	6	240	0	0	0	0
2010	38	3,800	5	200	0	0	0	0
2011	41	4,100	6	240	0	0	0	0
2012	40	4,000	6	240	0	0	0	0
2013	42	4,200	5	200	0	0	0	0
2014	42	4,200	6	240	0	0	0	0
2015	35	3,500	6	240	0	0	0	0
2016	35	3,500	5	200	0	0	0	0
2017	41	4,100	5	200	0	0	0	0
2018	37	3,700	5	200	0	0	0	0
2019	37	3,700	5	200	0	0	0	0
2020	39	3,900	9	360	0	0	0	0
2021	40	4,000	5	200	0	0	0	0
2022	39	3,900	5	200	0	0	0	0
Total	1,502	178,560	260	11,920	94	26,616	60	1,635

助成金実績一覧表

（単位：万円）

年号	海外受入		特別助成		人数計	金額合計
	助成人数	助成金額	人数	助成金額		
1987	0	0	0	0	26	2,200
1988	0	0	0	0	54	6,040
1989	0	0	0	0	59	6,860
1990	0	0	0	0	69	10,590
1991	0	0	0	0	84	14,510
1992	0	0	0	0	78	14,080
1993	0	0	0	0	80	14,311
1994	0	0	2	110	76	13,295
1995	0	0	1	50	74	9,240
1996	0	0	0	0	65	8,070
1997	0	0	1	30	60	7,115
1998	0	0	0	0	63	6,650
1999	0	0	4	130	67	6,420
2000	0	0	3	142	55	5,302
2001	0	0	3	120	53	5,010
2002	0	0	0	0	48	4,400
2003	0	0	0	0	44	4,200
2004	0	0	0	0	50	4,700
2005	0	0	0	0	50	4,700
2006	0	0	0	0	52	4,840
2007	5	1,000	0	0	61	6,240
2008	5	1,000	0	0	57	5,780
2009	3	600	0	0	39	3,840
2010	0	0	0	0	43	4,000
2011	0	0	0	0	47	4,340
2012	0	0	0	0	46	4,240
2013	0	0	0	0	47	4,400
2014	0	0	0	0	48	4,440
2015	0	0	0	0	41	3,740
2016	0	0	0	0	40	3,700
2017	0	0	0	0	46	4,300
2018	0	0	0	0	42	3,900
2019	0	0	0	0	42	3,900
2020	0	0	0	0	48	4,260
2021	0	0	0	0	45	4,200
2022	0	0	0	0	44	4,100
Total	13	2,600	14	582	1,943	221,913

36th Financial Report

Balance Sheet

As of March 31, 2023

（Unit：JP Yen）

Account	Amount
Ⅰ Assets	
1. Current Assets	
Current Assets Total	20,972,711
2. Fixed Assets	
(1) Basic Fund	
Basic Fund Total	1,100,000,000
(2) Specific Assets	
Specific Assets Total	-
(3) Other Long - term Assets	
Other Long - term Assets Total	85,037,657
Fixed Assets Total	1,185,037,657
Assets Total	1,206,010,368
Ⅱ Liabilities	
1. Current Liabilities	
Current Liabilities Total	41,126,432
Liabilities Total	41,126,432
Ⅲ Equity（Net Assets）	
1. Designated Net Assets	
Designated Net Assets Total	1,000,010,000
（Amount Appropriating to basic Fund）	(1,000,000,000)
(Amount Appropriating to specific assets)	0
2. General Net Assets	164,883,936
（Amount Appropriating to）	(100,000,000)
Equity Total（Net Assets）	1,164,883,936
Liabilities & Equity Total	1,206,010,368

Statement of Net Assets

From April 1 st, 2022 to March 31, 2023

（Unit：JP Yen）

Account	Amount
Ⅰ General Net Assets Changes	
1. Ordinary income & Expenditure	
(1) Ordinary income	
Interest from basic fund	17,442,402
Donation	40,010,594
Other Income	649,205
Ordinary Income Total	58,102,201
(2) Ordinaty Expenditure	
Project Expense	10,396,837
Grant Expense	41,000,000
Novartis Research Grant	39,000,000
Research Meeting Grant	2,000,000
Administrative Expense	3,956,142
Ordinary Expenditure Total	55,352,979
Ordinary Balance without Appraisal Profit or Loss	2,749,222
2. Nonrecurring Profit & Loss	
Nonrecurring Balance of Current Period	0
General Net Assets Ending Balance	164,883,936
Ⅱ Designated Net Assets Changes	
Designated Net Assets Change	(10,594)
Designated Net Assets Ending Balance	1,000,000,000
Ⅲ Net Assets Balance Ending Balance	1,164,883,936

第36期（2022年度）財務報告

貸借対照表

2023年3月31日現在

（単位：円）

科　　目	金　額
Ⅰ資産の部	
1．流動資産	
流動資産合計	20,972,711
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	－
⑶ その他固定資産	
その他固定資産合計	85,037,657
固定資産合計	1,185,037,657
資産合計	1,206,010,368
Ⅱ負債の部	
1．流動負債	
流動負債合計	41,126,432
負債合計	41,126,432
Ⅲ正味財産の部	
1．指定正味財産	
指定正味財産合計	1,000,010,000
（うち基本財産への充当額）	(1,000,000,000)
（うち特定資産への充当額）	(0)
2．一般正味財産	164,883,936
（うち基本財産への充当額）	(100,000,000)
正味財産合計	1,164,883,936
負債及び正味財産合計	1,206,010,368

正味財産増減計算書

2022年4月1日から2023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	17,442,402
受取寄付金	40,010,594
雑収益	649,205
経常収益　計	58,102,201
⑵ 経常費用	
事業費	10,396,837
支払助成金	41,000,000
ノバルティス研究奨励金	39,000,000
研究集会助成金	2,000,000
管理費	3,956,142
経常費用　計	55,352,979
当期経常増減額	2,749,222
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	164,883,936
Ⅱ指定正味財産増減の部	
当期指定正味財産増減額	(10,594)
指定正味財産期末残高	1,000,000,000
Ⅲ正味財産期末残高	1,164,883,936

[Board of Trustees] 5 trustees, 2 auditors As of July 1, 2023

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 July 1, 2023**

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 July 1, 2023

Post	Name	Title
Chairman	Yasuteru Urano, Ph.D.	Professor, University of Tokyo Graduate School of Pharmaceutical Sciences
Member	Masaki Ieda, M.D., Ph.D.	Professor, Keio University School of Medicine
	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
	Yasushi Sakata, M.D., Ph.D.	Professor, Osaka University Graduate School of Medicine
	Osamu Takeuchi, M.D., Ph.D.	Professor, Kyoto University Graduate School of Medicine
	Osamu Nureki, Ph.D.	Professor, University of Tokyo Graduate School of Science
	Akiko Hayashi-Takagi, M.D., Ph.D.	Team leader, RIKEN Center for Brain Science
	Yu Hayashi, Ph.D.	Professor, University of Tokyo Graduate School of Science
	Yasuyuki Fujita, M.D., Ph.D	Professor, Kyoto University Graduate School of Medicine

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　　2023年7月1日現在（敬称略）

職 名	氏 名	現　　職
代表理事	高田　邦昭	群馬県公立大学法人 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　　2023年7月1日現在（敬称略）

職 名	氏 名	現　　職
評議員長	清水　孝雄	公益財団法人 微生物化学研究会 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
	柴﨑　正勝	公益財団法人 微生物化学研究会 理事長 東京大学名誉教授
	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］

2023年7月1日現在（敬称略）

職 名	氏 名	現　　職
選考委員長	浦野　泰照	東京大学 大学院薬学系研究科 教授
選考委員	家田　真樹	慶應義塾大学 医学部 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
	坂田　泰史	大阪大学 大学院医学系研究科 教授
	竹内　　理	京都大学　大学院医学研究科　教授
	濡木　　理	東京大学　大学院理学系研究科　教授
	林（高木）朗子	理化学研究所　脳神経科学研究センター　チームリーダー
	林　　悠	東京大学 大学院理学系研究科　教授
	藤田　恭之	京都大学　大学院医学研究科　教授

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。

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ご寄付は、随時受付けております。詳しくは、財団事務局までお問合せください。

（E-メール：foundation.japan@novartis.com）

事務局より

2023年度も、おかげさまで財団年報を発行する運びとなりました。2020年に世界中に大きな影響を及ぼした新型コロナウイルス感染症はいまだ収束しない状況ですが、ワクチンやデジタルの発展により、新しいスタイルのもとで かなりコロナ前の状況に戻った感があります。

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

当財団は1987年9月の財団設立以来、助成件数は**総数で1,943件、総額で約22.2億円**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

2023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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