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Contents

Introduction

Akimichi Kaneko, MD, PhD
Chairman of the Board of Trustees

We have compiled here the research accomplishments of the grantees of the NOVARTIS Foundation (Japan) for the Promotion of Science in FY2016. The Foundation was originally established on September 4, 1987 with the generous donation of JPY 1 billion from Ciba-Geigy AG, Switzerland, for the purpose of "contributing to academic development and thus improving public health and welfare of the people by subsidizing to promote creative research and international exchange in the fields of biological and life sciences and related chemistry". Since then, the Foundation has granted nearly JPY 2 billion to approximately 1,700 research cases in 30 years. Research funds provided by the Foundation may be only a part of the cost of the entire research, however, we have continued to subsidize in belief that we could contribute to the development of excellent research. Joy of grantees could be felt in the acknowledgment of the papers received by the Secretariat, and we are pleased with our humble contribution.

The Foundation supports creative research in the fields of biological and life sciences, related chemistry and information science. Numerous entries submitted are examined by specialized researchers in a strict, fair and impartial manner. While we value originality as selection criteria, considerations are given to those start-up laboratories in urgent needs of research funds, as well as age and women researchers. In each case, we believe that excellent research is selected. In fact, we are informed that the quality of the research funded by the Foundation is highly esteemed by researchers in Japan, proving our purposes have not been wrong. We hope that these researches will lead the development of life sciences, and eventually to the development of new drugs and therapies.

Speaking of 2016, the Kumamoto earthquake occurred on April 14th and 16th was observed the maximum seismic intensity 7. Kumamoto and Oita prefectures had 267 deaths (including direct death and related deaths). A complete destruction of houses or a semi-destruction exceeded 43,000 buildings. It suffered extensive damage. I prayed for the reconstruction and awarded five Novartis research grants to Kumamoto and Oita researchers as earthquake disaster frame. I hope that it helped the research in a harsh.

This Annual Report summarizes the excellent research outcomes. It is an outstanding achievement accomplished within a limited period of one year. We are deeply grateful to everyone who supported the activities of the Foundation, including the selection committee members who have selected these excellent researches.

はじめに

代表理事　金子　章道

ここに2016年度にノバルティス科学振興財団研究助成金を受けられた方々の研究報告を収録いたしました。当財団は1987年９月４日、スイス、チバガイギー社からの10億円のご寄附をもとに、「生物・生命科学および関連する化学の領域において、創造的な研究ならびに国際交流への助成を行うことにより、学術の振興を図り国民の健康と福祉の向上に寄与する」ことを目的に設立されました。爾来30年間に1,700件近く、金額にしておよそ20億円の助成を行ってまいりました。当財団が提供する研究費は、研究全体に要する費用の一部かも知れませんが、優れた研究の発展に少しでも寄与できればと考え、助成して参りました。事務局に寄せられた論文等の謝辞からも受賞者の喜びが伝わり、多少なりとも貢献できているものと嬉しく思っております。

当財団は、生物・生命科学、関連する化学および情報科学の領域における創造的な研究を助成しております。数多く寄せられた応募を専門の研究者により厳正に公正かつ公平に審査しております。選考基準と致しまして独創性を重視しておりますが、研究室を新たに立ち上げ研究資金に逼迫しているケースや年齢、女性研究者にも配慮しており、いずれも優れた研究が選択されたものと存じます。事実、当財団で助成を受けた研究の質が高いと国内の研究者の間で評価されていることを聞くにつけ、我々の目指すところは間違っていないことを確信する次第であります。このような研究が生命科学の発展、ひいては新しい治療薬の開発や治療法の開発に発展することを期待しております。

2016年と言えば、４月14日及び16日に発生した最大震度７を観測する熊本地震にて、熊本県と大分県は死者267人（直接死、関連死などを含め）、住宅の全壊・半壊は43,000棟を超えるなど、甚大な被害を受けました。その復興を祈念しまして、震災枠として熊本及び大分の研究者にノバルティス研究奨励金、5件を授与しました。厳しい環境の中、研究の一助になられたものと思います。

この年報にはこのような優れた研究の成果をまとめております。１年間と限られた時間の中で達成した立派な業績であります。なお、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をはじめ、財団の活動を支えて下さっている関係者の皆様に深く感謝いたします。

II.

Reports from the Recipients of Novartis Research Grants

Regulatory function of stress responsive splicing controlled by long non-coding RNA

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Summary Abstract

Satellite III long noncoding RNA (Sat III lncRNA) is the architectural RNA to construct nuclear stress bodies (nSBs) that are formed under heat stress condition. We identified multiple protein components of nSBs that include SR splicing factors and their modifier protein. Sat III knockdown study revealed that nSBs are required for efficient phosphorylation of SR proteins that control splicing of specific mRNAs in the stress recovery phase.
Key Words：noncoding RNA, stress response, splicing regulation

Introduction

Sat III lncRNA is a primate-specific lncRNA which is transcribed from the Sat III repeats in pericentromeric regions. Sat III repeats are transcriptionally silent in normal unstressed condition, however, it is transcribed under heat stress. Sat III lncRNA recruits specific RNA-binding proteins (RBPs) including specific SR proteins and SWI/SNF chromatin remodeling complexes to assemble a massive ribonucleoprotein complex called nuclear stress body (nSB) (Ref1). nSBs are detectable for several hours even after the stress removal. nSBs presumably regulate pre-mRNA splicing under certain stress conditions. However, the precise structure and function of nSBs remains uninvestigated.

Results

1. Identification of nSB protein components by ChIRP-MS

We first isolated nSBs by ChIRP (Chromatin Isolation by RNA Purification) from the heat shocked HeLa cells. ChIRP was carried out using antisense oligonucleotide (ASO) complementary to the repeat sequence of Sat III lncRNA. Silver staining of the proteins in the ChIRP fraction with Sat III ASO revealed that multiple proteins were specifically precipitated under heat stressed cells but not from normal unstressed cells. Mass spectrometric analysis (ChIRP-MS) identified multiple RNA binding proteins (RBPs) including Serine/Arginie rich splicing factors (SR proteins), HNRNP proteins and Scaffold attachment factors as the Sat III-interacting proteins or the nSB components. Most of the identified nSB proteins are localized in nuclear speckles under normal unstressed conditions, suggesting that nuclear speckle are partially remodeled and its components relocate to nSBs by interacting with Sat III lncRNA upon heat stress. Interestingly, in addition to the RBPs, their modifier proteins were identified as the nSB components. Immunofluorescence combined with RNA-FISH confirmed that one modifier protein was co-localized with Sat III lncRNA in nSBs.

2. nSBs control SR protein re-phosphorylation in post-stress recovery phase

SR proteins are known to be rapidly dephosphorylated upon heat stress and gradually rephosphorylated after the stress removal. Here, we hypothesized that nSBs regulate the phosphorylation state of the nSB-localized SR proteins by recruiting the modifier protein. We investigated the effect of Sat III knockdown on phosphorylation state of SR proteins during and after heat stress. The phosphorylation state was monitored by loading the proteins on Phos-tag SDS-PAGE. Sat III knockdown caused marked delay in the modifier-dependent rephosphorylation of nSB-localized SR proteins after stress removal. However, it hardly affected their stress-induced dephosphorylation, suggesting that Sat III lncRNA facilitates rephosphorylation of nSB-localized SR proteins in post-stress recovery phase by recruiting the modifier protein. This result raised an intriguing possibility that Sat III lncRNA controls pre-mRNA splicing after stress removal by controlling the phosphorylation state of specific SR proteins.

3. nSBs control pre-mRNA splicing in stress recovery phase

Recent studies have revealed that multiple partially-unspliced pre-mRNAs that retain specific intron(s) are localized in the nucleus. Some of these nuclear retained pre-mRNAs undergo splicing upon heat stress that leads to production of mature mRNAs. We investigated the role of Sat III lncRNA in the heat shock-responsive intron removal from the nuclear retained partially-unspliced pre-mRNAs. In the control HeLa cells, the nuclear pool of these partially-unspliced pre-mRNAs were mostly depleted by the heat shock-induced splicing and conversely restored within a few hours after the stress removal. In contrast, this restoration was markedly delayed in the Sat III knockdown cells. Moreover, we confirmed that nSB-localized SR proteins and the enzymatic activity of the modifier protein were also required for the rapid restoration of the nuclear pool of the partially unspliced pre-mRNAs. These data suggest that nSB is required for efficient recovery of accumulation of partially unspliced pre-mRNA through modification of SR proteins upon stress removal. RNA-seq analysis identified multiple pre-mRNAs that are analogously controlled by nSBs during stress recovery phase through modification of the nSB-localized SR proteins.

Discussion & Conclusion

Our findings suggest that Sat III lncRNA-dependent nSB accelerates restoration of the pool of specific nuclear-retained pre-mRNAs, which was depleted upon heat stress beforehand, through facilitating rephosphorylation of nSB-localized SR proteins by specific modifier protein in the post-stress recovery phase. Sat III lncRNA as the architectural RNA of nSBs is primate specific lncRNA which lacks in other mammalian species including rodents such as mouse. It is intriguing to pursue the pattern of splicing control in heat stress condition as well as stress recovery phase in mouse cells. Sat III lncRNA is transcribed from Satellite III repeats in pericentromiric region which is silenced in unstressed cells. The repeated sequence may correspond to the recognition motifs of specific SR proteins raising the interesting possibility that evolution of satellite repeats gives some advantage to produce lncRNAs to capture specific splicing regulatory proteins under heat stress.

References

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一般の皆様へ

21世紀のポストゲノム時代に入り、ヒトゲノムの75% もの領域から正体不明なノンコーディングRNA（lncRNA）が産生されていることが明らかになり、その機能に注目が集まっている。Sat IIIと呼ばれるlncRNAは、熱ストレスが細胞に加わった際に合成され、核内ストレス体という顆粒状の核内構造体を組み立てる機能を果たしている。実施者は、今回この核内ストレス体の果たす機能を調べた結果、Sat III lncRNAが核内ストレス体内に係留しているSRタンパク質という制御因子のリン酸化状態をコントロールして、遺伝子発現過程の一段階であるRNAスプライシングを制御していることを発見した。このメカニズムは、lncRNAが細胞のストレス状態からの回復を効率よく行うために働いていることを示しており、ヒトの身体のストレス遭遇時に重要な役割を果たしている可能性が浮上した。

How is recombination suppression achieved in the first place between sex chromosomes?

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Summary Abstract

A fundamental question concerning sex chromosome evolution is what has led to the lack of recombination between the pair of chromosome. Large-scale chromosomal inversions have been assumed to be a major cause of the suppressed recombination. In this study, we characterized the sex-determining region of a pair of closely-related species of fish, and identified a novel sex-determining gene. Moreover, we found that their sex-determining regions exhibit differential recombination patterns, despite that their sequences are very similar each other. Our results are not consistent with the inversion hypothesis and, thus, suggest epigenetic mechanisms as a potential trigger of the recombination suppression.
Key Words：Evolution, recombination suppression, sex chromosome

Introduction

A major question concerning sex chromosome evolution is what has led to the lack of recombination between X and Y (or Z and W) chromosomes. Large-scale chromosomal inversions have been assumed to be a major cause of the suppressed recombination[1]. However, recent reports including ours suggest that recombination suppression is variable near the sex-determining locus, implying that the suppression is initiated by mechanisms other than large-scale inversions, and that the inversions may be the effect rather than cause of recombination suppression. Since sex-limited chromosomes of classic model species (such as mammalian Y and Drosophila Y) are enriched for rearrangements and repeats elements, research on newly formed sex chromosome systems is needed to understand how the initial recombination suppression was established.

Results

1) In order to understand how recombination suppression was initially established, we searched for newly formed sex chromosome systems in many fish species, and found a pair of closely-related species (Carangidae) that potentially show differential recombination patterns around the sex-determining locus.

2) To know whether the recombination suppression around the sex-determining locus has developed in the two species, we examined extents of linkage disequilibrium across the sex-determining locus by performing genome-wide (five individuals for each sex and species) sequencing and sex-determining-region-wide sequencing (approximately 50 individuals for each sex and species). We found that species A exhibits no sign of suppression of recombination around the sex-determining region, while species B shows a marked decrease in recombination ratio in the region spanning approximately 300 Kb. This result indicates that the sex chromosome systems of these two closely-related species is indeed suitable for studying an initial phase of recombination suppression between a pair of sex chromosomes in vertebrates.

3) As a first step to establish the sex chromosomes of the two species as a model system in the field of sex chromosome evolution as well as to conduct rigorous analyses of the sex-determining region in the near future, we performed genome-wide and region-wide association assay for identifying the sex-determining gene of the two species. We found that a single nucleotide polymorphism (SNP) in a steroidogenic gene Hsd17b1 is the sole polymorphisms almost perfectly associated with phenotypic sex in both species[2]. This sex-determining SNP causes a non-synonymous change from glycine on the W chromosome to glutamic acid on the Z near the catalytic center of Hsd17b1. Subsequent biochemical and molecular dynamics analyses suggested that the amino acid substitution disrupts the hydrogen-bond network among steroids and the catalytic residues of the Hsd17b1. Consequently, inter-conversion between 17-keto and 17β-hydroxy steroids is attenuated in the Z-type HSD17B1 protein. In situ hybridization of developing gonads indicated that Hsd17b1 mRNA is expressed in both undifferentiated and differentiating gonads of both genotypic sexes. In the differentiating ovary, both Z- and W-type Hsd17b1 is expressed in pre-granulosa cells, whereas Cyp19a1a, another key component of estrogens synthesis, is predominantly expressed in pre-theca cells. In the differentiating testis, only Z-type is expressed in pre-Sertoli cells, and no induction of Cyp19a1a was observed. Based on these results, we conclude that homozygosity for the Hsd17b1 allele on the Z chromosome results in testis formation due to the reduced enzymatic activity of its gene product and the resultant shortage of estrogens, whereas Z/W heterozygosity promotes the development of ovaries due to the active-type Hsd17b1 on the W chromosome.

4) To know whether the suppression of recombination between the Z and W chromosomes in species B is due to large-scale inversions, we performed targeted genome sequencing of the sex-determining regions from two species by using long-reads technologies, and constructed contigs/scaffolds of the targeted region from Z and W chromosomes. We then compared the contigs/scaffolds within species (between Z and W) as well as between species (e.g. between Z of species A and W of species B). We found no evidence of the large-scale inversions in those sequences of both species, although accumulation of repeats elements was observed in species B. This result clearly indicates that the decreased recombination ratio observed in species B is not initiated by the large-scale inversions.

Discussion & Conclusion

In this study, we first showed that a pair of closely-related species (Carangidae) exhibit differential recombination patterns around the sex-determining locus. We then identified a novel master-sex-determining gene and its precise genomic position in these species. We finally obtained a strong evidence suggesting that large-scale inversions and sequence differences between sex chromosomes are not the initial mechanisms that reduce recombination. One possible mechanism is an epigenetic modification around the sex-determining locus as the sequence of Z and W are very similar. Since the region required for the epigenetic analyses is sufficiently small, and the location of the sex-determining gene has been identified in the pair of closely-related species, these fishes will be an excellent material to study the effect of epigenetic modifications on the evolution of reduced recombination in sex chromosomes.

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一般の皆様へ

ヒトのＹ染色体は著しく退化しており、大多数の遺伝子を失っています。このような退化したＹ染色体は多くの動植物にみられます。この性染色体の退化は「組み換え抑制」てによってもたらされますが、「その原因は染色体間の逆位である」と説明されてきました。しかし、この説は、ヒトやハエの退化しきった性染色体をもとにした説であり、真の原因はわかっていません。我々は、退化直前・直後の性染色体をもつ魚類群を発見しました。これらを調べたところ、逆位が組み換え抑制のトリガーではなさそうだということがわかりました。抑制がある種とない種で、性決定領域のDNA配列は類似していることから、エピゲノム機構が、組み換え抑制に関わる可能性が高いと考えられました。

Identification of novel neural processing mechanism by using transcriptome analysis

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Summary Abstract

Temperature acclimation is essential system for living and proliferation of animals, yet their neural mechanisms have not been well known. We are using temperature experience-dependent cold acclimation of nematode C. *elegans,* as a model for studying temperature acclimation. ASJ sensory neuron that has been known as a light sensing neuron senses and memorizes temperature in cold acclimation. In this study, we conducted RNA-sequencing analysis of ASJ by using single cell RNA-sequencing analysis.
Key Words：Temperature memory, acclimation, C. elegans

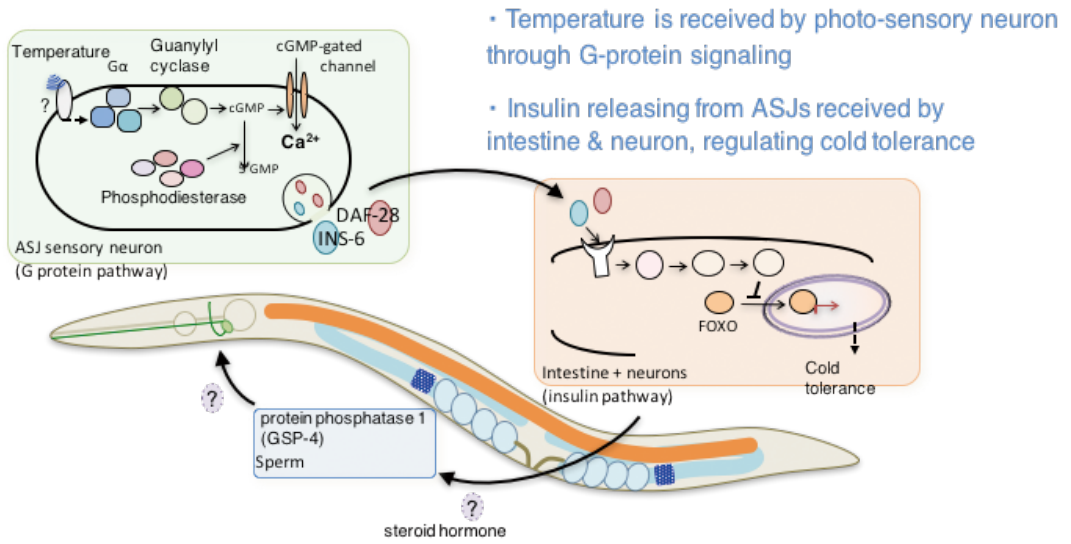
Introduction

Cold tolerance in *C. elegans* is a phenomenon that 20°C-cultivated animals cannot survive at 2°C, whereas 15°C-cultivated animals can survive at 2°C. ASJ sensory neuron that has been known as a light and pheromone sensing neuron senses temperature and regulates cold tolerance through insulin signaling (Ohta, Ujisawa et al., *Nature commun,* 2014). Our previous analyses suggest that cold tolerance is regulated by tissues network containing neurons, intestine and sperm, in which a feedback regulation from sperm to ASJ thermosensory neuron is essential (Sonoda, et al., *Cell Reports,* 2016). However, we have not known thermo-sensing molecules on ASJ and how ASJ is controlled by sperm signaling remain poorly understood.

Results1

Cold tolerance of *C. elegans* is regulated by a pair of head sensoryneruon, ASJ (Figure1)(Ohta, Ujisawa et al., *Nature commun,* 2014; Ujisawa et. al., *PLOS ONE,* 2016; Sonoda, et al., *Cell Reports,* 2016). In the ASJ sensoryneuron, temperature is received by the unidentified-temperature receptor that activates multiple trimeric G protein alpha subunits (Gα)(Figure1). Gα activate two types of guanylyl cyclases, which increase concentration of cGMP in ASJ neuron, then opens cGMP-gated cation channel composed by TAX-4 and TAX-2. Increment of intracellular calcium concentration regulates secretion of insulin like molecules from synaptic region of ASJ neuron. These insulins encoded by *daf-28* and *ins-6* genes are received by the intestine and neuron, in which insulin-signaling pathway negatively regulates cold tolerance thorough FOXO-type transcriptional factor encoded by *daf-16* gene (Figure1). The insulin signaling in intestine affects sperm that regulates ASJ neuronal activity. On these sequential molecular signaling, a secretory signaling from sperm to head sensoryneuron has not been known.

Figure1　Tissue network regulating cold tolerance in *C. elegans*



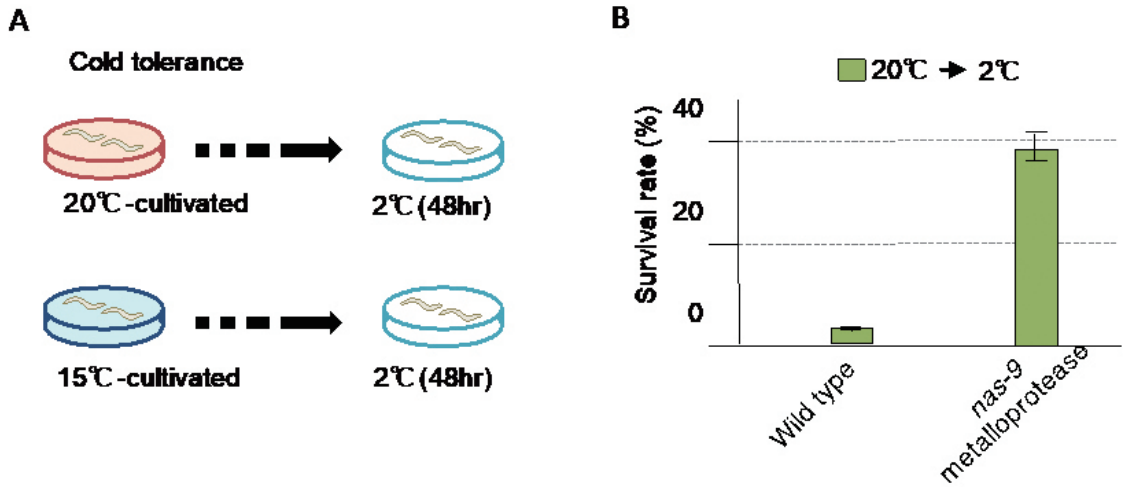
[Ohta*, Ujisawa* et al., *Nature commun,* 2014] *co-first author
[Sonoda, Ohta, Maruo, Ujisawa, Kuhara, *Cell reports*, 2016]
[Ujisawa et al., *PLOS ONE,* 2016]

To identify new genes involved in the temperature sensation and feedback from sperm to ASJ in temperature tolerance, we conducted RNA-sequencing analysis comparing mRNA expression levels between ASJ and a gustatory neuron by using single cell RNA-sequencing analysis (Sugi & Ohtani, *BBRC*2014). PAB-1 is a polyA binding protein that binds with polyA site of mRNA. We expressed PAB-1 gene in ASJ sensoryneuron specifically, by using the ASJ specific promoter *trx-1*. This PAB-1 protein was tagged with 3*FLAG-tag for immunoprecipitation assay. The transgenic animal containing extrachromosomal array, *trx-1p:: 3*FLAG-pab-1,* were cultivated at 20 degree. Then mRNA in ASJ was in vivo cross-linked with FLAG-tagged PAB-1 protein. We homogenized these animals and isolated mRNA binding with FLAG-tagged PAB-1 protein by anti-FLAG-tag with magnetic beads. By using this mRNA from ASJ, we constructed cDNA library for RNA sequencing analysis by using next generation DNA sequencer. Also, cDNA library from a gustatory neuron was constructed as a control analysis against ASJ. Because, the concentrations of mRNA from ASJ or ASE were very low levels, we amplified these mRNA to cDNA by using whole transcriptome amplification kit.

Results2

By comparing gene expression levels between ASJ and a gustatory neuron in 20°C cultivated-animals, we found the expression levels of ~600 genes such as neuropeptides, neuropeptide receptors, insulin were altered. We measured cold tolerance phenotypes of many mutants defective in those genes, and found that mutants defective in collagen, metalloprotease, cation channels showed abnormal cold tolerance. In these mutants, mutant animals defective in metalloprotease encoded by *nas-9* gene showed strong abnormality in cold tolerance (Figure2). After cultivation at 20 degree, wild-type animals can not survive at 2 degree (Figure2A, B). By contrast, about 40% of *nas-9* mutant animals can survive at a degree, after cultivation at 20 degree (Figure2B). Previously reported, *nas-9* encodes an astacin-like metalloprotease and a *nas-9::GFP* promoter fusion reporter construct is expressed in embryos and in the hypodermis though larval to adult stage. However, expression of *nas-9* gene in ASJ was not reported. Our RNA sequencing analysis revealed that astacin-like metalloprotease NAS-9 is expressed in ASJ. We therefore hypothesized that NAS-9 functions in ASJ thermosensory neuron and is involved in neural function or development of ASJ neuron, which maybe required for cold tolerance.

Figure2　　*nas-9* mutant shows abnormal cold tolerance



Discussion & Conclusion

Temperature tolerance system is important for animal, but its mechanisms are intricate. In *C. elegans,* sensoryneuron senses temperature and temperature information is transmitted to downstream tissues through insulin signaling. In the nervous system, ASJ sensoryneuron acts as a thermosensory neuron in cold tolerance.

The results in this study showed that important molecules for cold tolerance in thermosensory neuron. Especially, astacin-like metalloprotease NAS-9 could function in ASJ and could be involved in neural function or development of ASJ neuron, which may be required for cold tolerance. We believe that the molecular and cellular analysis of cold tolerance in *C. elegans* should shed light into understanding fundamental mechanisms of temperature tolerance and acclimation in animals including human.

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一般の皆様へ

温度は生物の生死に直結する環境情報である。本申請者はこれまでに、*C. elegans* の低温耐性を指標に、温度応答のシンプルな実験系を確立した (Ohta et al., *Nature commun,* 2014)。具体的には、頭部の感覚ニューロンが温度を感知し、インスリンを分泌することで、腸などに働きかけ、温度耐性を獲得させる。さらに、腸が精子に働きかけ、精子が頭部の温度受容ニューロンをフィードバック制御する (Sonoda, Ohta et al., *Cell reports,* 2016)。本研究を通じて我々は、低温耐性に関して、温度受容ニューロンで機能する新規の分子を同定した。

Analysis of starch grain formation using live imaging technique

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Summary Abstract

Conventionally, the iodine staining has been used to observe of starch grains. This iodide staining method can observe the shape of starch grains, but cannot visualize amyloplast, which is an plant organelle that synthesizes starch grains. In this study, transgenic plants expressing green fluorescent proteins accumulating in amyloplast were produced in barley and rice (*AmyloTarget-GFP* plants). The *AmyloTarget-GFP* plants produced in this study can directly visualize amyloplasts and at the same time visualize starch grains indirectly. It will be an important research tool for analyzing mutants related to starch grains in the future.

Key Words：Starch grains, Rice, Barley, Green fluorescent protein, Imaging

Introduction

Starch grains are particles formed by starch synthesized in plant cells. The starch grains are synthesized inside the plant organelle called amyloplast which is one type of plastid. Since amyloplast is almost occupied by starch grains, it is believed that shape of starch grains should be the shape of amyloplast, but there are rare cases in which amyloplast is directly visualized. In order to clarify the shape of amyloplasts synthesizing starch grains, this study used fluorescent proteins to visualize amyloplast in barley and rice and observe them to obtain new findings.

Results

The morphological diversity of starch grains has been reported over 100 years ago. The diversity is remarkable especially in the endosperm of Poaceae families[1)]. Morphologically, starch grains are classified as either compound or simple starch grains[2)]. Rice starch grains in endosperm are formed as assemblies of small starch particles[3)]. This is compound starch grains. On the other hand, barley develops simple starch grains that is composed of a single starch particle. Both rice and barley are important crops, but the morphology of starch grains is in contrast. Therefore, comparative imaging of starch grains development of the two important crops will lead to clarify the mechanism that defines the shape of the starch grains. So, I used rice and barley to construct transgenic plants that visualize starch grains by green fluorescent proteins (GFP).

First, transit peptide (AmyloTarget) that effectively transport proteins to amyloplasts were identified. Then, the nucleotide sequence encoding AmyloTarget was fused to *GFP* gene. The *AmyloTarget-GFP* gene was inserted downstream of the promoter and transformed into rice and barley. The transformed plants will be referred to as *AmyloTarget-GFP* plants. The rice *AmyloTarget-GFP* plants were grown at 28-30°C and the barley *AmyloTarget-GFP* plants were grown for 13-15°C. Significant GFP fluorescence signals were observed in rice and barley with *AmyloTarget-GFP* gene introduced. In the case of rice, GFP fluorescence was observed in the state of brown rice. The presence or absence of *AmyloTarget-GFP* gene was evaluated based on the presence or absence

of a fluorescent signal, and a homozygote was isolated from the segregation ratio. The developing seeds were focused because it is the synthesis stage of starch grains. Thin sections were prepared from developing seeds and observed by confocal laser scanning microscope. In the case of barley, it was found that a plurality of simple starch grains was present in a single amyloplast at the early developing stages. In the later stage, it was observed that large starch grains and small starch grains were present in the same amyloplast. This result implies that amyloplast containing several starch grains exists in the endosperm of barley. In the case of rice, it was found that amyloplast having several compound starch grains exists. This result also means that amyloplast containing several starch grains is developed in rice. Transmission electron microscopic observation of developing seeds of non-transgenic rice and barley also gave images showing similar characteristics. That means the results is not the effect of transformation.

In the *AmyloTarget-GFP* plants, GFP is transported inside the amyloplast, but it does not penetrate inside the starch grains. Therefore, the part of the starch grains inside the amyloplast are black spots. This result implies that starch grain can be visualized indirectly in *AmyloTarget-GFP* plants. In the *AmyloTarget-GFP* plants, GFP fluorescence was also observed in chloroplasts of leaves in both rice and barley, and the chloroplasts could be visualized. This means that the AmyloTarget-GFP plants produced in this study can be useful research tools not only for amyloplasts but also for morphological analysis of chloroplasts.

Discussion & Conclusion

In this study, *AmyloTarget-GFP* plants were successfully constructed in rice and barley. In *AmyloTarget-GFP* plants, amyloplast can be directly visualized, and starch grains can be indirectly visualized in living endosperm cells. I believe that *AmyloTarget-GFP* plant is important research tool to clarify the mechanism to differentiate between compound and simple starch grains. Sequential observation will be needed to clarify when and how the morphological differentiation occur during seed development. In addition, I have already isolated several rice and barley mutants defective in starch grain morphologies[3]. Several mutants had genetic lesions in starch biosynthetic enzymes. Introduction of *AmyloTarget-GFP* gene into these mutants, amyloplasts and starch grains can be visualized in mutant backgrounds. Therefore, the observation system constructed in this study should also be important when analyzing starch-related mutants.

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一般の皆様へ

澱粉粒とは、植物細胞内で合成された澱粉が形成する粒子のことである。澱粉粒は、アミロプラストという植物オルガネラの内部で合成される。澱粉粒の形状は植物種によって多様性を示し、その形状は澱粉の利用用途と精製効率を規定する重要形質と考えられている。しかし、澱粉粒の形状が決定される仕組みは未解明である。本研究では、蛍光タンパク質を用いて、生きた細胞内でアミロプラスト並びに澱粉粒を可視化する観察系を構築した。今後、澱粉粒やアミロプラストを形態学的に解析する上で、極めて重要な研究ツールになり得るだろう。

The role of RNA binding protein PSF in prostate cancer progression

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Summary Abstract

New therapeutic approaches are necessary for treating hormone-refractory prostate cancer. Activation of androgen receptor (AR) and its variants expression along with the downstream signals are mostly important for disease progression. Our high-speed sequence analyses identified global PSF-binding transcripts. Interestingly, mRNAs of spliceosome-related genes are putative primary targets of PSF. Their gene expressions are upregulated by PSF in hormone-refractory prostate cancer. Moreover, PSF coordinated these spliceosome proteins to form a complex to promote AR splicing and expression. Thus, targeting PSF and its related pathways implicates the new therapeutic possibility for hormone-refractory prostate cancer.
Key Words：Androgen receptor, prostate cancer, RNA-binding protein, PSF

Introduction

Androgen receptor (AR) regulates many genes central to the identity and behavior of prostate cancer cells (1). AR functions in a ligand-dependent manner in many prostate cancers, and androgen deprivation therapy is effective in inhibiting tumor growth. However, most of the patients acquire resistance to this therapy, and eventually suffer from castration-resistant prostate cancer (CRPC) due to abberant expression and enhanced activity of AR and its variant, AR-V7 (2-5). PSF has a unique structure possessing both DNA- and RNA-binding domains, implicating in transcription and nuclear RNA processing (6, 7). However, the transcriptional and post-transcriptional specific targets and the clinical significance of these proteins in prostate cancer progression still remain to be elucidated.

Results

To explore other functions of PSF in prostate cancer, we next analyzed RNAs bound with PSF using deep-sequence analysis. First, we performed RNA immunoprecipitation coupled with deep sequencing (RIP-seq) in LNCaP cells treated with vehicle or DHT. By mapping sequence tags to RefSeq, GENCODE and NONCODE, we identified putative PSF target genes (Fold > 2, P-value < 10-5). We further performed RIP-seq in CRPC model cells (LTAD and 22Rv1). In LTAD and 22Rv1 cells, we found more binding genes, which significantly overlapped with those of LNCaP cells. In addition, we observed that DHT treatment changed the binding transcripts of PSF. We found that androgen-regulated genes such as CAMKK2 are enriched among DHT-specific target genes of PSF. In contrast to the PSF targets identified by ChIP-seq, pathway analysis showed that spliceosome genes are assumed to be the primary targets of PSF at RNA level in LNCaP cells, suggesting this gene cluster is specifically regulated by PSF as an RNA-binding protein. Prostate cancer-associated signals were also significantly included in these pathways.

To understand how PSF effects on the binding transcripts, we performed directional RNA-seq to investigate the gene regulation by PSF. These results revealed that PSF mainly increases the

expression of its binding transcripts. In addition, knockdown of PSF resulted in a marked decrease in DHT-induced expression of AR target genes. Taken together, non-coding signals associated with prostate cancer, spliceosome genes, and AR-regulated genes by binding to these gene transcripts to increase stability.

We next investigated the PSF function in CRPC model cells. Importantly, the pathway analysis of PSF-binding genes in 22Rv1 cells indicated the importance of PSF in induction of spliceosome genes, as observed in LNCaP cells. In addition, the interaction of PSF with prostate cancer-associated signals was also enhanced. For example, the binding of PSF to the introns of SchLAP1 and AR) was enhanced in LTAD and 22Rv1 cells compared with LNCaP cells, suggesting the role of PSF in expressing these important signals in CRPC.

We then investigated the role of PSF in AR splicing and post-transcriptional regulation. First, we confirmed the binding of PSF with AR and AR-V7, an AR-splicing variant associated with CRPC development, by RIP-assays. In addition, western blot analyses showed that AR-V7 protein expression was detected in VCaP and 22Rv1 cells. Interestingly, PSF expression was also upregulated in these cell lines compared with LNCaP and LTAD, in which AR-V7 protein could not be detected. To determine whether PSF induces AR-V7 protein production or not, we performed qRT-PCR and western blot analysis by using siRNAs targeting PSF and its associated factor, NONO. Surprisingly, PSF knockdown in 22Rv1 cells inhibited full-length AR and AR-V7 mRNA and protein dramatically. Analysis of mRNA stability of AR and AR-V7 by inhibiting transcription indicated that PSF and NONO are involved in the post-transcriptional regulation of these mRNAs.

We further investigated whether upregulation of spliceosome genes could enhance splicing activity cooperating with PSF and NONO in CRPC. Our immunnoprecipitation and western blotting analysis indicated that PSF interacts with these spliceosome components, and was responsible for the complex formation of spliceosome. In addition, immunofuorescnece analysis showed that this interaction occurred in the nucleus. RIP-assay also indicated that these factors binding to AR transcripts and act as an integrator for PSF-mediated splicing of AR transcripts. Taken together, these results revealed that PSF also orchestrated its target splicing factors at protein levels to form a complex for splicing and protein expression in CRPC.

Discussion & Conclusion

We indicated the importance of PSF in the regulation of splicing machinery in the progression of aggressive prostate cancer. Our discovery revealed a novel mechanism in which a broad range of splicing components were aberrantly regulated for cancer progression. The wide-ranging upregulation of the splicing pathway in metastatic prostate cancer could affect the splicing complexes in cancer. RNA splicing is mediated by an assembly, rearrangement and disengagement of a set of small nuclear ribonucleoprotein (snRNP) complexes (U1, U2 and either U4/5/6 or U11/U12) or other proteins onto the pre-mRNAs (8, 9). The broad coverage of the wide spectrum of these components in our study suggests the involvement of PSF in enhancing this machinery. Interestingly, frequent mutations of splicing pathway have been reported in several malignancies by whole-exome sequencing (10-12). These clinical analyses provide an intriguing insight into the mechanism of cancer progression by splicing machinery since RNA splicing system has essential cellular roles for the diversity of protein species using a limited number of genes. Loss of splicing activity by mutations would induce severe developmental abnormality and tumorigenesis (13). Conversely, such mutation has been rarely identified in prostate cancer (14). Enhanced splicing machinery could be

indispensable for the cancer progression in some types of malignancies (15). Moreover, the present study would illustrate the specificity of PSF function and splicing machinery in prostate cancer.

In summary, our global analysis of PSF functions revealed its target signals based on the RNA-binding ability in addition to cell cycle regulator control by epigenetic silencing of the transcription with histone modification. Importantly, we proposed a new mechanistic link between RNA-binding proteins and AR as well as prostate cancer-associated signals, indicating the clinical and biological impact of PSF in the development of abnormal splicing machinery in hormone-refractory prostate cancer. Considering the potential function of AR and AR-variants in the development of metastatic CRPC, PSF and its associated factors could have the potential for therapy targets.

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一般の皆様へ

前立腺がんは世界的にみて男性に発生するがんとして最も多いがんです。男性ホルモンであるアンドロゲンによりがんの成長、病気の進行を促進することが知られておりアンドロゲンを抑制する薬剤が治療に用いられるものの治療抵抗性に陥ることが問題となっています。治療抵抗性の出現にはアンドロゲンの受容体の発現上昇が知られていますが、その機序がよくわかっていません。遺伝子の発現にはDNAからの転写により生成されるRNAから蛋白合成を通じて行われます。本研究はRNA結合タンパク質であるPSFがアンドロゲン受容体およびRNAの成熟に関与する遺伝子群のRNAに作用することでその安定性、発現を促進していることを明らかとしました。RNA成熟に関与する遺伝子群の発現上昇が引き起こされアンドロゲン受容体の活性化、がんの悪性化につながる新たな作用機序を見出しています。

Selective information routing in the hippocampal network

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Summary Abstract

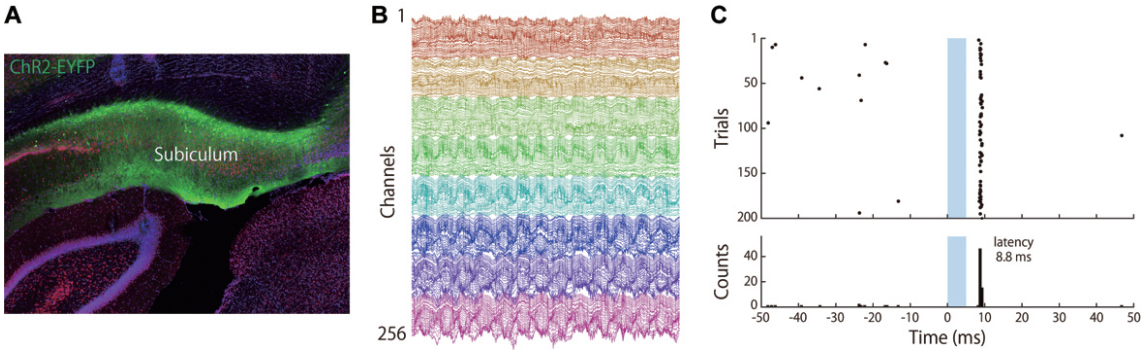
The subiculum is a major output hub of the hippocampal formation. However, information outflow from the subiculum to various downstream regions remains poorly characterized. This research involved the use of a combination of large-scale recordings and optogenetics to establish a method for the antidromic identification of projection neurons in freely behaving rats. This method allowed us to investigate information outflow from the subiculum to each downstream region.
Key Words：hippocampus, subiculum, optogenetics, routing, memory

Introduction

The hippocampal formation, which comprises the hippocampus proper, dentate gyrus, and subiculum, is crucial for learning and memory. Historically, although most studies have focused on the hippocampus proper, recent evidence has highlighted the substantial contribution of the subiculum to interregional connectivity and behavioral performance. In fact, from an anatomical point of view, the subiculum is a major output hub of the hippocampal formation. However, information outflow from the subiculum to various downstream regions remains poorly characterized. Thus we investigated the target-specific information outflow from the subiculum using large-scale recordings and antidromic identification of projection neurons in freely behaving rats.

Results

Although the subiculum is a major output structure in the hippocampal formation (Naber and Witter, 1998), the type of information transferred from the subiculum to each downstream region remains elusive. The main reason for this gap is that by using currently available electrophysiological recording methods we cannot classify the recorded neurons based on the brain region(s) to



which each neuron sends axon(s) (Ciocchi et al., 2015; Saiki et al., 2018). We overcame this limitation by combining large-scale recordings and optogenetics, thus establishing a method to simultaneously record from over 100 neurons and identify the target brain areas of approximately 10% of the recorded neurons (Fig 1). For this purpose, we used adeno-associated viruses to express

channelrhodopsin (ChR2) in the subicular neurons in freely behaving rats. Simultaneously, we implanted optic fibers in the target brain areas of the subiculum (the nucleus accumbens, retrosplenial cortex, intralaminar and anteroventral thalamic nuclei, and medial mammillary body) and stimulated the axons of ChR2-expressing subicular neurons using blue light. The spikes induced by photo-stimulation at the axons retrogradely propagated into the cell bodies in the subiculum. By recording from the subiculum using high-density silicon probes (up to 256 channels), we detected retrogradely propagated spikes and thereby identified the neurons directly projecting into that target area. The collision test was performed to ensure that the spikes recorded at the cell bodies during photo-stimulation were indeed back-propagated spikes generated at the axons and not indirectly generated via synaptic interactions. The collision test is based on the following rationale: if a spike generated at the axon of a given neuron by blue-light stimulation occurs within several milliseconds before or after a spike spontaneously generated at the cell body of that neuron, the spike generated at the axon propagates to the cell body and that generated at the cell body propagates to the axon, thus resulting in a collision; therefore back-propagated spikes generated at the axon are not recorded at the cell body. When blue light-induced spikes are due to synaptic interactions, collisions will not occur. Therefore, blue light-induced spikes can be detected at the cell body even if spikes are generated at the soma at very similar time points as light-induced spikes. To ensure the efficacy of the collision test, we first putatively identified the neurons projecting into the target area. We then selected the channel at which largest spike waveform of those neurons were recorded and used ON-LINE spike detection algorithm to identify the spikes recorded from these channels. Using these spikes, we triggered blue-light stimulation at the target area, thus speeding up the collision test. So far, we have been able to identify the target areas of approximately 10% of the recorded neurons. To increase this proportion, we are currently working to increase the efficacy of this method by optimizing the following: ChR2 expressions in the subiculum, blue light power, optical stimulation sites, and spacing between silicon probe recording sites, thus increasing the number of recorded neurons.

Moreover, we established recording systems that enable recording from free-moving rats during various behavioral tasks, thus revealing the type of information represented by individual subicular neurons. We established an alternation task with more than several seconds of delay, which is a hippocampus-dependent memory task. We also established behavioral tasks using zig-zag and radial mazes, the open field test, and a linear track to reveal the information represented by individual subicular neurons.

Fig 1. Channelrhodopsin (ChR2) was expressed in the subiculum neurons using adeno-associated virus, (B) then we recorded from the subiculum using a 256 channels silicon probe, (C) and stimulate a projection target brain region using blue light. By detecting spikes generated at the axon by light stimulation and back-propagated to the soma with short latency (<15 ms) and jitter (<1 ms), we classified neurons by their projection target regions.

Discussion & Conclusion

We have established a method for antidromic identification of projection neurons in freely behaving rats by combining large-scale recordings and optogenetics. The use of high-density silicon probes (up to 256 channels) for large-scale recordings enabled us to simultaneously record from over 100 subicular neurons. In addition, we expressed ChR2 in subicular neurons and stimulated target brain areas using blue light. The detection of blue light-induced back-propagated spikes at the cell bodies in the subiculum allowed us to identify the target areas of approximately 10% of the recorded subicular neurons. We also established behavioral tasks that allow us to characterize the information represented by subicular neurons. Using this developed method to identify the target areas of the recorded neurons and behavioral tasks, we are currently investigating target-specific information outflow from the subiculum.

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一般の皆様へ

海馬と呼ばれる脳の領域は、個人的な経験を思い出す能力であるエピソード記憶に重要な役割を果たします。本研究では、海馬からどの脳領域へどのような情報が伝達されるのかを明らかにするための基盤的な技術を開発しました。今後は開発した技術を用いて、海馬と他の脳領域がどのように情報をやり取りして記憶をサポートしているのかを明らかにしたいと考えています。アルツハイマー病などの認知症では、ごく初期から海馬に変性がみられます。海馬における情報処理のメカニズムを少しでも明らかにして、将来的に認知症の新規治療法を確立するための基盤を作りたいと考えています。

Chemical approach to developing anticancer agents of marine alkaloid origin

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Summary Abstract

Agelastatin A (AA) is a natural antitumor alkaloid isolated from the marine sponge *Agelas dendromorpha.* We have established a new method for the chemical synthesis of AA and its analogs. Biological assessment of AA analogs has led to the discovery of novel compounds that exert antiproliferative activity against cancer cell lines. Structure-activity relationship (SAR) studies on AA analogs have provided important insights into the molecular design for the further development of AA-based medicinal leads.

Key Words：natural product, chemical synthesis, anticancer, agelastatin A

Introduction

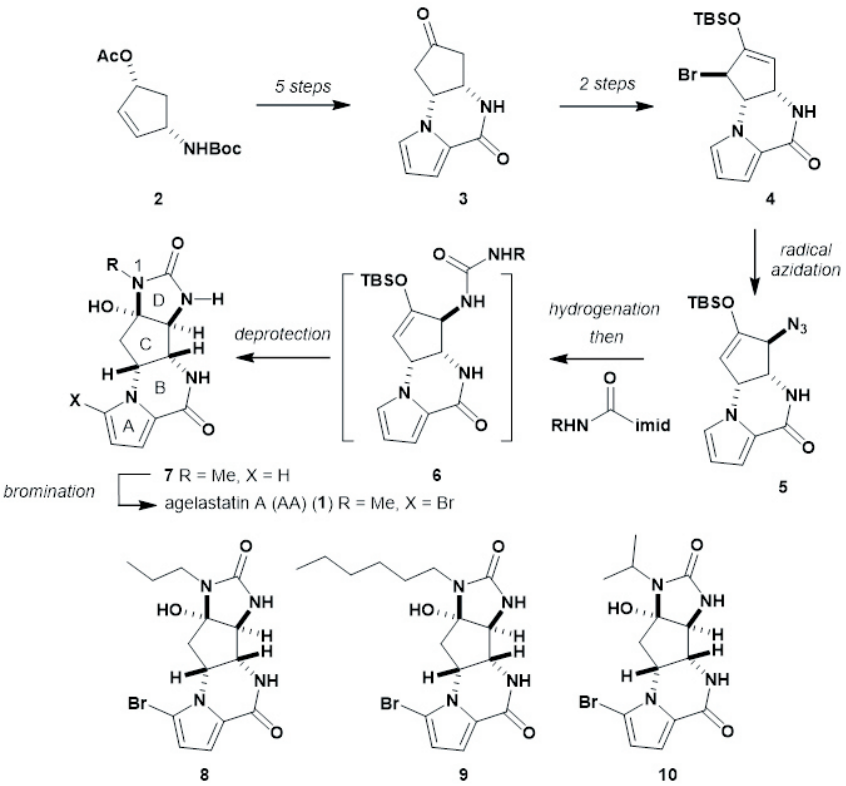
Agelastatin A (AA), a tetracyclic alkaloid isolated from the marine sponge *Agelas dendromorpha,* has shown potent antiproliferative activity against various cancer cell lines by inhibiting the Wnt/β-catenin pathway responsible for cellular processes. Moreover, AA exhibits anti-osteopontin (OPN) activity and has been touted as an anti-OPN agent.[1] As various cancers, particularly aggressive and metastatic cancers, significantly express OPN, there has been avid interest in the development of AA derivatives as a new therapeutic agent for such cancers.

Results

1) Development of new divergent synthetic routes to AA and its analogs:

Our previous structure-activity relationship (SAR) studies on AA have revealed that the modification of the N_1-substituent on the D-ring system yields new analogs with potent biological activity.[2,3] Therefore, we endeavored to establish a new synthetic protocol that streamlines access to D-ring analogs bearing various N_1-substituents. Our study on the chemical synthesis commenced with known amino alcohol derivative **2** (>99% ee) that serves as the C-ring motif of the AA molecule (Scheme 1). Starting material **2** was subjected to five-step synthetic manipulations to produce tricyclic ketone **3** that corresponds to the ABC ring system of AA. Then, a sequential transformation involving enolization and bromination was applied to ketone **3** to deliver TBS enol ether **4** that is ready for the installation of the pivotal nitrogen functionality of the D-ring system. Whereas the key nitrogenation reaction was extremely challenging, we eventually devised a new means that features radical azidation to deliver azide **5**, which bears the necessary functionalities to fully access the D-ring system. Hydrogenation of compound **5** afforded amine (structure not indicated), which was further subjected to carbamoylation and deprotection to afford **7**. The final bromination of **7** smoothly furnished AA **(1)**. Furthermore, the synthetic protocol wherein various carbamoylation reagents were employed eventually culminated in the facile syntheses of novel AA analogs **(8, 9,** and **10)** with such N_1-substituents as *n*-propyl, *n*-hexyl, and *i*-propyl groups.

Scheme 1 *A new synthetic route to agelastatin A (AA) and AA analogs*

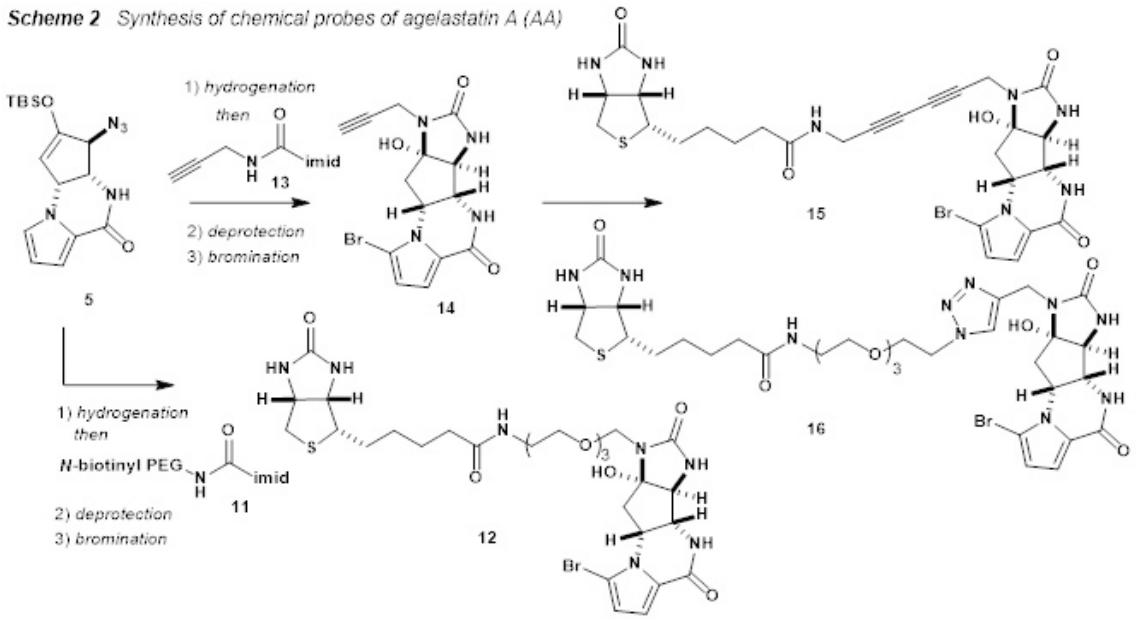


2) Identification of novel potent AA analogs by SAR studies:

New AA analogs **8, 9,** and **10** synthesized by the above-mentioned protocol were tested for their *in vitro* cytotoxicity toward various cancer cell lines, including DU145 (prostate cancer cells). The SAR study suggested that the structural modification of the N_1-substituent with linear alkyl chains was somewhat tolerable and AA analogs 8 and 9 obtained by the structural modification retained their biological activity. In contrast, the introduction of a bulky alkyl substituent at the N_1-position led to significant loss of activity. These SAR studies provide important insights into the molecular design for the further development of AA-based lead compounds.

3) Synthesis of chemical probes to identify molecular target underlying biological activity:

Having established a new protocol to access AA analogs with varying N_1-substituents, we made further efforts to synthesize chemical probes that would enable identification of the molecular target responsible for the biological activity of AA. Common intermediate **5** was subjected to reduction, carbamoylation with **11**, deprotection, and bromination to afford biotinylated compound **12**. In addition, compound **5** was coupled with alkyne fragment **13** to provide propargyl derivative **14**, which was reacted with an alkyne fragment (structure not shown) to furnish compound **15**. Third chemical probe **16** was synthesized by connecting propargyl derivative **14** to an azide fragment (structure not shown). However, the biological assessment of chemical probes **12**, **15**, and **16** revealed that none of these derivatives exhibited any promising antiproliferative activity. This result provides an additional insight into the SAR of AA analogs, namely, the introduction of linear polar substituents at the N_1-position may be unfavorable to the biological function of AA.



Discussion & Conclusion

The present study was undertaken to develop new anticancer leads derived from the marine natural alkaloid agelastatin A (AA). We established divergent synthetic routes to AA and its analogs, wherein a radical azidation reaction enabled facile construction of the nitrogen-containing molecular architecture of AA. Furthermore, AA analogs that exhibit antiproliferative activity against cancer cell lines were identified by the SAR study. Three chemical probes that possess a biotin tag on N_1-nitrogen were also synthesized. However, biological assessment of the probes revealed their lack of antiproliferative activities, suggesting that such structural modifications are deleterious to the biological activity of AA.

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一般の皆様へ

海綿から見出されたアゲラスタチンＡは，抗がん剤の開発において大変魅力的な物質として興味を集めています。これまで我々は，アゲラスタチンＡの化学構造を人工的に改変し、抗がん剤のリード化合物を創り出す研究を進めてきました。本研究では、こうした創薬研究の基盤を成す化学合成法の開発、化学合成によって得た化合物の中から有望な物質を見出すこと、そして、アゲラスタチンＡの作用機序の解明に役立つ分子プローブの合成に取り組みました。その結果，各種誘導体の新規合成法の開発並びにリード化合物の設計指針となる知見を得ることに成功しました。

Catecholamine-induced senescence of endothelial cells and bone marrow cells promotes cardiac dysfunction in mice

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Summary Abstract

In the present study, we found that catecholamine infusion induced senescence of endothelial cells and bone marrow cells, and promoted cardiac dysfunction in mice. Continuous infusion of isoproterenol induced cardiac inflammation and cardiac dysfunction. Expression of p53, a master regulator of cellular senescence, was increased in the cardiac tissue and bone marrow cells of these mice. Suppression of cellular senescence by genetic deletion of p53 in endothelial cells or bone marrow cells led to improvement of isoproterenol-induced cardiac dysfunction. Our results indicate that catecholamine-induced senescence of endothelial cells and bone marrow cells plays a pivotal role in the progression of heart failure. Suppression of catecholamine-p53 signaling is crucial for inhibition of remodeling in the failing heart.

Key Words：Chronic inflammation, p53, cellular senescence

Introduction

Previous studies have suggested a central role of cellular senescence in the progression of pathologic changes in the failing heart. It is well known that the sympathetic nervous system is activated in patients with heart failure, and this change is associated with poor clinical outcomes. Sympathetic activation increases the levels of various catecholamines, such as epinephrine and norepinephrine, but the contribution of these catecholamines to cellular senescence associated with heart failure remains to be determined.

Results

To investigate whether catecholamine infusion induced cellular senescence, we administered isoproterenol to mice via an infusion pump, leading to the development of cardiac hypertrophy associated with cardiac systolic dysfunction. Under these conditions, there was an increase in p53 and the transcript of its target molecule, p21 (*Cdkn1a*), in cardiac tissues. We previously detected p53-induced senescence of endothelial cells and bone marrow cells in a murine model of LV pressure overload[1]. These findings led us to analyze p53 expression in bone marrow cells under hyperadrenergic conditions in the present study. After continuous infusion of isoproterenol, there was a marked increase of p53 and its transcript p21 (*Cdkn1a*) in bone marrow cells. These results suggested that the hyper-adrenergic state associated with heart failure promotes p53-induced cellular senescence in cardiac tissue and in the bone marrow. Chronic sterile inflammation develops in patients with heart failure and promotes pathologic changes[2-4]. In agreement with this report, we found that isoproterenol infusion increased the infiltration of mononuclear cells and Mac3 positive cells into the left ventricle, and was associated with increased LV expression of transcripts for

inflammatory markers. Isoproterenol infusion also increased transcripts for integrin alpha-L (*Itgal*) in bone marrow cells. These results suggested that activation of adrenergic signaling per se contributes to elevation of integrin expression by bone marrow cells and to increased cardiac tissue expression of intercellular adhesion molecule-1 (ICAM-1), which may promote sterile inflammation and remodeling in the failing heart.

Our data indicated that activation of adrenergic signaling resulted in up-regulation of p53 in both cardiac tissue and bone marrow. In addition, transcripts of *Icam1* and *Itgal* were respectively increased in cardiac tissue and bone marrow by a hyperadrenergic state. We previously reported that p53 positively regulates the expression of these adhesion molecules at the transcriptional level, inducing cardiac inflammation and remodeling in a murine model of LV pressure overload[1]. To obtain further direct evidence that p53 expression in endothelial cells and/or bone-marrow cells contributes to progression of catecholamine-induced heart failure, we performed isoproterenol infusion in endothelial cell (EC) - or bone marrow cell (BM)-p53 knockout (KO) mice. EC-p53 KO mice were generated by crossing Tie-2 Cre mice with $Trp53^{flox/flox}$ mice, and $Tie2\text{-}Cre^{+}$ $Trp53^{flox/flox}$ (EC-p53 KO) mice and their littermate controls ($Trp53^{flox/flox}$ mice) were analyzed. Infusion of isoproterenol led to cardiac dysfunction and dilatation in control mice, while these changes were ameliorated in EC-p53 KO mice. We generated a BM-p53 KO model by transplanting bone marrow cells from systemic p53 KO mice into wild-type mice after irradiation, while control mice were generated by transplanting bone marrow cells from wild-type mice into irradiated wild-type mice. Isoproterenol infusion caused systolic dysfunction along with cardiac dilatation in the control groups, while such changes were ameliorated in BM-p53 KO mice. These results suggested that up-regulation of p53 signaling in endothelial cells and bone marrow cells by a hyper-adrenergic state induces cardiac dysfunction.

Discussion & Conclusion

In this study, we showed that catecholamine-induced senescence of endothelial cells and bone marrow cells had a pivotal role in the progression of pathological changes associated with heart failure. In a murine model of isoproterenol-induced heart failure, infusion of isoproterenol led to an increase of p53 expression in both cardiac tissue and bone marrow, while genetic suppression of p53 in these organs ameliorated cardiac dysfunction.

While β-blockers were initially contraindicated for patients with heart failure, these drugs have since become first-line therapy for this critical condition. Carvedilol is a non-selective β-blocker with lower affinity for the β1-adrenergic receptor and higher affinity for the β2-adrenergic receptor. Among various β-blockers (including selective and non-selective drugs), carvedilol is recognized as first-line therapy for heart failure[5, 6]. Our findings suggest that β-blockers like carvedilol may have a beneficial effect via suppression of β2-adrenergic receptor signaling and prevention of cellular senescence in the hyper-adrenergic state associated with heart failure.

In coclusion, inhibition of catecholamine-induced cellular senescence is important for suppression of pathologic changes in the failing heart.

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一般の皆様へ

本研究で私たちは、心不全に伴って心臓で炎症が惹起される機序について明らかにしました。このような炎症の亢進が、心不全悪化につながっていることから、今後新たな治療の標的となりうると考えられます。

Modulation of three-dimensional chromatin structure by Piwi-piRNAs

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Summary Abstract

The piRNA (PIWI-interacting RNA) pathway in *Drosophila* ovarian somatic cells represses transposable elements (TEs) transcriptionally to maintain the genome integrity. Here, we described the link between piRNA mediated silencing and regulation of chromatin structure. We performed Hi-C experiment, capable of detecting 3D structure of the genome, together with series of ChIP-seq and RNA-seq analyses. This revealed that piRNAs silence their target TEs by dynamic regulation of chromatin 3D structure coupled with modulation of histone marks. Additionally, we obtained a list of candidate proteins, such as insulators, which may play a key role in this regulation.
Key Words：Chromatin structure, Epigenetic regulation, Transcriptional regulation, RNA silencing, non-coding RNA

Introduction

The piRNA pathway in *Drosophila* ovarian somatic cells represses TEs transcriptionally to maintain the genome integrity. The Piwi-piRNA complex establishes heterochromatic H3K9me3 marks on TEs and their neighboring regions to silence them. Additionally, we have recently shown that the linker histone H1 is necessary for Piwi-piRNA to repress TEs. We proposed a model in which Piwi-piRNA complexes exert TE silencing through H1 association with specific chromatin regions, resulting in modulation of chromatin accessibility.

Results

The piRNA pathway in *Drosophila* ovarian somatic cells represses TEs transcriptionally to maintain the genome integrity [Iwasaki et al., Annu Rev Biochem (2015)]. The Piwi-piRNA complex establishes heterochromatic H3K9me3 marks on TEs and their neighboring regions to silence them. Additionally, we have recently shown that the linker histone H1 is necessary for Piwi-piRNA to repress TEs [Iwasaki et al., Mol Cell (2016)]. We proposed a model in which Piwi-piRNA complexes exert TE silencing through H1 association with specific chromatin regions, resulting in modulation of chromatin accessibility.

In line with this and the fact that some non-coding RNAs are known to effect chromatin boundaries [Cohen et al., WIREs RNA, (2014)], we have analyzed the involvement of Piwi-piRNA on the 3D chromatin organization of *Drosophila* OSC (Ovarian Somatic Cells) cell line, using Hi-C (Chromatin conformation capture sequencing) method [Lieberman-Aiden et al., Science (2009)]. This revealed that depletion of Piwi induces long-range interactions within a chromosome arm. Also, we analyzed TADs (Topologically associating domains) of OSCs using Hi-C data, and revealed the decrease of intra-TAD interaction and increase of inter-TAD interaction upon loss of Piwi. Importantly, the decrease of intra-TAD interaction and increase of inter-TAD interaction correlated with the density of Piwi-piRNA target TEs in each TAD. This suggests that Piwi is essential for maintaining the

structure of chromatin, which possibly is mediated by the regulation of target TEs.

Additionally, we performed ChIP-seq experiment of H3K9me3, H3K27Ac, and H3K4me3 histone marks upon depletion of Piwi. This revealed that H3K9me3 repressive marks were correlated with decrease of intra-TAD interaction, where H3K27Ac and H3K4me3 active histone marks were inverse correlated with decrease of intra-TAD interaction induced by Piwi knock down. Importantly, we analyzed the transcription level of the genes harbored in each TADs by using RNA-seq analysis. This also suggested that decrease of intra-TAD interaction upon Piwi knock down was inverse correlated with average expression level of the genes located in TADs. These results suggest that Piwi modulate chromatin level in a manner, which couples histone marks, and also transcription levels.

We further performed the Hi-C experiment using *Drosophila* ovary, in order to see whether chromatin modulation examined by using OSC cell line could still be seen *in vivo*. We could observe the decrease of intra-TAD interaction and increase of inter-TAD interaction in Piwi mutant flies, but in much stronger level than in OSCs. This may be due to the fact that in case of using mutant files, we can observe the effect of Piwi on TADs prior to the primary formation of TADs. We will further analyze the data to discuss *in vivo* effect of Piwi on chromatin structure.

Also, in order to screen for the protein, which is involved in this modulation of chromatin structure by Piwi, we performed ChEP (Chromatin Enrichment for Proteomics) analysis [Kustatscher et al., Nat Prot (2014)], to obtain a list of proteins whose chromatin association level is affected by depletion of Piwi. This analysis revealed that Piwi might affect on the chromatin binding of insulator proteins, which are known as the genetic boundary elements. We have already raised several antibodies for these candidate insulator proteins. We will further analyze the impact of Piwi on these factors and discuss how Piwi can modulate 3D chromatin structure.

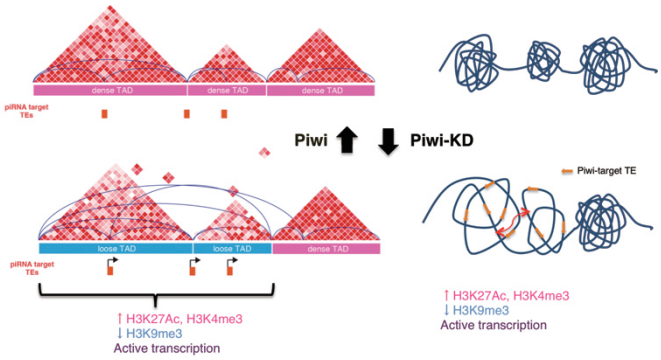

Fig. Piwi-piRNA mediated chromatin structure regulation model

Discussion & Conclusion

Our combined Hi-C, ChIP-seq, and RNA-seq data analysis using OSC cell line suggests that Piwi is essential for maintaining the structure of chromatin, which possibly is mediated by the regulation of target TEs. The results also suggest that Piwi modulate chromatin level in a manner, which couples histone marks, and also transcription levels. Further analysis performed using *Drosophila* ovary, suggested the similar decrease of intra-TAD interaction and increase of inter-TAD interaction in Piwi mutant flies, but in much stronger level. This may be due to the fact that in case of using mutant files, we can observe the effect of Piwi on TADs prior to the primary formation of TADs. We will further analyze the data to discuss *in vivo* effect of Piwi on the chromatin structure. Also, we obtained insulator proteins as candidates from ChEP analysis. This analysis suggested that Piwi might affect on the chromatin binding of insulator proteins, which are known as the genetic boundary elements. We have already raised several antibodies for these candidate insulator proteins. We will further analyze the impact of Piwi on these factors and discuss how Piwi can modulate 3D chromatin structure.

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一般の皆様へ

生物の設計図であるゲノムは、自身のコピー数をどんどん増やしてしまう「利己的な遺伝子」として知られるトランスポゾンの転移により、傷ついてしまいます。piRNA と呼ばれる小さなRNA が、このトランスポゾンを抑え込む働きをしており、この制御が正常に機能しないと不妊等が引き起こされることが知られています。そこで本研究では、piRNA がどのようにトランスポゾンを抑制するかを解析しました。研究成果として、piRNA によるトランスポゾンの抑制においては、核内でゲノム（クロマチン）がどのように折りたたまれているかが制御されることが重要だということが分かりました。

Catalytic Synthesis of Spiroketals Using Water as the Oxygen Source Triggered by Rearrangement of Internal Alkynes

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Summary Abstract

A ruthenium-catalyzed synthesis of [5,6]-spiroketals that involves a very rare 1,2-carbon migration of internal alkynes is reported. Mechanistic studies revealed that this reaction proceeds via a disubstituted vinylidene complex as the keyintermediate by 1,2-carbon migration of the eneynones.
Key Words：Spiroketal, Internal alkyne, Vinylidene, Ruthenium, Rearrangement

Introduction

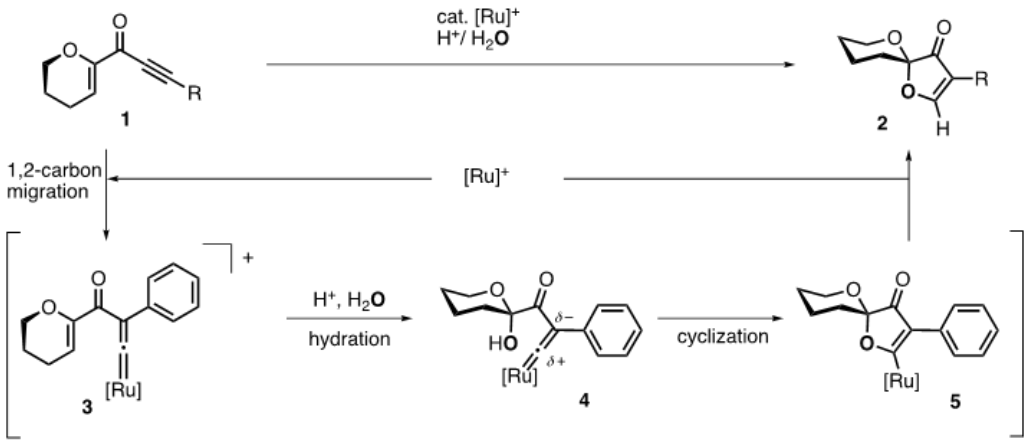
Catalytic processes that involve the vinylidene rearrangement of internal alkynes with a carbon substituent on their alkyne terminus by 1,2-carbon migration has not yet been reported though experimental[1] and theoretical[2] investigations into stoichiometric internal alkyne-to-vinylidene rearrangements have only recently been reported.

Meanwhile, we have developed the first catalytic reaction that involves the vinylidene rearrangement of internal alkynes by 1,2-carbon migration.[3] These results encouraged us to develop new and useful reactions that involve 1,2-carbon migration. Herein, we report a ruthenium-catalyzed synthesis of [5,6]-spiroketals, which are core structures of biologically active natural compounds such as γ-rubromycin and berkelic acid.[4]

Results

Scheme 1 shows working hypothesis for the catalytic synthesis of [5,6]-spiroketals **2** that involves vinylidene rearrangement of eneynone **1**. A cationic ruthenium complex affects the internal alkyne-to-vinylidene rearrangement of **1**, and the vinylidene complex 3 is formed. Hydration of dihydropyran moiety in 3 under an acidic condition generates hemiacetal **4**. Subsequent oxycyclization of **4**, in which the hydroxy group nucleophilically attack to the electrophilic α-carbon atom of a vinylidene ligand, and [5,6]-spiroketals are formed. Finally, the protonation (decomplexation) of **5** furnishes the desired [5,6]-spiroketals **2** with concomitant regeneration of the ruthenium catalyst.

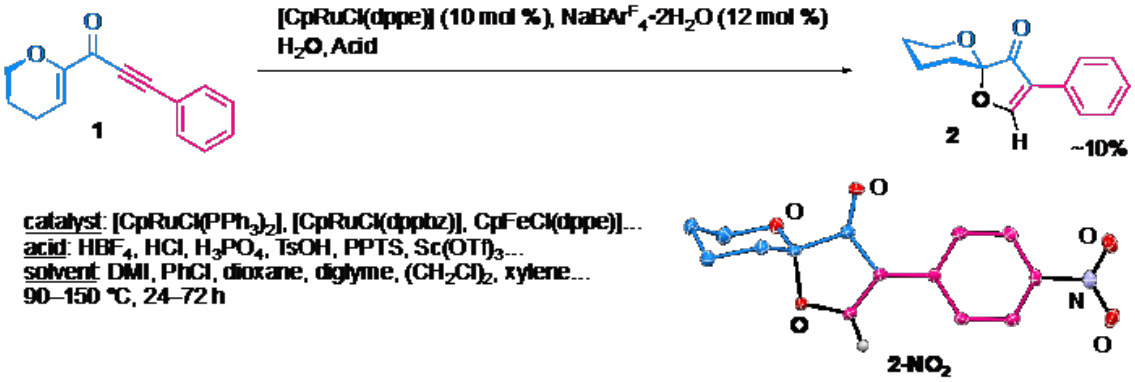
Scheme 1. Working hypothesis



Initially, I investigated the reaction of **1** using [CpRuCl(dppe)]/$NaBAr^F_4{\cdot}2H_2O$ (Ar^F = 3,5-$(CF_3)_2C_6H_3$) as the catalytic system, which was found to be highly active in the previous studies.[3] When a mixture of **1**, [CpRuCl(dppe)] (10 mol%), and $NaBAr^F_4{\cdot}2H_2O$ (12 mol%) was stirred under heating conditions, the desired

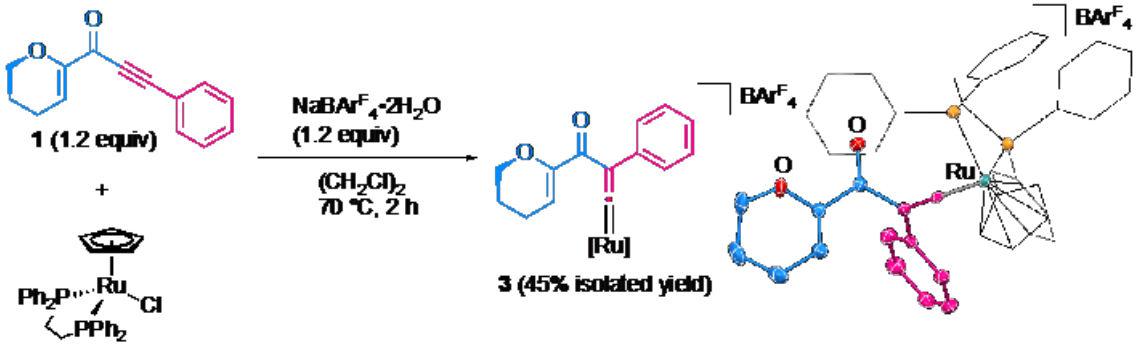
1,2-aryl migration/hydration/cyclization product, [5,6]-spiroketal **2**, was formed, albeit in ca. 10 % yield (Scheme 2). The [5,6]-spiroketal structure was unambiguously determined by a single-crystal X-ray diffraction analysis of **2-NO$_2$**. Unfortunately, screening of catalysts, acids, and solvents did not improve the yield of the product.

Scheme 2. Ru-catalyzed synthesis of [5,6]-spiroketal 2 involving 1,2-carbon migration



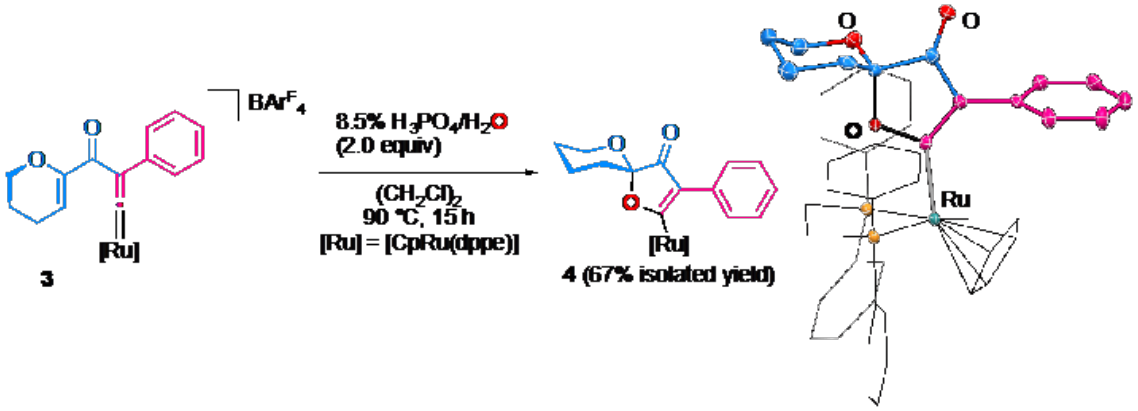
To gain insight into the underlying mechanistic aspects of the present reaction, I examined the stoichiometric reaction between **1**, [CpRuCl(dppe)], and $NaBAr^F_4{\cdot}2H_2O$ at 70 °C in PhCl (Scheme 3). After stirring for 2 h, vinylidene complex 3 was isolated in 45% yield. The molecular structure of **3** was unambiguously determined by a single-crystal X-ray diffraction analysis that confirmed the anticipated 1,2-carbon migration to form the corresponding disubstituted vinylidene intermediate. The isolation of the vinylidene complex implies that the hydration/cyclization of **1** proceeds slower than the vinylidene rearrangement of **1** under the applied reaction conditions.

Scheme 3. Stoichiometric internal alkyne-to-disubstituted vinylidene rearrangement of **1** on $[CpRu(dppe)]^+$ that leads to vinylidene complex **3**



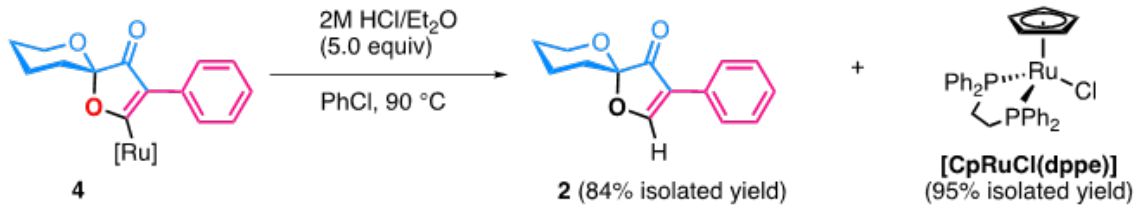
Next, hydration of vinylidene complex **3** was examined. When a solution of **3** was stirred at 90°C in the presence of H_3PO_4 (8.5% aqueous solution, 2 equiv), [5,6]-spiroketal complex **4** was isolated in 67% yield (Scheme 4). The molecular structure of **4** was unambiguously determined by a single-crystal X-ray diffraction analysis. The formation of **4** from **3** indicates that the vinylidene rearrangement of internal alkynes is the crucial step in the present catalytic synthesis of [5,6]-spiroketals.

Scheme 4. Hydration/cyclization of **3** that leads to [5,6]-spiroketal complex **4**.



The final step of the catalytic cycle was also confirmed (Scheme 5). Thus, reaction of ruthenium complex **4** with HCl afforded the desired [5,6]-spiroketal **2** in 84% yield as well as [CpRuCl(dppe)] in 95% yield.

Scheme 5. Decomplexation of **4** using HCl that leads to **2** and **[CpRuCl(dppe)]**



Discussion & Conclusion

I have demonstrated a ruthenium-catalyzed formation of [5,6]-spiroketals, which proceed via a very rare 1,2-carbon migration of the internal alkynes. Stoichiometric reactions revealed that the disubstituted vinylidene complex that is directly derived from the internal alkyne is the key intermediate in this spiroketal synthesis. This study shows that the vinylidene rearrangement of internal alkynes by 1,2-carbon migration can be integrated into useful catalytic reactions that involve carbon-carbon bond formation reactions. Further studies directed toward the catalytic formation of various spiroketals that involve 1,2-carbon migration is ongoing.

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一般の皆様へ

本研究では、これまでの常識では考えられなかったルテニウム触媒をもちいる内部アルキンの1,2−転位を起点とする［5,6］−スピロケタールの触媒的新規合成法の開発に挑戦しました。［5,6］−スピロケタールは、ベルケリン酸やγ-ルブロマイシンなど抗腫瘍生物活性物質の活性を担う重要な骨格です。今後の薬理学研究に向けてスピロケタールライブラリーを構築するためには、それらを自在に合成できる新たな反応の開発が必要不可欠です。低収率ではありますが、［5,6］−スピロケタール新しい合成法の開発に成功し、段階的に反応を行うことによって反応機構についても明らかとしました。

Molecular analysis for the pathophysiological significance of brain hypoxic response and application for the CNS diseases

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Summary Abstract

Chronic cerebral hypoperfusion is manifested in a wide variety of CNS diseases, including neurodegenerative and mental disorders that are accompanied by chronic mild-hypoxic condition and resultant cognitive impairment. Here, we used a chronic cerebral hypoperfusion mouse model to investigate whether transient receptor potential ankyrin 1 (TRPA1), a Ca2+-permeable cation channel, which can respond to mild hypoxic conditions. When male mice were subjected to chronic cerebral hypoperfusion, cognitive dysfunction and white matter injury at day 14 were observed only in TRPA1-knockout mice compared but not wild-type mice, suggestting that hypoxia-sensitive TRPA1 may play a protective in chronic cerebral hypoperfusion-related disorders.
Key Words：Hypoperfusion, Hypoxia, CNS diseases, TRPA1

Introduction

Chronic cerebral hypoperfusion, resulting in an inadequate supply of blood to the brain and inducing hypoxic condition, is elicited by ageing and a variety of risk factors such as atherosclerosis, hypertension, and obesity. It has been previously suggested that chronic cerebral hypoperfusion is highly related to dementia diseases such as Alzheimer's disease and vascular dementia, and other CNS diseases. Therefore, the precise molecular mechanism of chronic cerebral hypoperfusion-induced cognitive impairment needs to be identified.

Results

In this study, we investigated the pathophysiological role of TRPA1, a Ca2+-permeable channel that is expressed in neuron, glial cells and endothelial cells and is involved in responding to hypoxic conditions, using a mouse chronic cerebral hypoperfusion model with bilateral common carotid artery stenosis (BCAS) by the 0.18 mm internal diameter microcoils, as previously described (Temma et al., 2017). Cognitive assessment was performed by novel object recognition test. Experiments were conducted in a dimly illuminated situation (30 Lx), with mice being habituated to the black box for 3 days (10 min a day) before the training. In the training session, two different objects (a yellow triangular prism and a blue quadrangular pyramid) were placed in the box and mice were allowed to freely interact with the objects for 10 min. Six hours after the training session, the test session was performed, and the blue quadrangular object was replaced by a wooden ball to serve as a novel object. Exploratory time was defined as the time spent exploring the blue quadrangular object in the training session and the wooden ball in the test session, and was considered an indicator of locomotor activity. Exploratory preference was defined as the ratio of the exploratory time versus the total time spent exploring both of the objects, and was considered to be an indicator of recognition memory as previously described (Miyanohara et al., 2018).

When mice were subjected to BCAS, the rCBF 60 min after the operation was successfully decreased to approximately 60% compared with the sham operation, with the change not differing between the wild-type (WT) and TRPA1-knockout (TRPA1-KO) mice. Exploratory preferences to the two different objects were around 50%, with no significant difference being observed in the training sessions in both genotypes. In the test session, exploratory preferences to the novel object after the training session and interval did not differ between the groups at day 14 after BCAS, whereas at day 14 there were significant decreases in BCAS-operated TRPA1-KO mice, but not in BCAS-operated WT mice. At day 28, there were significant decreases in both genotypes. These results suggest that blockade of TRPA1 can accelerate the development of cognitive impairment induced by chronic cerebral hypoperfusion.

Next, we have checked tissue injury in BCAS-operated mice. White matter damage is a characteristic of a variety of CNS diseases, and is associated with cognitive impairment in dementia cases and other psychological disorders. To evaluate the involvement of TRPA1 in white matter injury due to chronic cerebral hypoperfusion, myelin staining was performed in the corpus callosum at day 14 after BCAS. In BCAS-operated TRPA1-KO mice, there was a significant decrease in myelin density. By contrast, such a decrease was not observed in BCAS-operated WT mice.

To investigate whether stimulation of TRPA1 affects chronic cerebral hypoperfusion-induced cognitive impairment, we systemically administered cinnamaldehyde (CA), a pungent compound in cinnamon and also a TRPA1 agonist, into BCAS-operated mice. In the test session of the novel object recognition test, daily CA-treated mice showed significant reductions in cognitive disturbance when compared with vehicle-treated mice.

Discussion & Conclusion

Within CNS, TRPA1 is expressed in expressed in neuron, glial cells (presumably astrocyte and oligodendrocyte) and cerebral endothelial cells and sensitive to hypoxic conditions. Here we demonstrate that cognitive dysfunction and white matter injury at day 14 were observed only in TRPA1-KO mice but not in WT mice. Moreover, daily treatment with a TRPA1 agonist CA significantly improved cognitive perturbation. Our data indicate that TRPA1 plays a protective role in the development of chronic cerebral hypoperfusion-induced cognitive impairment, and propose a new hypothesis in which TRPA1 stimulation specifically improves the pathology with chronic cerebral hypoperfusion. These findings shed light on the understanding of the mechanisms of chronic cerebral hypoperfusion-related CNS diseases, and are expected to provide a novel therapeutic molecule for cognitive impairment in CNS diseases, including dementia.

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TRPM2 Channel Aggravates CNS Inflammation and Cognitive Impairment via Activation of Microglia in Chronic Cerebral Hypoperfusion.

一般の皆様へ

脳血流の軽度な低下は、脳梗塞や心不全にかかったり、継続的なストレスを受けたりすることで起き、さらに高血圧・糖尿病・脂質異常症などの生活習慣病による動脈硬化により慢性化されます。これまでに我々は、軽度な脳血流の低下であっても長く続くことで白質傷害を主に介して、軽度な認知機能障害になることを示してきましたが、本研究により低酸素感受性を有する TRPA1チャネルがこの軽度な認知機能障害に対して保護的な役割を果たしていること、並びにこの TRPA1を刺激することにより病態を改善できる可能性を示すことができました。今後はこの TRPA1チャネルを介した保護機構をより詳細に解析したいと思います。

Elucidation of the onset mechanism of myeloproliferative neoplasms through the erythropoietin receptor

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Summary Abstract

In myeloproliferative neoplasms (MPN), erythropoietin (Epo)-independent signaling through Epo receptor (EpoR) can be caused by JAK2 V617F mutant. Previously, we found that phosphorylation of three tyrosine residues, Y343, Y460, and Y464 in EpoR, are required for the JAK2 mutant to exhibit its oncogenic activity. In the present study, to understand how JAK2 mutant induced cellular transformation through the phosphorylation of EpoR, we purified EpoR complex in cells expressing JAK2 V617F mutant and identified the some components of EpoR complex such as STAT5, Grb2 PI3K and CIS.

Key Words：myeloproliferative neoplasms (MPNs), JAK2 V617F mutant, EpoR, STAT5

Introduction

A somatic mutation in JAK2, V617F, has been identified in the majority of patients with MPNs. Although we found that phosphorylation of EpoR was critical for JAK2 mutant-provoked cellular transformation, the mechanisms by which EpoR contributes to JAK2 mutant-induced oncogenic signaling. It is expected that the phosphorylation of tyrosine residues in EpoR provides a platform for the recruitment and activation of signaling mediators which are critical for JAK2 mutant-provoked transformation. Therefore, to define the signaling mechanisms by which the combination of the JAK2 mutant and EpoR causes the pathogenesis of MPN, we tried to identify the interacting proteins with EpoR.

Results

In Ba/F3 cells expressing the JAK2 V617F mutant, eight tyrosine residues in the intracellular domain of EpoR were phosphorylated. We performed a mutational analysis of the eight EpoR tyrosine residues and found that the Y343F/Y460F/Y464F mutant in which Y343, Y460 and Y464 were substituted with phenylalanine completely lost the ability to induce JAK2 mutant-induced cell proliferation and tumor formation. These results indicated that phosphorylation at Y343, Y460, and Y464 in EpoR was required for the JAK2 mutant to exhibit its oncogenic activity (Ueda et al. J Biol Chem 2017).

In order to investigate the roles of phosphorylated tyrosine residues in EpoR, we constructed EpoR mutants such as 8YF in which all eight tyrosine residues was substituted with phenylalanine, 7YF (7YF-Y343, 7YF-Y460, 7YF-Y464) harboring one intact tyrosine residue and 5YF-Y343/460/464 harboring three intact tyrosine residues and examined the effects of these mutants on JAK2 mutant-induced transformation.

We showed that EpoR recruited a number of signaling molecules such as a transcription factor, STAT5, an adaptor protein, Grb2, phosphoinositide 3-kinase (PI3K) and a suppressor of cytokine

signaling, CIS in Ba/F3 cells expressing JAK2 mutant. We next investigated whether EpoR mutants could recruit these molecules.

Whereas EpoR associated with STAT5, Grb2, and PI3K, 8YF failed to associate with these molecules. Interestingly, not only EpoR but also 7YF-Y343, 7YF-Y460, and 5YF-Y343/460/464 interacted with STAT5, suggesting that the phosphorylated Y343 and Y460 are critical for the recruitment of STAT5 in cells expressing the JAK2 mutant. Both 7YF-Y460 and 5YF-Y343/460/464 interacted with Grb2, suggesting that the phosphorylation of Y460 seemed to be sufficient for the recruitment of Grb2 in cells expressing JAK2 mutant. Furthermore, these EpoR mutants failed to associate with PI3K, suggesting that PI3K-Akt pathway seemed to be dispensable for JAK2 mutant-induced oncogenic signals (Fig. 1).

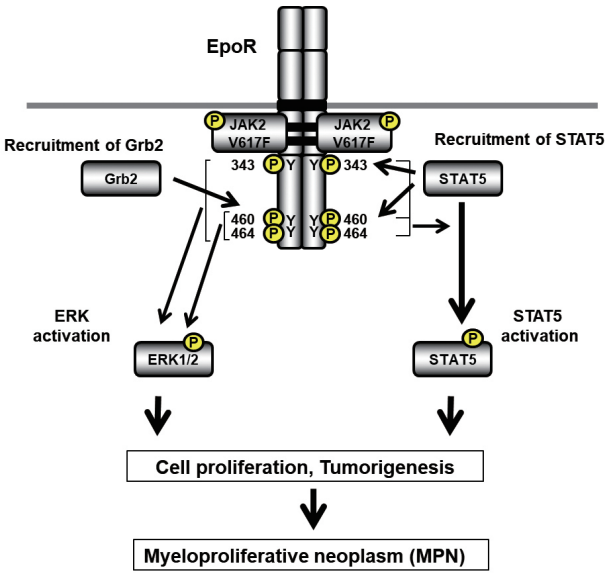

Fig. 1 JAK2 V617F mutant exhibits transforming activity through the phosphorylation of Y343, Y460, and Y464 in EpoR

Previous study reported that activation of STAT5 and ERK1/2 through Grb2 was involved in cell proliferation and tumor formation. We next examined the influence of mutations in tyrosine residues in EpoR on JAK2 mutant-induced activation of STAT5 and ERK1/2. As a result, it was revealed that three tyrosine phosphorylation at Y343, Y460 and Y464 are necessary for the full activation of STAT5. In addition, whereas the co-expression of EpoR and 5YF-Y343/460/464 with the JAK2 mutant significantly induced the phosphorylation of ERK1/2, the co-expression of 8YF and 7YF mutants failed to induce the phosphorylation of ERK1/2. Interestingly, co-expression of 6YF-Y343/464 and 6YF-Y460/464 induced the phosphorylation of ERK1/2 to the same extent as EpoR. These results suggest that the phosphorylation of Y464 is insufficient for the activation of ERK1/2, but the phosphorylation of either Y343 or Y460 in addition to Y464 could induce the activation of ERK1/2 in the cells expressing the JAK2 mutant (Fig. 1). We also found that CIS interacted with phosphorylated Y401 in EpoR, resulting in the inhibition of JAK2 mutant-provoked cellular transformation.

Collectively, we demonstrated that three tyrosine residues in EpoR, Y343, Y460 and Y464, are essential for cellular transformation through their mediation of activation of STAT5 and ERK1/2. We propose that the targeted disruption of this pathway has therapeutic utility for managing MPN. Furthermore, we showed that CIS functions as a novel type of tumor suppressor in JAK2 mutant-induced tumorigenesis by interacting phosphorylated EpoR.

Discussion & Conclusion

Several studies have reported that the physical interaction with EpoR is indispensable for the JAK2 mutant to induce the pathogenesis of MPN, however; the JAK2 mutant-induced signaling pathway through EpoR has not been elucidated. In this study, we first found that three tyrosine residues in EpoR, Y343, Y460 and Y464, are essential for cellular transformation through their mediation of activation of STAT5 and ERK. We also found that CIS prevented the JAK2 mutant-induced tumorigenesis by interacting phosphorylated EpoR at Y401.

Collectively, the results of this study and previous findings indicate the importance of EpoR as a suitable target for therapeutic drugs against MPN and also that the functional role of EpoR in oncogenic signaling induced by the JAK2 mutant needs to be elucidated in more detail.

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一般の皆様へ

慢性骨髄増殖性腫瘍 (MPN) の効果的な治療薬を開発する上で、未だ不明な点の多い MPN の発症メカニズムを解明し、理解することが重要です。今回の研究では、MPN の原因となることが知られているチロシンキナーゼ JAK2変異体が、サイトカイン受容体 EpoR を介して STAT5や ERK などのシグナル伝達分子を活性化し、細胞増殖や腫瘍形成を誘導するメカニズムを明らかにしました。本研究を通して、EpoR が MPN 治療薬の標的分子となりうる可能性が高いことが示されました。

Induction and visualization of adult cardiomyocytes proliferation through growth factor receptor activation

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Summary Abstract

Cardiac myocytes lose their cell cycle activity after birth. After myocardial infarction, cardiac function is impaired due to loss of cardiac myocytes. In this project, we tried to develop novel strategy to induce cell cycle activity in mature cardiac myocytes, thus increase their number, improve contractile function. For this purpose, we succeeded in generating mouse line conditionally overexpressing growth factor receptor. It is currently ongoing work to examine if adult cardiac myocytes can activate cell cycle activity by this gene modulation.
Key Words：Cardiac myocytes, Proliferation, Growth factor receptor, Gene-targeted mouse

Introduction

Heart is an integral organ to maintain our life. Among various types of cells in heart, cardiac myocytes play major role in heart beating. However, soon after birth, cardiac myocytes lose proliferative potential. Thereby, once heart loses cardiac myocytes through myocardial infarction, cardiac myocytes are not capable of proliferating, leading to contractile dysfunction. In this research, we attempted to induce proliferative potential in adult cardiomyocytes by overexpressing growth factor receptor in cardiac myocytes, trying to develop novel regenerative therapy for post infarct heart failure.

Results

Soon after birth, cardiac myocytes lose cell cycle activity, cease proliferating whithin the first week postnatally. Loss of cardiac myocytes due to myocardial infarction leads to heart contractile dysfunction. To prevent heart failure after myocardial infarction, we attempted to postnatlly activate cell cycle activity, thereby increase cell number of cardiac myocytes. For this purpose, we try to generate mouse line conditionally overexpressing one of the growth factor receptor.

First, we designed a novel targeting vector to target into Rosa locus, where we deleted transcription initiation sites so that endogenous transcription for Rosa lncRNA loci does not disturb transcription of our transgene which is driven by CAG promoter. To overexpress the transgene selectively and specifically in cardiac myocytes in heart, we put floxed-Neo-polyA casssette between CAG promoter and our transgene, then planned to cross with cardiac myocyte specific troponin T-Cre mouse line. We also put 2A sequence at the carboxyl-terminus, followed by histone fused mCherry coding sequence (H2B-mCherry), which enable us to visualize nuclei of cardiac myocytes overexpressing the growth factor receptor.

We optimized targeting strategy to ES cells using Crispr/Cas9. By utilizing pEGxxFP psystem, we confirmed double strand break efficiently happens in our target Rosa locus with our gRNAs. As the size of gene we would like to knock-in was 9 ~ 10 kbp, we estimated that the efficiency of

proper targeting might be very low. At this time point, it was not clear how long homologous arm is necessary for knock-in strategy with Crispr/Cas9. Generally, a couple of hundreds of nucleotide sequence were considered to be sufficient to knock-in EGFP coding sequence with Crispr/Cas9, however, thinking of the large size of our knock-in gene, we prepared 2 kbp of homologous sequence each side, total 4 kbp of homologous arm. Next, we performed gene-transduction to ES cells by lipofection. To select transfected ES cells, we used Puromycin for 48 hours, as Puro resistant gene expresses by the plasmid co-transfected with our targeting vector and Crispr/Cas9-gRNA expressing vector. Although our targeting vector contained Neo resistant gene, we did not use Neomycin (G419) for selection, thinking of future application of our targeting approach. For future design of targeting vector, we would not like to put Neo resistant gene with a promoter intervened with FRT sequence, so that we do not have to take out the resistant gene with Flpase, and avoid extra consumption of time.

After all, we succeded in obtaining two positive ES clones among from 32 clones we picked, followed by injection of ES cells into 8-cell stage embryos. We obtained chimeric mice, then succeeded in germline transmission, obtained conditionally overexpressing mouse line. This mouse line was crossed with cardiac myocyte specific Troponin – T Cre mouse.

We sectioned heart tissue, then immunostained with anti-troponin-T antibody to label cardiac myocytes, anti-PDGFR-alpha antibody to label fibroblasts, and CD31 antibody to label endothelial cells. By immuno-staining, we confirmed that H2B-mCherry was expressed selectively and specificlly in cardiac myocytes, as we expected. In future experiments, we will dissociate cardiac myocytes from postnatal heart to define the change of the number of cardiac myocytes by overexpression of the growth factor receptor. We will also inject EdU intrapertoneally to assess the cell cycle activity, confirm how our transgene promote cell cycle activity.

Discussion & Conclusion

It has been very inefficient to knock-in large size of gene to targeted locus before Crispr/Cas9 was introduced. In this project, by optimizing suitable culture conditions, designing proper targeting vector, and utilizing Crispr/Cas9, we succeeded in efficiently obtaining gene-targeted ES cell lines. By injectiing of ES cells into 8-cell stage embryos, we generated novel conditionally overexpressing knock-in line, which might activate cell cycle, even in adult cardiac myocytes. Our knocked-in gene can also be overexpressed in any cell lineage of any tissue by using suitable Cre mouse line, which would be commercially available. By future experiments, we will reveal whether growth factor receptor activation might be able to modulate cell cycle activity, and induce proliferation of mature cardiac myocytes. This result might open the door for the possiblity of promissing novel therapeutic strategy for heart failure after myocardial infarction.

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一般の皆様へ

心筋細胞は生後細胞周期の休止期に入りその後増殖することはない。そのため心筋梗塞で心筋細胞が失われると心機能障害をきたし心不全を発症してしまう。本研究ではマウスで成長因子受容体シグナルを活性化することにより、成熟心筋細胞の増殖を誘導しうるかどうかを明らかにすることを目的とした。本研究の結果、心筋細胞で成長因子受容体を過剰発現させ、その細胞を1細胞レベルで直接可視化できるマウスの作製に成功した。今後、このマウスを用いて、成熟心筋細胞の増殖を誘導することが可能であれば、梗塞後心不全に対する新規治療の開発への展望が開けるだろう。

Analysis of the function and genomic abnormality of tumor suppressor gene PHLDA3 in neuroendocrine tumors - Towards development of a novel genetic diagnosis method for neuroendocrine tumors -

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Summary Abstract

We have found that: the PHLDA3 gene encodes a novel repressor of oncogene Akt and functions as a tumor suppressor gene in NET of lung and pancreas (1, 2); in these NET, the function of PHLDA3 is frequently lost due to genomic abnormality; and cases in which PHLDA3 function is lost are shown to be highly malignant and have poor prognosis (2, 3). Based on our findings, we perform: Genomic analysis using human NET samples with clinical information; and Analysis of NET model animals.

Key Words：NET, PHLDA3, Akt

Introduction

Neuroendocrine tumors (NET), which is a rare cancer, occurs in various organs of the whole body. Due to its rareness, the mechanism of tumorigenesis is not sufficiently elucidated, and there are no effective diagnostic methods or treatments.

In this project, we will analyze the world's largest NET cohort / new NET mouse model, and clarify the tumor-suppressing pathway common to NET across organs, and also clarify the relationship between patient prognosis, drug efficacy and genomic abnormality. From the results obtained, we will develop a novel diagnosis and treatment method, and respond to unmet medical needs of NET patients.

Results

We have proceeded with the genomic analysis using human NET samples with detailed clinical information, and the analysis of NET model animals. Specifically, we used specimens of: 150 cases of Pancreatic NET; 203 cases of Pituitary NET; 69 cases of Colorectal NET; and 30 cases of Thyroid NET. And, we have performed the genomic analysis focusing on whether or not any PHLDA3 genetic abnormality exists. Although the results are still preliminary, we have obtained results that is reasonable for considering that all of the analyzed NETs have PHLDA3 genetic abnormality, and thus PHLDA3 is a tumor suppressor gene common to all NETs (3, 4).

1. Genomic analysis of the NETs

We hold the world's largest pancreatic, pituitary, colon, thyroid and parathyroid NET specimen cohort with over 450 cases. Using the specimens, analysis of PHLDA3 gene abnormality (LOH, methylation, point mutation), comprehensive whole exon sequence analysis, and RNA-seq have been performed.

Through these analyses, in addition to PHLDA3 genetic abnormality, we have identified genes mutated together with PHLDA3. We are now analyzing the intracellular pathway involved in NET onset.

2. Analysis of NET model animals

We have established an unique pancreatic NET model mice (PHLDA3 and MEN1 double-deficient mice) that mimic the genomic abnormality of human NET, and model mice of pituitary and thyroid NET are currently being prepared. The pancreatic NET model mice and model cell lines derived from the model mice have been treated with drugs used for therapy of pancreatic NET, and the clinical predictability of these models are now being verified. At the same time, we are now exploring the usefulness of combination therapy using the inhibitors of the pathways identified in project 1.

3. Development of NET diagnostic method using the PHLDA3 gene

We have analyzed the relationship between genetic abnormality of PHLDA3 and patient prognosis / therapeutic drug response, and are now developing a new NET diagnostic method. We have also conducted a research to develop a diagnostic method based on the combination of genetic abnormality of genes found in project 1 and PHLDA3.

Discussion & Conclusion

We will continue to proceed the genomic analysis using human NET samples with detailed clinical information and the analysis NET model animals, for elucidating the essential condition of Neuroendocrine tumors (NET), which is a rare cancer. And we aim at further development of NET diagnostic method using PHLDA3 gene for a novel prognosis and treatment option.

As for ethical considerations, we will compliance to the ethical guidelines related to study of human genome and gene analysis, and the ethical guidelines related to epidemiological study. With reference to the genetic recombination experiments, we obey “Act on securing biodiversity by regulation on the use of genetically modified organisms, etc.” and related laws and regulations. With reference to the animal experimentation, we compliance with basic guidelines and perform the experimentation according to the animal examination code with due consideration for the experiment animals.

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一般の皆様へ

神経内分泌腫瘍は全身の様々な臓器に生じますが、希少がんであるため研究開発は遅れており、有効な診断法・治療法がありません。

私たちは、肺と膵臓の神経内分泌腫瘍において、PHLDA3 遺伝子は高頻度にゲノム異常によって機能が失われていることを発見し、*PHLDA3* 遺伝子が肺と膵臓の神経内分泌腫瘍において、がん抑制遺伝子として機能している事を明らかにしました。さらに、PHLDA3 機能が失われている症例は悪性度が高く予後不良である事が示されました。これらの知見を元に、神経内分泌腫瘍のヒトや動物のサンプルを解析し、その本態を解明し、治療と診断に貢献したいと考えております。

Disease specific regulation of the histone methylation in acute myeloid leukemia

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Summary Abstract

In the present study, we demonstrate the role of Lysine demethylase 4B (KDM4B)-mediated epigenetic regulation in the disease progression of acute myeloid leukemia (AML).
Key Words：Epigenetics, Hematologic malignancy

Introduction

KDM4B is an epigenetic regulator that functions as a histone demethylase for di- and tri-methylated H3K9 as well as di- and tri-methylated H3K36 and H1.4K26. Although KDM4B has so far been reported to promote tumor development in several types of cancers, the tissue specificity and context dependency still remain largely uncertain.

Results

To investigate the role of KDM4B in hematopoiesis, Kdm4b flox/flox (fl/fl) mice were crossed to transgenic mice expressing Cre recombinase from the interferon-responsive Mx1 promoter (Mx1-Cre mice). To induce Cre expression, Mx1-Cre/ Kdm4b fl/fl mice and littermate controls (Kdm4b fl/fl) were injected with polyinosine-polycytosine, which mimics a viral infection. With this protocol, Cre-mediated recombination through the loxP sites effectively excised exon 5 of the Kdm4b gene and generated a Kdm4b-null allele (Kdm4bΔ). Western blot analysis showed that Cre induction brought a clear reduction in the amount of Kdm4b protein in bone marrow (BM) cells. We characterized hematopoietic stem cell (HSC) compartment in the BM of $Kdm4b^{\Delta/\Delta}$ and control mice using surface marker analysis, and found similar absolute cell numbers between these two genotypes. There was no significant difference in hematopoietic colony forming capacity in semisolid media by use of c-Kit+ HSC/progenitor cells from BM of $Kdm4b^{\Delta/\Delta}$ and control mice.

We found the upregulation of KDM4B in AML with 8;21 chromosomal translocation. When transcription factor AML1 is fused to ETO (a nuclear protein that interacts with co-factor molecules) by the 8;21 translocation [t(8;21)], the fusion gene AML1-ETO activates or inactivates target gene expression through recruitment of co-factors, resulting in the disease progression of AML. To examine the role of Kdm4b in clonogenecity mediated by AML1-ETO, we retrovirally transduced the AML1-ETO to c-Kit+ BM progenitor cells and subsequently performed serial hematopoietic colony forming assay. As a result, the colony-replating capacity of $Kdm4b^{\Delta/\Delta}$ cells transduced with AML1-ETO was significantly reduced compared to that of the AML1-ETO-transduced controls. A previous study revealed that AE9a, a splice isoform of AML1-ETO exhibited more potent leukemogenic activity in mice. Based on this finding, we also assessed the impact of Kdm4b on AE9a-mediated clonogenecity by serially colony replating. Kdm4b deficiency in c-Kit+ BM cells transduced with AE9a significantly decreased number of colonies at the successive plating rounds. Thus, these results suggest that Kdm4b is required for robust clonogenic potential in t(8;21) leukemia.

Next, we examined the effect of KDM4B suppression on human AML cells using SKNO1 cell line, a human t(8;21) leukemia cell line model. Two different lentiviral shRNA constructs with the puromycin resistance gene were designed to effectively decrease the KDM4B protein in SKNO1 cells. Notably, stable knockdown of KDM4B altered cell morphology and led to markedly decreased cell proliferation, coupled with increased cell death. We then performed RNA sequencing to identify the molecular basis of the cellular impact by KDM4B knockdown. Previous reports showed that the AML1-ETO fusion is crucial for proliferation of human t(8;21) AML cell lines including SKNO1. We applied Gene Set Enrichment Analysis (GSEA) to our gene expression data based on reported gene sets consisting of genes induced by AML1-ETO in human CD34+ hematopoietic stem cells. As a result, we found that KDM4B knockdown cells showed a significant reduction of the AML1-ETO-inducible gene expressions. Thus, a role of KDM4B promoting AML1-ETO-mediated gene regulation is suggested, which may partly explain the mechanism underlying antiproliferative effect by KDM4B knockdown in SKNO1 cells.

Discussion & Conclusion

Our results highlight KDM4B-mediated epigenetic regulation contributing to the disease progression of AML and suggest KDM4B as a potential therapeutic target for t(8;21) leukemia.

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Epigenetic regulation by a histone demethylase in leukemia with 8;21 chromosomal translocation [abstract]. In: Consortium of Biological Sciences 2017; 2017 Dec 6–9; Kobe, Japan; Abstract 1P-1020.

一般の皆様へ

急性骨髄性白血病で高発現を認めるエピゲノム制御因子が白血病の病態にどのように関与しているかを明らかにする研究を進めています。

Treatment of metastatic tumors by systemic injection of tumor-retargeted oncolytic herpesviruses

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Summary Abstract

The route of administration of oncolytic herpes simplex virus (HSV) in the majority of clinical trials to date has been direct injection into the tumor. This appears to be one of the factors that may limit the therapeutic potential of oncolytic HSV as a single agent against systemically metastatic tumors. We have successfully established an HSV platform fully retargeted to tumor-associated antigens that enable restriction of virus infection at the stage of entry. This achievement may prove to be a major breakthrough that facilitates treatment of metastatic tumors via systemic injection of oncolytic HSV.
Key Words：Oncolytic virotherapy, Cancer, Herpes simplex virus

Introduction

Oncolytic virotherapy is a novel therapeutic modality for malignant diseases that exploits selective viral replication in cancer cells but not in normal cells. Herpes simplex virus (HSV) is a promising agent for oncolytic virotherapy due to its broad cell tropism and the identification of mutations that favor its replication in tumor cells. However, the route of administration of oncolytic HSV in the majority of clinical trials to date has been direct injection into the tumor. This appears to be one of the factors that may limit the therapeutic potential of oncolytic HSV as a single agent against systemically metastatic tumors.

Results

Entry of HSV is initiated by the binding of glycoprotein D (gD) to one of its receptors, herpesvirus entry mediator (HVEM) or nectin-1. This interaction results in a conformational change in gD, triggering sequential activation of gH and gB to execute fusion between the viral envelope and cell membranes. For virus retargeting to epidermal growth factor receptor (EGFR)-, carcinoembryonic antigen (CEA)-, or epithelial cell adhesion molecule (EpCAM)-expressing cells, we mutated or deleted gD residues essential for binding to the natural entry receptors, inserted an EGFR-, CEA-, or EpCAM-specific single-chain antibody (scFv), and observed specific virus entry into cells expressing the cognate target antigen. We combined the retargeted gD alleles with hyperactive gB mutations D285N/A549T identified through directional evolution experiments and found that these viruses entered the target cells around 100-fold more efficiently than the wild-type-gB versions without raising off-target entry. In addition, we found that not only initial entry but also subsequent cell-to-cell spread of our retargeted viruses was stringently dependent on the cellular expression of the cognate target antigens by carrying out experiments that distinguish between these two similar, but distinct entry processes. One of the EGFR-retargeted viruses efficiently killed a panel of EGFR-expressing human tumor lines in vitro and reduced the growth of human tumors in nude mice.

Toxicity experiments involving intracranial virus inoculation demonstrated symptom-free animal survival at a dose that was 100,000-fold higher than a dose of wild-type virus that was fatal.

Then, we sought to determine whether the EGFR-retargeted virus would preferentially accumulate in EGFR-positive human tumors in nude mice. Following the establishment of subcutaneous flank tumors, equal amounts of the wild-type virus and the EGFR-retargeted virs were administered by tail vein injection. The animals were sacrificed 2 days later and the biodistribution of each virus in various organs including the tumor, brain, lung, heart, liver, spleen, and kidney, was determined by qPCR for the viral genome. As a result, the number of the wild-type virus genomes per 100 ng tissue DNA was comparable to or lower in the tumors than in most of the other tissues from the same animals. In contrast, the EGFR-retargeted virus was detected at 100–1,000-fold higher levels in the tumors than in other tissues. These results indicated that the EGFR-retargeted virus preferentially homed to the human EGFR-positive tumor tissue. Furthermore, we observed the intravenous injection of the EGFR-retargeted virus reduced the growth of human EGFR-positive tumors in the same mouse model.

Discussion & Conclusion

Our successful establishment of HSV platforms fully retargeted to tumor-associated antigens that enable restriction of virus infection at the stage of entry may prove to be a major breakthrough that facilitates treatment of metastatic tumors via systemic injection of oncolytic HSV. In addition, this novel technology will allow researchers to pursue strategies of manipulating the HSV genes involved in viral replication and/or cell-to-cell spread towards augmentation of oncolytic activity, instead of its attenuation. Furthermore, the receptor-retargeting technology may prove to be useful to overcome the issue that the oncolytic HSVs currently used are likely to be ineffective for tumors that do not express gD receptors at sufficient levels.

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一般の皆様へ

がん細胞だけに特異的に侵入し、静脈内投与可能な標的化腫瘍溶解性ウイルス療法が開発できれば、原発巣のみならず全身の転移巣にも有効な治療法となりえる。私たちは、単純ヘルペスウイルス（HSV）にがん細胞表面抗原を認識する単鎖抗体を組み込むことにより、標的細胞のみに侵入可能な標的化 HSV の構築に独自に成功した。この標的化 HSV は、現在臨床開発が進められているウイルス複製の段階でがん選択性を発揮する HSV に比して、より静脈内投与に適していると考えられる。

Revealing cell membrane functions through in-vitro analysis by tip-enhanced Raman spectroscopy in liquid

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Summary Abstract

In this research, we constructed tip-enhanced Raman spectroscopy (TERS) applicable in liquid environment, which enables label-free imaging of membrane components through Raman analysis at nanoscale. With the constructed system, we have obtained Raman spectrum of lipid bilayers with metallic TERS tips. This would be a promising result to realize a powerful technique that visualizes how membranes behave at nanoscale in a physiological condition without any labeling.
Key Words：Raman spectroscopy, Tip-enhanced Raman spectroscopy, cell membranes

Introduction

Membrane of a cell is not just a frame of cell but an important platform that possesses channel proteins, receptors and catalytic areas to maintain our health. Also, membrane components work as a trigger for various medicines. It is therefore essential to understand how membranes function with tiny molecules. For this aim, it is required to develop an analytical technique that can analyze “real” membranes at nanoscale in a physiological condition. TERS is a promising for this purpose because of label-free analysis through Raman spectroscopy and nanoscale spatial resolution through near-field light localized at a metallic tip.

Results

We have constructed TERS applicable in liquid environments by combining Raman spectroscopy with an atomic force microscopy available in liquid conditions. In the system, a z-polarizer was installed to generate z polarization at a metallic tip so that near-field light is efficiently excited by oscillating plasmon along the tip axis. A pinhole was also located to drastically reduce scattering background through confocal configurations.

In TERS, metallic tips also play a very important role because localization and intensity of near-field light (spatial resolution and sensitivity) are strongly dependent on structure of metallic tips. Slight differences in the metallic tip structures largely changes property of near-field light so that it is crucial to develop a practical method to fabricate metallic tips that show strong enhancement and localization of near-filed light with high reproducibility. For this aim, we utilized electron beam deposition technique and sputtering technique for tip fabrication. We first deposited an amorphous carbon tip, which is extremely sharp with tip diameter of less than 10 nm, by electron beam deposition under atmosphere of organic molecule precursors. We then coated it with silver by sputtering technique. Through these processes, silver rod-like structures were obtained at the tip apex. By changing exposure time of electron beam and sputtering, we can precisely control the length and thickness of the rod structure, which means precise control of spatial resolution and near-field light intensity. The dependence on the length and thickness was also confirmed through finite difference time domain methods.

To make liquid-TERS system more practical, we developed a technique to recycle tips. By dipping silver-coated tips in HNO3 solution for a hour, and then treating them by plasma etcher over night, silver coating was entirely removed and cleaned. We confirmed that we can re-fabricate silver rod-like structure on recycled tips.

Another issue in the liquid-TERS is contamination of metallic tips. Metallic tips are easily contaminated with biological molecules floating in an observation pool, which gives unwanted Raman signals and disrupt measurements. To avoid this problem, we applied sodium chloride in the observation buffer. Since chloride ions have strong affinity to silver, they are strongly attached on the surface of silver-coated tips by removing contaminations from the tips. We clearly observed decrease of Raman signals from contaminations after dipping metallic tips in 100 mM sodium chloride solution for 30 minutes. Since 100 mM of sodium chloride is quite similar with physiological environments, we can get another unique advantage that this method does not disturb cell membrane activities.

With the constructed setups and developed techniques, we first tried fluorescent measurements. We successfully imaged fluorescent beads over the diffraction limit of light. DNA labeled with fluorescent molecules was also imaged, which proved that this technique is applicable for biological samples. Moreover, imaging rate was several seconds, which implies that this technique even has a potential for dynamic imaging of molecules in liquid.

Since we confirmed performance of the constructed TERS system, we then performed TERS imaging with carbon nanotubes in liquid with nanoscale spatial resolution. Further, we demonstrated TERS measurements of lipid bilayers. We successfully obtained Raman signals when metallic tip is in contact to the lipid bilayers. This result is highly promising to realize nano-analytical technique to observe “real” cell membranes at work in physiological environments.

Discussion & Conclusion

We have constructed TERS system working in liquid environments, which was confirmed with super-resolution fluorescent imaging of several samples. Promising results on lipid bilayers with TERS was also obtained. Besides, we made huge progresses in metallic tip fabrication techniques, which is highly important to make TERS system more practical. However, there are still some rooms that we can work on to improve our TERS system. For instances, cell membrane has 3D structure so that it will be required to track tip positions in z-directions by piezo control and feedback systems. Metallic tips still did not give consistent enhancement for TERS measurements of cell membranes. By optimizing these things, we believe that our liquid-TERS system contributes to reveal cell membrane functions at nanoscale details in the future.

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一般の皆様へ

細胞膜は細胞同士を隔てる単なる仕切りではなく、細胞外からの情報をやり取りしたり、必要な物質を細胞内に輸送したり、私たちの健康を維持するために必要な機能を沢山持っています。また、多くのウィルスや薬も細胞膜を足掛かりとして作用するので、細胞膜の様々な現象を単分子レベルで解明することは、創薬の発展にもとても重要です。しかし、ありのままの細胞膜を単分子レベルで観察する手法は非常に困難です。私たちは、これを実現すべく生体環境で使える先端増強ラマン散乱顕微鏡という装置を開発しました。単分子レベルの分解能でありのまま細胞膜を観察できるため、今後細胞膜の研究にますます役立つことと期待しています。

Elucidation of the immune-nerve-metabolic systems network in cardiac homeostasis and failure

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Summary Abstract

In the present study we identified a novel heart-brain-kidney network that control adaptive response to pressure overload to the heart. In the heart cardiac resident macrophages are essential for cardioprotection and amphiregulin is a key mediator produced by the cardiac macrophages. We have also analyzed the mechanism that confers cardioprotection by amphiregulin.
Key Words：Heart failure, Macrophage

Introduction

Heart failure is a complex clinical syndrome characterized by cardiac function that is insufficient to meet systemic demand. The prevalence of heart failure is rapidly increasing partly due to aging society so that heart failure is now considered as a global pandemic. In addition to abnormalities intrinsic to the heart, dysfunction in other organs and systemic factors greatly affect the development and consequences of HF. For instance, nearly half of chronic HF (CHF) patients also have chronic kidney disease (CKD), which increases their rate of cardiovascular mortality, suggesting cardiorenal linkage via mechanisms still poorly understood. Moreover, previous studies have mainly focused on the signaling mechanism that regulate cardiomyocyte hypertrophy, recent studies have been unraveling crucial roles played by nonmyocytes in the response to cardiac stress. In the present study we aim to elucidate the function of cardiac macrophages in the maintenance of cardiac homeostasis.

Results

We found that there are at least two subpopulations of macrophages in the myocardium. In response to pressure overload induced by transverse aortic constriction (TAC) only cardiac resident $Ly6C^{lo}$ macrophages were increased. In a series of experiments we found that cardiac $Ly6C^{lo}$ macrophage activation and proliferation are regulated by a heart-brain-kidney network. Pressure overload in the heart activates renal collecting duct epithelial cells via the sympathetic nervous system. The renal collecting duct epithelial activation triggers communication between collecting duct epithelial cells, renal $CD11b^{+}F4/80^{hi}Ly6C^{lo}$ macrophages and endothelial cells. Activated renal endothelial cells then produce and secrete CSF2, which stimulates the cardiac $Ly6C^{lo}$ macrophages essential for the proper cardiac adaptive response to pressure overload. This dynamic heart-brain-kidney interplay is essential for the body's response to cardiac stress. Dysfunction of this network impaired proper adaptive response to pressure overload and mice developed heart failure.

Our results indicate that cardiac $Ly6C^{lo}$ macrophages are necessary for a proper cardiac adaptive response to pressure overload. We identified amphiregulin (AREG) as a key cardioprotective mediator produced by cardiac $Ly6C^{lo}$ macrophages. In the heart cardiac $Ly6C^{lo}$ macrophages are the major source of AREG and expression of AREG is upregulated by TAC. $Ly6C^{lo}$ macrophages that

lack Areg failed to protect the heart from heart failure induced by TAC when they were transplanted into the heart. Inhibition of AREG signaling using a neutralizing antibody significantly reduced cardiomyocyte hypertrophy, cardiac function and overall survival in wild-type mice after TAC without affecting cardiac Ly6Clo macrophage numbers. Conversely, recombinant AREG rescued collecting duct epithelial cell-specific *Klf5* knockout mice that develop heart failure after TAC from the heart failure phenotype. Collectively, these results demonstrate that AREG is important for cardioprotection. Mechanistically, AREG is the major mediator secreted from cardiac Ly6C^{lo} macrophages that induces a hypertrophic response in cardiomyocytes.

To further elucidate the cardioprotective function of cardiac Ly6C^{lo} macrophages and the interaction between cardiomyocyte, macrophages and other nonmyocytes, we analyzed transcriptome and epigenome in cardiac macrophages by RNA-seq, ChIP-seq, and ATAC-seq. We found that epigenome in cardiac macrophages are dynamically modulated by cardiac stress. Using in vitro coculture systems and in vivo analyses we also found that the interactions between cardiomyocyte, macrophages and other nonmyocytes, including neurons, shape the epigenome in cardiac macrophages. Moreover, we identified candidates of transcription factors and histone modifiers that are important for shaping the cardiac macrophage-specific epigenome.

We also elucidated additional mechanisms, by which AREG confers cardioprotection. In addition to activate the hypertrophic program in nearby cardiomyocytes, our results indicate that AREG has multiple functions that are essential for cardiac homeostasis in the steady state as well as cardiac stress. Consequently, while *Areg* knockout mice did not show cardiac phenotype in the steady state, cardiac homeostasis was already modulated. Upon various cardiac stress *Areg* knockout mice developed heart diseases, such as heart failure.

Discussion & Conclusion

Our results demonstrate that cardiac resident macrophages are a key player in the maintenance of homeostasis and adaptive response to cardiac stress. In the present study we analyzed the effects of AREG on cardiomyocytes and found that the cytokine crucially contributes to the maintenance of cardiac homeostasis by multiple mechanisms. In addition, we found that local cell communication with cardiomyocytes and nonmyocytes is essential for the generation of cardiac macrophage-specific epigenome. Moreover, cardiac macrophages are regulated by the heart-brain-kidney network. The signaling mechanisms identified in the present study appear to be important as potential therapeutic targets for heart failure.

一般の皆様へ

心不全は急速に増加しており、新しい治療法を開発することは喫緊の課題です。私たちは、心臓に存在するマクロファージ（免疫細胞の一種）に注目して、その機能の解析を行いました。心臓マクロファージは心臓へのストレスに対して心臓を守る機能を持っています。また、アンフィレグリンという分子を作ることが大事なことを見いだしました。心臓マクロファージの機能は、心臓－脳－腎臓をつなぐネットワークによって制御されています。また、心臓の中では、心臓に存在する心筋細胞等の細胞との関連によって制御されています。本研究で同定したメカニズムは今後の心不全治療法の開発に役立てることができると考えています。

Roles of synaptic plasticity and replay during sleep in memory consolidation

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Summary Abstract

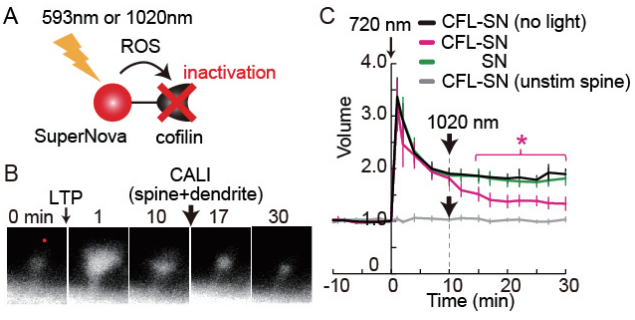
To elucidate roles of synaptic plasticity and replay during sleep in memory consolidation, we developed a new optical method for erasing hippocampal LTP and memory. In this system, cofilin was fused with SuperNova (CFL-SN) and light was illuminated to inactivate cofilin. structural LTP induced in the single dendritic spine in the hippocampal slice culture expressing CFL-SN was cancelled by light irradiation. Furthermore, we showed memory was erased by light irradiated on CA1 neurons expressing CFL-SN through optic fiber. This method is a potential tool to know when and where synaptic plasticity occur during sleep and memory consolidation.
Key Words：memory consolidation, Cofilin, CALI, synaptic plasticity

Introduction

Memory is retained in the hippocampus for up to one week after initial learning. It is then transferred to the neo-cortex where it becomes permanently stored. This process is called memory consolidation. Although it has been speculated that LTP-like mechanism during sleep is involved in this process, there has been no information when and where it happens. We previously found that in the initial phase of LTP, cofilin is transported to the spine and persistently accumulates at there. Therefore, we assume that cofilin-dependent synaptic plasticity during sleep plays an important role in memory consolidation.

Results

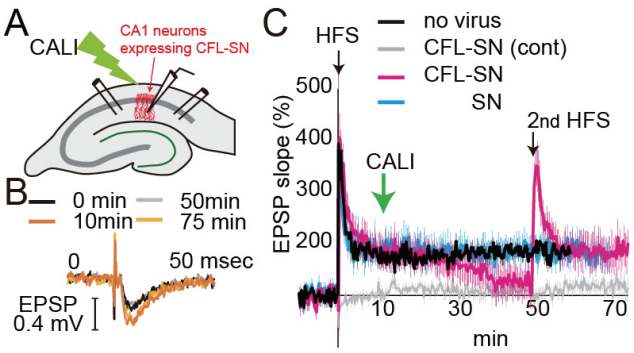
In order to inactivate cofilin, we employed a genetically encoded photosensitizer protein, SuperNova. To validate this system, a persistent enlargement of the spine (structural LTP) was induced with two-photon (2P) uncaging of MNI-glutamate in the single dendritic spine in the hippocampal slice culture, which express SN-fused cofilin (CFL-SN), and 559 nm laser was subsequently irradiated. Followed by laser irradiation on the single spine 10 min after LTP induction, the spine volume gradually decreased. Decrease in the spine volume was also observed up to



Erasure of sLTP by optically inactivating cofilin(A) Structure of fusion proteins. (B) GFP image. Ten min after LTP induction by photouncaging of glutamate, CALI was carried out. (C)Summary.

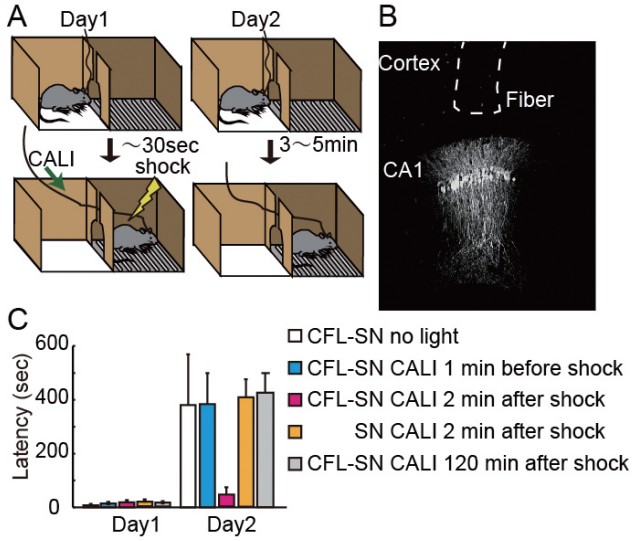
30 after respectively after LTP induction but not at 50 min after. Furthermore, the laser irradiation on the spine 1 min before sLTP had no effect on the spine enlargement. The laser irradiation on the spine without LTP induction had no effect on the spine volume. These results indicate cofilin is critical for maintenance of LTP up to 30 min after induction, and the inactivation of cofilin had negligible effect on LTP induction and the volume of unstimulated spine .

We confirmed electrically recorded LTP is canceled by the illumination. Cofilin-SN was overexpressed and field EPSP was recorded from stratum radiatum of CA1. Light from Hg lamp (520-550 nm, 500 uW, 3 min) was illuminated 10 min after HFS. After illumination, EPSP slope gradually decreased. LTP could be induced again 40 min after CALI and potentiation of EPSP was observed, indicating the cancellation of LTP by CALI is not due to non-specific impairment in synaptic transmission.



Electrically recorded LTP was canceled by CALI (A)Field recording and CALI(B) The averaged epsp.(C)Time course of slope of EPSP.

To test this system in memory formation, we expressed CFL-SN in hippocampal neurons by injecting AAV-floxed-CFL-SN into CA1 region of CaMKII-Cre mouse, then implanted an optical fiber above the virus injection region. Memory was examined by Inhibitory Avoidance (IA) task. Memory was not impaired just by overexpress of CFL-SN in CA1 neuron. In contrast, memory was significantly impaired when 593 nm laser light was delivered 2 min after an electrical shock at day 1. On the other hand, memory was not impaired when the light delivered 1 min before the electrical shock. Illumination 2 hours after the shock had no effect on memory. These data are consistent with those from slice culture. This new method can specifically erase memory shortly (2 min) after memory formation without any effect on subsequent induction of memory.



(A) IA test and CALI.(B)Distribution of cofilin-SN expressed by AAV vector in CA1 pyramidal neurons (immunostaining). (C). Results of IA test.

Discussion & Conclusion

We developed a new optical method for erasing hippocampal LTP and memory. By using this system, structural LTP induced in the single dendritic spine in the hippocampal slice culture expressing CFL-SN can be cancelled by light irradiation. Furthermore, Memory in vivo can be erased by light irradiated on CA1 neurons expressing CFL-SN through optic fiber. By testing the effect of CALI at different time points after the learning, we can identify the time window when NMDAR-dependent LTP process takes place. We can test the involvement of LTP-like mechanism in memory consolidation. By monitoring EEG, the timing of replay can be detected as ripple/sharp wave. We can detect ripple/sharp wave (as both awake and sleep) and sleep status (REM or non-REM). Light can be delivered whenever the certain brain status are observed. For example, we can induce CALI, when sharp wave is observed with delay (for example, 1 min) to test the involvement of replay in inducing LTP locally at hippocampus. In this experiment, the effect of LTP erasure will be monitored by behavior and also EEG itself, such as frequency and amplitude of replay (both awake and sleep).

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一般の皆様へ

記憶は学習後、海馬に短期的に保持されたのち皮質に移行し長期的に保存されます。これは記憶の固定化と呼ばれ、我々の日常において重要な機能です。しかしその詳細なメカニズムは分かっていません。我々は記憶の固定化において LTP（シナプス長期増強）が重要な役割を担っていると考えました。本研究では光照射によって LTP を解除する方法を開発しました。光照射によって LTP の解除することで、LTP が生じる詳細な場所と時間を知ることが可能になりました。したがいまして本技術は、記憶の固定化の過程（特に睡眠中）のいつどこで LTP が生じて固定化が完了するかを知ることができる画期的な技術と言えます。

Study on the Mechanism of Sex Determination in Germline Stem Cells

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Summary Abstract

Foxl3 is a switch gene acting in germline stem cells to determine the sexual fate of germ cells (to become eggs or sperm) in the teleost fish, medaka. The possible candidate genes downstream of *foxl3* were isolated. In addition, we successfully showed that germ cells posses an innate character to feminize the body. The character is independent of commitment of germ cells to gametogenesis.
Key Words：Germ Cells, Foxl3, Sex Determination

Introduction

Germline stem cells are the cells that produce eggs or sperm but they are sexually indifferent even though they are in mature ovary or testis. For the first time in vertebrates, we identified a switch gene, *foxl3*, for the germline stem cells to commit to either eggs or sperm. Although understanding of the switch mechanism will definitely contribute a lot to promotion of the studies on regenerative and reproductive medicine and animal husbandary, the detail of the switch mechanism is largely unknown. For this purpose using teleost fish, medaka, we will focus on character of germline stem cells and address the target genes by *foxl3* in germ cells.

Results

Downstream Targets of Foxl3.

We have generated transgenic medaka that recapitulated the expression of *foxl3* by fluorescence, EGFP, and reared medaka *foxl3* mutant with the transgene. The transgenic *foxl3* mutant medaka does not express the functional Foxl3 protein but the germ cells that would otherwise express foxl3 are labeled with EGFP. With the transgenic and *foxl3* mutant medaka, germ cells were isolated by a cell sorter.

By comparing transcriptomes between germ cells with *foxl3* expression and those without *foxl3*, DEG(+) (transcripts upreguated in wildtype germ cells) and DEG(-)(transcripts upreguated in *foxl3* mutant germ cells) genes were picked up as the candidates regulated by *foxl3*. The comparison allowed us to obtain approximately, over 200 candidate genes.

To further screen the candidate genes, we have employed the iDamIDSeq method to identify the cis-element (binding sequence) of Foxl3. For this purpose, Dam-methylase gene was linked *foxl3*. The RNA transcribed from the fused genes was injected into medaka fertilized eggs. One day after injection, the methylated DNA was analyzed at the level of entire genome and compared with that in control embryos.

We have examined genome sequences of the candidate gene (DEG+ and DEG- genes) and selected the genes with the *cis*-element. This process allowed us to obtain much smaller number of the candidate genes.

Next we examined if Foxl3 may bind to the *cis*-element in the candidate genes by a ChIP method. Because many germ cells expressing foxl3 were required for this purpose, we used germ cells isolated from *meioC* mutant. As a result, a specific binding to the *cis*-element could be confirmed.

Characterization of Germ Cells:

Previously we have been showing that germ cells have a feminizing effect on the body in medaka. In other words, medaka cannot be female even if it does not have a Y chromosome. Germ cells are prerequisite for developing female tissue structures. However, there are many different developmental steps that germline stem cells have to go through (PGCs - germline stem cells – cystic germ cells – meiotic germ cells (follicles)) to become sperm or eggs. Then the question arose which step of germ cells are responsible for feminizing the body.

To address this question, we have generated several medaka mutants in which one of the developmental steps was impaired. Initially we expected follicles to be responsible for the feminizing power of germ cells. Unlike our expectation, medaka mutants that cannot develop follicles (*figla* mutant) or cystic germ cells (*meioC* mutant) can become female. Then we generated the mutant that shows the defect in germ cells at an earlier step (*dazl* mutant). PGCs of this mutant do not develop beyond the step of germline stem cells. Approximately a half of the XX mutant still shows normal female.

We have also examined the feminizing role of germ cells on the process of male to female sex reversal. *Gsdf* mutant exhibits male to female sex reversal. The XY medaka with mutations in both gsdf and meioC displayed female phenotype. This indicates that germ cells are important for the process of male to female sex reversal.

Discussion & Conclusion

The switch mechanism that drives germline stem cells to develop into oogenesis or spermatogenesis is critically important to promote studies on reproduction and regenerative medices. However, the details on the mechanism are not in mystery. In this study, the candidate genes regulated by Foxl3 protein have been successfully obtained. These genes are expected to have a critical role on the switch mechanism. In additions, we have revealed an innate character of germ cells. The conventional view on germ cells was that germ cells were very passive cells regulated by somatic cells and specialized cells that only develop into gametogenesis. Our results are providing a novel view that germ cells have a positive role on feminization and, hence, germ cells are more than gametogenesis. .

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一般の皆様へ

生殖幹細胞が卵になるか精子になるかの仕組み（生殖細胞の性決定）を理解することは、基礎生物学のみならず、生殖医学や再生医学、さらには育種分野においても極めて重要である。にもかかわらず、この仕組みはほとんどわかっていない。今回の研究により、この生殖細胞の性決定を司る遺伝子の候補を絞り込むことができた。さらには今まで知られていない生殖細胞の特性も明らかにできた。生殖細胞は単に卵や精子になるだけではなく、メスになるためには必要不可欠の細胞であり、この特性は生殖細胞が本来もつ特性（すなわち自身が卵になったり精子になったりすることとは関係ない）であることを示すことができた。

ROR1-mediated caveolae formation and caveolar endocytosis in lung cancer

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Summary Abstract

The receptor tyrosine kinase-like orphan receptor 1 (ROR1), a transcriptional target of the lineage-survival oncogene NKX2-1/TTF-1, sustains pro-survival signaling from multiple receptor tyrosine kinases (RTKs) in lung adenocarcinomas. In addition to its kinase-dependent role, ROR1 functions as a scaffold protein to facilitate interaction between CAV1 and CAVIN1, and consequently maintains caveolae formation. Here, we report that ROR1 possesses a novel scaffold function indispensable for efficient caveolae-dependent endocytosis. CAVIN3 was found to bind with ROR1 at a site distinct from those for CAV1 and CAVIN1, a novel function required for proper CAVIN3 subcellular localization and caveolae-dependent endocytosis, but not caveolae formation itself. Furthermore, evidence for a mechanistic link of ROR1-CAVIN3 interaction as well as consequential caveolae trafficking with RTK-mediated pro-survival signaling towards AKT in early endosomes was found in lung adenocarcinoma cells.

Key Words：Caveolae, Endocytosis, Survival signaling, ROR1, CAVIN3, Lung cancer

Introduction

We previously reported that ROR1 is a transcriptional target of the lineage-survival oncogene NKX2-1/TTF-1 that shows frequent gene amplification and overexpression in lung adenocarcinomas, where it sustains a favorable balance between pro-survival PI3K-AKT and pro-apoptotic ASK1-p38 signaling axes in both ROR1 kinase-dependent and independent manners. In addition, an unexpected finding obtained in our recent study revealed that this RTK plays a role as a scaffold protein for CAV1 and CAVIN1, facilitating their association at the plasma membrane. This kinase-independent function of ROR1 maintains CAV1 expression by preventing its lysosome-dependent degradation as well as consequential caveolae formation, which in turn sustains pro-survival signaling toward AKT from multiple RTKs, including EGFR, MET, and IGF-IR.

In the present study, we found that ROR1 interacts with CAVIN3 and sustains caveolae-mediated endocytosis. Furthermore, we provide evidence of a mechanistic link of the ROR1-CAVIN3 interaction with PI3K-AKT pro-survival signaling downstream of EGFR and IGF-IR in EEA1-positive endosomes in lung adenocarcinoma cells.

Results

We initially analyzed the effects of siROR1 treatment on caveolae-dependent endocytosis by treating NCI-H1975 cells with cholera toxin. In contrast to marked internalization of AlexaFluor 488-conjugated cholera toxin B in siControl-treated cells, ROR1 knockdown significantly increased the number of cells with membrane staining and conversely decreased that of those with intracellular staining, reflecting inhibited internalization. Recent studies have shown that CAVIN3 is localized

in both surface-associated caveolae and internalized caveolae, and plays a role in trafficking of caveolae vesicles inside cells [1, 2]. We speculated that ROR1 may cooperate with CAVIN3 in caveolae-dependent endocytosis. Two-color immunofluorescence staining revealed the co-existence of punctate signals of ROR1 and CAVIN3. These findings demonstrated that ROR1 and CAVIN3 co-localize in caveolae structures in lung adenocarcinoma cells, and play a role in caveolae-dependent endocytosis.

We investigated whether the CAVIN3-binding region of ROR1 is required to sustain the caveolae structure and/or CAVIN3 subcellular compartmentalization. NCI-H1975 cells were stably introduced with either siROR1-resistant wild-type ROR1 (WTm) or that lacking the CAVIN3-binding region (ΔTK1m), then treated with siROR1. WB analysis combined with sucrose density-gradient centrifugation demonstrated a marked difference in terms of CAVIN3 subcellular compartmentalization between siROR1-treated WTm and ΔTK1m transfectants. We then analyzed the caveolae structure using electron microscopic observations of ultrathin sections and found that siROR1 treatment did not appreciably affect the number of those structures in ROR1-ΔTK1m-expressing cells as compared to ROR1-WTm-expressing cells. The present findings thus indicated that an interaction of ROR1 with CAVIN3 is required to sustain CAVIN3 subcellular compartmentalization in caveolae, while it is not necessary for caveolae formation *per se* in lung adenocarcinoma cells.

It has been shown that CAVIN3 plays a role in caveolae dynamics through its interactions with CAVIN1 and CAV1, while the association between caveolae and actin may involve the motor protein MYO1C [3, 4, 5]. We then investigated whether deletion of the CAVIN3 binding region of ROR1 affects complex formation among CAVIN1, CAVIN3, and MYO1C. We found that unlike ROR1-WTm, CAVIN3-binding-deficient ROR1-ΔTK1m could not sustain the interaction of CAVIN3 with either CAVIN1 or MYO1C. These results suggest that the CAVIN3-binding region of ROR1 is required for interactions of CAVIN3 with CAVIN1 and MYO1C in lung adenocarcinoma cells.

We therefore examined which binding region(s) of ROR1 are required for caveolae-dependent endocytosis. To this end, NCI-H1975 cells were reconstituted with various mutant forms of siRNA-resistant ROR1. ROR1-ΔTK1m-reconsituted cells exhibited significantly impaired uptake of CTB-488 into cytoplasm, which was similarly observed in ROR1-ΔTK2+3m (the lacking the CAVIN1-binding region) and ROR1-ΔS/T2m (the lacking the CAV1-binding region) cells. We also quantified the internalization of transferrin-488 and found lack of any significant difference between ROR1-WTm- and -ΔTK1m-reconstituted NCI-H1975 cells. The present findings thus clearly indicated that ROR1 plays a role as a scaffold protein for caveolae components, including CAV1, CAVIN1, and CAVIN3, which are crucially involved in caveolae formation and caveolae-dependent endocytosis in lung adenocarcinoma cells.

Together with our previous notion of ROR1-mediated sustainment of pro-survival signaling in lung adenocarcinoma cells, the present findings led us to speculate that the CAVIN3 interacting function of ROR1 may also be involved in this regard. NCI-H1975 and PC-9 cells were accordingly treated with siRNAs against either ROR1 or CAVIN3, which resulted in inhibition of proliferation to a similar extent. We also observed that CAVIN3 knockdown significantly decreased EGF- and IGF-I-induced AKT phosphorylation in NCI-H1975 cells to a degree similar to that seen with siROR1 treatment.

Notably, reconstitution with ROR1-ΔTK1m did not rescue EGF- or IGF-I-induced AKT phosphorylation, in contrast to that with ROR1-WTm in NCI-H1975 cells. Accumulated evidence strongly suggests that membrane receptor signaling pathways elicit signals from various endosome compartments [6, 7]. In proximity ligation assay (PLA), we observed that both EGF- and IGF-I-stimulation resulted in readily detectable fluorescence signals with a punctate-like distribution in ROR1-WTm-reconsituted NCI-H1975 cells, indicating robust interaction of a phosphorylated form of AKT with EEA1, a specific marker involved in signaling in early endosomes. In marked contrast, neither EGF- nor IGF-I-stimulated interaction between EEA1 and phosphorylated AKT was elicited in ROR1-ΔTK1m-reconsituted NCI-H1975 cells. EEA1 knockdown significantly decreased AKT phosphorylation in a manner similar to that seen with siROR1 treatment.

Discussion & Conclusion

Recent studies have shown that CAVIN3 couples caveolae to the intracellular transport machinery by linking the CAVIN1/CAVIN3 complex to actin filaments with MYO1C. Co-localization of ROR1 and CAVIN3 on actin filaments was observed by super-resolution structured illumination microscopy, thus the CAVIN3-binding region of ROR1 appears to be necessary for the interaction of CAVIN3 with not only CAVIN1 but also MYO1C. These findings indicate that ROR1 is a key scaffold protein for at least 3 essential components in the caveolae complex, i.e., CAV1, CAVIN1, and CAVIN3, for maintaining caveolae formation and dynamics. However, we think that it is unlikely that ROR1 is invariably involved in all tissues, based on its negligible expression in normal human adult tissues. It is possible that there may be other yet unidentified molecule with similar functions in a given cell state and lineage.

In summary, the present study revealed a novel function of ROR1 in lung adenocarcinoma cells. ROR1 interacts with CAVIN3 and MYO1C, and has a crucial role as a scaffold protein to sustain caveolae-dependent endocytosis and RTK-mediated pro-survival signaling towards AKT in early endosomes. Future studies for development of novel therapeutic strategies focused on inhibition of the multifaceted scaffold functions of ROR1 are thus warranted, which may ultimately help to reduce the intolerable number of deaths due to this devastating cancer.

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一般の皆様へ

これまで私達は、リネジ生存癌遺伝子であるTTF-1によって転写活性化されるROR1が、EGFRやMET、IGF-IR等の多様な受容体の活性化の維持に寄与することで、肺腺がんにとっての重要な生存シグナルを担うことを明らかにしてきた。

本研究では、受容体としてのROR1の新たな機能に着目し、肺腺がん細胞におけるカベオラの詳細な生理機能の解明を目的とした。

本研究により、ROR1はカベオラ依存的なエンドサイトーシスによって生じたエンドソームにおいて様々なアダプター蛋白質をリクルートし、肺腺がん細胞の生存シグナルを経時的、及び空間的に制御していることが判明した。

本研究の成果は、ROR1とエンドサイトーシスに関わるCAVIN3との相互作用を標的とした、これまでにない肺腺がんの生存シグナルを特異的に抑える独自性の高い革新的な阻害剤の開発につながると期待される。

Regulation of energy metabolism by environmental cue and epigenome

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Summary Abstract

The mechanisms by which environmental cues regulate energy metabolism in adipocytes via epigenome are not fully understood. Genome-wide analyses of transcriptome and epigenome in adipocytes revealed that chronic cold stimuli facilitates induction of genes involved in thermogenesis and lipid metabolism in beige adipocytes via demethylation of histone H3 lysine 9 (H3K9). On the other hand, excess glucose facilitates induction of genes involved in glucose metabolism in white adipocytes via demethylation of H3K9 and promotes lipid accumulation.
Key Words：Metabolic syndrome, epigenome, adipocytes, metabolism

Introduction

Adipose tissues play a central role in the regulation of whole-body energy homeostasis. Dysfunction of adipose tissues is associated with onset of metabolic syndrome and diabetes (1). White adipocytes are specialized for fat storage as an energy source, while brown adipocytes and beige adipocytes are specialized for fat burning and thermogenesis upon acute and chronic cold stress, respectively. The epigenome is acquired genetic information consisted of DNA methylation and histone post-translational modifications. Environmental cues could alter epigenome via as yet poorly understood mechanisms and regulate adipocytes fate and function (2).

Results

To reveal the epigenetic mechanisms by which chronic cold stimuli facilitates formation of beige adipocytes, immortalized preadipocytes derived from stromal vascular fraction of inguinal subcutaneous white adipose tissue from 3- to 6-week-old mice were induced for beige adipocytes and subjected to genome-wide analysis of transcriptome by RNA-sequencing (RNA-seq). RNA-seq analysis upon differentiation of beige adipocytes revealed that genes involved in thermogenesis and lipid metabolism (e.g. *Ucp1, Cidea, Cpt1b, Elovl3*) are upregulated by β-adrenergic signaling and PPARγ (Peroxisome Proliferator-Activated Receptor γ) signaling. Inhibition of β-adrenergic signaling by propranolol or removal of PPARγ agonist prevented induction of genes involved in thermogenesis and lipid metabolism. Epigenome analysis in beige adipocytes by chromatin immunoprecipitation (ChIP)-PCR showed that repressive histone H3 lysine 9 (H3K9) di-methylation on *Ucp1* and *Cidea* is removed during beige adipocytes differentiation by pro-longed β-adrenergic signaling and the action of histone H3K9 demethylase JMJD1A. Functional analysis of JMJD1A revealed that β-adrenergic signaling and PPARγ signaling are integrated by phosphorylation of JMJD1A at Ser265 via protein kinase A (PKA) to induce beige adipocyte formation. JMJD1A was recruited to beige-selective genes during differentiation of beige adipocytes upon activation of β-adrenergic signaling. Phosphorylation-resistant S265A mutation prevented JMJD1A interaction

with PPARγ, PGC-1α (PPARγ coactivator 1α), and PRDM16 (PRD1-BF1-RIZ1 homologous domain containing 16), recruitment to beige-selective genes, histone H3K9 demethylation, induction of beige-selective genes, as well as beige adipocyte formation in vivo. On the other hand, phospho-mimetic S265D mutation facilitated JMJD1A interaction with PPARγ, PGC1-α, and PRDM16, induction of beige-selective genes, and beige adipocyte formation. Interestingly, in brown adipocytes, which are responsible for acute response to cold exposure, rapid induction of genes involved in fat burning and thermogenesis depends on PKA-phosphorylation of JMJD1A but does not depend on epigenome alteration (i.e. histone demethylation by JMJD1A). These results indicate that pro-longed cold stress is transmitted to adipocytes epigenome via phosphorylation of and histone demethylation by JMJD1A to induce the formation of fat-burning beige adipocyte for chronic adaptation to cold (3).

To understand the epigenetic mechanism how extracellular glucose regulates epigenome, fate, and function of adipocytes, genome-wide analysis of transcriptome was performed using 3T3-L1 white adipocyte cell line. RNA-seq analysis revealed that genes involved in glucose metabolism (e.g. *Slc2a4, Hk2, Gpi1*) are upregulated in the presence of excess extracellular glucose. Furthermore, genes involved in citrate cycle enzymes as well as citrate cycle metabolites are elevated during white adipocytes differentiation. Upregulation of genes involved in glucose metabolism depends on elevation of key metabolites for epigenome regulation (i.e. acetyl-CoA and α-ketoglutarate). Acetyl-CoA is required for histone acetylation reaction, while α-ketoglutarate is required for histone demethylation reaction. Depletion of glucose or inhibition of citrate cycle by knockdown of isocitrate dehydrogenase 3β (IDH3β) prevented upregulation of genes involved in glucose metabolism. Genome-wide analysis of epigenome in white adipocytes cell line by ChIP-sequencing showed that repressive H3K9 di-methylation on *Slc2a4* and *Hk2* is removed in the presence of excess extracellular glucose. Inhibition of citrate cycle by knockdown of IDH3β inhibited elevation of α-ketoglutarate and prevented demethylation of H3K9 on *Slc2a4* and *Hk2* during differentiation of white adipocytes. These results suggest that glucose availability is transmitted to epigenome via cellular metabolism to characterize fat-storing white adipocytes.

Discussion & Conclusion

Environmental cues alter cellular epigenome in adipose tissue and promote formation of either beige adipocytes responsible for fat burning or white adipocytes responsible for fat accumulation through poorly understood mechanisms. This study revealed that histone H3K9 demethylase JMJD1A senses pro-longed cold stress via PKA-phosphorylation at Ser265 to induce the formation of beige adipocytes by integrating β-adrenergic signaling and PPARγ signaling. This study also revealed that excess extracellular glucose alters epigenome of white adipocytes to induce genes involved in glucose metabolism and facilitate fat accumulation. Thus, therapeutic target of Ser265-phosphorylation of JMJD1A might prove to be a novel target for the treatment of metabolic syndrome via promoting beige adipocytes formation.

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一般の皆様へ

脂肪組織は全身のエネルギー代謝を担う重要な組織で、脂肪組織の機能破綻はメタボリックシンドロームの発症と関係しています。白色脂肪細胞は脂肪を蓄える一方で、ベージュ脂肪細胞は脂肪を燃焼し熱を産生します。環境は後天的なゲノム修飾であるエピゲノムを介して、脂肪細胞の性質を決めると考えられています。本研究により、寒さの刺激は脂肪を燃焼するベージュ脂肪細胞を作り、過剰なエネルギーは脂肪を蓄える白色脂肪細胞を作ることが分かりました。

Mitochondrial dynamics and antiviral innate immunity

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Summary Abstract

Mitochondria act as a platform for antiviral innate immunity, and the immune system depends on activation of the retinoic acid-inducible gene I (RIG-I) dependent signaling pathway via an adaptor molecule, mitochondrial antiviral signaling (MAVS). In this study, we revealed that RIG-I-mediated antiviral innate immunity requires mitochondrial dynamics.
Key Words：Mitochondria, RNA virus, innate immunity, cellular function

Introduction

Mitochondria are crucially involved in numerous cellular processes, including apoptosis and calcium homeostasis. Mitochondria also have a unique role in innate immunity against RNA viruses. Mitochondrial-mediated antiviral immunity depends on activation of the RIG-I signaling pathway and MAVS, a downstream adaptor of RIG-I at the mitochondrial outer membrane (MOM), has a key role in the signal transduction. Upon viral infection, MAVS recruits various types of effectors at the MOM, and the orchestrated "MAVS signalosome" is the primary unit governing antiviral innate immunity. Although the role of the MAVS signalosome in mitochondria to provide a molecular platform that facilitates signal transduction is well characterized, insight into how the organelle functions to facilitate antiviral immunity has remained unclear.

Results

To elucidate the molecular basis of mitochondrial dynamics coupled with the RIG-I signaling pathway, we speculated that optic atrophy 1 (OPA1), a mediator of mitochondrial fusion, has a role in antiviral innate immunity because of our previous evidences on Mitofusin (Koshiba et al. **Sci. Signal.** 2011). As reported previously, we observed that *OPA1*$^{-/-}$ cells contained less mitochondrial DNA (mtDNA) with completely lacked mitochondrial fusion, and had a disrupted cristae morphology (Yoshizumi et al. **Sci. Rep.** 2017). These phenotypic defects were restored when a *WT OPA1* isoform gene (L-OPA1; variant 1), but not an inactive mutant *K301A* or short isoform (*S-OPA1*), was re-introduced into null cells. Strikingly, although the *OPA1*$^{-/-}$ cells exhibited severely impaired SeV-induced production of IFN-β and IL-6, the immune responses were substantially restored by recovery of the mtDNA level in rescued cells (Yoshizumi et al. **Sci. Rep.** 2017). Given that OPA1 is important for mtDNA stability and the antiviral immune response, we used histochemical staining for cytochrome *c* oxidase (complex IV) activity to directly assess the OPA1 function involved in mitochondrial respiration. As expected, the COX staining pattern of the *V1(WT)*-rescued OPA1$^{-/-}$ cells showed full recovery of the respiration defect, whereas *K301A* and *S-OPA1* failed to rescue the COX activities (Yoshizumi et al. **Sci. Rep.** 2017). Taken together, these results indicate that functional actions of OPA1 are linked to the improvement of mitochondrial dynamics and the induction of mitochondrial-mediated antiviral innate immunity.

Discussion & Conclusion

The findings of our study using cell biological approaches provided new insight into how mitochondria facilitate antiviral immunity through its dynamic properties. Mitochondria are believed to have evolved from organisms such as α-proteobacterium, and the discovery of their role in host-cell defense was unexpected. It is not surprising, however, that cells gaining respiratory function through a symbion could greatly advance not only through enhanced energy production, but also through safeguarded host defense against other infectious pathogens, especially in vertebrates.

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一般の皆様へ

近年の研究から、ミトコンドリアには今までに知られていない新たな生理機能として、RNA ウイルスに対する自然免疫に密接に関わる仕組みが備わっていることが明らかになってきた。しかしながら、このミトコンドリアを介した自然免疫の作動はどのような機構により調節されているのか？不明な点が多く残されている。本研究では、ミトコンドリアの形態的な特徴に着目し、ダイナミックな構造特性が自然免疫応答に必要であることを明らかにした。

Analysis of epigenetic regulation toward establishment of a stable culture method of neural progenitor cells

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Summary Abstract

During development, uncommitted progenitor cells continuously change their characteristics and gradually acquire their specific functions. The purpose of this research is to reveal the molecular mechanisms of the transition of the progenitor characters from the epigenetic point of view. We particularly focused on a histone modification enzyme, a DNA methyltransferase and an ubiquitin ligase, and analysed their functions by means of forced expression in chick embryos.
Key Words：Neural tube, sonic hedgehog (Shh), chromatin modification

Introduction

Any functional cells originate from the uncommitted naïve cells. The uncommitted stem cells are converted into the progenitor state by the inductive signals and then further differentiate into the postmitotic cells that have their unique characteristics. Importantly, the transition from uncommitted to postmitotic state is irreversible; the postmitotic functional cells cannot revert to the less-differentiated state. We postulated that this irreversibility is due to the chromatin modification of the specific regions of the genome. Based on this hypothesis, we focused on a histone modification enzyme, DNA methyltransferase, and an ubiquitin ligase that are highly expressed during the neural development.

Results

The neural tube, formed as a narrow tissue along the rostral-caudal axis in the dorsal part of the embryo, is the embryonic organ of the central nervous system, and is comprised of a number of types of neural progenitor and postmitotic neurons. These cells originated from the uncommitted cells generated in the neuroepithelium during gastrula stages, and gradually acquire unique characteristics by signal molecules emanating from the specific regions of the neural tube. Sonic hedgehog (Shh), produced in the floor plate region, is one of such signal molecules, and forms a gradient from ventral to dorsal. The different types of neural progenitor cells are generated depending on the concentration of Shh. However, we have shown that the timing of the signal – when the progenitor cells receive the signal molecule – is also important to determine the cell fate (Sasai et al., 2014). This theory is called “competence”, or how the progenitor cells respond to a signal, and is assumed as one of the critical theory in the determination of the cell fate as well as another theory “induction’ which represents the importance of the kind of the signal molecules.

We hypothesized that the competence is regulated by the activities of chromatin modifying enzymes, including DNA methyltransferases, histone modifiers and ubiquitin ligases.

First, we focused on the DNA methyl transferase DNMT3. DNMT3 *per se* is expressed in the whole neural tube, but has been shown to regulate the expression of the Arx gene, expressed in the

floor plate region of the neural tube. We inferred that DNMT3 is essential for limiting the expression area of Arx in the floor plate, and electroporated the siRNA targeting DNMT3 in the neural tube of the chick embryos. As the result we found the Arx gene expression aberrantly in the dorsal area, which is supposed to express the interneuron progenitor genes.

Next we analysed the importance of a polycome-type of the transcription factor. This factor, which we call vPTF after ventral polycome transcription factor, is expressed only in the progenitor regions of the neural tube, and we hypothesized that this factor is involed in the maintenance of the progenitor state. To address this question, we performed the forced expression of vPTF in the neural tube, and consequently found the progenitor region is expanded in the postmitotic neuronal area. These two results suggest that the chromatin modifying enzymes play critical roles in the determination of the differentiate state during the neural diufferentiation.

Further analysis will reveal the mechanisms by which the expression and activities of these chromatin modifying enzymes are regulated. The possible upstream factors include kinases and scaffold proteins. We will perform a systematic screening searching for such interacting proteins and will elucidate the whole picture of the epigenetic regulation that controls the temporal shift in the response to the signal molecules, and consequently makes the differentiation irreversible. Furthermore, the maintenance of the progenitor state by manipulating the chromatin state will make it possible to culture the progenitor cells stably. The ultimate goal of this research is to develop the technology to maintain the progenitor state, which will generate the specific functional neurons more efficiently.

Elucidating the regulatory mechanism of histone modification status in colorectal cancer stem cells to maintain stemness and its application for drug development

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Summary Abstract

In this study, we demonstrated that the histone methyltransferase SUV39H1 was significantly overexpressed in colorectal cancer cells. Given that inhibition of SUV39H1 resulted in the growth inhibition of cancer cells, SUV39H1 appears to be an ideal molecular target to develop anti-cancer agents against colorectal cancer stem cells.
Key Words：Cancer stem cell, Histone modification

Introduction

In late years, the importance of epigenetics in human tumorigenesis has been attracting attention, and it is revealed that histone modifications play a central role in epigenetic alteration of cancer cells. On the other hand, the significance of cancer stem cells in human cancer recently becomes clear; it is suggested that development of anti-cancer drugs targeting cancer stem cells is a useful tool to treat cancer effectively. In the present study, we tried to clarify the importance of histone modifications to maintain the stemness of cancer stem cells, and to develop anti-cancer drugs targeting cancer stem cells,

Results

The difficulty in expanding cancer-initiating cells *in vitro* is one of the major obstacles for their biochemical characterization. Dr. Okamoto in National Cancer Center Research Institute, who is a collaborator of this project, previously found that Rho kinase (ROCK) inhibitors greatly facilitated the establishment of spheroids from primary colon cancer (1). Following the procedure established, we dissected and cultivated primary cancer cells under spheroid culture conditions to isolate and expand cancer cancer-initiating cells from primary colon cancer in this study. Then we purified cancer stem cells from operatively extracted specimens of colorectal cancer using a Rho kinase inhibitor; Subsequently, we conducted subculture of those cells to clarify their physiological characteristics. To identify the histone modification enzyme that is specifically up-regulated in cancer stem cells, we performed transcriptome analyses using the microarray. In this case, we compared expression levels of histone modification enzymes between CD44-positive cells (cancer stem cell group) and CD44-negative or low expressing cells (control cells), and identified that the histone lysine methyltransferase suppressor of variegation 3-9 homolog 1 (SUV39H1) and Protein arginine N-methyltransferase 5 (PRMT5) were significantly overexpressed in colorectal cancer stem cells. Since overexpression of SUV39H1 was observed in colorectal cancer stem cells from 4 different patients and we observed big differences of SUV39H1 expression levels between CD44-

positive cells and CD44-negatibe cells, we validated its expression by quantitative real-time PCR. Consequently, elevated expression of SUV39H1 was observed in all cases of cancer stem cells.

The Su(var)3-9, Enhancer-of-zeste, Trithorax (SET) domain-containing proteins constitute the major group of histone lysine methyltransferases. SET domain-containing proteins are characterized by the SET domain consisting an approximately 130-140 amino acid, evolutionary well conserved sequence motif, which was initially discovered in the Drosophila proteins Su(var)3-9, Enhancer-of-zeste and Trithorax (2). In addition to the SET domain, SUV39 family proteins possess pre-SET (N-SET) and post-SET (C-SET) domains at the amino and carboxyl boundaries of the SET domain respectively (3, 4). Unlike the pre-SET and SET domain, the post-SET domain does not have some specific structure, but is proximal to the active site (5). The post-SET domain includes three conserved cysteine residues that are reported to be crucial for histone lysine methyltransferase activity in the SUV39 family proteins. Substitution of these cysteine residues completely abolished the enzymatic activity of DIM-5 and SETDB1 (6, 7). SUV39H1 is a member of the suppressor of variegation 3-9 homolog family and the first histone lysine methyltransferase identified. This enzyme specifically methylates lysine 9 of histone H3 (H3K9). Trimethylation of H3K9 represents a specific tag for epigenetic transcriptional repression by recruiting heterochromatin protein 1 (HP1) proteins to methylated histones. Mainly functions in heterochromatin regions, thereby playing a critical role in the establishment of constitutive heterochromatin at pericentric and telomere regions. Although it is known that SUV39H1 functions as the organization of heterochromatin and the segregation of chromosome, functions of the enzyme in cancer cells are still unknown. Therefore, we tried to clarify the significance of SUV39H1 in cancer cells.

We designed small interfering RNA (siRNA) targeting SUV39H1 in order to specifically suppress the expression of SUV39H1, and transfected the siRNA into two independent cancer cell lines with two control siRNAs (siEGFP and siLuciferase). Subsequently we measured viability of cancer cell lines using Cell Counting Kit-8 (CCK8), which allows sensitivity colorimetric assays for the determination of cell viability in cell proliferation and cytotoxicity assays. As a result, we confirmed that SUV39H1 inhibition repressed the proliferation of cancer cells.

Discussion & Conclusion

Our expression profile analysis clearly indicated that the histone lysine methyltransferase SUV39H1 was significantly overexpressed in colorectal cancer stem cells. In addition, knockdown of SUV39H1 resulted in the proliferation of cancer cells. These result imply that SUV39H1 appears to be an ideal target for the treatment of colorectal cancer, targeting on colorectal cancer stem cells. Indeed, it is known that histone methyltransefrases and demtehylases have been attracting attention as a novel type of target to develop anti-cancer drugs (8). Moreover, some chemical compound, which may inhibit SUV39H1, has already been developed (9).In the present study, we identified that the inhibition of SUV39H1 is likely to be a novel way to cure colorectal cancer, targeting colorectal cancer stem cells. In this case, still detailed molecular mechanisms of how SUV39H1 contributes of stemness functions of colorectal cancer stem cells are unknown; therefore, we need to clarify this points. Further detailed molecular analysis may explore the significance of SUV39H1 in colorectal cancer stem cells, and its importance as a target to cure colorectal cancer.

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一般の皆様へ

本研究代表者は、ヒストンメチル化異常の細胞がん化における重要性を世界に先駆けて明らかにし、その後も常に世界最先端の研究を行ってきた結果、ヒストンを含めたタンパク質メチル化は非常に多様性のある現象であることを解明してきた。一方、最近ヒトがんにおけるがん幹細胞の重要性が明らかになってきており、がん幹細胞を標的とした分子標的治療薬を開発することにより、効果的ながん治療を行うことができる可能性が示唆されている。本研究は、大腸がん幹細胞における、タンパク質メチル化の重要性を明らかにし、ヒストンメチル化関連酵素を標的とした、革新的な新規分子標的治療薬を創生することを目的として施行した。

Lymphoma progression promoted by the breakdown of the system for convergence of inflammation

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Summary Abstract

We have identified TFL, a critical modulator for the convergence of inflammation, from a patient with Follicular Lymphoma. In this proposed study, we found that the prognosis of murine lymphoma model is modulated by the TFL deficiency and identified a candidate inflammatory molecule that is critically regulated by TFL in this process.
Key Words：TFL, lymphoma, inflammation, convergence

Introduction

Malignant Lymphoma is a kind of hematologic malignancies and is characterized by the features of inflammation such as fever, body weight loss and night sweat. The aim of this study was to examine whether the impaired convergence of inflammation could cause lymphoma progression, particularly by focusing on the molecule TFL.

Results

We have previously reported the translocation of the immunoglobulin light chain κ locus gene with a possible tumor suppressor gene, TFL, in transformed follicular lymphoma (1, 2). Subsequently, we generated TFL knockout (TFL-/-) mice. Experimental autoimmune encephalitis (EAE) induced in TFL-/- mice demonstrated persistent severe paralysis. Central nervous system (CNS)-infiltrated CD4+ T cells in TFL-/- mice contained a higher proportion of IL-17 producing T cells than TFL+/+ mice during the resolution phase of EAE. Thus, TFL is a novel gradual and persistent posttranscriptional regulator of the inflammatory cytokines and attenuates the excessive inflammation (3, 4).

In this proposed study, we first crossed TFL-/- mice with murine lymphoma model vavP-Bcl2 transgenic (Bcl2Tg) mice to generate double mutant mice. The survival of TFL-/-Bcl2Tg mice was significantly altered compared with TFL+/+Bcl2Tg littermate mice. We could confirm that TFL is a critical regulator of the lymphoma progression. We are currently evaluating the pathological involvement of lymphoma in the organs of these mice.

We noticed that B lymphoid lineage cells with abnormal surface markers existed in the bone marrow of the Bcl2Tg mice. We sorted this particular cell population and compared the gene expression profiles between TFL+/+Bcl2Tg and TFL-/-Bcl2Tg mice. This analysis picked up several genes which drastically changed in the absence of TFL. Among these, we noticed that the factor X, which is well known as an inflammatory soluble factor, was included. The protein level of factor X was extremely high in the blood of TFL-/-Bcl2Tg compared to TFL+/+Bcl2Tg mice at approximately one year of age. We are now trying to confirm that B lymphoid lineage cells with

abnormal surface markers in the bone marrow are the major producers of factor X by culturing the sorted cells.

To clarify whether TFL could degrade factor X via the posttranscriptional pathways, we generated dual-luciferase reporter vectors with 3'UTR of factor X and IL-2 as a control. This vector was transfected into the several hematopoietic cell lines including lymphoid and myeloid lineages. In this system, IL-2 was degraded in most cell lines by TFL. Whereas TFL degraded factor X preferentially in lymphoid cells.

These results suggest that TFL is a key regulator of the inflammatory feature of lymphoma, which may influence the disease progression.

In the study of gene profiles of B lymphoid lineage cells with abnormal surface markers in the bone marrow as mentioned above, the levels of some microRNA were found to be altered as well as factor X in the absence of TFL. TFL is known to be located at P-bodies where many microRNAs are processed in the cell body (2). We would like to expand our research to identify the regulatory mechanism of microRNA by TFL in the control of lymphoma.

Discussion & Conclusion

The usual treatments for malignancies are traditional cytotoxic drugs and recent molecular targeted agents. The prognosis of lymphoma is getting better because of the combination of these therapies. But in case of high aged patients, current therapeutic strategy is sometimes not enough or intolerable. Targeting the inflammatory mechanism could be a novel and patient friendly therapy. Based on our results, TFL may play a critical role to control the production of inflammatory factors, which may compromise the patient status. We believe that TFL is a good candidate molecule as a target for a novel therapy.

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一般の皆様へ

我々がリンパ腫の患者さんから独自に発見したTFLという分子は、炎症の収束に必須の分子でした。この分子がないと、一旦起こった炎症が収束せずに続いてしまうのです。悪性リンパ腫は血液悪性腫瘍の一種ですが、発熱や体重減少、寝汗など全身性炎症症状が特徴です。マウスモデルを用いた本研究で、我々はマウスリンパ腫モデルの予後がTFL分子の有る無しで変化することと、その変化に重要なTFLによってコントロールされている可能性の高い分子を見いだしました。成因のわかっていない悪性リンパ腫の進展を癌という観点ではなく炎症収束機構の破綻と捉える考え方ができる可能性を示唆しており、通常用いられている抗がん剤ではなく炎症を標的としたリンパ腫治療の可能性を探る糸口になるかもしれません。

Role of CRIMI as a novel regulator for osteoblast functions revealed sing osteoporotic mutant mice

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Summary Abstract

imla mice are novel mutants that exhibit reduced bone mass. By whole exome sequencing and linkage analysis, we identified the causative gene, *Crim1*, for an osteoporosis-like phenotype in *imla* mice. Furthermore, we showed that *Crim1* mRNA was expressed in an osteoblast cell line. There results strongly suggest that Crim1 is involved in the regulation of osteoblast differentiation and/or function.

Key Words：osteoporosis, mutant mice, whole exome sequencing, Crim1

Introduction

We identified a mouse mutant that exhibits microphthalmia, hindlimb syndactyly, and osteoporosis from an ENU mouse mutagenesis screening (Fig. 1). These phenotypes were shown to be inherited as an autosomal recessive trait. We named this mutant allele as *imla* (Iwate microphthalmia with limb anomalies) and tried to identify it by whole exome sequencing.

Results

We identified 71 mutations that might change the coded protein functions by whole exome sequencing. By searching for MGI mouse database, we knew that the phonotypes of *Crim1* knockout mice were very similar to those of *imla* homozygotes, suggesting that *Crim1* was the causative gene. The identified *Crim1* mutation, c.418T> A, was a missense mutation that causes a Cys-to-Ser substitution at position 140 (p.Cys140Ser). Linkage analysis showed that the two markers located near *Crim1* region completely linked to imla phenotypes. These results strongly suggest that imla is the *Crim1* mutation, p.Cys140Ser.

Imla mice exhibited an osteoporosis-like phenotype, suggesting that Crim1 is involved in bone homeostasis. Indeed, *Ctim1* mRNA was modestly expressed in osteolastic cell line MC3TC-E1, and Its expression gradually increased during differentiation (Fig. 2). The secretion of p.Cys140Ser mutant protein that were over-expressed in COS7 cells was severely inhibited compared to the wild-type CRIM1 protein.

Figures

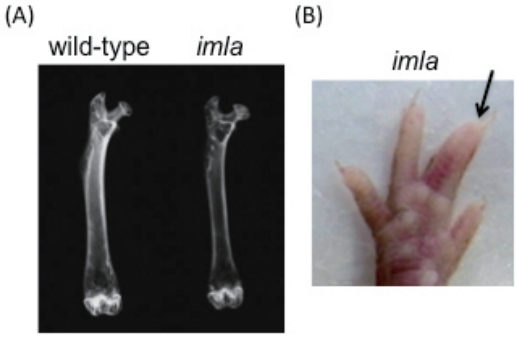

Fig. 1 Phenotypes of *imla* mutant mice.
(A) osteoporosis, (B) syndactyly

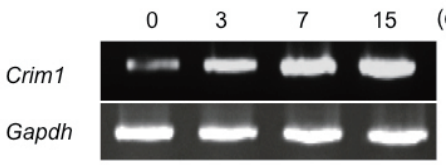

Fig. 2 *Crim1* mRNA expression
in osteoblastic cell line MC3T3-E1 cells

Discussion & Conclusion

Crim1 is the causative gene for an osteoporosis-like phenotype in *imla* mice, and its mRNA is expressed in osteoblast cell line. There results strongly suggest that Crim1 is involved in bone homeostasis by regulating osteoblast differentiation and/or function.

一般の皆様へ

私たちは骨粗鬆症を発症する新規変異マウスを同定し、*imla* マウスと命名しました。本研究では全エクソーム解析と連鎖解析という方法を用いて、*imla* マウスにおける骨粗鬆症の原因遺伝子として Crim1を同定しました。Cim1は骨を作る骨芽細胞に発現しており、骨芽細胞の機能を調整することによって、骨量を制御している可能性が示されました。今後は骨量制御における CRIM1の役割を明らかにし、CRIM1を標的とした骨粗鬆症治療薬の創薬研究基盤の確立を目指して、研究を展開します。

Peroxisome biogenesis disorders: Molecular mechanism of athogenesis in the cerebellum

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Summary Abstract

A model mouse for peroxisome biogenesis disorders (PBD) shows upregulation of a neurotrophin (NT) and a dominant-negative isoform of NT receptor, TrkB-T1, in cerebellum, where abnormal dendritic arborization and axonal swelling of Purkinje cells are evident. In this study, we addressed involvement of peroxisomal metabolic abnormality in upregulation of NT. We found that abnormal cytosolic reductive state in peroxisome-deficient cells is likely responsible for the upregulation of NT. In addition, primary culture of cerebellar neurons suggested that the malformation of Purkinje cells in PBD model mouse is caused by a combination of the elevated NT and prominent expression of TrkB-T1.

Key Words：Peroxisome, peroxisome biogenesis disorder, neurotrophin, TrkB, cerebellum

Introduction

Peroxisome is an organelle participating in various anabolism and catabolism such as β-oxidation of very-long-chain fatty acids and biosynthesis of ether phospholipids. Patients with peroxisome biogenesis disorders (PBDs) manifest dysmorphogenesis of central nervous system (CNS) such as abnormal arborization of Purkinje cells in cerebellum. We found that secretion of a neurotrophin (NT) was increased in peroxisome-deficient astrocytic cell line. The expression of NT and a dominant-negative isoform of NT receptor, TrkB-T1, were also elevated in cerebellum of our PBD model mouse. We investigated how and why peroxisome deficiency leads to the elevation of NT and subsequent dysmorphogenesis of cerebellum. (99 words)

Results

Secretion of NT is elevated in the peroxisome-deficient RCR-1 cell, an astrocyte-like cultured cell line derived from rat embryonic cerebellum. To investigate how deficiency of peroxisome biogenesis in RCR-1 cells enhances the *NT* expression, several peroxisome metabolisms including synthesis of plasmalogens and fatty acid β-oxidation were assessed. Restoration of plasmalogens in peroxisome-deficient RCR-1 cells by the treatment with hexadecylglycerol did not alter the elevated *NT* expression. Similarly, the deficiency of peroxisomal fatty acid β-oxidation generated in RCR-1 cells by knockdown of acyl-CoA oxidase 1 catalyzing the first step of β-oxidization showed no increment of the *NT* mRNA level. Taken together, these results suggested that the upregulation of NT mRNA level is not induced by the depletion of plasmalogens and the impaired peroxisomal fatty acid β-oxidation. Cytosolic redox potential in the peroxisome-deficient cells is more reductive than normal cells (Yano et al., 2010). Interestingly, *NT* expression was lowered by treatment with 3-amino-1,2,4-triazole, a catalase inhibitor, of the peroxisome-deficient RCR-1 cells but not control cells. Accordingly, these results suggested that abnormal cytosolic redox state is likely involved in the elevation of *NT* expression.

Our PBD model mouse, *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse established here, manifests the malformation of cerebellum including abnormal arborization and axonal swelling of Purkinje cells. NT expression is elevated around Purkinje cell layers of cerebellum in the *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse. To investigate whether the malformation of the cerebellum in *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mice is a consequence of the elevated NT protein level, we performed primary culture of cerebellar neurons in the absence or presence of NT. Purkinje cells with swollen axons were more frequently detected upon treatment with NT of the cells from *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse. Moreover, the development of dendrites in the *Pex14*-defective Purkinje cells was affected in the presence of NT. In the wild-type Purkinje cells, the axonal formation and dendritic arborization were not altered by the NT treatment. Such dysmorphologies of Purkinje cells in vitro were in good agreement with those observed in the cerebellum of *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse. Taken together, these results suggested that an elevated level of NT induces the dysregulation of NT signaling pathway only in the peroxisome-deficient Purkinje cells, giving rise to the axonal swelling and the abnormal dendritic arborization. In the cerebellum of *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mice, TrkB-T1 is significantly elevated relative to that in the wild-type mice. Therefore, the malformation of Purkinje cells in *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse is most likely due to a combination of elevated NT and prominent expression of TrkB-T1 (Fig.1).

We investigated how the protein level of NT is elevated in cerebellum of *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse. *NT* mRNA level was not altered in the cerebellum, suggesting that cerebellar cells are less likely involved in the upregulation of NT in *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse. In contrast, mRNA and protein levels of NT were upregulated in inferior olivary nucleus (ION) of *Pex14*$^{\Delta C/\Delta C}$ mutant mice. Given these results together with the fact that climbing fiber projects from ION to Purkinje cells, we suggest that the elevated NT protein in the Purkinje cell layer is most likely delivered via climbing fibers (Abe et al., 2018).

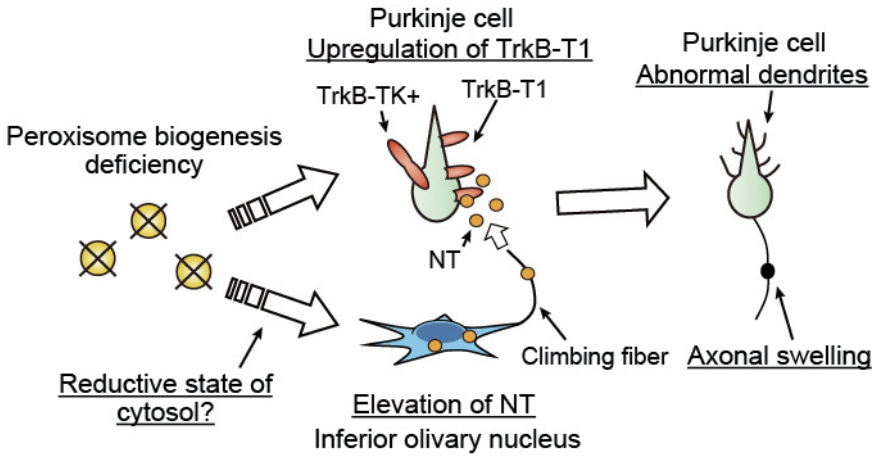

Fig.1 Schematic model of malformation of Purkinje cells caused by peroxisome biogenesis deficiency. Peroxisome biogenesis deficiency leads to upregulation of TrkB-T1 in Purkinje cells and elevation of NT in inferior olivary nucleus under reductive condition of cytosol. Excess amount of NT is secreted via climbing fiber, followed by abnormal development of dendrites and axonal swelling of Purkinje cells.

Discussion & Conclusion

In the developmental stage of the cerebellum, NT immunoreactivity is evident around Purkinje cells, whereas expression of *NT* mRNA is undetectable. We, therefore, suggest that the NT protein in the Purkinje cell layer is delivered via climbing fibers from the ION, where *NT* mRNA is strongly expressed. Indeed, the expression of *NT* mRNA is elevated in the ION neurons of *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse. On the other hand, Purkinje cells in *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse predominantly express TrkB-T1, which induces dominant-negative inhibition of NT signaling. In the primary culture of cerebellar neurons, the dendritic development of Purkinje cells from *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse is compromised and swollen axons were increased by the NT treatment. Therefore, a combination of elevated NT from ION neurons via climbing fiber and prominent expression of TrkB-T1 in Purkinje cells is likely responsible for the malformation of Purkinje cells (Fig.1). Further studies would be required to elucidate the molecular mechanisms underlying the transcriptional upregulation of the *NT* and *TrkB-T1* in the ION and the Purkinje cells, respectively, of *Pex14*$^{\Delta C/\Delta C}$ BL/ICR mouse.

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一般の皆様へ

細胞小器官ペルオキシソームの形成障害は、大脳や小脳における神経発生・分化障害・神経変性を特徴とする先天性致死疾患ペルオキシソーム欠損症をもたらすが、その発症メカニズムは不明である。我々は病態発症の分子機構解明を目的として、ペルオキシソーム欠損症モデルマウスを作出し、検証を進めるなかで小脳における神経栄養因子 NT の発現量増加を見出している。また、このモデルマウス小脳の神経初代培養実験により、過剰な NT が小脳プルキンエ細胞の形態異常を導くことやサイトゾルの還元化がその発現量増加に関与することを明らかにした。これらの研究成果は、未だ治療法が確立していないペルオキシソーム欠損症の治療法開発にも繋がるものと期待される。

Optically transparent bamboo-cellulose/collagen nanocomposite having antibacterial action

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Summary Abstract

The potential of bacterial (*Staphylococcus aureus*) and fungal attacks (*Corious versicolar and Tyromyces palustris*) against moso bamboo shoot skin (*Phyllostachys pubescens Mazel*) extracts were evaluated. Dichloromethane and hexane extracts from bamboo shoot skin had antibacterial activity, and Dichloromethane extracts shown growth inhibitory effect against *Tyromyces palustris*. Cellulose nanofibers could be obtained from bamboo shoot skin. These results suggest the possibility of production of nanocomposite having antibacterial action from bamboo shoot skins that are mainly discarded at present.

Key Words：Bamboo shoot skin, cellulose, nanofiber, antibacterial activity

Introduction

Bamboo grows widely in zones with a wet season ranging from the tropics to temperate areas and an attractive material due to their rapidity of growth. They are fast-growing renewable biomass and some bamboo species may provide higher biomass yield than dedicated energy crops such as corn stover, switchgrass or miscanthus. This study focused on moso bamboo shoot skins for the production of cellulose nanofiber having antibacterial activity.

Results

In this experiment, we evaluated the antibacterial and anti-fungal activity of extracts from moso bamboo shoot skin. Moso bamboo shoot skins were serially extracted with n-hexane, dichloromethane, and methanol for 24 h at 180 rpm.

The results of antibacterial assay are shown in fig. 1. The cfu of dichloromethane extract was <10, n-hexane was 1.0 x 10, methanol was 9.0 x 10, and control was 1.2 x 10^6. The dichloromethane and n-hexane extract showed strong antibacterial activity.

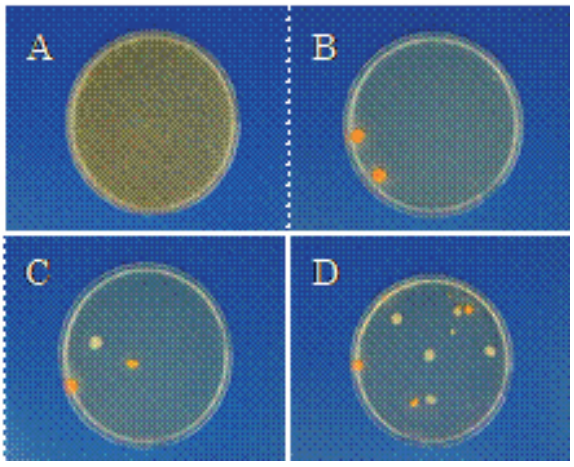

Fig.1. Antibacterial assay of bamboo shoot extract of control(A), n-hexane(B), dichloromethane(C), methanol (D).

The results of anti-fungal assay are shown in Fig.2. The dichloromethane extracts shown growth inhibitory effect against Tyromyces palustris. However, there was no effect on Corious versicolar.

Finally, we successfully obtained cellulose nanofiber from bamboo shoot skin.

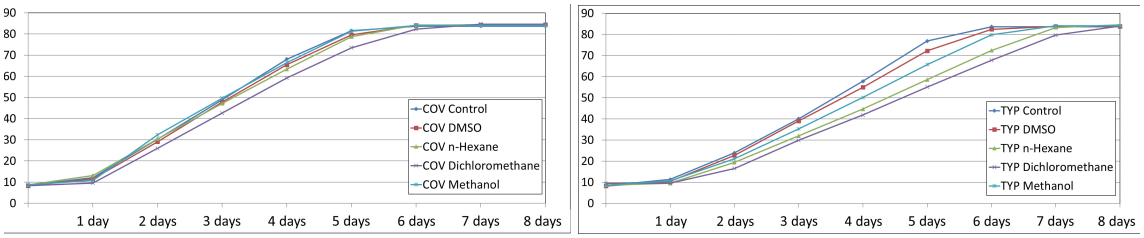

Fig.2. Antifungal assay (growth rate) of bamboo shoot extract against *Tyromyces palustris* (left) and *Corious versicolor* (right)

Discussion & Conclusion

It was reported that 2,6-dimethoxy-pbenzoquinone was isolated from the skin of bamboo trees as an antibacterial compound (1). It was also reported that bamboo shoot skin has antibacterial activity against *S. aureus* (2). However, 2,6-dimethoxy-p-benzoquinone was not detected in extracts of bamboo shoot skin by high-performance liquid chromatography analysis (2). It was suggested that antibacterial compounds other than 2,6-dimethoxy-pbenzoquinone are present in bamboo shoot skin. In this study, we found that bamboo shoot skin has anti-fungal activity against *T. palustris* not only *S. aureus.* Moreover, we could obtain cellulose nanofiber from residues after extracting the active compounds of bamboo shoot skin. Currently, bamboo shoot skins are either burned or composted. This study indicates the possibility of bamboo shoot skins as antibacterial nanofiber materials.

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一般の皆様へ

竹材の表皮や筍皮の細胞には抗菌性があることが経験的に知られており、古来より食品の保存や薬品などに用いられてきました。本研究では、このような竹の持つ抗菌・抗酸化成分を利用した抗菌ナノセルロース医療用材料の創製を目的に、抗菌成分の抽出、活性試験、および抽出残渣からのセルロースナノファイバー製造を行いました。その結果、抽出した成分からは黄色ブドウ球菌のようなバクテリアのみならず、真菌類への阻害効果が示唆されるなど、新たな効果が発見され、また抽出残渣からはセルロースナノファイバー製造に成功しました。高い生体適合性を有しながらも機械的特性の低さからこれまで利用が制限されてきたコラーゲンなどの医療用ゲル材料補強に、今回得られた筍皮由来抗菌成分およびナノファイバーを用いることで、幅広い用途展開につなげて行きたいと考えています。

Establishment of therapeutic genome editing techniques applicable for post-mitotic neurons

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Summary Abstract

The goal of our research is to develop a novel therapeutic strategy to activate the potential of neurons to elongate their nerve fibers and to re-build a new neural network in the injured central nervous system (CNS). In the adult mammalian CNS, it is well known that injured nerve fibers exhibit very limited regeneration ability. The evidence is now emerging that such inability of regeneration is not simply due to poor intrinsic regeneration ability of adult neurons, but rather due to the inhibitory environment. If such inhibition can be overcome, nerve regeneration would be greatly enhanced. In this research project, we have established genome editing techniques applicable for activation of intrinsic nerve growth-promoting factors of post-mitotic neurons.
Key Words：nerve regeneration, genome editing, gene therapy, central nervous system

Introduction

Precise wiring patterns are one of the remarkable features of neurons, and establishment of the proper neuronal network requires control of synapse formation. Fluorescence imaging of spatio-temporal localization of synaptic proteins at single-cell resolution in highly complicated neural networks is prerequisite for analyzing the cell biological mechanisms of synaptogenesis. Ectopic expression of fluorescent-tagged proteins is a commonly used, simple and easy way. However, it is unsuitable for observing synaptic proteins, because it tends to cause alterations in synaptic morphology and plasticity, due to aberrant localization and amount of the expressed proteins. Several methods other than ectopic expression, including generation of recombinant antibody-like probes or conditional knock-in mice sparsely expressing fluorescently tagged proteins, have been recently developed. However, there has been no method that provides high-throughput tools for examination of various endogenous synaptic proteins in primary cultured post-mitotic neurons.

Results

In tis study, by combining the CRISPR/Cas9-mediated genome editing technology with electroporation techniques, we tried to modify endogenous proteins of interest *in vivo* in the mouse retina and brain and *in vitro* in the rat neuron primary cultures by inserting the reporter-tagged cDNAs into the target gene loci. Unlike cultured cell lines which can proliferate infinitely, or fertilized eggs which give rise to adult bodies after many cell divisions, neurons *in vivo* do not clonally expand. Therefore, it is almost impossible to use the correctly targeted neurons for further studies after genotyping. To overcome this problem, we planed to develop a method to identify the correctly targeted neurons without genotyping. We focused on the fact that there is often no promoter activity around the exons encoding the C-termini of proteins. We expected that the knock-in targeting vectors, which correspond to the genomic DNAs encoding the C-termini of proteins, do

not express fluorescent reporters by themselves, but that the reporter genes are turned on when the targeting vectors are correctly integrated into the genome by CRISPR/Cas9-mediated homologous recombination.

Through several optimizations, our results show that the efficiency of CRISPR/Cas9-mediated gene knock-in in the rodent neural tissues is unexpectedly high, reaching up to ~10% of the transfected cells at some gene loci, and that the "C-terminal fusion approach" worked well with few false-positive signals.

Optimization of *in vivo* gene targeting technology in the mouse brain

To examine if the genome-editing technology can be optimized to be applicable to neurons in the mammalian brain, we performed optimization of gene targeting technology to modify endogenous proteins *in vivo* in the mouse brain. A post-synaptic protein, PSD-95 was chosen as a target, and the CRISPR gRNA was designed to target the genome sequence around the stop codon of the PSD-95 gene. The targeting vector was designed to express a PSD-95-mClover C-terminal fusion protein from the endogenous locus. Embryonic day 14 (E14) mouse brains were co-electroporated *in utero* with PSD-95 targeting vector (mClover), CAG-tdTomato, and the CRISPR construct (CAG-Cas9/U6-gRNA (PSD-95)). When the transfected brains were harvested and examined at adult (Postnatal day 65), a PSD-95-mClover fusion protein were detected only in the presence of the CRISPR construct, showing that endogenous PSD-95 was correctly tagged with mClover in the mouse brain by *in vivo* gene targeting.

Optimization of *in vitro* gene targeting technology in the rat neuron primary cultures

To examine if the genome-editing technology can be optimized to be applicable to *in vitro* differentiated neurons prepared from the mammalian brain, we challenged to modify endogenous synaptic proteins in neuron primary cultures prepared from E18 rat cortex and hippocampus. PSD-95 and a pre-synaptic protein, Synaptophysin (Syp), were chosen as targets, and the CRISPR gRNAs were designed to target the exons containing the stop codons of the *PSD-95* and Syp genes. The targeting vectors were designed to express PSD-95-mCherry and Syp-EGFP fusion proteins from the endogenous loci. We observed CRISPR/Cas9-dependent gene knock-in at both gene loci in the neuron primary cultures prepared from rat cortexes and hippocampi. Fluorescent signals were detected only in the presence of the CRISPR construct, and their expression patterns were identical to those of endogenous PSD-95 and Syp detected with the antibodies.

Discussion & Conclusion

At present, there have been no reports demonstrating the regeneration of nerve fibers from the injured mature neurons in the central nervous system (CNS) by direct gene therapy. If the strategy developed in this research project works and planned nerve fiber regeneration successfully occurs, it will overturn the old dogma that CNS neurons cannot be regenerated in the adult mammalian brain. It will further provide new insight into a non-invasive strategy in regenerative medicine without the need for cell transplantation.

References

Daiki Ohama, Takahiko Matsuda, and Izumi Oinuma (2018)
Differential regional and subcellular localization patterns of afadin splice variants in the mouse central nervous system. *Brain Research*（印刷中）

一般の皆様へ

もし、本研究期間に至適化に成功した成熟後神経細胞へのノックイン手法により中枢神経回路の可塑性ともいえる再生現象が引き起こされれば、「中枢神経自らは再生しない」という科学的常識を覆し、生物学の教科書にある「常識」を書き換えるほどのインパクトを与えるとともに、幹細胞移植に依らない非侵襲的再生治療法の実現のための基盤研究として、iPS 細胞開発以来の大きく、新たな視点が与えられる。

Vascular intraluminal pressure-dependent switching machinery of ngiogenic mode

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Summary Abstract

This research aimed to clarify a mechanobiological machinery for regulating angiogenesis in a vascular intraluminal pressure-dependent way. Through the research, 1) we found that perivascular pericytes might potently switch on and off angiogenesis by controlling vascular intraluminal pressure load, and 2) identified a mechanism of how vascular intraluminal pressure inhibits angiogenic branch elongation by endothelial cells.
Key Words：Angiogenesis, Mechanobiology, Intraluminal pressure

Introduction

Angiogenesis is a vital reaction to increase new blood vessels. It potently contributes not only to tissue construction and homeostasis but also to the pathogenesis of various diseases. Blood vessels have a lumen structure, and thereby the vascular walls are constantly exposed to mechanical stimuli by blood flow. However, it still remains unclear whether or not and how blood flow affects angiogenesis. Just recently, we identified that vascular intraluminal pressure load could cause inhibition of angiogenesis at a certain condition. This research aimed to clarify a mechanobiological machinery which regulates angiogenesis in a vascular intraluminal pressure-dependent way.

Results

We identified that vascular intraluminal pressure load could inhibit branch elongation during endothelial cell driven angiogenesis so far. On the other hand, a recent report showed that blood flow enhanced lumen formation (Ref. 1). From two findings, we hypothesized that there is astute machinery that can switch two different angiogenic modes, branch elongation and lumen formation, by spatiotemporally controlling vascular intraluminal pressure. Especially, this research focused to clarify how exposure to vascular intraluminal pressure load is controlled in angiogenic endothelial cell sprouts during the branch elongation mode.

1) Analyses using an *in vivo* murine retinal angiogenesis model

We assumed a possibility that emerging vessels structurally avoid excessive exposure to vascular intraluminal pressure. First we checked the pattern of vascular lumen formation in emerging vessels of P4 mice retina by immunohistochemically detecting lumen with ICAM2 and endothelial sprouts with CD31. We found that lumen was not formed continuously from the proximal lumen. Instead, once vascular lumen in the angiogenic front was formed isolated from the proximal lumen where blood flows, and it then fused to the proximal one. The result suggests that a possible mechanism to avoid excessive intraluminal pressure load in just emerged branch.

Next we checked whether or not pericytes contribute to the characteristic pattern formation of vascular lumen in the emerged branch. We inhibited pericyte coverage over endothelial sprouts of P4 mice retina using anti-PDGFRbeta neutralizing antibody as previously reported (Ref. 2). We found that vascular lumen formation was not significantly changed in the absence of pericyte coverage. However, vascular outgrowth was delayed and the vasculature was dilated, suggesting that pericyte might contribute to control of vascular intraluminal pressure load in other ways.

2) Analyses using an *in vitro* angiogenesis model using microfluidic device

One of the reasons why blood flow-dependent mechanisma of angiogenesis regulation remained not addressed was due to lack of useful methods. Therefore, we firstly developed an *in vitro* analyzing system to assess impacts of vascular intraluminal pressure load on angiogenesis by applying a recently established original assay system with a microfluidic technology (Ref. 3, 4). Using this new system, we have ever identified that hydrostatic pressure load inhibits angiogenic branch elongation of endothelial cells.

From *in vivo* results, pericytes are supposed to contribute to control of vascular intraluminal pressure load. Therefore, we checked the effect of pericyte addition on *in vitro* angiogenesis upon the hydrostatic pressure load. We found that pericyte addition could mostly cancel hydrostatic pressure load-dependent inhibition of branch elongation, suggesting that pericyte regulates angiogenesis at least via control of vascular intraluminal pressure load.

Finally, we addressed to dissect molecular and cellular mechanisms governing vascular intraluminal pressure-dependent inhibition of angiogenic branch elongation by endothelial cells. We found that vascular intraluminal pressure generates vascular wall tension, which induces adequate F-actin formation failure and subsequent antero-posterior polarity loss in angiogenic endothelial cells. Eventually, endothelial cells failed to elongate the branch due to loss of directed migration. We also checked contribution of YAP/TAZ, a well known molecular transducer of mechanical stimuli, but the expression pattern in angiogenic sprouts was not significantly affected by hydrostatic pressure load.

Discussion & Conclusion

In this research, we found pericyte to be a possible controller, which switches angiogenic mode by regulating vascular intraluminal pressure load to endothelial cell vascular wall. This seems at least not due to spatiotemporal regulation of vascular lumen formation. Furthermore, we found an inhibitory role of vascular intraluminal pressure on angiogenesis, in which vascular wall tension causes failure of directed collective endothelial cell migration. Although in the present research it remains uncovered the mechanism of how pericyte controls vascular intraluminal pressure load, the present findings provide new insights to angiogenic mechanism from the point of view mechanobiology and also provide a basis to develop epochal diagnostic tools and therapeutic strategies those target angiogenesis in various diseases.

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一般の皆様へ

血管新生は、もともとある血管から新しく血管をつくりだす現象である。我々の体が作られる時のみでなく、その後の修復過程や様々な病気の発症や進行時に見られる極めて重要な現象であり、その全貌を十分に理解する必要がある。今回の研究では、血管内腔を流れる血液（血流）により生じる内腔圧が血管新生を抑制するメカニズムを明らかにした。また、血管をとりまく細胞ペリサイトが血管内腔圧の作用を調節することで血管新生を制御する新しい血管新生メカニズムが示唆された。本研究は、血管新生の理解に貢献すると共に、血管新生を標的とする新たな治療法創生に資する。

Novel therapeutic strategy for heart failure targeting PKG/TORC signaling and its intracellular localization.

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Summary Abstract

PKG1α can inhibit mTOR complex in redox manner via new phosphorylation site on TSC2, by which preventing PKG1α oxidation blunts pathological hypertrophy.
Key Words：cyclic GMP, redox regulation, cardiac hypertrophy, mTOR

Introduction

Cyclic GMP-dependent protein kinase (PKG1α) transduces NO- or natriuretic peptide-related signaling to regulate physiological processes in cardiovascular system [1,2]. The primary enzyme activation occurs when cGMP binds to the regulatory domain [3]. However, it's recently reported that PKG can be also stimulated by oxidation at C42, forming an intramolecular disulfide bond [4,5]. We sought to examine the role of PKG redox modulation on cardiac hypertrophy and heart failure, focusing on TSC2 (tuberin), a new candidate of PKG substrate, which negatively regulates mechanistic target of rapamycin (mTOR) signaling.

Results

We have shown that preventing C42 oxidation in PKG1α can blunt adverse cardiac remodeling induced by cardiac pressure overload [6]. Our recent phospho-proteome analyses with rat cardiomyocytes revealed a new PKG phosphorylation site on TSC2, so we decided to elucidate the unknown function in the development of pathological cardiac remodeling. TSC2, an upstream master repressor of TORC1, is known to be enhanced by AMP-activated protein kinase (AMPK) or glycogen synthase kinase-3β(GSK-3β), whereas can be suppressed by Akt or ERK1/2 activation [7]. We thereby hypothesize that the PKG phospho-site potently activates TSC2 nearby GSK-3β phosphorylation site. To test the hypothesis, we first checked if the downstream of mTORC1 signaling was modulated in neonatal rat cardiomyocytes expressing either redox insensitive PKG1α ($PKG1\alpha^{C42S}$) or wild-type PKG1α ($PKG1\alpha^{WT}$). The results showed endothelin-1 (ET1) stimulation increased phosphorylation levels in p70S6K and elF4E binding protein-1(4EBP1) in cells expressing $PKG1\alpha^{WT}$, while the levels were significantly blunted in ones expressing $PKG1\alpha^{C42S}$ despite no remarkable changes in the upstream signaling such as Akt, ERK1/2, AMPK, or GSK-3β. These results indicate that some unknown upstream factors including TSC2 play an important role to substantially mediate mTORC1 signaling pathways to regulate balance of protein synthesis and the recycling. We also made sure ET1-induced fetal gene upregulations such as Nppb and regulator of calcineurin-1 were also attenuated in cells expressing $PKG1\alpha^{C42S}$ compared to $PKG1\alpha^{WT}$, which findings corroborate the critical role of PKG/TSC2/mTORC1 to blunt pathological cardiac hypertrophy in redox manner. We produced the mouse model harboring TSC2 phosphorylation mutation at PKG site in order to check the reproducibility in vivo as well. We suppose that examining in vivo study with the valuable

mice will provide the significance of phosphorylation site on mTORC1 regulation and the anti-hypertrophic effects against cardiac pressure overload.

We have shown PKG redox modulation can control its localization and the targeting. PKG normally distributes throughout the cell at rest. However, it acutely moves to outer plasma membrane after stimulation upon hormone or mechanical stress. Preventing PKG oxidation enables to remain there after the translocation, whereas oxidized PKG stays in the cytosol. We tested if PKG localization can modulate autophagy in neonatal rat cardiomyocytes expressing PKG^{WT} or PKG^{C42S}. The confocal immunofluorescence so far showed cells expressing $PKG1\alpha^{WT}$ substantially inhibits autophagy-proteolysis followed by mTOR hyper-activation and thus more hypertrophy, recapitulating our in vitro results.

Discussion & Conclusion

In cell model, we demonstrated that pathological hypertrophy signaling was enhanced with ET1 stimulation and that the responses were accompanied by mTORC1 activation along with an enhanced protein synthesis and the decreased degradation. We also found the consequent responses were attributed to the inhibition of negative regulator TSC2 which was the novel PKG target. Moreover, PKG appeared to activate TSC2 by phosphorylation at unknown site S1365 in redox-dependent manner. These results should be finally confirmed in vivo mouse model such as phospho-mimetic or phospho-silenced TSC2 mutants exposed to sustained cardiac pressure overload. Our study and the findings would provide helpful therapeutic strategy for heart failure through novel PKG/TSC2/mTORC1 signaling pathway.

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一般の皆様へ

サイクリック GMP 依存性プロテインキナーゼ (PKG) の活性は、サイクリック GMP の結合により惹起される従来の調節機構以外に、酸化還元 (レドックス) によるサイクリック GMP 非依存性の調節機構がわかり、心血管における意義が注目されている。本研究は、細胞の成長や増殖、生存を調節する哺乳類ラパマイシン標的蛋白質 (mTOR) のシグナルを抑制するチュベリン (TSC2) 上に、新規の PKG リン酸化部位を見出し、病的心肥大における同部位のリン酸化の意義を明確にすることを目的にした先駆的な研究です。本研究成果を契機に、心不全治療における新たな分子標的戦略の発展が期待されます。

Virus-mediated suppression of host innate immune response

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Summary Abstract

The innate immune system is a first line of defense against viral infection. On the other hand, many viruses have evolved to escape host innate immune response. In this study, we focused on the host and virus interaction, and revealed new molecular mechanism of how virus escape host innate immune response.

Key Words：Innate immunity; virus

Introduction

The innate immune system is essential for protection against viral infection especially at early phase of viral infection (Oshiumi H et al 2016). However, many viruses have evolved to escape host innate immune response. Previously, we have revealed that extracellular vesicles (EV) released from hepatocytes infected with hepatitis B virus (HBV) suppressed the innate immune response of macrophages (Kouwaki T et al 2016). But, underlying mechanism of EV-mediated suppression remains elusive. In this study, we focused on the molecular mechanism and found that microRNAs within EVs were important for the suppression.

Results

Extracellular vesicles (EVs) were collected from cell culture medium of hepatocytes infected with or without hepatitis B virus (HBV). We determined microRNA expression levels in collected EVs and found that miR-21 and miR-29a expression levels in EVs were increased by HBV infection. miR-21 and miR-29a are known to suppress IL-12 expression. Thus, we investigated whether EVs released from HBV-infected hepatocytes could reduce IL-12 expression in macrophages. Interestingly, we found that IL-12 expression of macrophages was reduced by adding EVs into cell culture medium.

To reveal the underlying mechanism, we examined whether EVs delivered those microRNAs into macrophages. RNAs within EVs were stained with SYTO RNA select dye, which is a membrane-permeable dye and emits green fluorescence. EVs stained with the dye were added to cell culture medium of macrophages, and then cells were incubated for 6 hrs. Interestingly, we observed green fluorescence at the cytoplasm of macrophages, suggesting that RNAs within EVs were transferred to the macrophage cytoplasm.

Considering that EVs deliver RNAs into the cytoplasm of macrophages, we reasoned that intracellular microRNA levels would be increased after incubation of macrophages with EVs. As expected, we observed significant increase of intracellular miR-21 and miR-29a levels after incubation of macrophages with EVs. Collectively, these data suggest that EVs deliver miR-21 and

miR-29a from HBV-infected hepatocytes to macrophages, thereby suppressing IL-12 expression in macrophages.

Considering that HBV increased miR-21 and miR-29a levels in EVs, it is expected that viral proteins increased miR-21 and miR-29a levels. To test this hypothesis, viral proteins were over-expressed in hepatocytes, and microRNA levels in EVs were subsequently determined. As expected, we could determine viral proteins that increased miR-21 and miR-29a levels in EVs.

To test the effect of host innate immune response on miR-21 and miR-29a expression in EVs, we investigated whether stimulation with pathogen-associated molecular patterns (PAMPs) increased miR-21 and miR-29a expression in EVs. PolyI:C, CL097, and non-methylated CpG DNA are PAMPs and are recognized by TLR3, TLR7, and TLR9, respectively. We have previously shown that TLR3, TLR7, and TLR9 are involved in the recognition of HBV. We found that TLR3 could increase miR-21 levels. But, TLR7 and TLR9 failed to increase the expression. Although TLR3 increased miR-21 levels in EVs, the viral protein-mediated increase of miR-21 and miR-29a expression in EVs were more significant. Taken together, our data suggest that viral proteins play a major role in the increase of miR-21 and miR-29a expression levels in EVs released from HBV-infected hepatocytes.

Sorting of microRNA into EVs requires several proteins. hnRNPA2B1 is a protein required for sorting of microRNAs with EXO-motif into EVs. To investigate whether hnRNPA2B1 is involved in the sorting of miR-21 and miR-29a into EVs after HBV infection, we performed knockdown assay using siRNA for hnRNP2AB1 and control. siRNA for hnRNPA2B1 and control were transfected into HBV-infected hepatocytes. 4 days after transfection, EVs were collected from cell culture medium, and microRNA levels were subsequently determined. Interestingly, miR-21 and miR-29a levels were decreased by siRNA for hnRNPA2B1. Taken together, our data suggest that viral proteins increased the sorting of miR-21 and miR-29a into EVs via hnRNPA2B1 and that released EVs deliver miR-21 and miR--29a into the cytoplasm of macrophages, thereby attenuating the production of IL-12 required for the activation of NK and Th1 cells.

Discussion & Conclusion

An accumulating body of evidence has shown that EVs deliver functional RNAs, such as microRNA and mRNA, from donor to recipient cells, thereby mediating intercellular communications. However, the role of EVs in viral escape from host innate immune response has not been fully elucidated. In this study, we revealed a novel molecular mechanism of EV-mediated suppression of host innate immune response by viral proteins. Our observations suggest that HBV utilizes EVs to suppress host innate immune response.

HBV persistently infects human hepatocytes. It is still unclear whether EVs plays a major role in the persistent infection. Further studies using patient samples are required to assess the in vivo role of EVs in pathogenesis.

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一般の皆様へ

自然免疫応答は、ウイルス感染初期の生体防御に必須の役割を果たす。しかし、多くのウイルスはこの自然免疫応答を抑制する能力を進化の過程で獲得しているため、ヒトに感染し病気を引き起こす。B型肝炎ウイルスは、ヒトの肝がんの主要な原因の一つである。本研究では、B型肝炎ウイルスが細胞外小胞を利用して自然免疫応答を抑制するメカニズムを解明することで、B型肝炎ウイルスが持続感染するメカニズムの一端を解明した。今後、この過程を阻害するような、新たなB型肝炎治療薬開発につながると期待される。

Treg Related Exosomal microRNA Involving to the Spatial and Chronologic Evolution of CRC

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Summary Abstract

In recent years, the immune response ton cancer has attracted attention. Regardless of the presence of an immune response mechanism, we can't overcome colorectal cancer yet. It is suggested that the local immune response of colorectal cancer is not functioning normally, so the elucidation of the immune response and the reaction of tumor cells in colorectal cancer is an important issue.

We are focusing on regulatory T cell and microRNA, and try to elucidate these problems.

Key Words：microRNA, regulatory T cell, colorectal cancer

Introduction

Regulatory T cell (Treg) cells maintain the balance of immune self-tolerance and homeostasis via limiting aberrant or excessive inflammation[1]. MicroRNAs (miRNAs) have emerged as important regulators in many physiological and pathological processes including development, differentiation, metabolism, immunity, cell proliferation and apoptosis[2]. Previous studies suggest that miRNAs play important roles in the function of Treg cells[3, 4].

Results

We collected 58 samples (11 cases: multi-region, 3cases: single-region) from the primary site of colorectal cancer.

Samples were stored at -4℃ and separated to tumor infiltrating lymphocyte (TIL). TIL included 5.0x10^5 – 3.5x10^7 cells, so we were able to enforce cell sorting analysis. We tried to separate effector Treg and non-Treg from TIL, but it was difficult to determine the cut off line of CD25. So we'll add to fluorescent antibody and determine the cut off line of CD25. As we mentioned above, our study is amid at the experiments.

Discussion & Conclusion

By referring to miRNA group of high expression in effector Treg and non-Treg, we implement an enrichment analysis using online miRNA tool miRAA to identify pathway related to the Treg function. Then, we disclose the prognostic significance of isolated serum miRs preoperatively. Regulatory T cell might play a crucial role in cancer progression via the immune evading system. We expect the revelation of data about epigenetic regulators, such as micro RNA, which should be the most powerful diagnostic and therapeutic tool in the future.

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一般の皆様へ

近年、癌に対する免疫応答が注目されるようになり、悪性黒色腫等では、抗 PD-1抗体であるニボルマブが臨床応用されています。しかし、適応は一部の癌腫に限定されており、大腸癌では臨床試験の段階です。

免疫応答機構の存在に関わらず、大腸癌の克服に至っていないのは、大腸癌の局所での免疫応答が正常に機能していないことが示唆せれ、大腸癌における免疫応答と腫瘍細胞の反応機序の解明は重要な課題です。

そこで、我々は、がん抗原に対する免疫応答を制御する制御性 T 細胞に注目し、研究を進めています。

さらに、免疫機構で、重要な役割を担う遺伝子・タンパク群の中で、情報伝達分子の役割を担う microRNA に注目し、制御性 T 細胞と microRNA の関連性を解明しようと考えています。

Elucidation of the sialyl-Tn antigen-mediated survival mechanism of hypoxic tumor cells

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Summary Abstract

Intratumoral hypoxia induces Sialyl-Tn (sTn) antigen production, which activates integrin signal leading to the enhanced tumor invasion and metastasis. sTn antigen elongates the lifetime of integrin molecules that increased protein abundance of integrins and resulted in the enhancement of the integrin signal. Moreover, the signal activated the NF-kB, which elevated transcription of superoxide dismutase (SOD) 2. This allowed tumor cells to avoid the ischemia-reperfusion stress and consequently apoptosis. These findings indicated that the hypoxia induced sTn antigen is a functional molecule important for the survival of tumor cells in tumor tissues and metastasis.
Key Words：Cancer, hypoxia, glycosylation, Sialyl-Tn antigen

Introduction

In the process of tumor development, extensive tumor cell growth and insufficient blood circulation cause hypoxic conditions, which activate a transcription factor HIF-1. This regulates various gene expressions regarding tumor growth, metastasis, angiogenesis, anti-apoptosis and so on. However, details of molecular mechanism of the hypoxia induced tumor metastasis and survival have not been well understood. Recently, we revealed that a glycan tumor marker molecule, Sialyl-Tn (sTn) antigen, is synthesized under hypoxic conditions and facilitates metastasis and survival. The present study address to reveal the molecular mechanism underlying the sTn antigen-mediated tumor development under hypoxic conditions.

Results

We addressed to reveal the mechanism of the enhanced tumor invasion. Immunoblot analyses revealed that sTn antigen was highly expressed on α2 and β1 integrins, which work as a collagen receptor to stimulate cellular motility. sTn antigen expressing cells exhibited facilitation of collagen-mediated cell adhesion and invasion, which were mediated by activation of FAK (Focal adhesion kinase) downstream of integrin signal. The up-regulation of FAK activity was attributed to the elevation of protein abundance of α2 integrin without transcriptional augmentation in sTn antigen expressing cells. Cell surface protein dynamics analyses revealed that the lifetime of α2 integrin carrying sTn antigen was elongated on the cell surface that resulted in the increase of protein abundance of α2 integrin. Moreover, the hypoxia-induced tumor invasion was suppressed by genetic attenuation of ST6GalNAc-I (enzyme synthesizing sTn antigen) or α2 integrin. These results rationally explain that sTn antigen evokes the facilitation of tumor invasion and metastasis through integrin signal in the presence of collagen. Analyses of gene expression profile of sTn antigen expressing cells revealed that expression level of some antioxidant enzymes, encompassing HO-1 (Heme oxygenase 1) and SOD2 (Superoxide dismutase 2), were increased. Consistent with

this, sTn antigen expressing tumor cells acquired resistance to oxidative stress and avoids the oxidative stress-induced apoptosis. Inhibition of HO-1 by using SnPP-IX or inhibition of SOD2 by genetic attenuation increased intracellular oxidative stress levels in sTn antigen expressing cells at steady state. These results indicate that sTn antigen induces anti-oxidative enzymes, HO-1 and SOD2 and reduces intracellular oxidative stress levels. This may contribute to the survival of tumor cells in tumor microenvironments. Analyses of the mechanism of the sTn antigen-induced expression of HO-1 revealed that sTn antigen activates integrin and the down stream signal, AKT. AKT activation stimulates nuclear translocation of a transcription factor NRF2. The nuclear NRF2 binds to the promoter region of HO-1 gene and activates transcription of HO-1. Indeed, α2 integrin knock down attenuated the activation levels of AKT and the binding of NRF2 to HO-1 promoter. Besides, analyses of the mechanism of the sTn antigen-induced expression of SOD2 revealed that sTn antigen activates NF-κB, which binds to the enhancer region of SOD2 gene and transactivates SOD2 expression in sTn antigen expressing cells. *In vitro* ischemia-reperfusion experiments revealed that the cellular superoxide production by ischemia-reperfusion challenge was significantly reduced and the resulting apoptosis were mostly abolished in sTn antigen expressing cells. Challenge of an ST6GalNAc-I inhibitor compound abolished sTn antigen expression. This resulted in the reduction of SOD2 expression and elevation of superoxide production under ischemia- reperfusion challenge conditions. Indeed, levels of oxidative stress and number of apoptotic cells were significantly reduced in sTn antigen positive regions in tumor tissues. These overall results indicate that the hypoxia-related sTn antigen production induces expression of anti-oxidative enzymes to enable tumor cells to survive in high oxidative stress environments in tumor tissues. This must contributes to increase the rate of the hypoxia-induced tumor metastasis. The ST6GalNAc-I inhibitor effectively attenuates the sTn antigen-induced SOD2 expression and survival of tumor cells in tumor tissues that suggests it can be the lead of the next generation of antitumor drugs.

Discussion & Conclusion

It has been well recognized that the cellular anti-oxidative activity is important for formation of distant tumor metastasis, since tumor cells are exposed to high oxidative stress in blood stream. Consistent with this, metastatic tumor cells acquires intracellular metabolic changes and anti oxidative enzymes to reduce cellular oxidative stress levels. However, the molecular mechanism of these alterations has not been well understood. Our study clearly indicates that intratumoral hypoxia induces sTn antigen production in tumor cells that facilitates autonomous cellular motility, leading to metastasis. Moreover, sTn antigen induces anti-oxidative enzymes, which eliminate reactive oxygen species generated in ischemia-reperfusion cycles in tumor tissues that allows tumor cells survive in tumor microenvironments. These sTn antigen-mediated cellular alterations facilitates tumor survival and metastasis in the process of tumor development. Pharmacological attenuation of the sTn antigen production can be a rational target to suppress tumor metastasis that may contribute to develop the next generation of anti-tumor drugs in the future.

一般の皆様へ

がんが転移する過程においてがん細胞は酸化ストレスに曝されるため死滅します。しかし、この酸化ストレスへの耐性を獲得した一部のがん細胞は生存し転移を果たします。これまで、この酸化ストレスへの耐性獲得のメカニズムは殆ど明らかになっていませんでした。私たちはがん細胞が作り出すシアリル Tn 糖鎖抗原ががんの浸潤能を活性化するとともに抗酸化酵素の産生を誘導することで、がん細胞の生存と転移を直接促進することを明らかにしました。この発見はがん細胞の転移の仕組みを明らかにするのみならず、新しい抗がん剤の開発にとって重要な知見であります。

III.

Reports from the Recipients of Grants for International Meetings

Report on Research Meeting

1. Name of Research Meeting / Conference
   The 6th Symposium on *ATP1A3* in Disease

2. Representative
   Masayuki Sasaki

3. Opening period and Place
   Opening period: September 21, 22. 2017
   Place: The Palace Hotel Tachikawa, Tachikawa city, Tokyo

4. Number of participants / Number of participating countries and areas
   Number of participants: 120 (48 from overseas, 72 from Japan)
   Number of participating countries (14; USA, UK, France, Germany, Netherlands, Denmark, Belgium, Norway, Sweden, Italy, Spain, Australia, India, Japan)

5. Total cost
   ¥7,225,000

6. Main use of subsidy
   Part of Meeting room: ¥301,180
   Cotton Tote bag print: ¥98,820

7. Result and Impression

   The 6th Symposium on *ATP1A3* in Disease was held at the Palace Hotel Tachikawa in Tachikawa, Tokyo. Forty eight participants were welcomed from oversea countries, and 72 participants were from Japan. Participants consisted of medical doctors (neurologists or pediatricians), basic scientists (pharmacology, molecular biochemistry, or molecular genetics), and family members of patients with *ATP1A3*-related neurological disorders.

   Fifteen speakers were invited from Europe, the USA, and Australia. These speakers were the top class specialists for *ATP1A3*-related neurological disorders, basic biochemistry, or molecular genetics. These speakers gave participants the up-to-date information about *ATP1A3*-related neurological disorders, for example, Alternating Hemiplegia of Childhood (AHC), Rapid-onset Dystonia-Parkinsonism (RDP), Cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) or recurrent encephalopathy with cerebellar ataxia (RECA), especially in the fields of clinical diagnosis (semiology and classification) and treatments, and basic science of molecular biochemistry of the gene and pump protein (Na/K transporting pump ATPase).

   Seven speakers were invited from Japan. They gave participants from all over the world the latest topics on the new treatment methods using iPS cells and the mechanism of hereditary Parkinson disease which is related to autophagy.

   In this Symposium, participants discussed how we were able to utilize these advanced technique effectively in the development of the treatment methods for *ATP1A3*-related neurological disorders.

The social gatherings were held in lunch time and dinner time in this Symposium. All participants became acquainted with each other, and some international collaborative studies were started since this Symposium. Most attendants expressed that they were satisfied with the contents of this Symposium. And they gave kind applause to the host members when the Symposium was closed. Most attendants expressed that they would like to participate in the next Symposium which would be held at Chicago, the USA in 2018.

8. Additional description

Report on Research Meeting

1. Name of Research Meeting / Conference
   29th International Symposium on Chirality [Chirality 2017; ISCD-29]

2. Representative
   Chair: Toshikazu Takata (Tokyo Institute of Technology, Japan)
   Co-Chair: Toru Asahi (Waseda University, Japan)
   Honorary Chair: Yoshio Okamoto (Nagoya University, Japan, Harbin Engineering University)

3. Opening period and Place
   July 9–12, 2017, Waseda University International Conference Center, Tokyo, Japan

4. Number of participants / Number of participating countries and areas
   355 person from 24 countries and areas

5. Total cost
   JPY 30,000,000

6. Main use of subsidy
   The subsidy was allocated for a part of the traveling costs and accomodation fee of Invited speakers and Lecturer of Short Couse:

Expense item		JPY
travel expenses & accomodation fee	Prof. Wojciech Dzwolak (University of Warsaw, Poland)	150,000
	Prof. Nina Berova (Columbia University, USA)	130,000
	Prof. Takeo Kawabata (Kyoto University, Japan)	52,020
	Prof. Hiroaki Sasai (Osaka University, Japan)	53,700
Printing costs	Program & Abstract book printing costs (as part of 972,000 yen)	14,280

7. Results and Impression
   International symposium on chirality have been held since 1988. This symposium has been focused on “Exploring the Chirality of molecule or molecular aseembly” and provides a forum for broad scientific and technological exchange among researchers from all over the world. The intention is to emphasize the significance and multidisciplinary character of cutting-edge research on important chirality aspects, and accordingly to put together researchers and students from various disciplines such as chemistry, physics, and life science in academic, industrial, and governmental communities for discussing research findings and promoting research networking among them. At this meeting, 82 lectures including plenary, keynote, invited, and general ones, and 184 excellent poster presentations were programmed. As the plenary speakers, we invited Prof. Hisashi Yamamoto, President of the Chemical Society of Japan, he is a representative organic chemist of Japan and Prof. Andrew J. Gellman, Carnegie Mellon University, he is a pioneer of chiral metal surface science. As the invited speakers, we invited Prof. W. Dzwolok (biophysical scientists), Prof. T. Kawabata (organic chemist), Prof. H. Sasai (organic chemist), and other 31 speakers of various scientific and technological fields. In addition to these

presentations, chirality 2017 provided Short Course lectures as technical seminar for participants for free, and we invited Prof. Nina Berova (analytical scientists) as one of the lecturer of short course by this fundation. On the opening ceremony, winner of the 2017 Chirality Medal, Prof. Takuzo Aida (the university of tokyo) presented the Award Lecture. Despite the hot weather everyday, a lot of participants visited the venue from early morning, and active discussion was seen in each lectures. Social programs to promote the personal contact among the participants were provided for three days during this symposium and we got favorable reviews. On the closing ceremony, 10 awardees of student poster presentation competision were hornored by chairman, subsequently Chiraity 2018 was introduced by the next chairman of the meeting. As the related meetings, domestic meeting of chirality, MC 2018, and asia area meeting, MC Asia 2018, were also preannounced. (333 words)

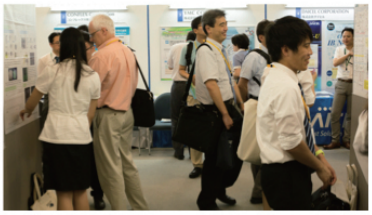
8. Additional description

Opening Ceremony　メインホール Hall A


Short Course 会場


Prof. N. Berova


Prof. H. Sasai


ポスター会場


Welcome Reception（大隈ガーデンハウス）


Prof. T. Kawabata


第２会場 Hall B


Prof. W. Dzwolak

Report on Research Meeting

1. Name of Research Meeting / Conference
   The 15th International Conference on Na,K-ATPase and Related Transport ATPases

2. Representative
   Hiroshi Suzuki, Haruo Ogawa, and Chikashi Toyoshima

3. Opening period and Place September 24th to 30th, 2017
   Lake Biwa Otsu Prince Hotel (Otsu city, Shiga prefecture)

4. Number of participants / Number of participating countries and areas 139 people from 20 countries and areas

5. Total cost 30,060,000 yen

6. Main use of subsidy
   Financial assistance for transportation to students and young post-doc researchers

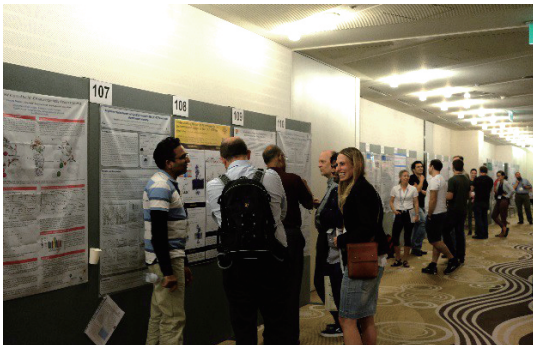
7. Result and Impression
   We are delighted to report that "The 15th International Conference on Na,K-ATPase and Related Transport ATPases", held from September 24th to 30th, 2017 in Otsu, has been a great success. The conference is a historic international meeting, concerning molecules belonging to the P type ATPase family represented by sodium pump. The P-type ATPase family is essential for life and extremely important in medicine. It consists of an ion pump group that actively transports ions such as calcium, sodium, potassium, proton, copper, manganese and the like, and a flippase group that produces asymmetric distribution of membrane phospholipids. All these couple the chemical energy of ATP and the transport. It is an important research topic, as P-type ATPases are deeply implicated in serious diseases such as muscle/myocardial disorder, cancer, diabetes, immune abnormality, neurological abnormality *etc.* This conference has been hold every three years in various countries of the world since 1973, and this is the second time the conference is held in Japan. The last time was at Sapporo in 1999. There are 139 participants from 20 countries, mainly from Europe and the North America. We invited Professor Toshio Ando (Kanazawa University) as a special lecturer, who is the leading expert of developing the high speed AFM in Japan. Professor Poul Nissen (Aarhus university, Denmark) and professor Chikashi Toyoshima (The university of Tokyo) gave keynote lectures on structural biology of P-type ATPases. There are 8 sessions in the conference, and thirty leading scientists in this field gave presentations of their latest findings as invited speakers. Sixteen authors were selected from their abstracts through a peer review by committee members, and they had opportunities to present their works as oral presentations. There are 160 posters, and active discussions were taken place during the conference. We introduced "1 min flash talk" as a new attempt, so that every poster presenters, including young researchers and students, had a chance to give a one-minute oral presentation. This attempt was highly appreciated by many researchers.

8. Additional description

We sincerely appreciate your understanding of our conference and support. Thanks to your support, we could offer 40,000 yen as a travel assistance for ten excellent poster presenters (all of them are students).

会場案内看板

講演会場の様子



ポスター会場の様子

記念撮影

Report on Research Meeting

1. Name of Research Meeting / Conference
   31st Annual Meeting of the Japanese Society for Biological Sciences in Space (JSBSS)

2. Representative
   Akihisa Takahashi, ph.D.
   Gunma University Heavy Ion Medical Center

3. Opening period and Place
   September 20th to 22nd, 2017
   "Gunma Kaikan" (2-1-1 Oote-machi, Maebashi, Gunma, 371-0026, Japan)

4. Number of participants / Number of participating countries and areas
   200 (1-USA / 2-China / 1-Indonesia / 1-Iran)

5. Total cost
   2,500,000 JPY

6. Main use of subsidy
   Venue rental fee

7. Result and Impression
   The year of 2017 is the 60th anniversary of The "Sputnik 1" orbited the Earth, the first major milestone in space exploration. Forty-eight years following the first moonwalk, now it is possible for humankind to spend more than six months in the Outer Space, which accommodated inside of the "International Space Station." Next, we have challenging dreams that we will get back to the Moon, going to the Mars, and will be able to live in the Space permanently with a high quality of life. However, the plans still have so many obstacles which are needed to be anticipated and settled biologically. We believe that by investigating a life from outer Space may lead us to the discovery of unknown biological functions which are hidden in the global environment. Therefore, our "Biological Sciences in Space" is expected to solve these obstacles. This conference aimed to continue to provide a forum for discussion about fundamental and timely issues and trends as well as for developing collaborations through the exchange of research results and ideas in the field of space life sciences.
   In this meeting, we invited Dr. M. Hada (Senior Research Scientist /Preview A & M University) from USA. She is specialist of bio-dosimetry using by chromosome aberration assay for risk evaluation of the space radiation in the Astronaut, and gives a speech to us about "Chromosome aberrations induced by acute and chronic dose rate GCR simulated exposure". In addition, we asked Dr. S. Furukawa (JAXA) to give a special lecture about "The International Space Station (ISS) and space medical biology". As a doctor and astronaut, he has personally experienced extreme stresses during a five-and-a-half month stay in Space in 2011. He suffered, for example, from skeletal muscle atrophy due to zero gravity, a dull headache induced by body fluid shifts, biological rhythm dysfunction triggered by the enclosed environment, space radiation exposure, microbial risks, etc. He sensed the synergistic interactions among these factors and suspected that

they might also be relevant challenges on Earth. We were able to know these valuable experience and seeds of science. We have special session about “lunar farm, space experiment, and manned space program” and 4 symposiums about (1) gravity-response in plants, (2) gravity-response in animals, (3) space radiation effect, and (4) astrobiology. There were total 74 presentations.

We enjoyed all sessions and also discussion. I think we had a fruitful discussion for our future collaborative research.

8. Additional description

Thank you for your assistance. I think the 31st JSNSS meeting succeeded.

Report on Research Meeting

1. Name of Research Meeting / Conference
   International Conference on
   "The Origin of Life –Synergy among the RNA, Protein and Lipid Worlds –"

2. Representative
   Prof. Masayuki Imai (Tohoku University)
   Prof. Tadashi Sugawara (Kanagawa University)

3. Opening period and Place
   May 29 -30, 2017
   The main auditorium, Graduate School of Mathematical Sciences,
   The University of Tokyo

4. Number of participants / Number of participating countries and areas
   172/ 4 countries (India, Japan, UK, USA)

5. Total cost
   Facility Fee: 217,700-
   Abstract: 76,986-
   Part-time job fee: 94,640-
   Invitation Fee: 1,791,987-
   Overseas
   Prof. Szostak 1,007,060-
   Prof. Deamer 421,349-
   Prof. Chen 203,182-
   Prof. Rajamani* 19,700-
   Domestic
   Prof. Ichikawa (Osaka Univ.) 60,840-

   Coffee Break: 79,856-

   Total: 2,181,313-

   *Prof. Rajamani (Indian Institute of Science, Education, and Research) was invited by ELSI (Earth-Life Science Institute, Tokyo Institute of Technology) from 5/22 to 6/9 and ELSI supported her travel expense. We supported her accommodation fee from 5/28 to 5/30.

6. Main use of subsidy
   Invitation Fee: 80,540-
   Prof. Rajamani and Prof. Ichikawa
   Abstract: 76,986-
   Part-time job fee: 94,640-

   Total: 252,166-*

*The difference from the original estimation (400,000-) originated from the invitation fee of Prof.
Rajamani, which was supported by ELSI.

7. Result and Impression

This international conference was organized to encourage communications among synthetic biologists, synthetic chemists and non-equilibrium physicists to reveal the mystery of the origin of life. For this purpose we invited representative researchers at the forefront of this topic. The conference was started by a keynote talk of Prof. Sugawara on a vesicle based model protocell, that is a most advanced synthetic protocell including self-reproduction, self-maintenance, and evolution abilities. In the second keynote talk, Prof. Deamer, who is a pioneer of the astrobiology, showed a most plausible scenario of the origin of life in the prebiotic era, which is synthesis of biopolymer at hydrothermal fields and encapsulation of the biopolymer within membranous compartments. In the third keynote talk Prof. Szostak, who is winner of Nobel Prize in 2009, discussed plausible pathway for the transition from chemical evolution toDarwinian evolution, where the main unsolved problems are the chemistry of nucleotide activation and a means of carrying out multiple sequential rounds of RNA replication. In the final keynote lecture, Prof. Kaneko addressed theoretical approach toward protocells, which includes i) Thermodynamic realizability of an autonomously reproducing protocells, ii) Waste problem, iii) Diversity, iv) Kinetic origin of information, and v) Compartmentalization. From these keynote lectures one can extract the essence of the origin of life based on his/her own background. In the conference participants had a lot of discussions to exchange their research results and polish up their ideas, which suggests activity of this research field. In fact, at first, we estimated that the participants of this conference will be around 50 people, but the actual number of participants was 172 people, which indicates a great success of this international conference. Finally I show impressions of this conference presented by foreign invited speakers;

Prof. Deamer: “Thank you for all the work you did that made this such a successful conference. It was a pleasure to meet you and the other Japanese scientists, and to see how we are using parallel approaches to answer one of the great remaining questions of biology: How can RNA-based life begin on habitable planets? I hope you enjoyed the talks as much as I did.”

Prof. Szostak: “Thanks again for inviting us all to this very nice conference.”

Prof. Chen: “Thank you very much for the invitation to the conference and for your hospitality in Tokyo. It was a wonderful program of talks and I learned a lot during the symposium. It was also very nice to meet both of you after reading much about your work.”

Prof. Rajamani: “I wish to convey my most sincere thanks for having invited me to the wonddddderful conference that you both organized. It was a truly rejuvenating meeting and it indeed is an honour to be a part of the wonderful group of speakers that you both brought together for the conference...:):)”

8. Additional description

31st Grant Report (FY2017)

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 2017 are as follows.

31st Novartis Research Grant: 41Researchers (JPY 1 mil.),	Subtotal JPY 41 mil.
Research Meeting Grant: 5 Meetings (JPY 0.4 mil.),	Subtotal JPY 2.0 mil.
	Total JPY 43.0 mil.

31st Novartis Research Grant (FY2017)

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	Inagaki Takeshi	Institute for Molecular and Cellular Regulation, Gunma University	professor	Epigenetic and Metabolic Changes on transdifferentiation of white adipocytes
2	Takahashi Yasufumi	Kanazawa University	Associate Professor	Development of super resolution chemical imaging microscopy
3	Suzuki Takayuki	Nagoya University	Lecturer	Elucidation of molecular bauplan to determine the positioning of the posterior body structure along the anteroposterior axis
4	Kondoh Kunio	National Institute for Physiological Sciences	Assistant Professor	Neural circuits that regulate glucose metabolism between hypothalamus and peripheral organs.
5	SiewKee Low	Japanese Foundation for Cancer Research	Group Leader	Exploratory analysis to evaluate mutation spectrum of circulating tumor DNA from metastatic breast cancer treated with aromatase inhibitors
6	Matsumoto Yoshinori	Okayama University	Assistant Professor	Investigation of the new signaling pathway in osteoblastogenesis
7	Nomura Seitaro	The University of Tokyo Hospital	Assistant Professor	Heart Cell Molecular Atlas Project to dissect the regulatory mechanisms of circulatory homeostasis
8	Isotani Ayako	Nara Institute of Science and Technology	Associate Professor	Development of the new organ lack model
9	Yoshiba Satoko	The Univarsity of Tokyo, Faculty of Pharmaceutical Sciences	Assistant Professor	Molecular mechanism for the initial assembly of human procentriole
10	Kanai Ryuta	The University of Tokyo	Research Associate	Structural basis for inactivation of Na+,K+-ATPase by β-amyloid
11	Matsumura Shigeyoshi	Graduate School of Science and Engineering, University of Toyama	Assistant professor	Evolutionary development of fluorescent RNA sensors by droplet technology
12	Shimada Midori	Yamaguchi University, Joint Faculty of Veterinary Medicine	Professor	Functional Analysis of Chk1 Targets for Breast Cancer Treatment

#	Name	Institution	Title	Research Project
13	Matsuo Takuya	Nagoya University, Center for Gene Research	Lecturer	Revealing a new red light signaling pathway for resetting the circadian clock in green algae
14	Matsuzaka Takashi	University of Tsukuba	Associate Professor	Elucidation of the role of fatty acid elongase Elovl6 in the pancreatic β-cell
15	Osafune Kenji	Center for Osafune lab, CiRA, Kyoto University	Professor	Development of regenerative therapy and novel therapeutic drugs for renal anemia using human iPS cell-derived erythropoietin-producing cells
16	Nakajima Kazunori	Keio University	Professor	Mechanism that controls the neuronal survival/death in the developing brain
17	Nakao Shu	Ritsumeikan University	Assistant Professor	Direct reprogramming into cardiac pacemaker cells for a novel regenerative medicine for arrhythmias
18	Urabe Daisuke	Toyama Prefectural University	Professor	Structure determination of formosalide A by the combined technology of the NMR calculation and total synthesis
19	Watanabe Kazunori	Okayama University	Assistant Professor	Development of apoptosis induction method with spatiotemporal control using microRNAs.
20	Nemoto Tetsuhiro	Graduate School of Pharmaceutical Sciences, Chiba University	Professor	Study on the enantioselective total synthesis of dragmacidin E: a natural product with high potential for medicinal chemistry researches
21	Kumano Hironori	University of Yamanashi	Research Associate	Understanding the neural mechanism underlying flexible decision making by the combination of neurophysiology and deep neural networks
22	Asai Masashi	Tokyo University of Science, Faculty of Pharmaceutical Sciences	Assistant Professor	Drug development of DYRK1A inhibitors: targeting excess phosphorylation in Alzheimer's disease
23	Murata Takahisa	The University of Tokyo	Associate Professor	Development of combination therapy targeting on tumor endothelial cells
24	Baba Yoshihiro	Medical Institute of Bioregulation, Kyushu University	Professor	Molecular mechanism of store-operated Ca2+ entry signalosome and its physiological significance
25	Suzuki Hiromu	Sapporo Medical University	Professor	Decoding the epigenome program which defines normal and tumor endothelial cells
26	Kondo Shigetada	Osaka Prefecture University	Professor	Elucidation of long non-coding RNA family induced by molecular targeting anti-cancer drugs
27	Shindo Takayuki	Shinshu University Graduate School of Medicine	Professor	Development of anti-metastatic therapy based on the regulation of vascular integrity
28	Akiyama Taishin	RIKEN Center for Integrative Medical Science	Team leader	Mechanisms underlying commitment to thymic epithelial cells preventing onset of autoimmune diseases
29	Kimura Keiji	University of Tsukuba	Associate Professor	Regulation of cellular functions by the nuclelous as a stress sensor
30	Nakagawa Osamu	National Cerebral and Cardiovascular Center Research Institute	Director	Tras-omics approach to elucidate cellular signaling pathways in vascular development, morphogenesis and disease
31	Saitoh Hisato	Kumamoto University	Professor	Studies on formation and function of extracellular DNA fibers
32	Ibi Daisuke	Meijo University	Assistant Professor	5-HT2A receptor agonists in treatment to psychiatric disorders

#	Name	Institution	Title	Research Project
33	Teh OoiKock	Hokkaido Univerity Institute for International Collaboration	Assistant Professor	RopGEF in the regulation of planar cell polarity in Plants
34	Muroi Yoshikage	Obihiro University of Agriculture and Veterinary Medicine	Associated Professor	A novel oxytocin neuronal system for regulating lactating behavior in mice
35	Ohigashi Izumi	Institute of Advanced Medical Science, University of Tokushima	Associate Professor	Molecular mechanism for the bifurcation of thymic epithelial cells
36	Nakatsuka Atsuko	Okayama University Hospital	Assistant Professor	The role of Heat shock protein 40 Member C1 in hepatic lipid metabolism
37	Kawada Kenji	Kyoto University	Lecturer	Novel treatment strategy targeting CCR1-positive myeloid cells in colorectal cancer
38	Takehana Yusuke	Nagahama Institute of Bio-Science and Technology	Associate Professor	Evolution of sex determination systems
39	Tainaka Kazuki	Brain Research Institute, Niigata University	Specially Appointed Professor	Design principle for specific chemical probes toward 3D neuropathology
40	Miyanishi Hiroshi	University of Miyazaki	Assistant Professor	Molecular basis of osmoregulatory ionocytes
41	Hayashi Shinichiro	National Center of Neurology and Psychiatry	Section Chief	Clarification of molecular mechanisms underlying the maintenance of satellite cell stemness and its therapeutic application for Muscular Dystrophy

FY2017 Research Meeting Grant

(JPY 400 thousand x5 = 2.0 million)

#	Meeting	Date (Place)	Institution / Title	Name
1	6th Symposium on ATP1A3 in Disease	2017.9.21-22 (Tokyo)	National Center of Neurology / Director of the Dep. of Child Neurology	Sasaki Masayuki
2	The 29th International Symposium on Chirality, Chirality2017; ISCD-29	2017.7.9-12 (Tokyo)	Tokyo Institute of Technology / Professor	Takata Toshikazu
3	The 15th International Conference on Na,K-ATPase and Related Transport ATPases	2017.9.24-30 (Ohtsu)	IMCB, The Univ. of Tokyo / Associate Professor	Ogawa Haruo
4	31st Annual Meeting of Japanese Society for Biological Sciences in Space (JSBSS31st)	2017.9.20-22 (Gunma)	Gunma University Heavy Ion Medical Center / Professor	Takahashi Akihisa
5	International Symposium on The Origin of Life	2017.5.29-30 (Tokyo)	Tohoku University / Professor	Imai Masayuki

第31期（2017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。2017年度は、下記の総額4,300万円の助成事業を実施しました。

第31回ノバルティス研究奨励金	41件（1件100万円）	4,100万円
研究集会助成	5件（1件 40万円）	200万円
	総額	4,300万円

第31回ノバルティス研究奨励金（2017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	稲垣　毅	群馬大学 生体調節研究所	教授	脂肪細胞の形質制御における代謝エピジェネティクス
2	高橋　康史	金沢大学 ナノ生命科学研究所	准教授	超解像度ケミカルイメージング顕微鏡の創成
3	鈴木　孝幸	名古屋大学大学院 生命農学研究科 附属鳥類バイオ サイエンス研究センター	准教授	体の前後軸形成における下半身の位置を決めるバウプランの分子実体の解明
4	近藤　邦生	生理学研究所	助教	視床下部と末梢臓器を結ぶ、糖代謝を制御する神経回路の解明
5	ロー シューキー	公益財団法人がん研究会 がん研究所	グループリーダー	アロマターゼ阻害薬治療を受けた転移性乳がん患者における血中循環腫瘍ゲノムの解析
6	松本　佳則	岡山大学	助教	骨粗鬆症克服を目指した新たな骨芽細胞シグナルネットワークの解明
7	野村征太郎	東京大学 医学部附属病院	特任助教	心臓細胞アトラスによる循環恒常性制御機構の解明
8	磯谷　綾子	国立大学法人奈良先端 科学技術大学院大学	准教授	新規臓器欠損モデルの開発
9	吉場　聡子	東京大学大学院 薬学系研究科・薬学部 生理化学教室（北川研究室）	助教	中心小体におけるカートホイール構造構築の分子機構の解明
10	金井　隆太	東京大学	助教	βアミロイドによる Na+,K+-ATPase の不活化機構の構造的解明
11	松村　茂祥	富山大学 大学院理工学研究部（理学）	助教	ドロップ・テクノロジーを用いた RNA 蛍光センサーの進化的創出
12	島田　緑	山口大学 共同獣医学部	教授	がんの病態形成に関わる Chk1 標的因子の機能解明と革新的治療法の確立
13	松尾　拓哉	名古屋大学 遺伝子実験施設	講師	緑藻の概日時計をリセットする未知の赤色光受容伝達経路の解明
14	松坂　賢	筑波大学	准教授	膵β細胞における脂肪酸伸長酵素 Elovl6 の役割の解明
15	長船　健二	京都大学 iPS 細胞研究所 長船研究室	教授	ヒト iPS 細胞由来エリスロポエチン産生細胞を用いた腎性貧血に対する再生医療と新規治療薬の開発

No.	氏　名	所　属	職　位	研究課題
16	仲嶋　一範	慶應義塾大学	教授	発生期脳神経細胞の生死を決める機構
17	中尾　　周	立命館大学	助教	リプログラミング技術を応用した心臓ペースメーカー細胞の誘導法の確立
18	占部　大介	公立大学法人 富山県立大学	教授	NMR 計算と全合成を基盤とした稀少ポリケチドホルモサリド A の構造決定
19	渡邉　和則	岡山大学	助教	マイクロ RNA を用いた時空間制御可能なアポトーシス誘導法の開発
20	根本　哲宏	千葉大学大学院薬学研究院	教授	未開拓創薬有用天然物：ドラグマシジン E の不斉全合成研究
21	熊野　弘紀	山梨大学	特任助教	神経生理学と深層学習の組み合わせによる柔軟な判断の神経基盤の解明
22	浅井　　将	東京理科大学薬学部	助教	ダウン症関連遺伝子 DYRK1A を標的とした新規アルツハイマー病治療薬の開発
23	村田　幸久	東京大学	准教授	癌内皮細胞を標的とした抗癌剤感受性増大法の開発
24	馬場　義裕	九州大学 生体防御医学研究所	教授	ストア作動性 Ca2+ 流入シグナルソームの分子機序と生理的意義
25	鈴木　　拓	札幌医科大学	教授	正常と腫瘍血管内皮を規定するエピゲノムプログラムの解読
26	近藤　茂忠	大阪府立大学	教授	がん分子標的薬によって誘導される悪性腫瘍化 RNA ファミリーの解明
27	新藤　隆行	信州大学 大学院医学系研究科	教授	血管の統合性制御に基づく、転移抑制法の開発
28	秋山　泰身	理研 統合生命医科学研究センター	チームリーダー	自己免疫疾患の発症を抑制する胸腺上皮細胞への分化決定機構の同定
29	木村　圭志	筑波大学	准教授	ストレスセンサーとしての核小体を介した細胞機能の制御
30	中川　　修	国立循環器病研究センター 研究所 分子生理部	部長	血管発生・形態形成とヒト血管病におけるシグナル伝達機構：多階層オミックス解析による研究
31	斉藤　寿仁	熊本大学 大学院先端科学研究部	教授	細胞外 DNA の形成機序とその生理作用に関する研究
32	衣斐　大祐	名城大学	助教	5-HT2A 受容体刺激薬の精神疾患治療薬としての有用性
33	鄭　　恵国	北海道大学 国際連携機構	助教	Developmental Biology in Plants
34	室井　喜景	帯広畜産大学	准教授	エネルギー状態に基づき授乳行動を制御する新規オキシトシン神経機構の解明
35	大東いずみ	徳島大学 先端酵素学研究所	准教授	胸腺上皮細胞分岐分子機構の解析
36	中司　敦子	岡山大学病院 腎臓・糖尿病・内分泌内科	助教	肝脂質代謝における Heat shock protein 40 Member C1 の意義
37	河田　健二	京都大学	講師	CCR1 陽性骨髄由来細胞をターゲットにした新たな大腸癌治療
38	竹花　佑介	長浜バイオ大学	准教授	性決定システムの進化機構の解明
39	田井中一貴	新潟大学脳研究所	特任教授	3 次元神経病理学に向けた特異的ケミカルプローブの設計戦略
40	宮西　　弘	宮崎大学	助教	浸透圧調節を担う塩類細胞における分子基盤の構築
41	林　晋一郎	国立研究開発法人 国立精神・神経医療研究センター 神経研究所	室長	筋衛星細胞の未分化性維持機構の解明と筋ジストロフィー治療法への応用

2017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。2017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.	研究集会名	開催日 （開催地）	所属・職位	氏　名
1	第６回 ATP1A3遺伝子関連疾患に関する国際シンポジウム	2017.9.21-22 （東京）	国立精神・神経医療研究センター病院／小児神経診療部長	佐々木征行
2	第29回不斉に関する国際会議	2017.7.9-12 （東京）	東京工業大学／教授	高田十志和
3	第15回ナトリウムポンプと関連イオンポンプに関する国際会議	2017.9.24-30 （大津）	東京大学 分子細胞生物学研究所／准教授	小川　治夫
4	日本宇宙生物科学会第31回大会	2017.9.20-22 （群馬）	群馬大学重粒子線医学研究センター／教授	高橋　昭久
5	国際シンポジウム　「生命の起源」	2017.5.29-30 （東京）	東北大学／教授	今井　正幸

31st Financial Report

Balance Sheet

As of March 31, 2018

(Unit：JP Yen)

Account	Amount
IAssets	
1.Current Assets	
Current Assets Total	19,859,149
2.Fixed Assets	
(1) Basic Fund	
Basic Fund Total	1,100,000,000
(2) Specific Assets	
Specific Assets Total	634,194
(3) Other Long - term Assets	
Other Long - term Assets Total	79,971,399
Fixed Assets Total	1,180,605,593
Assets Total	1,200,464,742
II Liabilities	
1.Current Liabilities	
Current Liabilities Total	52,245,214
Liabilities Total	52,245,214
III Equity(Net Assets)	
1.Designated Net Assets	
Designated Net Assets Total	1,000,634,194
(Amount Appropriating to basic Fund)	1,000,000,000
2.General Net Assets	147,585,334
(Amount Appropriating to)	100,000,000
Equity Total(Net Assets)	1,148,219,528
Liabilities & Equity Total	1,200,464,742

Statement of Net Assets

From April 1, 2017 to March 31, 2018

(Unit：JP Yen)

Account	Amount
I General Net Assets Changes	
1.Ordinary income & Expenditure	
(1) Ordinary income	
Donation	40,108,000
Ordinary Income Total	55,420,100
(2)Ordinaty Expenditure	
Project Expense	53,492,407
Grant Expense	43,000,000
Novartis Research Grant	41,000,000
Research Meeting Grant	2,000,000
Administrative Expense	14,748,656
Ordinary Expenditure Total	68,241,063
Ordinary Balance without Appraisal Profit or Loss	△ 12,820,963
2.Nonrecurring Profit & Loss	
Nonrecurring Balance of Current Period	
General Net Assets Ending Balance	147,585,334
II Designated Net Assets Changes	
Designated Net Assets Change	△ 108,000
Designated Net Assets Ending Balance	1,000,634,194
III Net Assets Balance Ending Balance	1,148,219,528

第31期（2017年度）財務報告

貸借対照表

2018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	19,859,149
2．固定資産	
(1) 基本財産	
基本財産合計	1,100,000,000
(2) 特定資産	
特定資産合計	634,194
(3) その他固定資産	
その他固定資産合計	79,971,399
固定資産合計	1,180,605,593
資産合計	1,200,464,742
Ⅱ負債の部	
1．流動負債	
流動負債合計	52,245,214
負債合計	52,245,214
Ⅲ正味財産の部	
1．指定正味財産	
指定正味財産合計	1,000,634,194
（うち基本財産への充当額）	1,000,000,000
2．一般正味財産	147,585,334
（うち基本財産への充当額）	100,000,000
正味財産合計	1,148,219,528
負債及び正味財産合計	1,200,464,742

正味財産増減計算書

2017年4月1日から2018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	
(1) 経常収益	
受取寄付金	40,108,000
経常収益　計	55,420,100
(2) 経常費用	
事業費	53,492,407
支払助成金	43,000,000
ノバルティス研究奨励金	41,000,000
研究集会助成金	2,000,000
管理費	14,748,656
経常費用　計	68,241,063
当期経常増減額	-12,820,963
2．経常外増減の部	
当期形状外増減額	
一般正味財産期末残高	147,585,334
Ⅱ指定正味財産増減の部	
当期指定正味財産増減額	-108,000
指定正味財産期末残高	1,000,634,194
Ⅲ正味財産期末残高	1,148,219,528

List of Board Members

[Board of Trustees] 5 trustees, 2 auditors As of Oct 1, 2017

Post	Name	Title
Chairman	Akimichi KANEKO	Kio University; Emeritus Professor, Keio University
Trustee	Shigetaka ASANO	Visiting Professor, MD, School of Medicine, Kobe University; Emeritus Professor, University of Tokyo
	Masao ENDOH	Emeritus Professor, MD, Yamagata University
	Kuniaki TAKATA	President, Gunma Prefectural College of Health Sciences President, Gunma Prefectural Public University Corporation
	Masao TORII	President, Novartis Holding Japan K.K.
Auditor	Tokuzo NAKAJIMA	Certified Public Accountant
	Masanori FUSE	Financial Advisor, Finance & Administration Div., Novartis Pharma K.K.

[Board of Councilors] 10 councilors As of Oct 1, 2018

Post	Name	Title
Chairman	Tsuneyoshi KUROIWA	Member of the Japan Academy; Emeritus Professor, University of Tokyo
Councilor	Norio AKAIKE	Head, Kumamoto Kinoh Hospital Clinical Research Center Visiting Professor, Kumamoto University, Graduate School of Medicine and Pharmaceutical Research Emeritus Professor, Kyushu University
	Hiroyuki KAWASHIMA	Former Professor, Graduate School of Medical & Dental Sciences, Niigata University
	Masakatsu SHIBASAKI	Director, Microbial Chemistry Research Center, Microbial Chemistry Research Foundation
	Akihiko NAKANO	Deputy Director, Photonics Engineering Research Center & Team Leader, Live Cell Super-Resolution Imaging Research, RIKEN
	Tadanori MAYUMI	Emeritus Professor, Osaka University
	Miwako MORI	Emeritus Professor, Hokkaido University
	Toyoshi FUJIMOTO	Professor, MD, Graduate School of Med, Nagoya University
	Masamitsu IINO	Specially appointed Professor, MD, Nihon University Emeritus Professor, University of Tokyo
	Tohru HIROSE	Director, Division Head Japan Development, Novartis Pharma K.K.

[Grantee Selection Committee] 20 members As of Oct 1, 2018

Post	Name	Title
Chairman	Masafumi TAKIGUCHI	Professor, MD, Center for AIDS Research, Kumamoto University
Member	Koichiro KUWAHARA	Professor, MD, School of Medicine, Shinshu University
	Masanobu OHSHIMA	Professor, Cancer Research Institute, Kanazawa University
	Yosuke TAKAHAMA	Professor, Institute for Genome Research, Tokushima University
	Eisuke NISHIDA	Director, RIKEN Center for Biosystems Dynamics Research
	Mitsuyasu HASEBE	Professor, National Institute for Basic Biology
	Haruhiko BITO	Professor, MD, Graduate School of Medicine, University of Tokyo
	Jun YAMASHITA	Professor, MD, Center for iPS Cell Research and Application, Kyoto University
	Motoko YANAGITA	Professor, MD, Graduate School of Medicine, Kyoto University
	Masafumi YANO	Professor, MD, School of Medicine, Yamaguchi University
	Hirokazu ARIMOTO	Professor, MD, Graduate School of Life Sciences, Tohoku University
	Hiroyuki TAKEDA	Professor, Graduate School of Science, University of Tokyo
	Eiji HARA	Professor, Research Institute for Microbial Diseases, Osaka University
	Makoto HAYASHI	Team Leader, Plant Symbiosis Reseach Team, RIKEN Center for Sustainable Resource Science
	Hirokazu HIRAI	Professor, MD, Head of Department of Neurophysiology and Neural Repair, Gunma University
	Kouichi FUKASE	Professor, Graduate School of Science, Osaka University
	Michisuke YUZAKI	Professor, School of Medicine, Keio University
	Akihiko YOSHIMURA	Professor, Department of Microbiology and Immunology, Graduate School of Medicine, Keio University
	Hiroshi Ito	Professor, Department of Graduate School of Medicine, Keio University
	Takashi Ohshima	Professor, Drug Discovery and Development Center, Graduate School of Pharmaceutical Sciences affiliated Company-Academia-Government collaboration, Kyushu University
	Yoko Hamazaki	Professor, Graduate School of Medicine, Kyoto University

公益財団法人ノバルティス科学振興財団

役 員 名 簿

2018 年 10 月 1 日現在 (順不同、敬称略)

職　名	氏　名	現　　職	就任年月日	常勤・非常勤
代表理事	金子　章道	慶應義塾大学名誉教授	2018年6月25日	非常勤
理 事	浅野　茂隆	神戸大学大学院医学系研究科客員教授 東京大学名誉教授	2018年6月25日	非常勤
	遠藤　政夫	山形大学名誉教授	2018年6月25日	非常勤
	高田　邦昭	群馬県立県民健康科学大学学長 群馬県公立大学法人理事長	2018年6月25日	非常勤
	鳥居　正男	ノバルティスホールディングジャパン (株) 代表取締役社長	2018年6月25日	非常勤
監 事	中嶋　徳三	中嶋徳三公認会計士事務所 公認会計士	2016年6月10日	非常勤
	布施　正則	ノバルティスファーマ (株) 企画管理本部 ファイナンシャルアドバイザー	2016年6月10日	非常勤

評 議 員 名 簿

2018 年 10 月 1 日現在 (順不同、敬称略)

職　名	氏　名	現　　職	就任年月日	常勤・非常勤
評議員長	黒岩　常祥	日本学士院会員 東京大学名誉教授	2016年6月10日	非常勤
評 議 員	赤池　紀扶	医療法人社団寿量会熊本機能病院 臨床研究センター所長、学術顧問 熊本大学大学院医学薬学研究部客員教授 九州大学名誉教授	2016年6月10日	非常勤
	川島　博行	元新潟大学大学院医歯学総合研究科教授	2016年6月10日	非常勤
	柴崎　正勝	公益財団法人微生物化学研究会 微生物化学研究所長	2016年6月10日	非常勤
	中野　明彦	理化学研究所 光量子工学研究センター 副センター長 生細胞超解像イメージング研究チーム チームリーダー	2016年6月10日	非常勤
	眞弓　忠範	大阪大学名誉教授	2016年6月10日	非常勤
	森　　美和	北海道医療大学客員教授 北海道大学名誉教授	2016年6月10日	非常勤
	藤本　豊士	名古屋大学大学院研究科教授	2016年6月10日	非常勤
	飯野　正光	日本大学医学部特任教授 東京大学名誉教授	2016年6月10日	非常勤
	廣瀬　　徹	ﾉﾊﾞﾙﾃｨｽ ﾌｧｰﾏ (株) 取締役開発本部長	2016年6月10日	非常勤

選 考 委 員 名 簿

2018 年 10 月 1 日現在 (順不同、敬称略)

職　名	氏　名	現　　職	就任年月日	常勤・非常勤
選考委員長	滝口　雅文	熊本大学エイズ学研究センター教授	2015年6月26日	非常勤
選考委員	桑原宏一郎	信州大学医学部教授	2015年6月26日	非常勤
	大島　正伸	金沢大学がん進展制御研究所教授	2015年6月26日	非常勤
	西田　栄介	理化学研究所生命機能科学研究センター センター長	2015年6月26日	非常勤
	長谷部光泰	自然科学研究機構基礎生物学研究所教授	2015年6月26日	非常勤
	尾藤　晴彦	東京大学大学院医学系研究科教授	2015年6月26日	非常勤
	山下　　潤	京都大学 iPS 細胞研究所教授	2016年6月10日	非常勤
	柳田　素子	京都大学大学院医学研究科教授	2016年6月10日	非常勤
	矢野　雅文	山口大学医学部医学科教授	2016年6月10日	非常勤
	有本　博一	東北大学生命科学研究所教授	2016年6月10日	非常勤
	武田　洋幸	東京大学大学院理学系研究科教授	2017年6月19日	非常勤
	原　　英二	大阪大学微生物病研究所教授	2017年6月19日	非常勤
	林　　誠	理化学研究所環境資源科学センター チームリーダー	2017年6月19日	非常勤
	平井　宏和	群馬大学大学院医科学系研究科教授	2017年6月19日	非常勤
	深瀬　浩一	大阪大学大学院理学研究科教授	2017年6月19日	非常勤
	柚﨑　通介	慶應義塾大学医学部教授	2017年6月19日	非常勤
	吉村　昭彦	慶應義塾大学大学院医学研究科教授	2017年6月19日	非常勤
	伊藤　　裕	慶應義塾大学大学院医学部教授	2018年6月25日	非常勤
	大嶋　孝志	九州大学大学院薬学研究院附属産学官連携創薬育薬センター教授	2018年6月25日	非常勤
	濵﨑　洋子	京都大学大学院医学研究科教授	2018年6月25日	非常勤

事 務 局 便 り

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

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事務局より

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2018年3月に財団設立30周年記念式典を開催し、また8月には30周年記念誌も刊行できました。前事務局長の田中基晴氏の貢献に感謝します。

事務局は、今後とも財団の設立目的である学術の進展に寄与するべく、研究助成を中心とした公益事業に邁進して参ります。

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。

2018年10月吉日
事務局長　岩見　文明

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