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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 FY2015. 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 29 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.

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2015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29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。

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

LY6E; a Conductor of malignant tumor growth through modulation of the PTEN/PI3K/Akt/HIF-1 axis

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Summary Abstract

Lymphocyte antigen 6 complex, locus E (LY6E) has been implicated in the malignant progression of various types of cancers; however, the underlying mechanism remains unclear. Here, we identified LY6E as an activator of HIF-1 and revealed their mechanistic and functional links in malignant tumor growth[(1)]. The aberrant overexpression of LY6E increased HIF-1α gene expression principally at the transcription level. This, in turn, led to the expression of the pro-angiogenic factors, VEGFA and PDGFB, through decreases in the expression levels of PTEN mRNA and subsequent activation of the PI3K/Akt pathway. The LY6E-HIF-1 axis functioned to increase tumor blood vessel density and promoted tumor growth in immunodeficient mice. LY6E expression levels were significantly higher in human breast cancers than in normal breast tissues, and were strongly associated with the poor prognoses of various cancer patients. Our results characterized LY6E as a novel conductor of tumor growth through its modulation of the PTEN/PI3K/Akt/HIF-1 axis and demonstrated the validity of targeting this pathway for cancer therapy.

Key Words : tumor hypoxia; hypoxia-inducible factor 1 (HIF-1); Lymphocyte antigen 6 complex, locus E (LY6E); PTEN/PI3K/Akt/HIF-1 Axis; angiogenesis

Introduction

Accumulating evidence has shown that HIF-1 plays critical roles in radioresistance of cancers and tumor recurrence post-radiotherapy[(2)] and eventually causes death of cancer patients. Clinical studies have demonstrated consistent data that HIF-1 could be used as an adverse prognostic factor for not only local tumor recurrence but also distant tumor metastasis in cancer patients. These findings justify targeting HIF-1 for cancer therapies[(2)].

HIF-1, a transcription factor composed of an α-subunit (HIF-1α) and a β-subunit (HIF-1α), its activity is known to depend on the expression levels of HIF-1α protein[(2)]. Under normoxic conditions, HIF-1α protein is actively degraded through the hydroxylation- and subsequent ubiquitination-mediated proteolysis reactions. On the contrary, HIF-1α becomes active under hypoxic conditions because of the inactivation of the hydroxylases, and then, interacts with its binding partner, HIF-1β. Resultant heterodimer, HIF-1, binds to its cognate enhancer sequence, the hypoxia-responsive element (HRE), and induces transcriptions of various genes related to the escape from hypoxia (invasion and metastasis of cancer cells) as well as the improvement of oxy-gen-availability (angiogenesis) and adaptation of cellular metabolism to hypoxia (metabolic reprogramming)[(2)].

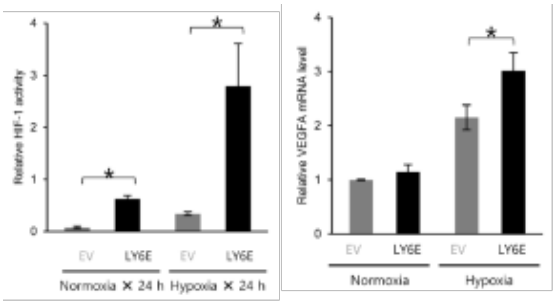
Results

In order to explore novel genes which are responsible for the HIF-1-mediated tumor malignant progression, we recently established a new genetic screening method[(1,3,4)] and found that

overexpression of lymphocyte antigen 6 complex, locus E (LY6E) functions in the activation of HIF-1[(1)]. In the present study, we analyzed both the molecular mechanisms underlying the LY6E-mediated activation of HIF-1 and the involvement of the LY6E-HIF-1 axis in malignant progression of cancers.

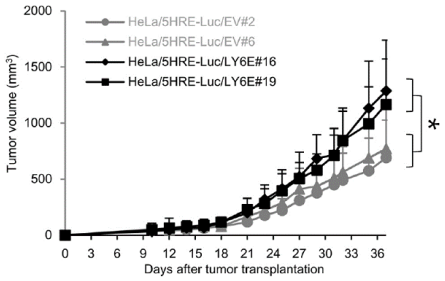
Forced expression of LY6E using a plasmid-based expression vector for LY6E increased HIF-1α gene expression principally at the transcription level (Figure 1).

Fig. 1



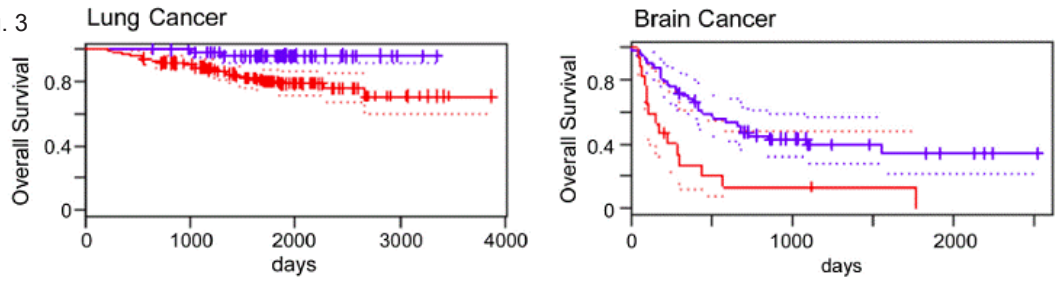
This, in turn, led to the expression of the pro-angiogenic factors, VEGFA and PDGFB, through decreases in the expression levels of PTEN mRNA and subsequent activation of the PI3K/Akt pathway. The LY6E-HIF-1 axis functioned to increase tumor blood vessel density and promoted tumor growth in immune-deficient mice (Figure 2).

Fig. 2



LY6E expression levels were significantly higher in human breast cancers than in normal breast tissues, and were strongly associated with the poor prognoses of various cancer patients (Figure 3).

Fig. 3



Discussion & Conclusion

Our results characterized LY6E as a novel conductor of tumor growth and tumor radioresistance through its modulation of the PTEN/PI3K/Akt/HIF-1 axis and demonstrated the validity of targeting this pathway for cancer therapy.

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一般の皆様へ

がんには個性があり、治療が効きやすいタイプや効きにくいタイプ、転移をし易いタイプやしにくいタイプなど、患者さんによって患っているがんのタイプは様々です。私達は、がんの悪性度と治療抵抗性を左右する原因遺伝子の一つとして、LY6E を発見しました。そして LY6E 蛋白質を豊富に含むがんは増殖が極めて速く、治療がなかなか効きにくいこと、そして結果的に患者さんの生存期間が短くなってしまうことを確認しました。この研究結果を基に、LY6E を標的とする新たな治療法の開発に向け、研究を続けています。

Control of host immune responses by virulent cell-wall lipids of *Mycobacterium tuberculosis* through innate lipid pattern-recognition receptors.

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Summary Abstract

Mycolic acid (MA) and Phenolic glycolipid (PGL) are lipids identified as virulent lipids of *Mycobacterium tuberculosis* that suppress host immunity. We identified ITAM-coupled innate immune receptors, MAR and PGLR, that recognize MA and PGL, respectively. Macrophage response to MAR or PGLR was markedly impaired in MAR or PGLR-deficient cells, respectively. In addition, MAR-deficient cells as well as mice showed enhanced Mincle-mediated innate immune response essential for host protection to *M. tuberculosis*. Thus, MAR acts as a negative regulator of Mincle and may contribute to immune evasion of *M. tuberculosis* through the recognition of MA.
Key Words：Tuberculosis, innate immunity

Introduction

M. tuberculosis contains various unique lipids in the cell walls that act on the activation or suppression of immunity. Although structure and function of such lipids have been extensively studied, the host receptor for them was not well understood. Mycolic acid (MA) and Phenolic glycolipid (PGL) are lipids identified as virulent factors of *M. tuberculosis* that suppress host immunity. We have identified novel ITAM-coupled receptors expressed on innate immune cells, MAR and PGLR, that recognize MA and PGL, respectively. In this study, we aim to clarify the role of these receptors in the pathogenesis of tuberculosis.

Results

We found that macrophage stimulation with the glycosylated MAs, TDM and GMM, produced the macrophage chemoattractant MCP-1 as well as the inflammatory cytokine TNF and inducible nitric oxide synthase (iNOS), both of which are known to be essential for tuberculosis control in macrophages, while macrophage stimulation with the non-glycosylated MAs, free MA and GroMM, induced MCP-1 but only few or no production of TNF and iNOS. The non-glycosylated MA-induced chemokine production required MAR but not Mincle, while the responses to the glycosylated MAs required Mincle but not MAR. Interestingly, MAR could bind to the glycosylated MAs albeit it is weaker than Mincle, but its binding appeared to inhibit Mincle-mediated response as MAR deficiency enhanced TDM-induced MCP-1, TNF and iNOS productions. Consistent with these results, MAR-deficient mice showed accelerated elimination of *Mycobacterium bovis* BCG *in vivo*.

Similar to MA, we found that PGL induced MCP-1 but not TNF and iNOS by macrophages *in vitro*, and the MCP-1 production is completely dependent on PGLR.

Together, these results suggest that the mycobacterium virulent lipids, non-glycosylated MAs and PGL, may show its immnosuppressive activity through their host receptors, MAR and PGL, respectively.

Discussion & Conclusion

Therapy with anti-TNF antibodies for rheumatoid arthritis and inflammatory bowel disease promotes relapse of tuberculosis, which suggests that suppression of host TNF production is effective means of avoiding immunity and reactivation. We identified MAR as a pattern-recognition receptor for non-glycosylated MAs, known as virulent lipids with immunosuppressive activity. Interestingly, MAR is DAP12-associated receptor that induce Mφ chemotactic factor MCP-1 upon stimulation, while not inducing TNF and NO production. Given enhanced TNF production in response to mycobacterium lipids in MAR-deficient Mφ, a therapeutic strategy can be expected to enhance the anti-tuberculosis immune response by inhibiting the binding of the lipid ligand to MAR or its signaling, thereby promoting elimination and dormancy of *M. tuberculosis*. On the contrary, by stimulating this inhibition pathway, development of anti-inflammatory/immunosuppressive drugs with a new concept can also be expected.

一般の皆様へ

結核は世界人口の1/3が罹患する世界最大の細菌感染症であり、年間200万人を超える人が命を失い続けています。しかし、結核を薬で完治させることは難しく、効果的なワクチンも未だに存在しません。結核を克服するためには、宿主免疫による結核菌の認識と応答の仕組みと、結核菌が免疫を回避する仕組みの両方を分子レベルで理解する必要があります。我々の研究によって結核菌の病原性脂質による免疫抑制の分子機構が明らかになれば、新しい概念での結核の予防薬や治療薬の開発に繋がるものと期待しています。

Research of glycosylation that dynamically regulates innate immunity

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Summary Abstract

Innate immunity is the first line of defense to combat invaded pathogens. In this study, we found that glucosaminoglycan (GAG) regulates immune activity. GAG suppresses hyper-activation of the Toll pathway in the steady state. Interestingly, pathogen infection reduces the expression of a GAG synthesizing enzyme to enhance immune responses. These results indicate that GAG plays an important role in both suppression of runaway inflammation and enhancement of immune responses.
Key Words：Glycosylation, Glucosaminoglycan, Innate immunity

Introduction

Innate immunity is the first line of defense to combat invaded pathogens. Delayed and/or inadequate innate immune responses can result in failure to combat pathogens, whereas excessive and/or inappropriate responses cause runaway inflammation. Therefore, immune responses are tightly regulated from initiation to resolution and are repressed during the steady state.

Glycosylation is well known to control activities of target proteins. Innate immunity is also regulated by glycosylation (1). However, only a few glycans have been reported to be involved in innate immunity so far. Glycosylation dependent regulation of innate immunity remains largely unrevealed.

Results

First, we screened the *Drosophila* RNAi library to identify what glycosylenzymes are involved in innate immune responses and found that the immune responses are hyper-activated in mutants of *tout-velu* (*ttv*) and *brother of tout-velu* (*botv*) genes required for synthesis of glucosaminoglycan (GAG). GAG is a long sugar chain with a tetra-saccharides at the reducing end and disaccharide repeats extended to the non-reducing end. Since Ttv and Botv are enzymes to generate disaccharide repeats, most part of GAG are deleted in *ttv* and/or *botv* mutants. Thus, our results suggest that GAG suppresses activation of innate immune responses in the steady state.

We next investigated what tissue requires GAG to regulate innate immune responses. To approach this question, we knocked down *ttv* gene in several immune tissues including the lymph glands, lymphoid cells and fat bodies. The lymph glands contain most immune precursor cells and a part of mature lymphoid cells. The mature lymphoid cells are released into body fluid. The lymphoid cells play various roles in immune responses such as release of humoral immune factors and anti-microbial peptides, phagocytosis and encapsulation. When invaded pathogens are recognized by humoral immune factors, humoral signals activate expression of anti-microbial peptides in the fat bodies. Our result showed that immune responses in the fat bodies were hyper-activated when *ttv* was knocked down in the lymph glands. This result raised two possibilities. One is that a large number of immune precursor cells may differentiate into mature cells in *ttv* knockdown, causing

hyper-activation of innate immune responses. The other is that the lymph glands in *ttv* knockdown may send excess signal to fat bodies. To distinguish these two possibilities, we examined whether precursor cells differentiate into mature cells in the lymph glands in which *ttv* was specifically knocked down. Mature cells were hardly found in the lymph glands. This result precludes the former possibility. However, the possible signal from the lymph glands to the fat bodies has not been identified. This indicates that there is a new signal sent from the lymph glands to the fat bodies.

Taken together, GAG in the lymph glands suppresses activation of immune responses in the fat bodies during the steady state. This raises a next question. When pathogens invade into bodies, GAG still continues suppression of immune responses or not. We measured transcriptional change of seven genes encoding GAG synthesizing enzymes with pathogen invasion. Among the genes, mRNA level of *botv* by pathogen challenge was reduced to ~60% compared to the steady state. To examine whether 60% reduction is significant level for immune activation, we measured immune response level in heteroallelic mutant of *botv* (*botv*+/-) in which *botv* mRNA is expected to be reduced to ~50%. In the mutant, immune response level was significantly enhanced, suggesting that 60% reduction of *botv* mRNA is enough to activate immune responses. These results suggest an interesting regulatory machinery that GAG suppresses hyper-activation of immune responses in the steady state and once pathogens invade into bodies, expression of Botv, the GAG synthesizing enzyme is reduced to enhance immune responses.

Discussion & Conclusion

Immune responses are tightly regulated from initiation to resolution and are repressed during the steady state. The current study demonstrates that GAG in the lymph glands suppresses activation of immune responses during the steady state and once pathogens invade into bodies, expression of the GAG synthesizing enzyme is reduced to enhance immune responses for prompt elimination of pathogens. We have previously reported that galactose-containing glycans suppresses hyper-activation of innate immune responses in the steady state as well (1). Taken together with the report, the current study strongly suggests that more than one glycosylation machineries play an important role in regulation of innate immunity.

References

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一般の皆様へ

免疫は私達の身体を守るのに重要な役割を果たしています。その免疫は、感染していないときには働かないように抑えられていなければなりません。一方、いったん病原体などが感染したときには免疫は十分に活性化されなければなりません。本研究では、その免疫の動的な制御に糖鎖が重要な役割を果たしていることを見出しました。非感染時には糖鎖が免疫の活性化を抑え、感染時には糖鎖が減少することで免疫を十分に活性化させていました。将来的には、糖鎖を使った免疫の活性化を制御する技術が期待されます。

Development of therapy for multiple sclerosis by regulation of neural circuit formation factor LOTUS expression

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Summary Abstract

Multiple sclerosis (MS) is recognized as an autoimmune demyelinating disorder of the central nervous system with axonal degeneration induced by Nogo receptor-1 (NgR1)-mediated signaling. We identified lateral olfactory tract usher substance (LOTUS) as an endogenous antagonist of NgR1. We therefore examined the relationship between LOTUS and experimental autoimmune encephalomyelitis (EAE), an animal model for MS. Disease severity in EAE was improved in LOTUS-deficient mice, whereas that was increased in LOTUS-overexpressing mice. Furthermore, the binding of LOTUS to the CD4-positive T-cells from EAE mice increased IL-17 and IFN-γ secretions with cell proliferation of T-cell. These findings suggest that LOTUS may be involved in EAE induction through inflammation response.
Key Words：LOTUS, Multiple sclerosis, EAE, Therapy, Inflammation

Introduction

Multiple sclerosis (MS) is generally recognized as an autoimmune demyelinating disorder of the central nervous system (CNS), and it has been recently considered that axonal degeneration induced by Nogo receptor-1 (NgR1)-mediated signaling is an important pathological feature in the first and the secondary progressive phases of MS (Petratos et al., 2012). We previously identified lateral olfactory tract usher substance (LOTUS) as an endogenous antagonist of NgR1 (Sato et al., 2011; Kurihara et al., 2014). We also found that the LOTUS concentration in cerebrospinal fluid (CSF) was significantly decreased in patients with MS when compared with normal controls (Takahashi et al., 2015). Therefore, we examined the relationship between LOTUS expression levels in the CNS and disease activities in experimental autoimmune encephalomyelitis (EAE), an animal model for MS.

Results

Since variations in LOTUS levels in CSF were well correlated with disease activity in MS, it could be considered that low LOTUS concentrations in the CNS may be possibly involved in enhancement of NgR1 signaling, thereby loss of LOTUS may induce axonal degeneration in MS. Therefore, we predicted pathological condition of MS may grow seriously in accordance with decrease of LOTUS expression level. To address this issue, we analyzed disease activity of EAE in LOTUS-knocking out (LOTUS-KO) and LOTUS-overexpressing transgenic (LOTUS-Tg) mice. In contrast to our expectation, disease severity in onset of EAE was improved in LOTUS-KO mice, whereas that was slightly increased in LOTUS-Tg mice when compared with wild type mice. These surprising findings suggest that LOTUS may be involved in EAE induction with inflammatory response.

We next examined whether LOTUS binds to lymphocytes and induces cytokines secretion related to inflammation response. We found that LOTUS bound to lymphocytes from EAE mice and the

LOTUS binding to cultured lymphocytes from EAE mice significantly increased IL-17 and IFN-γ secretions, although the LOTUS binding did not change in the level of IL-2 secretion. Furthermore, MTT assay revealed that IL-17 secretion induced by LOTUS binding increased cell proliferation of lymphocyte. These findings suggest that binding of LOTUS to lymphocyte may be involved in inflammatory response in acute phase of EAE. We also confirmed these findings in CD4-positive T-cells from EAE mice. Namely, LOTUS bound CD4-positve T-cells and the binding of LOTUS to the T-cells increased IL-17 and IFN-γ secretions with cell proliferation.

Next, We examined whether LOTUS binds to lymphocyte from NgR1-knocking out (NgR1-KO) mice, since LOTUS binds to NgR1 and NgR1 is also expressed in lymphocytes. LOTUS bound to lymphocytes from EAE-induced NgR1-KO mice, indicating that LOTUS may interact with unknown binding partner in lymphocytes except for NgR1. This is also confirmed in CD4-positive T-cells from NgR1-KO mice. Thus, binding of LOTUS to unidentified molecule expressed in lymphocyte and CD4-positive T-cells may be involved in the induction of EAE and the association of LOTUS with the unidentified molecule may be a possible therapeutic target in acute phase of MS.

Discussion & Conclusion

It was found that the LOTUS concentration in CSF was significantly decreased in patients with MS (Takahashi et al., 2015). The reason why LOTUS expression is decreased in accordance with the disease activity of MS remains completely unknown. LOTUS expressed in the CNS may decrease in compensation for the damage LOTUS has given. As a step in elucidating molecular mechanism of the regulation of LOTUS expression, we are making an attempt to search for some factors that induce decrease of LOTUS expression. It is also necessary to examine whether EAE is induced by transplantation of lymphocytes sensitized to LOTUS in wild type mice in order to validate the EAE induction by LOTUS. In addition, identification of the binding partner of LOTUS in lymphocyte is required for development of future therapy for MS, because the association of LOTUS with the unidentified molecule may be a possible therapeutic target in acute phase of MS. In conclusion, binding of LOTUS protein in blood to lymphocytes may induce EAE induction and the association of LOTUS with unidentified molecule on lymphocytes may be a novel therapeutic target in MS.

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一般の皆様へ

国の難病に指定されている多発性硬化症に見られる神経変性は Nogo 受容体という分子が関与していると報告されているため、私達が発見した Nogo 受容体の働きを阻止する LOTUS という分子とこの病気との関連について病態モデルマウスを用いて調べたところ、予想に反して、LOTUS は血中のリンパ球と結合して炎症を引き起こすサイトカインの一種をリンパ球から分泌させて炎症を引き起こす可能性が示されました。即ち、LOTUS はこの病気の発症に関連することが示唆されました。LOTUS が引き起こす炎症を止めることでこの病気の発症や進行を抑制することができるのではないかと考え、その方策を明らかにするための研究を行っています。

Molecular mechanism of epigenetic dysregulation in myelofibrosis-initiating cells

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Summary Abstract

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic malignancies that originate from HSCs and are characterized by the excess production of mature myeloid cells and active extramedullary hematopoiesis. *HMGA2*, one of the polycomb target genes, has been implicated in the pathogenesis of MPNs including myelofibrosis. In this study, in order to understand the oncogenic function of Hmga2, we generated a novel conditional Hmga2 knock-in mouse.
Key Words : Myeloproliferative neoplasms (MPNs), Myelofibrosis (MF), Hematopoietic stem cells (HSCs), HMGA2, Epigenetics

Introduction

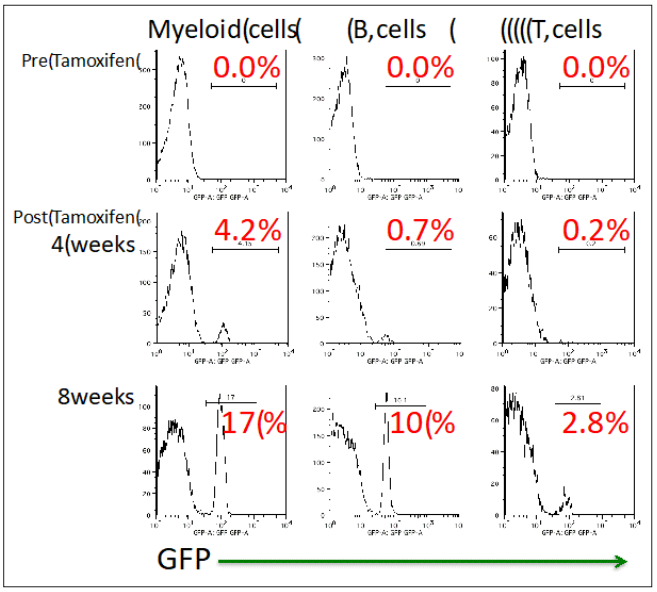
MPNs are clonal hematopoietic malignancies that originate from HSCs and are characterized by the excess production of mature myeloid cells and active extramedullary hematopoiesis. The identification of a somatic activating mutation in JAK2 ($JAK2^{V617F}$) in patients with MPN, including those with polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF), underlined the importance of the constitutive activation of the JAK-STAT signaling pathway in MPN pathogenesis (Levine at al. 2007). Recent genome-wide sequencing studies revealed frequent co-occurring mutations in epigenetic regulators such as *TET2*, *ASXL1*, and *EZH2* (Shih et al. 2012). Loss-of-function mutations in *EZH2* have been identified in approximately 10% of patients with PMF (Guglielmelli et al. 2011). We previously reported that *Ezh2*-deficient mice showed MPN-like phenotypes, such as increased platelet counts with EMH and myeloid-biased hematopoiesis at the expense of lymphopoiesis (Mochizuki-Kashio et al. 2011; Muto et al. 2013). We then attempted to determine how the loss of Ezh2 impacted on oncogene expression associated with the propagation of MF, and found that Hmga2, a potent oncogene, was higly rexpressed in MF stem/progenitor cells, relavant to enhanced expression of *HMGA2* in CD34+ stem/progenitor cells of patients with PMF. Here, we exmeined how Hmga2 enhances the funciton of MF-initiating cells and leads to promoting the development of MF via generating a novel conditional Hmga2 knock-in mice.

Results

We observed that the expression of *Hmga2* was significantly elevated upon the loss of Ezh2 in LSKs and MEPs regardless of the presence of $JAK2^{V617F}$, and its activation was more evident in MEPs, in which *Hmag2* physiologically undergoes transcriptional repression (data not shown). As the transduction of Hmga2 in wild-type HSCs has been shown to promote megakaryopoiesis (Oguro et al. 2012), we examined the effects of the ectopic expression of *Hmga2* in combination with $JAK2^{V617F}$ on the production of megakaryocytes. Indeed, the transduction of *Hmga2* in WT HSCs promoted the cell growth and increased the frequencies of megakaryocytes in culture in the presence

of SCF and TPO.

In contrast, overexpression of *Hmga2* in JAK2^{V617F} HSCs, which showed limited proliferation in culture, did not enhance cell growth. Instead, it markedly promoted the megakaryocytic differentiation of *JAK2*^{V617F} HSCs, and induced dysplastic megakaryocytes characterized by loose lobulation and various sizes in vitro (data not shown). These findings implicate the derepressed *Hmga2* in the expansion of abnormal megakaryocytes in aggressive MF mice. This result also appeared to be relevant to the activation of *HMGA2* in CD34^{+} cells in patients with PMF (Guglielmelli et al. 2011).



Given that Hmga2 appeared to promote the development of MF in vivo, we successfully generated a novel conditional Hmga2 knock-in mouse. To do this, we utilized a vector of Rosa26 loxP-STOP-loxP HA-tag-Hmga2 IRES-GFP to inject it into mouse ES cells and selected properly knocked-in clone mice. To understand how Hmga2 promotes the function of normal as well as cancer stem cells, we crossed this mouse with a transgenic mouse, which has a hematopoietic stem cells-specific Cre-ERT (in a collaboration with Prof Motomi Osato at NUS Singapore)(See a figure in the first page). We saw successful transduction of Hmga2 preferentially in myeloid cells, but not T-cells, which suggested the HSCs-specific induction of Hmga2 in vivo. We are now studying the molecular mechanisms of Hmga2 in the pathogenesis of MF.

Discussion & Conclusion

Hmga2, one of the polycomb-repressive complex 2 target genes de-repressed in the absence of Ezh2, markedly enhanced the megakaryocytic differentiation of HSCs in concert with JAK2^{V617F} in cultures. This result is consistent with previous findings in which the overexpression of *Hmga2* induced MPN in mice lacking *Hmga2* 3'UTR or *Bmi1/Cdkn2a* double knockout mice (Oguro et al 2012; Ikeda et al 2011), thereby strongly supporting the importance of *HMGA2* in the pathogenesis of PMF in patients harboring *EZH2* mutations. However, it remains unknown how Hmga2 promotes the initiation of MPN-stem cells, because of the lacking of mouse model, which can induce the expression of Hmga2 in a HSCs-specific manner. Once, we successfully got the Hmga2 knock-in mice, we will be able to understand how Hmga2 regulate the expression of target genes in stem cells via modulating chromatin formation.

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一般の皆様へ

原発性骨髄線維症とは骨髄の中で血小板を作る巨核球と顆粒球系細胞が腫瘍性に増殖して骨髄の線維化と造血障害に加えて、脾臓などで異所性造血を生じる予後不良ながんである。病気の原因として、遺伝子変異があり、約半数の患者でJAK2キナーゼの活性化変異があり、その他に共存する遺伝子変異としてエピゲノム制御因子であるEZH2やTET2が知られ、悪性度との関連が言われている。骨髄線維症マウスの造血幹細胞を採取して、患者の腫瘍細胞でも発現上昇が知られるHmga2を骨髄線維症の病態進展に責任のあるがん遺伝子として同定した。

今後、新規に作製したHmga2発現を造血幹細胞で特異的に誘導できるマウスを用いて、詳細な骨髄線維症の発症メカニズムを解明するとともに、標的遺伝子の検索を通した新規治療戦略の進展が期待できる。

Leukemogenesis caused by a defect of the ribosomal RNA processing

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Summary Abstract

In this study, we screened for the *DDX41* mutation of CD34-positive tumor cells and identified the p.R525H mutation in three cases among 23 AML/MDS patients. These patients commonly exhibited AML with peripheral blood cytopenias and low blast counts, suggesting that the mutation inhibits growth and differentiation of hematopoietic cells. Data from cord blood cells and leukemia cell lines suggest a role for DDX41 in the pre-ribosomal RNA processing, in which the expression of the mutant causes a certain ribosomopathy phenotype by suppressing MDM2-mediated RB degradation, thus triggering the inhibition of E2F activity. This study uncovered a pathogenic role of p.R525H DDX41 in the slow growth rate of tumor cells.
Key Words：DDX41, pre-rRNA processing, Acute Myeloid Leukemia

Introduction

Current comprehensive sequencing approaches led to the identification of rare but reproducible somatic gene mutations in myeloid malignancies. Among them, there is a somatic mutation in the *DDX41* gene encoding a DEAD-box type ATP-dependent RNA helicase. However, thus far, the detailed physiological function of DDX41, as well as the perturbation of the function by the mutant DDX41, has not been clarified. In this study, we propose a role for DDX41 as a pre-rRNA processing factor, in which the p.R525H mutation affects ribosome biogenesis. We found that ribosome biogenesis was widely affected when cells were transfected with DDX41 p.R525H, thus compromising cell cycle progression through impaired E2F function.

Results

(1) Identification of DDX41 p.R525H mutation in three AML patients.

We identified heterozygous somatic *DDX41* c.G1574A (p.R525H) mutation in three cases among 23 patients, which was confirmed by sanger sequencing. Intriguingly, these patients exhibited AML with peripheral and bone marrow cytopenias and low blast counts. According to our transcriptome data on patients' CD34-positive cells, almost half of the sequenced tags at c.G1574 were considered mutated in each patient. Therefore, almost all CD34-positive cells in these patients may harbor heterozygous *DDX41* p.R525H mutation. On the other hand, CD34-negative cells carrying the mutation were fewer compared with CD34-positive cells, suggesting a block in the differentiation of CD34-positive *DDX41* mutant cells into negative cells.

(2) Nuclear localization of DDX41 protein

We next clarified the localization of DDX41 protein. Ectopically expressed as well as endogenous DDX41 was mostly nuclear regardless of the p.R525H mutation and hardly co-localized with FLAG-STING, a protein shown to localize in ER. While previous reports described DDX41 as a cytosolic

DNA sensor that recognizes nucleic acids of pathogens, our study did not suggest this function based on the protein localization.

(3) Growth inhibition of p.R525H DDX41 expressing cells due to the impaired pre-rRNA processing

To investigate the molecular functions of DDX41 in hematopoietic cells, cord blood-derived human CD34-positive cells were transduced with wild-type (WT) or p.R525H DDX41. After a 30-day culture, p.R525H cells showed decreased proliferation compared with WT cells, accompanied by the suppression of mRNAs encoding ribosomal proteins. A GSEA analysis showed that gene expression pattern of p.R525H cells negatively correlated with ribosomal gene sets. These data suggest that a certain ribosomopathy may occur in the mutant DDX41 expressing cells.

These findings and a highly-conserved DEAD-box type RNA helicase domain of DDX41 led us to speculate that DDX41 protein might be involved in pre-rRNA processing. As expected from the position of the somatic mutation (p.R525), which is within the helicase core where the ATP binds and is hydrolyzed, the mutant helicase domain displayed a lower ATPase activity. In addition, Northern blot analysis probing ITS1 and ITS2 of pre-rRNA showed increased signals of 47S and 41S pre-rRNAs in THP-1 cells expressing p.R525H DDX41. Although the precise phase at which DDX41 takes part in the pre-rRNA processing has not been elucidated, this series of experiments suggests a role for DDX41 in the trimming of 5' ETS and/or ITS 2.

(4) Activation of RB pathway occurring in p.R525H DDX41 expressing cells

Recent studies on ribosomopathies revealed an activation of the MDM2-p53 pathway in the pathogenesis of the diseases. It is now widely recognized that RPL5 and RPL11, not incorporated into the 60S ribosome, preferentially bind to MDM2; therefore, inhibition of p53 by MDM2 is compromised, thus causing a stimulation of the p53 pathway. We initially assumed that the same defect might be involved in the growth impairment of p.R525H DDX41 cells. Nevertheless, GSEA did not indicate p53 activation in the cells.

On the other hand, GSEA instead revealed a negative enrichment of cell cycle-promoting genes regulated by the RB-E2F axis in p.R525H DDX41 cord blood cells. Cell cycle inhibition through the suppression of E2F activity was also detected in patient-derived samples. In our study, we found increased RPL5 and RPL11 bound to MDM2 in p.R525H DDX41 cells. Since the total amount of these ribosomal proteins was not apparently altered by the enforced expression of p.R525H DDX41, the mutant DDX41 might increase free ribosomal proteins that are not incorporated into the 60S ribosome; these proteins may eventually form a complex with MDM2.

Discussion & Conclusion

Here, we propose a mechanism of growth defect in hematopoietic cells triggered by p.R525H DDX41 occurring in the following order: (i) p.R525H mutant inhibits pre-rRNA processing; (ii) compromised ribosomal biogenesis as a result of impaired rRNA synthesis causes a release of ribosomal proteins that bind to MDM2; (iii) MDM2-mediated RB degradation is suppressed, thus eventually activating the RB pathway and resulting in the inhibition of E2F activity. Although this study uncovered a pathogenic role of p.R525H DDX41 in the slow growth rate of tumor cells, how the mutation induces AML development and inhibits cell differentiation is still not understood. Considering late occurrence of AML in patients harboring the mutation, it might require age-dependent epigenetic alterations or other somatic changes for this mutation to fully transform hematopoietic cells.

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一般の皆様へ

近年の遺伝子解析研究の飛躍的な進歩により、白血病や骨髄異形成症候群といった血液腫瘍の原因となる遺伝子異常は、ほぼ全て明らかにされたといえます。一方で、こうした遺伝子異常が実際にどのように血液細胞を腫瘍細胞へと変貌させるのか、という根源的な疑問は、遺伝子解析だけで解くことができないものであり、地道な分子生物学的研究を必要とします。こうしたなか私たちは、最近血液腫瘍の原因となることがわかった *DDX41* という遺伝子の変異に着目し、この変異により作られる異常なDDX41 が、リボソームと呼ばれる細胞内のタンパク質精製工場の機能を障害することを、多面的な解析手法により示してきました。今後さらに詳細なメカニズムを解明すべく、研究を続けていきたいと考えています。

Induction of antigen-specific regulatory T cells by dendritic cells in human

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Summary Abstract

To control autoimmune disease, allergy and graft rejection in a specific way, we have been working on inducting antigen specific regulatory T cells in mice and found that dendritic cells are critical antigen presenting cells for regulatory T cells to proliferate in an antigen-specific manner. In this study, we tried to translate our findings in mice into human.
Key Words：Regulatory T cells, Dendritic cells, antigen-specificity

Introduction

Regulatory T cells suppress important immune responses such as autoimmunity, anti-tumor immunity, graft rejection, allergy and responses to infection. Regulatory T cell-based therapy is promising, however, polyclonal regulatory T cells could lead to cancer and serious infection because of the global immune suppression. To control autoimmune disease, allergy and graft rejection in a specific way, we tried to induce antigen specific regulatory T cells using human cells.

Results

Regulatory T cells suppress important immune responses such as autoimmunity, anti-tumor immunity, graft rejection, allergy and responses to infection. Regulatory T cell-based therapy is promising, however, polyclonal regulatory T cells could lead to cancer and serious infection because of the global immune suppression. To control autoimmune disease, allergy and graft rejection in a specific way, we have been working on inducting antigen specific regulatory T cells in mice and found that dendritic cells are critical antigen presenting cells for regulatory T cells to proliferate in an antigen-specific manner. Thus, in this study, we tried to translate our findings in mice into human.

For the dendritic cell preparation, we first purified dendritic cell subsets from peripheral blood by flow cytometer. Human peripheral dendritic cell subsets were divieded into CD141+(BDCA3+) dendritic cells, CD1c+(BDCA1+) and BDCA2+ plasmacytoid dendritic cells. We were able to sort these dendritic cell subsets with >90% purity by flow cytometer. However, the cell numbers from 20ml blood were very low and were not enough for the titration or time course experiments.

Therefore, next, CD14+monycytes were purified from peripheral blood and monocyte-derived dendritic cells were generated in the culture with GM-CSF and IL-4. We determined the concentration of GM-CSF and IL-4, and were able to generate enough numbers of monocyte-derived dendritic cells. Monocyte-derived dendritic cells were treated with or without inflammatory cytokine cocktails, i.e., TNF-a, IL-1b, IL-6 and PGE2. Monocyte-derived dendritic cells were analyzed by flow cytometer and the phenotype was checked. We confirmed the cytokine cocktailes matured them

into hith CD86 and HLADR.

When monocyte-derived dendritic cells were cultered with CFSE-labeled allogeneic naïve CD4+T cells from peripheral blood, we confirmed that the dendritic cells could induce allogeneic T cell proliferation. Foxp3 is the transcription factor for differentiation of regulatory T cells. In addition to Foxp3, functional human regulatory T cells can be distinguished by the expression of CD45RA or CD127. Using these markers, we did time course analysis at several time points and determined the best condition for regulatory T cell induction.

We also found that effector T cells and regulatory T cells proliferated in the presence of FCS and could not detect the difference between allogeneic and syngeneic responses. Therefore, we chose a certain type of serum free medium and were able to reduce the non-specific proliferation to FCS.

Thus, using allogeneic systems, we were able to determine what type of dendritic cells could be used, how long cells should be cultured, and how many cells should be in the culture.

To determine the function of induced regulatory T cells, it is important to perform suppression assay in vitro. To set up the suppression assay in human cells, we first used naïve CD25highCD4+T cells and added into responder allogenic CFSE-labeled CD4+T cells. We determined the condition which the proliferation of responder T cells were suppressed by CD25highCD4+T cells. Using the same condition, we will be able to see the antigen specific suppression of induced regulatory T cells by titrating induced regulatory T cell numbers.

Collectively, in this study, we were able to determine the best condition to induce Foxp3+regulatory T cells using dendritic cells in human cells. We will use this condition and try to expand functional antigen specific regulatory T cells for the future therapy.

Discussion & Conclusion

It is very important to use antigen specific regulatory T cells to prevent global immune suppression. We were able to determine the best condition to induce Foxp3+regulatory T cells using dendritic cells in human cells by phenotype analysis. It seems that human dendritic cells derived from CD14+monocytes are able to induce antigen-specific regulatory T cells, although we still need to investigate the in vitro suppressive activity. We used allogeneic mixed leukocyte reaction, but other types of antigens are also needed to be investigated. We will continue this study and hope to translate into therapy in the future.

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一般の皆様へ

様々な免疫反応を抑制する制御性 T 細胞の抗原特異性を樹状細胞という特別な抗原提示細胞でコントロールする研究をこれまでマウスで行ってきた。抗原特異的な制御性 T 細胞を誘導する事ができれば、癌や感染症に対する免疫反応を抑制せずに、自己免疫疾患、アレルギー、移植片拒絶を治療する事が可能となる。本研究では、これをヒトの細胞に応用することを行った。ヒトの樹状細胞でも抗原特異的に制御性 T 細胞を誘導できる条件決定を行うことができた。

Study on the neuronal mechanisms for depression and anxiety induced by chronic stress and pain

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Summary Abstract

The present study demonstrated that the activation of neurons projecting from the BNST to the CeA increased anxiety-like behaviors, and that these neurons were Ih-positive type II neurons. Furthermore, the results showed that neurotransmission via CRF1 receptors within the BNST was continuously activated under the chronic pain condition, and thereby increased the excitatory inputs to typeII BNST neurons. These findings suggest that enhanced neuronal excitability in type II BNST neurons may be responsible for the increased anxiety level in chronic pain. Such a neuroplastic alteration under the pathological condition may be one of the underlying mechanisms of anxiety/depression disorders.

Key Words：Anxiety, Bed nucleus of the stria terminalis, Depression, Extended amygdala, Pain

Introduction

Mammals including humans are thought to have acquired and evolved neuronal mechanisms for negative emotion, such as depression and anxiety, as a biological defense system that protects themselves by suppressing their activities and raising vigilance against the surroundings, when they are in dangerous condition. Therefore, in order to understand the neuronal mechanisms of depressive disorders and anxiety disorders, it is necessary to clarify the neuronal mechanisms for negative emotion from the viewpoint as a biological defense system and to analyze the changes of such mechanisms in patients and disease model animals.

Results

The bed nucleus of the stria terminalis (BNST) together with the central amygdala (CeA) forms a forebrain functional unit described as an “extended amygdala”, which have been implicated in the regulation of negative emotional states such as anxiety, fear, and aversion. Although these two brain regions bidirectionaly send dense neural projections to each other, it remains to be elucidated the roles of these neural projections in the regulation of negative emotional states. In this study, we introduced channelrhodpsin2 into the BNST and activated the neuronal projection from the BNST to the CeA by applying photostimuation to the CeA. This activation increased anxiety-like behaviors in an elevated plus maze test. On the other hand, recent study reported that activation of the neural projection from the BNST to the lateral hypothalamus (LH) suppressed anxiety-like behaviors. Since activation of the neural projections from the BNST to these two brain regions (CeA and LH) showed the opposite effects on anxiety-like behaviors, we compared the electrophysiological properties of the BNST neurons projecting to these two brain regions. We injected retrograde tracers into the CeA or LH, and conducted whole-cell patch clamp recording from the BNST neurons labeled with retrograde tracers. Majority of the CeA-projecting BNST neurons showed hyperpolarization-

activated cation current (Ih current) in response to the hyperpolarizing current injection, while most of the LH-projecting BNST neurons did not show Ih current. These results suggest that CeA-projecting BNST neurons are Ih-positive type II neurons, and activation of these neurons increases anxiety-like behaviors, while LH-projecting BNST neurons are Ih-negative type III neurons and activation of these neurons suppresses anxiety-like behaviors.

The BNST is densely innervated with corticotropin-releasing factor (CRF) containing fibers. We previously reported that CRF depolarizes typeII neurons and enhances inhibitory synaptic transmission to typeIII neurons in the BNST. We also demonstrated the critical role of CRF neurotransmission within the BNST in aversive responses induced by formalin-evoked pain. However, the roles of CRF within the BNST in the regulation of negative emotions under the chronic pain condition remain to be elucidated. In this study, we examined the effects of CRF and a CRF type 1 receptor antagonist on the synaptic currents in the BNST neurons using the whole-cell patch-clamp recordings. Brain slices including the BNST were prepared from chronic pain model rats in which neuropathic pain was induced by spinal nerve ligation (SNL). In sham-operated rats, spinal nerves were exposed without ligation. Bath application of CRF significantly increased the amplitude of eEPSC in typeII neurons of the sham-operated rats, but not of the SNL model rats. By contrast, the amplitude of eEPSC was significantly decreased by bath application of NBI27914 (a CRF type 1 receptor antagonist) in typeII neurons of the SNL model rats, but not of the sham-operated rats. These data suggest that neurotransmission via CRF type 1 receptors within the BNST are continuously activated under the chronic pain condition, thereby increased the amplitude of eEPSC in typeII BNST neurons.

Discussion & Conclusion

The present study demonstrated that the activation of neuronal projection from the BNST to the CeA increased anxiety-like behaviors in an elevated plus maze test, and that the neurons projecting from the BNST to the CeA are Ih-positive type II neurons. Furthermore, the results showed that neurotransmission via CRF type 1 receptors within the BNST was continuously activated under the chronic pain condition, and thereby increases the excitatory inputs to typeII BNST neurons. These findings suggest that enhanced neuronal excitability in type II BNST neurons may be responsible for the increased anxiety level in chronic pain. Such a neuroplastic alteration under the pathological condition may be one of the underlying mechanisms of anxiety/depression disorders.

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一般の皆様へ

ヒトを含む哺乳類は、身を守るための生体防御システムとして、抑うつや不安などの負情動生成機構を獲得・進化させてきたと考えられます。したがって、うつ病や不安障害のメカニズムを理解するためには、生体防御システムとしての負情動生成機構を明らかにした上で、患者や病態モデル動物における変化を解析することが必要となります。私たちは、分界条床核と呼ばれる脳部位に着目して研究を進め、分界条床核から扁桃体中心核に投射する神経細胞の活性化が不安情動を引き起こすことを明らかにした。また、慢性痛時に分界条床核内シナプス伝達の可塑的変化が起こることも見いだしています。これらの神経機構がうつ病や不安障害に関与している可能性があります。

Pathogenesis of cardiomyopathy by RBM20 mutations modeled in genetically modified human iPS cells

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Summary Abstract

Point mutations in a splicing factor, RNA binding motif protein 20 (RBM20), are known to be responsible for cardiomyopathy. However, it remains elusive how these mutations lead to dysfunction of cardiomyocytes at the molecular level. We generated an RBM20 mutant allelic series in human iPS cells and differentiate them into cardiomyocytes. These iPS cell-derived cardiomyocytes (iPS-CMs) recapitulated the cellular phenotypes of cardiomyocytes in patients. Deep sequencing of transcripts of these iPS-CMs revealed that these point mutations are not simple loss-of-function mutations. Our study provides a key insight into pathogenesis of cardiomyopathy by the RBM20 point mutations.

Key Words：Cardiomyopathy, iPS cells, Genome editing, RBM20, Splicing

Introduction

Dilated cardiomyopathy (DCM) is the most common indication for heart transplantation[1-3]. Mutations in a cardiac-specific splicing protein, RBM20, account for up to 3% of observed DCM[4-6]. Interestingly, these mutations display a striking pattern of seven tightly clustered DCM-associated point mutations in RBM20[4,7]. As currently available rodent models are knockouts for Rbm20, they do not reveal the pathogenic mechanism caused by the RBM20 point mutations. Therefore, we introduced the RBM20 R636S and loss-of-function mutations into human iPS cells, and differentiated them into iPS-CMs. By transcriptomic analysis of these cells, we addressed the effects of the RBM20 mutations on cardiac splicing regulation.

Results

We introduced heterozygous and homozygous RBM20 R636S point mutations in human iPS cells derived from a healthy male patient, WTC11[8]. For this purpose, we applied a method that we developed recently based on digital-PCR and repeated rounds of limited dilutions without using any selection marker genes[8,9]. We also introduced a heterozygous 1-bp insertion mutation in the open reading frame of RBM20 by using the same method, which should result in loss-of-function mutations due to frameshifting. These iPS cell lines were then differentiated into cardiomyocytes by small molecules that modulate Wnt signaling[10]. The iPS-CMs were purified from other cell types by lactate treatment[11]. We performed immunofluorescent staining of Actinin in the iPS-CMs to visualize the sarcomeric structure that is a functional unit of cardiomyocytes. The mutant iPS-CMs displayed disarrayed sarcomere compared to WT iPS-CMs demonstrating that the mutant iPS-CMs faithfully recapitulated the cellular phenotypes of cardiomyocytes in DCM patients. To elucidate molecular pathogenesis behind the observed cellular phenotypes, total RNA purified from the iPS-CMs was subjected to a paired-end 100 bp deep sequence by illumina HiSeq. The obtained reads were mapped

onto the reference human genome, and the mapped data was then analyzed by AltAnalyze for genome-wide splicing profile[12]. AltAnalyze quantified the frequency of an exon being expressed as transcripts, so that we could analyze the splicing pattern based on how often each exon was included in mRNAs.

First, we compared the splicing profiles of wild-type (WT), heterozygous R636S mutant (R636S Het), and homozygous R636S mutant (R636S Homo) iPS-CMs. Because DCM patients are heterozygous for the R636S mutation, we expected to see splicing defects in R636S Het iPS-CMs. As we expected, R636S Het showed aberrant splicing compared to WT in genes associated with functions of cardiomyocytes including TTN, MYLK, and MEF2A (Figure 1). Some genes shifted their splicing patterns to more immature ones due to the mutation. Currently, we are analyzing how these splicing defects lead to the cellular phenotypes that we see in DCM patients. Interestingly, we observed distinct splicing phenotypes between R636S Het and R636S Homo iPS-CMs. For example, R636S Homo exhibited severer splicing defects than R636S Het in some genes including COL16A1, RYR2, CDC14B, GPBP1L1, and TTN, indicating an additive effect of the R636S mutation. However, both R636S Het and R636S Homo iPS-CMs showed splicing defects in some genes such as IMMT, NEO1, MICAL3, and DMD at a comparable level (Figure 1).

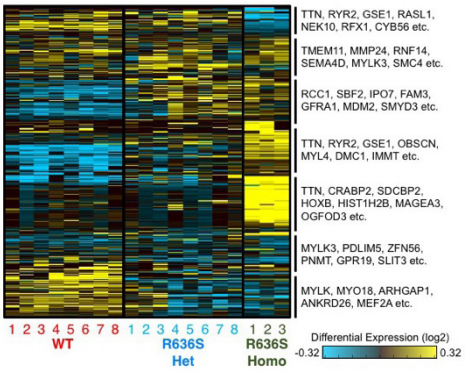

Figure 1. Splicing profiles of WT, R636S Het, and R636S Homo iPS-CMs
Total RNA of WT (8 samples), R636S Het (8 samples), and R636S Homo (3 samples) was analyzed by RNAseq for splicing patterns. The frequency for each exon being included in transcripts is color-coded (yellow and blue for high and low, respectively). Genes with altered exons are listed on the right.

Then, we compared the splicing profiles of WT, R636S Het, and the heterozygous 1-bp insertion mutant (1-bp Ins Het) to directly address whether the R636S mutation is a loss-of-function mutation. As a result, R636S Het and 1-bp Ins Het cells displayed rather different splicing patterns. Some exons upregulated in R636S Het cells were downregulated in 1-bp Ins Het, and vice versa. Many of them were genes involved in cardiomyocyte functions (Figure 2). If the R636S mutation were a simple loss-of-function mutation, R636S Het and 1-bp Ins Het mutations would have similar impacts on splicing regulation. Our results indicate that the R636S mutation is not a simple loss-of-function mutation.

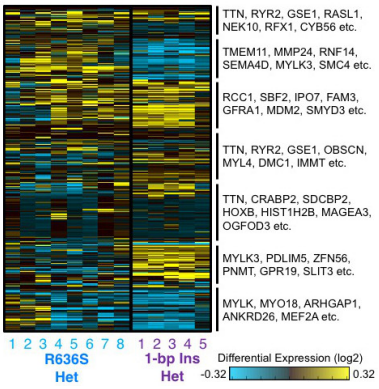

Figure 2. Splicing profiles of R636S Het and 1-bp Ins Het iPS-CMs
Total RNA of R636S Het (8 samples) and 1-bp Ins Het (3 samples) was analyzed by RNAseq for splicing patterns.

Discussion & Conclusion

If the pathogenic RBM20 point mutations were simple loss-of-function mutations, there would have been more frameshift and nonsense mutations distributed throughout the RBM20 gene. However, at least 7 pathogenic point mutations are accumulated in a stretch of only 5 amino acid residues, indicating that these point mutations may not be simple loss-of-function ones. Our findings based on RNAseq also support this hypothesis.

This stretch of amino acids is R634 to P638, RSRSP, in RBM20. This stretch is highly characteristic both in electric charge and structure, and two serines can be phosphorylated. Moreover, this stretch is located in the Arg-Ser (RS) domain of RBM20, which is suggested to be involved in protein

interactions. We speculate that these RBM20 point mutations have dominant negative effects by interfering with the protein interactions via the RS domain. We are now analyzing iPS-CMs with a homozygous RBM20 loss-of-function mutation to further elucidate molecular pathogenesis of DCM.

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一般の皆様へ

拡張型心筋症は、心臓移植の最多の要因である。近年、RBM20という遺伝子の変異が拡張型心筋症の原因であることが明らかとなったが、ラットなどのモデル生物を用いた研究では、心筋症発症機序の理解が十分ではなかった。そこで、私達はヒトiPS細胞にRBM20の変異を導入し、心筋細胞へと分化させ、詳細に解析することにより、これまでに知られていなかったRBM20の変異が心筋症発症につながる分子機構を解明した。本研究の成果が、新たな治療法の開発につながることが期待される。

Chemical investigation of the food-poisoning epidemics caused by the mushroom *Pleurocybella porrigens*.

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Summary Abstract

In autumn, 2004, 55 people got poisoned by eating an edible wild mushroom, *Pleurocybella porrigens* and 17 people among them died of acute encephalopathy. We have already reported the purification and characterization of a lectin from the mushroom (PPL). We have purified the lethal glycoprotein (B3), and the mixrture (PPL-B3) have showed protease activity and disrupted the blood-brain barrier in mice. However, B3 is insoluble with any organic solvent, and the primary structure of B3 is still unknown.

We have tried to obtain the gene of the lethal glycoprotein from *P. porrigens* by a yeast two-hybrid method. As a result, we selected 2 clones which might be truly positive.

Key Words：Mushroom, *Pleurocybella porrigens,*

Introduction

In autumn, 2004, 55 people got poisoned by eating an edible wild mushroom, *Pleurocybella porrigens* and 17 people among them died of acute encephalopathy. We have already reported the purification, characterization, and cDNA cloning of a lectin from the mushroom (PPL). We have purified the lethal glycoprotein (B3) which was extracted with boiling water, and the mixrture (PPL-B3) have showed protease activity and disrupted the blood-brain barrier in mice. Thus, the purpose of this research is to reveal structure of these proteins, complex formation mechanism and toxicity mechanism, and to clarify the mechanism of the onset of acute encephalopathy.

Results

A lethal toxic substance, named B3, has been isolated from boiling water-soluble part of *P. porrigens* and characterized by us in previous study. A lectin nemed PPL has been also isolated from water-soluble part of the mushroom and characterized, and its cDNA was obtained. The mixrture of PPL and B3 (PPL-B3) showed protease activity and disrupted the blood-brain barrier in mice. Furthermore, PPL-B3 complex showed novel exo-protease activity to cleave the substrates from both *N*- and *C*-terminus.

However, B3 is insoluble with any organic solvent, and the primary structure of B3 is still unknown. Thus, we have tried to obtain the gene of B3 from *P. porrigens* by a yeast two-hybrid method and have obtained 64 positive clones. Thus, we tried to obtain B3 gene in 64 positive clones by new screening method, and to express recombinant PPL in basidiomycete *Phanerochaete sordida* YK-624 in the present study.

We analyzed these gene sequences inserted in 64 positive clones, and 4 clones were selected by these information of transcriptome analysis and LC-MS/MS analysis of B3. These selected 4 genes were expressed using heterologous expression systems of *E. coli* BL21 and *P. sordida* YK-624.

However, all recombinants showed no protease activity although SDS-PAGE analysis of these clones revealed that each recombinant was successfully expressed. PPL-B3 complex showed novel exo-protease activity to cleave the substrates from both *N*- and *C*-terminus as described above. Thus, we developed a new protease assay for the selection of real positive clones by using fluorogenic and chromogenic substrate(s). As a result, we selected 2 clones which might be truly positive.

Occasionally PPL showed the protease activity when it contained a minor peak at m/z 13,000 in MALDI-TOF-MS. Therefore, we thought that this m/z 13,000 substance was concerned with the protease activity and named this substance PP-13,000. We isolated PP-13,000 by reversed phase liquid chromatography, and finally determined its primary structure of PP13,000.

Next, we tried heterologous expression of recombinant PPL by basidiomycete *P. sordida* YK-624. The glyceraldehyde 3-phosphate dehydrogenase gene promoter was used to drive the expression of the PPL gene in *P. sordida* YK-624. Furthermore, the signal peptide of lignin peroxidase which is an extracellular protein was used to secrete rPPL into extracellular region. The heterologous expression of rPPL by *P. sordida* YK-624 was confirmed by SDS-PAGE, lectin activity by the hemagglutination assay, indicating that the extracellular secretion of rPPL as active form was successful. Mixture of PP-13,000 and rPPL expressed in *P. sordida* YK-624 showed protease activity that hydrolyzed the substrates from both *N*- and *C*- terminal ends.

We previously analyzed a set of whole genome sequencing and transcriptome sequencing data in *P. porrigens* using the Illumina technology and released the compiled data from a web database A-WING[1, 2] Our results suggested that the genome of the mushroom had a unique structure and contained numerous novel genes[1], while the whole picture of the genetic information including the exact genome size and a full set of genes was not obtained because of the fragmented sequence assemblies. In the present study, we analyzed a draft genome sequence of *P. porrigens* to decode the unique genetic information. The improved genome and transcriptome assemblies constructed in this study allowed for characterization of the unique features in paralogous gene clusters and prediction of protein-coding genes.

Discussion & Conclusion

In present study, we obtained 2 positive clones by a yeast two-hybrid method. Furthermore, we succeeded in purification and primary structure determination of PP-13,000. These proteins might have caused the poisoning of *P. porrigens*. Further study is needed to clarify the toxicity mechanism of this mushroom.

rPPL was successfully expressed by using basidiomycete *P. sordida* YK-624 expression systems. We are now trying to elucidate the molecular mechanism for this acute encephalopathy using this rPPL.

The draft genome sequence and annotations obtained in this study were released at the web database A-WINGS20. Those will give aids to identify not only molecules that act on morphological and physiological traits in the mushroom, but also useful genes that control rare metabolite biosynthesis for drug development.

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(†; equal contribution)

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一般の皆様へ

スギヒラタケは東北、北陸、中部地方を中心に広く食用とされてきたキノコですが、2004年以降、スギヒラタケの摂食者が急性脳症を発病しました。本課題を遂行することによって、このキノコの毒性に関与する可能性がある毒性物質数種の構造決定を行う事ができました。この酵素は新たな酵素学として科学的価値だけでなく、様々な応用の可能性を秘めており、新たな酵素の創出も期待できます。さらにスギヒラタケ摂取患者に見られる病変は、既存の病気のものとは異なる未知の発生機序を強く示唆しています。今後も毒性メカニズムの解明のために、研究を継続する予定です。

Developing analysis of genome replication in single cell to elucidate molecular dynamics of DNA polymerases

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Summary Abstract

Synthesis by DNA polymerase is subjected to accidental posing due to many obstacles on genomic DNA: DNA damage, sequence-specific secondary structure, etc. The heterogenous nature of DNA replication arise at least from their stochastic behaviour of DNA polymerase. However, no studies have given insight into any clear specificity in terms of molecular dynamics of DNA polymerase. This study aim to develop a new experimental system to assess usage of DNA polymerase in a single cell in order to elucidate how multiple DNA polymerases cooperatively function to complete duplication of genome.
Key Words：DNA replication, Genome stability

Introduction

Duplication of cells must be faithfully carried out in proliferating cells. DNA replication is the stage in which cells are subjected to various DNA insults, which become truly dangerous and potentially causes cell death or genomic instability. The replication machinery is therefore endowed with plasticity that provides means to tolerate various replication stress. Each single cells initiate replication at different locations and various DNA polymerases are to be functionalised in response to accidental events in a course of DNA synthesis. In this study, we aimed to establish the experimental system to characterise DNA polymerase usage globally in a single cell and elucidate stochastic nature of DNA replication.

Results

To characterise functions of each DNA polymerase globally, we previously devised a new methodology to identify usage of DNA polymerase at genome wide level (Pu-seq) [1]. In this method, first, we established mutants of two major DNA polymerase: Polε and Polδ in which excessive ribonucleotides (rNMP) are incorporated into genomic DNA. Second, incorporated rNMP was mapped by high-throughput sequencing based technology. Finally, usage of these polymerases was calculated from abundancy of incorporated rNMP. Our analysis revealed that the division of labour of two polymerases is broadly maintained: Polε is responsible for leading strand DNA synthesis while Polδ is for lagging strand synthesis. Although this result provided the evidence that division of labour between two major DNA polymerases, their molecular dynamics remain unclear. The profiles of these polymerases were derived from a mass of cultured cells; however, behaviour of DNA polymerase is expected to be different in each single cell. This study therefore aims to develop the method to investigate the genome-wide profile of DNA polymerase in a single cell (single cell pu-seq). While this method is primarily being developed using fission yeast cells, we plan to apply the method in C.elegans germline cells to elucidate variation in the molecular mechanism of DNA

replication during the process of aging.

Regarding the development single cells Pu-seq, we required to examine rNMP incorporation in a single cell. As alkaline treatment cleaves DNA strands at incorporated rNMP, we are able to map rNMP by identifying location of 5' ends of cleaved fragments. Therefore, we aimed to perform whole genome amplification (WGA) from alkaline treated single cells. Among many WGA methods, multiple annealing and looping-based amplification: MALBAC method has been reported to cause relative lesser application bias [2]. After optimisation of this procedure, we successfully amplified genome DNA from alkaline treated single cells. However, further optimisation is still required to increase the amount of yielded DNA and probability of successful application before proceeding to preparation of DNA library for high-throughput sequencing.

To construct rNMP-incorporating DNA polymerase mutant in C.elegans, we utilised CRISPR cas9 system adapted in this organism [3]. In this system, donor single-stranded DNA bearing a desired mutation is co-injected with plasmids encoding Cas9 and a gRNA specific for the targeted locus into the C. elegans germline. An amino acid residue proximal or the steric gate in the catalytic site of Polε and Polδ was to change to a hydrophobic or small residue. As a result of multiple time injection, both desired Polε and Polδ mutants were isolated. In the case of the Polε mutant, we only integrated the mutation into one copy of Polε genes and thus worms carrying bi-allele mutation was screened after self-fertilisation. To examine whether these mutant polymerases incorporate rNMP into genomic DNA, we examined polymerase mutant worms in a condition where RNaseH2 was inactivated by RNA interference. In the absence of RNaseH2, rNMP incorporated by DNA polymerase would not be eliminated, resulting in its accumulation in genomic DNA. Consistent with this prediction, when RNaseH2 was inactivated in Polε or Polδ mutants, hatching rates of next generation were dramatically decreased. These results indicate that integrated Polε or Polδ mutations cause accumulation of rNMP during DNA replication in germline cells or early embryo, leading to failure of development.

Discussion & Conclusion

This study aims to elucidate DNA replication dynamics which is different among each single cell. To gain technical advance for this purpose, Using fission yeast cells, WGA prior to high-throughput sequencing has been optimized to achieve mapping of rNMP in single cells. While further optimisation is required to complete sequencing procedures of this experiment system, we successfully constructed mutants of Polε or Polδ in C.elegans which incorporate rNMP into genomic DNA. Thus, we currently prioritise the completion of single Pu-seq system and application of this system in c.elegans will follow to elucidate the mechanism of DNA replication, which is predicted to be variable in response to changes in metabolic status during process of ageing.

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一般の皆様へ

個々の細胞には個性があります．遺伝情報であるゲノムを複製するシステムはすべての細胞において，全く同じパターンで起きるわけではありません．複製が開始される場所，細胞に多種存在するＤＮＡを合成する酵素：ＤＮＡポリメラーゼの使われ方が異なっています．本研究では，１つ１つの細胞で，ゲノムＤＮＡ全体に渡り，個々のＤＮＡポリメラーゼ分子の役割を明らかにする実験系の構築を行いました．

A novel lincRNA, Linc-Heart, regulates cell cycle and proliferation of adult cardiomyocytes

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Summary Abstract

"Heart" is a dynamic tissue that maintains pumping by conserved contraction. However, once myocardial infarction (MI) occurs, the infarcted myocardium loses its capacity, resulting in the formation of scar tissue. The remaining myocardium is forced to compensate, often leading to heart failure and decreased survival. Meanwhile, long non-coding RNAs (lncRNAs) are transcribed RNA molecules of >200 nucleotides in length. Thousands of lncRNAs have recently been identified and many of them are expressed in a cell- and tissue-specific manner. However, the biological functions of these lncRNAs remain largely unknown. Intriguingly, a few recent studies have demonstrated some lncRNAs have crucial roles during developmental process. Thus, we hypothesize that there are unrecognized novel lncRNAs that control cell size, proliferation, and contractility of cardiomyocytes.

This study was designed to identify and characterize novel lncRNAs that have crucial roles in the heart. In particular, we focused on the role of lncRNAs of the regulation of cell biological systems such as cell size and cell cycle of cardiomyocyte after MI. In fact, we demonstrated that the expression of lncRNAs was dramatically changed after MI, and that a novel lncRNA, that we named "*linc-Heart*", can regulate cell cycle of adult cardiomyocytes. The findings in this study would lead to era with new therapeutic strategy for cardiac diseases by regulation of cell cycle in adult cardiomyocytes in the future.

Key Words：cardiomyocytes, cell cycle, long non-coding RNA, myocardial infarction, proliferation

Introduction

It is now recognized that the majority of the genome is actively transcribed to produce thousands of non-coding transcripts in many cell types and tissues.[1-3] A subset of these non-coding transcripts are classified as lncRNAs, transcribed RNA molecules greater than 200 nucleotides in length that do not encode proteins.[2-4] The functions of lncRNAs have recently been investigated in diverse biological processes such as stem cell pluripotency, immune responses, and cell-cycle regulation.[5-7] However, little is known about the expression and functional significance of lncRNAs in the heart.

Recently, a novel lncRNA, *Braveheart* (*Bvht*), has been defined as a critical regulator of cardiovascular commitment from embryonic stem cells.[8] *Bvht* represents the first lncRNA that defines cardiac cell fate and lineage specificity, linking the function of lncRNAs to cardiac development. In their study, the authors showed that the *Bvht* transcript is expressed in several adult mouse tissues including brain, colon, heart, and muscle with its highest expression in the heart. It will be important to further characterize the spatiotemporal expression of *Bvht* in development and disease. Most importantly, it will be essential to define the in vivo function of *Bvht* in mice, using genetic loss-of-function approaches.[9] Furthermore, another novel lncRNA, *Fendrr*, has been recently identified.[10] The lateral mesoderm-specific lncRNA, *Fendrr*, is essential for proper heart and body wall development in the mouse. Importantly, any lncRNAs having crucial roles in the maintenance

of adult heart functions have yet to be studied. It will be interesting to study whether novel lncRNAs might also participate in cardiac regeneration or be used to stimulate cellular reprogramming. Clearly, the discovery of *Bvht* and *Fendrr* will significantly impact the cardiovascular research field and human cardiovascular disease.

Therefore, the purposes of our study are 1) to identify key lncRNAs which regulate essential biological process in the heart, and 2) to explore the crucial functions of the identified lncRNAs in the heart.

Results

a) Myocardial infarction model

First of all, our important step is to screen potential candidate novel lncRNAs. To identify key lncRNAs which regulate essential biological process of adult heart, we used mouse model with MI. MI surgery was performed in male mice (C57BL/6J, 8 weeks old) by ligating left anterior descending artery under artificial ventilator and thoracotomy. Heart was dissected and myocardium from the border-zone area and that from the remote area was collected to obtain RNA at day 3 and day 14 after MI surgery, respectively.

b) LncRNA microarray screening

LncRNA microarray analysis was performed by using a lncRNA microarray kit (Agilent). RNA samples from myocardium after MI surgery or Sham surgery were used. Among more than 10,500 lncRNAs that the kit can measure, about 7,500 lncRNAs could be detected in our samples. The results of lncRNA microarray were further analyzed by Gene-Spring software (Agilent). The analysis of 3D PCA scores, hierarchical clustering, and profile plot of lncRNA expression suggested that RNAs from myocardium of border-zone area at 3 days after MI surgery (3POD) had the most dramatically altered lncRNA expression levels.

c) Primary characterization of potential candidate lncRNAs

Our purpose is to discover the potential lncRNAs that have crucial roles to maintain heart functions. Therefore, we focused on the specific lncRNAs whose expression levels are high in normal adult hearts compared to other tissues, and whose expression levels dramatically decrease or increase in myocardium following MI. At first, fold change analysis was performed, and we selected > 5.0 fold-changed lncRNAs in MI myocardium by qPCR. Next, we planned to confirm the expression levels of above lncRNAs by qPCR. Some of lncRNAs have high-homologous sequences as protein-coding mRNAs. Thus, we selected lncRNAs that don't have those homologous sequences and can be expected to be detected by qPCR independently. After measurement of expression levels by qPCR, the expression patterns of lncRNAs that could be detected by qPCR were compared to the results of lncRNA microarray, and lncRNAs whose expression patterns were similar between microarray and qPCR were selected for further analysis. The expression levels of candidate lncRNAs in several mouse tissues, such as brain, heart, lung, liver, kidney, and skeletal muscle, were measured by quantitative real-time PCR (qPCR) assays. The expression level of only 1 lncRNA was significantly greater in the heart compared to other tissues. Therefore, we decided to focus on this one as the potential candidate lncRNA, which we named "***linc-Heart***".

The expression pattern of *linc-Heart* in staged hearts was determined by qPCR. Intriguingly, we found that its expression level was low until post-natal day7, but after growing up, the *linc-Heart* was highly expressed in the adult heart.

We also checked the expression level of *linc-Heart* in several tissues by Northern blotting as well as qPCR, and estimated the size of *linc-Heart* transcripts. Northern blotting revealed that the expression level of *linc-Heart* was high in the heart compared to other tissues, and that its level was comparable between in 2 months-age heart and in 6 months-age heart. The predictive size of *linc-Heart* transcripts was 1.9Kb.

d) In vivo function analysis

To investigate the functions of "*linc-Heart*", 9 adeno-associated virus (AAV) containing *linc-Heart* (AAV-*linc-Heart*) was generated. Hearts injected with AAV-*linc-Heart* had high expression levels of *linc-Heart*. Hypertrophic hearts were created by transverse aortic constriction (TAC) surgery in 8-week old mice. Surprisingly, the size of cardiomyocytes in the hearts injected with AAV-*linc-Heart* was maintained within almost normal size, and the total count of cardiomyocytes was increased. Cell proliferation marker, ph3, was positive in the cardiomyocytes at post-TAC surgery day 3 hearts injected with AAV-*linc-Heart*.

Discussion & Conclusion

So far, nothing has been known about the functional significance of lncRNAs in the adult heart. Importantly, this study suggested that a novel lncRNA, *linc-Heart*, has a crucial role in the heart, which can regulate the cell cycle of cardiomyocytes in the adult heart. This lncRNA study is novel and will greatly impact cardiovascular biology and medicine. We will perform in vitro study to determine the detailed mechanisms mediated by *linc-Heart*, in near future.

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一般の皆様へ

本研究では成熟心筋細胞の細胞周期を調整する新規の lincRNA を同定し、linc-Heart と命名した。今後、linc-Heart が成熟心筋細胞の細胞周期を調整する詳細なメカニズムを解明し、心筋細胞周期の再活性化による病的心臓治療への応用の可能性を広げたいと考える。

本研究成果を応用することによって、これまでの試みから逸脱した全く新しい概念として、もともと存在している正常な成熟心筋細胞を細胞増殖させることにより治療する、という発想が可能な時代が到来する可能性がある。ヒト心筋梗塞後のリモデリング抑制や心筋症での心機能改善等へ応用できる可能性が広がり、臨床への将来的な貢献が多いに期待できる。

Thymic positive selection impacts T cell immune responses *in vivo*

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Summary Abstract

Thymic positive selection is generally recognized to shape antigen specificity repertoire of T cells, but its additional role in functional education of T cells has been recently suggested. Thymoproteasomes mediate positive selection of CD8 T cells by producing MHC class I-associated self peptides in thymic cortex. Herein, we report that the loss of thymoproteasomes affects *in vivo* T cell responses, including effector T cell differentiation and memory formation. This result suggest that thymoproteasome-dependent positive selection, an early step of T cell development, determines the fate of post-activated T cells.

Key Words：Lymphocyte, T cell, thymus, positive selection, proteasome

Introduction

In the thymus, early thymocyte repertoire is shaped through multiple selection steps to generate immunocompetent T cells. Positive selection is induced by low affinity engagement of antigen receptors with self-peptide-MHC complexes in thymic cortex and is recognized as a process to form an usefull antigen recognition repertoire. It is becoming evident that protein degradation machineries unique to thymic cortex facilitate the production of self peptides inducing positive selection[1]. Based on our previous finding that thymoproteasome-dependent positive selection determines the antigen responsiveness of mature CD8 T cells[2], the present study examined the effects of thymoproreasome-dependent positive selection on *in vivo* immune responses.

Results

1. Primary responses of T cells generated in thymoproteasome-deficient thymus

To generate monoclonal CD8 T cells positively selected in the absence of thymoproteasomes, lethally irradiated mice deficient of a proteolytic subunit of thymoproteasomes, β5t encoded by *Psmb11* gene, were reconstituted with bone marrow cells from *Rag1*$^{-/-}$OT-I transgenic mice[2-4]. Ovalbmin (OVA)-specific naïve OT-I T cells selected in the presence or absence of thymoproteasomes were transferred into Ly5.1 recipient mice. To activate donor OT-I cells *in vivo*, recipients were subsequently injected with bone-marrow-derived dendritic cells pulsed with OVA peptide and CpG adjuvant. At different time points after immunization, donor OT-I cells in recipient spleens were analyzed by flow cytometry. In both groups, donor cells similarly expanded by day 5 post immunization and then decreased overtime until day 30. This result indicats that CD8 T cells selected in thymoproteasome-deficient thymus normally generate primary antigen responses in terms of cell number.

2. Phenotype of thymoproteasome-independent T cells in effector and memory phases

T cells which are activated in response to antigens differentiate into several subsets of effector

T cells. Terminally differentiated effector cells ($KLRG1^{+}CD127^{-}$) are relatively short-lived and disappear overtime after the clearance of pathogens, while a part of memory precursor effector cells ($KLRG1^{-}CD127^{+}$) persist longer and ultimately differentiate into memory cells. Accordingly, we analyzed the expression of KLRG1 and CD127 in donor OT-I cells at different time points after immunization. OT-I cells positively selected in the absence of thymoproteasomes showed higher frequency of terminally differentiated effector cells than normally selected OT-I cells did 4 and 30 days after immunization, but not in other time points including day 5, 10 and 15. Together with the former finding that absolute donor cell number was similar between control and thymoproteasome-deficient groups, this result indicates that CD8 T cells selected in thymoproteasome-deficient thymus are biased to terminally differentiated effector cells in early expansion and memory phases.

3. Cytokine production by thymoproteasome-independent T cells

To understand the effect of thymoproteasome deficiency on the effector functions after activation in the periphery, at day 5 post immunization, the peak of response in cell number, production of effector cytokines was examined. Spleen cells from recipient mice were cultured in the presence of OVA peptide and brefeldin A, and intracellular cytokines were analyzed by flow cytometry. The frequency of cells producing IFNγ and TNFα was significantly decreased in thymoproteasome-independent OT-I cells in comparison with control OT-I cells. Thus, positive selection through thymoproteasomes affects the effector functions of post-activated mature T cells.

4. Memory responses of thymoproteasome-independent T cells

Functions of memory cells was investigated by testing the proliferative responses of donor OT-I cells following secondary immunization *in vivo*. At day 40 post primary immunization, the frequency and the absolute number of donor OT-I cells in the recipient spleens were similar between thymoproteasome-deficient and control groups. Four days after given secondary immunization, significant increase of donor OT-I cells was seen in control but not in thymoproteasome-deficient groups. In thymoproteasome-deficient group, donor cell numbers before and after secondary immunization were equivalent, indicating that secondary expansion was almost completely impaired in memory OT-I cells positively selected in the absence of thymoproteasomes. The data suggest that positive selection through thymoproteasomes dictates the functional quality of memory T cells during the early developmental stage of T cells in the thymus.

Discussion & Conclusion

The results of this study indicate that thymoproteasome-dependent positive selection instructs developing thymocytes to acquire optimal functions after maturation. Especially, impacts of thymoproteasome deficiency on immunological memory extends the significance of functional education through positive selection that we previously suggested in *in vitro* studies [2, 5]. Since its discovery in 1970's, positive selection has been understood as a process to shape the antigen recognition specificity repertoire. However, recent advances in our knowledge on thymic microenvironment and thymus-specific protein degradation machineries, including cathepsin L, thymus-specific serine protease, and constitutive autophagy [1], may provide opportunities to revisit the roles of positive selection in the immune system.

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一般の皆様へ

リンパ球のひとつであるT細胞は、病原体や癌細胞に対する防御に不可欠な役割を果たしています。T細胞は胸腺という器官で非常に複雑な過程を経て作られます。私たちは、胸腺に存在する特殊な酵素の解析を通し、胸腺での分化の過程が、成熟した後のT細胞の機能を決定づけることを明らかにしてきました。今回、ワクチンの基本原理としても重要な免疫記憶というT細胞の機能に、胸腺での分化過程が関わっている可能性を見出しました。機能的に優れたT細胞が作り出される仕組みを理解することは、免疫の異常が関係する様々な病気の予防や治療、優れたワクチンの開発に役立つと期待されます。

Role of junctophilin for the proper function and intracellular distribution of L-type calcium channels

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Summary Abstract

Specific localization of the L-type calcium channel (LTCC) to the junctional membrane (JM) of skeletal muscle is critical for the efficient excitation-contraction (EC) coupling; however, the targeting mechanism is still unclear. This study showed that the physical interaction between junctophilins (JPs) and the C-terminus of CaV1.1 subunits of LTCC is essential for the proper JM targeting of LTCC, the physical interaction between LTCC and ryanodine receptors (RyR) and therefore the efficient EC coupling in the skeletal muscle.
Key Words：L-type calcium channel, junctophilin, junctional membrane, skeletal muscle

Introduction

Skeletal muscle LTCC is localized to JM where the sarcolemma are closely apposed to the sarcoplasmic reticulum membrane. Although this JM targeting of LTCC is critical for the EC coupling by supporting the physical coupling between LTCC and RyR, its molecular mechanism is not fully elucidated. JPs are known to stabilize the JM complex by bridging the plasma membrane and sarcoplasmic reticulum. Here, we explored that JPs support the proper JM-targeting of LTCC by binding to the CaV1.1 subunit of LTCC and the physical interaction between LTCC and RyR, and thereby ensure the efficient EC coupling in the skeletal muscle.

Results

We first examined the effect of siRNAs against JP1 and JP2 on the localization and function of LTCC in cultured myotubes. Immunocytochemical analysis revealed that the JP1 or JP2 knockdown significantly inhibited the JM-targeting of LTCC. Calcium imaging analysis using a fluorescent calcium indicator Fluo-4 showed that the JP1 or JP2 knockdown significantly decreased the number of myotubes exhibiting a calcium transient in response to electrical stimulation. These results indicate that JPs contribute to the proper localization and function of LTCC in skeletal myotubes.

Co-immunoprecipitation studies revealed that $Ca_V1.1$, a pore subunit of skeletal muscle LTCC, physically interacted with JP1 and JP2 in the mouse skeletal muscle in vivo. Pull down assay with GST-fusion proteins bearing the cytosolic regions of $Ca_V1.1$ indicated that the proximal C-terminus of CaV1.1 is necessary for the binding of CaV1.1 with JPs. This JP binding domain (JPD) was well conserved between CaV1.1 and cardiac $Ca_V1.2$ which is also targeted to JM when expressed in cultured myotubes. However, JPD was not conserved in neuronal CaV2.1, which did not localize to JM when expressed in cultured myotubes. Alanine substitutions of several residues in JPD reduced the binding of the GST fusion proteins to JPs. The same alanine substitutions were introduced into the JPD of CaV1.1, and these mutant CaV1.1 were transiently expressed the channel subunit in cultured myotubes. Immunocytochemical analysis revealed that the JM-targeting rate of the mutant

$Ca_V1.1$ was significantly reduced compared with the wild-type. These data show that the physical interaction of $Ca_V1.1$ with JPs via the JPD is important for the proper JM-targeting of LTCC.

We next examined the effect of transient expression of transmembrane domain deletion mutant of JP1 (JP1ΔTM) in cultured myotubes. This mutant was not localized to JM but diffusely to the entire plasma membranes. Moreover, co-expression of JP1ΔTM inhibited the JM-targeting of LTCC. Thus, this mutant serves as a dominant negative molecule inhibiting the JM-targeting of LTCC. To investigate the physiological significance of the JM-targeting of LTCC in vivo, JP1ΔTM was next expressed in the tibialis anterior (TA) and flexor digitorum brevis (FDB) muscles of C57BL/6 mice by using the adeno-associated virus (AAV) vector system. Ten days after infection, the expression of JP1ΔTM was observed in >80% of muscle fibers. Interestingly, the contractile force and specific force of TA muscle were significantly decreased at any stimulation frequency between (1-200 Hz) with the JP1ΔTM-AAV injection. However, no changes in cross sectional area were observed between the muscles injected and not injected with JP1ΔTM-AAV. These results indicate that the JM-targeting of LTCC is essential for the efficient EC coupling of the skeletal muscle *in vivo*.

Furthermore, the effect of JP1ΔTM-AAV on twitch and tetanus calcium transients of FDB muscle fibers were analyzed. JP1ΔTM expression significantly decreased the peak amplitude of calcium transients. Immunocytochamical analysis showed that JP1ΔTM was localized more abundantly to the sarcolemma than the T-tubule membranes in FDB muscle fibers as was the case for myotubes. Compared with control FDB fibers, the JP1ΔTM expressed-fibers showed more abundant immunoreactivity of CaV1.1 in the sarcolemma than T-tubular membranes. Finally, the proximity ligation assay revealed that JP1ΔTM expression significantly decreased the physical coupling between LTCC and RyR. These data clearly show that JPs support the physical coupling between LTCC and RyR, and thereby, ensure the efficient EC-coupling in the skeletal muscle.

Discussion & Conclusion

It has been proposed that the proper JM-targeting of LTCC is critical for the efficient EC coupling of the skeletal muscle. It was also reported that the C-terminus of $Ca_V1.1$ and $Ca_V1.2$ subunits was necessary for the proper JM-targeting of LTCC in myotubes (1,2). However, the molecular mechanism underlying the JM-targeting is still not fully elucidated.

Our knockdown experiments showed that it is JPs that support the proper localization and function of LTCC. We also showed biochemically the physical interaction of CaV1.1 with JPs via its C-terminal JPD. Therefore, it is likely that the interaction of JPs and the C-terminus of $Ca_V1.1$ subunits ensures the proper intracellular localization and function of LTCC.

JP1ΔTM inhibited the JM-targeting of LTCC. By using this tool, we showed that JP1ΔTM expression decreased the specific force of mouse skeletal muscles by disrupting the JM targeting of LTCC and thereby the interaction between LTCC and RyR.

From these results, we conclude that the physical interaction between the C-terminus of CaV1.1 and JPs is essential for the proper JM-targeting of LTCC, the physical interaction between LTCC and RyR and therefore the efficient EC coupling in the skeletal muscle.

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一般の皆様へ

骨格筋が正常に収縮するためには，L 型カルシウムチャネルが結合膜構造と呼ばれる部位に集積することが必要不可欠である。しかし，その集積の詳細な分子メカニズムは明らかになっていない。本研究では，結合膜構造に存在するジャンクトフィリンと呼ばれる分子が，L 型カルシウムチャネルに結合し，正常な結合膜への局在化や機能を調節していることを見いだした。今回得られた結果は，骨格筋の収縮メカニズムを解明する上で，重要な知見のひとつであると考えられる。

Epigenetic regulation in cell reprogramming and chimera formation.

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Summary Abstract

Our study will be the first of its kind to demonstrate: 1) a simple epigenetic modifier (VitC) could help derive a stable cell line from the developing PGCs 2) distinct pluripotent state can arise from the germline, in addition to "naïve state" in mouse ES/ iPS cells and the "Primed state" in conventional mouse EpiSCs (Epiblast Stem Cells).
Key Words：Primordial Germ Cells, Reprogramming, Epigenetic, Chimera formation

Introduction

In the mice, primordial germ cells (PGCs), the common precursor of oocytes and spermatozoa, are specified from competent epiblast cells receiving inductive signals from the extra-embryonic ectoderm. Thereafter, PGCs increase in number while migrating through the developing hindgut and finally colonize the emerging gonads, where they initiate differentiation into either oocytes or spermatozoa. One unique property of progenitors of germ cell lineages is they are susceptible for being converted to pluripotent cell types without the need to introduce exogenous reprogramming factors as normally used for obtaining iPSCs from somatic cell types.

Results

The primordial germ cells could be converted to pluripotent embryonic germ cells (EGCs) with the combination treatments of LIF, FGF2 and SCF factors. This suggests epigenetic forces driven by the combination of these growth factors may contribute to reprogramming of PGCs to a pluripotent state. It is unknown, however, whether direct manipulation with epigenetic modifier could endow PGCs with pluripotent features. In this study, we examined the effect of Vitamin-C on freshly isolated PGCs. Vitamin-C, also know as L-ascorbic acid, has recently gained considerable attention largely due to its role in facilitating the efficiency as well as quality of iPSC reprogramming (Esteban et al, Cell Stem Cell, 2010; Stadtfeld, Nature Genetics, 2012). In addition, Blaschke et al (Nature, 2013) demonstrated that Vitamin-C promotes ICM-like DNA methylation state in mouse ESCs in a Tet-dependent manner. Intriguingly, when Vitamin-C was supplemented in mouse ESC culture the levels of several germline genes were elevated. This promoted us to test Vitamin-C's direct effect on freshly isolated *in vivo* PGCs. To our surprise, while there was no visible colony observed in control culture, Vitamin-C supplementation alone led to the emergence of alkaline phosphatase positive colonies and subsequently the derivation of stable cell line from freshly isolated PGCs. We designated this newly derived cell line as VcPGCs. To our surprise, these cells can be propagated indefinitely *in vitro* and demonstrated pluripotency using teratoma formation assay. More interestingly, the pluripotent cell lines generated with Vitamine-C share distinct features compared to conventional EGCs and thus may represent a distinct pluripotent state arising from the germline. VcPGCs are pluripotent as demonstrated by successful formation of teratomas comprising cells from all three primary germ

layers. Strangely, after injected into blastocysts, unlike EGCs, VcPGCs couldn't form chimeras, indicating they exist in a distinct pluripotent state than EGCs. This chimeric-incompetency, however, could be overcome by culturing VcPGCs in a culture media contained another chemical substances, suggesting distinct epigenetic forces contribute to the new pluripotent state.

Discussion & Conclusion

This goes well with the trending concept that pluripotency is not discrete but rather continuous states in early embryos and there are many different pluripotent states and each may harbor unique features and advantages. Our study will be the first of its kind to demonstrate: 1) a simple epigenetic modifier (VitC) could help derive a stable cell line from the developing PGCs 2) distinct pluripotent state can arise from the germline, in addition to "naïve state" in mouse ES/ iPS cells and the "Primed state" in conventional mouse EpiSCs (Epiblast Stem Cells)

一般の皆様へ

我々はこの研究を通じて、小分子化合物 L-Ascorbic Acid（ビタミン C）の添加により、将来精子や卵子の元となる " マウス始原生殖細胞（PGCs: primordial germ cells)" から、新規多能性幹細胞が樹立することを見出しました（vcPGC 細胞)。ビタミン C は、DAN 脱メチル化に機能する Tet ファミリーと協調的に機能することが広く知られており、マウス始原生殖細胞は DNA 脱メチル化によるエピジェネティック修飾の変化により、新規多能性幹細胞として再プログラム化されていると考えられます。本研究の成果は、細胞の初期化プロセスにおけるエピジェネティック修飾の役割を解明する、非常に有用なモデルとなることが期待されます。

Analysis of T2DM pathogenic mechanisms caused by susceptibility genes using human iPS cells

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Summary Abstract

We have previously shown that a mutation at the Kcnq1 locus reduces pancreatic β cell mass in mice via epigenetic modulation. We found that disruption of Kcnq1 results in reduced Kcnq1ot1 expression as well as increased expression of p57. However, these results were obtained from mice. Therefore, to confirm that these mechanisms correspond to human, we tried to induce the differentiation from hiPSCs into pancreatic β cells using reported methods. As a result, each marker was expressed in the cells and glucagon was expressed in the final stage, which suggested that hiPSCs differentiated into pancreatic endocrine cells. In addition, KCNQ1OT1 expression was significantly increased in the differentiated Glucagon+ cells. This may suggest that pathogenic mechanism of diabetes which is due to KCNQ1OT1 is specific to pancreatic islets.
Key Words：hiPSCs, KCNQ1, pancreatic beta cell

Introduction

Recently, susceptibility genes for Japanese type 2 diabetes (T2DM), such as KCNQ1, have been identified. However, most mutations, those may be related to T2DM onset, are on the intron and thus T2DM pathogenic mechanisms haven't been understood well. We have reported that increased expression of p57 in pancreatic islets significantly reduced pancreatic beta cell mass and induced abnormal glucose tolerance in Kcnq1 mutant mice in whose islets the expression of non-coding RNA Kcnq1ot1 was decreased. However, these results were obtained from mice. Therefore, to confirm that these mechanisms correspond to human, we decided to induce the differentiation into pancreatic beta cells from human iPS cells (hiPSCs) and analyze the effects of each SNP on gene expressions.

Results

At first, we analyzed mutations of KCNQ1 in 11 hiPSC lines including 201B7 by sequencing. As a result of analyzing mutations on 11 hiPSC lines, 8/11 had a KCNQ1 mutation. From these cell lines, we selected 201B7 cell line that had a KCNQ1 gene mutation and then tried to induce the differentiation into pancreatic beta cells. When the cells were cultured with ActivinA and CHIR99021, the expressions of SOX17 and FOXA2 were confirmed by RT-PCR and immunostaining. Moreover, cell clusters were in suspension culture with NOGGIN, KGF and EGF. PDX1 and NKX6.1 were expressed in the cells, which suggested that hiPSCs differentiated into pancreatic bud. We confirmed that mutations of KCNQ1 didn't inhibit the differentiation from hiPSCs into pancreatic bud. Next, we treated the differentiated cells with forskolin and Alk5 inhibitor. In the treated cells, the expression of Neurogenin3 was found by quantitative real-time PCR and immunofluorescence. Furthermore, MAFA and MAFB expressions were shown in the differentiated cells. To confirm the differentiation into pancreatic endocrine cells, we examined the

expressions of insulin and glucagon by real-time PCR and immunofluorescence. These examination resulted in that glucagon expression, but not insulin expression, was confirmed both by real-time PCR and immunofluorescence. These results suggested that hiPSCs differentiated into pancreatic endocrine cells.

Our previous report has shown that Kcnq1ot1 expression in pancreatic beta cells plays an important role in the maintenance of pancreatic beta cell mass in mice. Thus, we believe that KCNQ1OT1 expression is dependent on each tissues. To confirm the importance of KCNQ1OT1 in pancreatic beta cells, we compared the expression of KCNQ1OT1 between undifferentiated hiPSCs and differentiated glucagon positive cells. Real-time PCR showed that KCNQ1OT1 expression was increased in differentiated glucagon positive cells compared to hiPSCs. This result suggested that KCNQ1OT1 expression was expectedly important in pancreatic endocrine cells.

Discussion & Conclusion

We have achieved the differentiation from hiPSCs into pancreatic endocrine cells and clarified that KCNQ1OT1 expression was up-regulated in those cells. However, the challenge of differentiation from hiPSCs into insulin positive cells was unfortunately failed. We have to change the protocol about the differentiation into pancreatic β cells. Hereafter, we try to induce the differentiation from hiPSCs with SNP mutations into pancreatic β cells and analyze the effects of KCNQ1 SNP on gene expressions.

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一般の皆様へ

日本人2型糖尿病患者では、膵β細胞の脆弱性が問題であることが明らかになっており、その原因の一つとしてKCNQ1遺伝子の変異が注目されています。我々は、Kcnq1変異マウスではKcnq1ot1という分子の量が減ることによって膵β細胞量が減少することを報告しましたが、ヒトでも同様のことが起こっているかは不明です。そこでヒトiPS細胞を膵β細胞に分化させ、マウスの実験結果と比較することにしました。今回の研究では膵内分泌細胞への分化を達成しましたが、膵β細胞への分化誘導には至りませんでした。今後はプロトコールを改良し、さらに実験を進めていく予定です。

Molecular mechanism of plant cell growth via NIMA-related kinases

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Summary Abstract

NIMA-related protein kinase 6 (NEK6) promotes directional cell growth through phosphorylation of tubulin and the resulting destabilization of cortical microtubules in the model angiosperm *Arabidopsis thaliana*. The basal land plant *Marchantia polymorpha*, has a single NIMA-related protein kinase (MpNEK1), which directs tip growth of rhizoids (the early rooting system of land plants) through microtubule depolymerization. Thus, NEK-dependent mechanism of directional cell growth might be evolutionarily conserved and contributes to the early rooting system during the colonization of the terrestrial environment by plants.
Key Words：Directional cell growth, Cell polarity, microtubule, kinase

Introduction

Plant cortical microtubules align perpendicular to the growth axis to determine the direction of cell growth. However, it remains unclear how plant cells form well-organized cortical microtubule arrays in the absence of a centrosome. In this study, we investigated the functions of *Arabidopsis* NIMA-related kinase 6 (NEK6), which regulates microtubule organization during anisotropic cell expansion. To obtain insight into the roles of NIMA-related kinases in land plant evolution, we also analyzed the function of NIMA-related protein kinase 1 (MpNEK1) in the basal land plant (liverwort), *Marchantia polymorpha*.

Results

The *nek6* mutant shows the defects in growth directionality of epidermal cells and various organs (Fig. 1). Quantitative analysis of cell growth in the *nek6-1* mutant demonstrated that NEK6 suppresses ectopic outgrowth and promotes cell elongation in different regions of the hypocotyl of *Arabidopsis*. Because microtubules regulate growth direction of plant cells, we analyzed microtubules in *nek6* mutant. Loss of NEK6 function led to excessive microtubule waving and distortion (Fig. 2). These distorted microtubules were generated by detachment of microtubules from the cell cortex. The depolymerization of detached microtubules was significantly decreased in the *nek6-1* mutant, demonstrating that NEK6 depolymerizes distorted microtubules after detachment from the cortex.

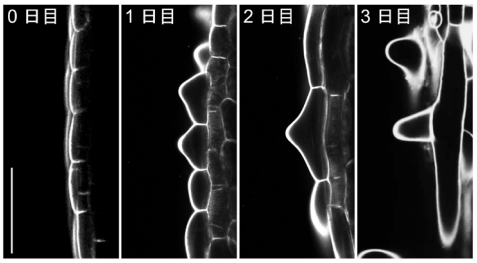

Fig.1. Growth polarity defect in *nek6* mutant

Live cell imaging showed that NEK6 localizes to the microtubule lattice and to the shrinking plus and minus ends of microtubules. Especially, NEK6 localized to the shrinking ends of microtubules detached from the cell cortex. To analyze gain-of-function phenotypes caused by *NEK6* overexpression, we used an estradiol induction system (an estrogen receptor-based XVE transactivator system, Fig. 3). In transgenic plants harboring estrogen-

inducible *NEK6*, estradiol treatment induced the expression of NEK6 approximately 100- to 1000-fold. The induced overexpression of *NEK6* destabilized cortical microtubules and suppressed cell elongation. This result support our hypothesis that NEK6 depolymerizes cortical microtubules.

Results

Furthermore, we identified five phosphorylation sites in □eta-tubulin that serve as substrates for NEK6 in vitro. Non-phosphorylatable alanine substitution of the phosphorylation site Thr166 promoted incorporation of mutant □eta-tubulin into microtubules (Fig. 4). This result suggests that Thr166 as a major regulatory site, which strongly affects microtubule localization of TUB4. To determine whether the kinase activity of NEK6 is indispensable for its function in directional cell expansion, the wild type and kinase-dead mutant NEK6-GFP fusion proteins were expressed in the *nek6-1* mutant under the control of *NEK6* promoter. The wild type NEK6-GFP complemented the ectopic outgrowth phenotype of *nek6-1*, whereas the kinase-dead mutant NEK6-GFP did not complement the *nek6-1* phenotype. This result demonstrates that NEK6 kinase activity is essential for directional cell expansion. Taken together, these results indicate that NEK6 promotes directional cell growth through phosphorylation of □eta-tubulin and the resulting destabilization of cortical microtubules.

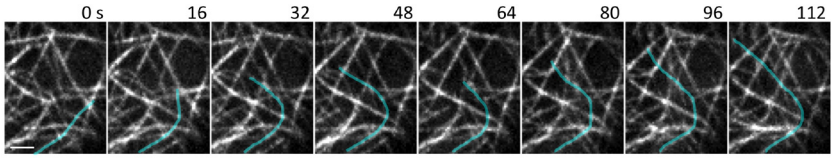

Fig. 2. Microtubule distortion in the *nek6* mutant

Tip growth, an essential process for plant morphogenesis and reproduction, is mediated by polarized accumulation of cellular materials coupled with turgor-pressure-driven growth. However, it remains to be solved how a single polarized growth site is determined and maintained. Here, we analyzed the function of NIMA-related protein kinase 1 (MpNEK1) in the liverwort, *Marchantia polymorpha*. We employed gene disruption by homologous recombination and found that Mp*nek1* knockouts showed a defect in growth directionality of rhizoid cells. In the wild type, rhizoid cells differentiate from the ventral epidermis and elongate by tip growth to form hair-like protrusions. In Mp*nek1* knockout mutants, rhizoids frequently changed their direction of growth and developed into a twisted and spiral morphology. MpNEK1-Citrine complemented the growth polarity defect and localized to the apical growing region in rhizoids. In the apical dome of rhizoids, the wild type exhibited fine microtubule meshwork, whereas tick microtubule cables extended into the apical dome of Mp*nek1* knockouts. Treatment with the microtubule-stabilizing drug taxol led to a phenocopy of Mp*nek1* knockouts. These results demonstrate that MpNEK1 directs tip growth of rhizoids through microtubule destabilization. Furthermore, MpNEK1 could rescue ectopic outgrowth of epidermal cells in *Arabidopsis nek6* mutant, strongly supporting evolutionarily conserved NEK-dependent mechanism of directional cell growth, which may contribute to the early rooting system during the colonization of the terrestrial environment by plants.

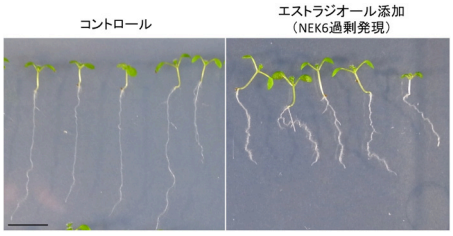

Fig. 3. Effect of NEK6 overexpression on plant growth

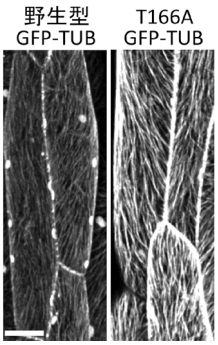

Fig. 4. T166A promotes microtubule localization of tubulin

Discussion & Conclusion

In this study, we showed that *Arabidopsis* NEK6 maintains polar plant growth by promoting longitudinal cell expansion and suppressing ectopic outgrowth. Both of these functions are mediated by NEK6-dependent microtubule disassembly. Our results demonstrate that NEK6 coordinates directional cell growth predominantly through depolymerization of cortical microtubules. NEK6 may participate in microtubule surveillance system to reduce excessive distortion of microtubules after detachment from the cortex. In the molecular level, NEK6 phosphorylates beta-tubulin incorporated into microtubules to destabilize existing microtubules.

Our functional analysis of Mp*NEK1* indicates its crucial role in directional growth of rhizoids. Mp*nek1* knockouts exhibited a defect in rhizoid growth directionality, but not in rhizoid elongation per se, implying specific function of Mp*NEK1* in the determination and maintenance of growth polarity. This is consistent with the role of AtNEK6 in directional expansion of epidermal cells. Actually, Mp*NEK1* could rescue outgrowth phenotype of *Arabidopsis nek6* mutant, clearly indicating the evolutionarily conserved NEK-dependent mechanism of cellular growth polarity. Therefore, land plant NEKs fundamentally regulate directional cell expansion and this primary function may have already been acquired during the early phase of land plant evolution.

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一般の皆様へ

植物は分裂組織から根や葉・花などの様々な器官を形成します。このプロセスでは、個々の細胞がどの方向にどれくらい成長するかが重要になります。植物細胞の成長方向は、細胞内の骨格である微小管が一定の方向に並ぶことで規定されます。しかし、微小管がどのようなしくみで一定の方向にきれいに並ぶのかわかっていません。私たちは、NEK というチューブリンタンパク質をリン酸化する酵素が、余分な微小管を除去して整理整頓することで、微小管がきれいに並び、細胞の成長方向が決定されることを見いだしました。このしくみは陸上植物の進化の初期に獲得されたと考えられます。

Molecular mechanism of overeating regulated by cellular stress response

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Summary Abstract

This study aims to identify the mechanism of bulimia induced by endoplasmic reticulum (ER) stress. Bulimia behavior began to appear within several hours after the administration of an ER stress agent and continued for approximately one day. The blood glucose level was slightly increased, that eating inhibitory hormone levels tended to be increased, and that eating acceleratory hormone levels were low after the ER stress loading. Based on these results, we suggest that ER stress-induced bulimia may not be caused by an abnormality of the hormones that regulate eating.
Key Words：ER stress & bulimia

Introduction

The detailed regulatory mechanism of feeding behavior on a full or an empty stomach has been elucidated by earlier research. However, much remains unknown about the mechanisms of bulimia and anorexia. We noticed an unexpected phenomenon: When a certain ER stress agent was administered to mice, bulimic behavior was induced. In our previous studies, we have already established our original tools for analyzing ER stress in live mice and at a molecular level. In this study, using these tools, we aim to discover a new possible role of cellular stress response molecules in the regulation of feeding behaviors.

Results

In this study, we aim to elucidate the relationship between the cellular stress response and feeding behavior at a molecular level. To this end, it is essential to investigate the dynamics of cellular stress response molecules in the brain area regulating feeding behavior (which has been studied very little so far). First, we need to compare the activation of important cellular stress molecule responses between bulimic model mice and normal mice. In essence, we have been performing conventional histological analysis using slices of part of the brain, including the feeding behavior center, and specific antibodies. We have also been approaching this problem by live imaging analysis in several kinds of mice using our original technology for visualizing cellular stress. At the same time, we have been examining whether bulimia is induced when an ER stress agent is injected in mice that lack cellular stress response specifically in the bulimic behavior center. By doing this, molecules responsible for inducing bulimia will be identified. Various knockout mice have either been produced in-house or purchased from other institutions and are already in use.

We have been investigating the activation status of neurons and molecules involved in feeding behavior in bulimic model mice, in parallel with previous experiments. Essentially, we have been comparing the functions of neurons in the hypothalamic paraventricular nucleus and the arcuate nucleus and the dynamics of leptin and ghrelin between bulimic model mice and normal mice. In

addition we have been investigating whether bulimic behavior of the relevant model mice is inhibited by stimulating leptin per se or leptinergic POMC/CART neurons. On the other hand, bulimia is reported to resemble narcotic addition; thus, functions of neurons involved in reward and pleasure should not be neglected. Therefore, we have also been analyzing the relevant bulimic model mice, focusing on dopamine receptors.

In our initial plan of experiments, we listed establishment of an ER stress-induced bulimic mouse model and investigation of dynamics of ER stress-related molecules in the feeding behavior center. Necessary data have been obtained from these experiments without problems, although not sufficiently. For example, with regard to the relationship between ER stress and bulimia, bulimic behavior caused by stress loading not only in mice of the single strain of C57BL/6 but also in several other strains of mice has been investigated to the level that warrants publication in journals. In addition, blood hormone levels and activation levels of ER stress-related molecules when bulimic behavior is induced have been investigated to a sufficient level to determine the next analytical strategy. Incidentally, the size of the stomach of mice to which an ER stress agent was administered 6 hours before testing is larger than that of mice to which physiological saline was administered. The stomach content of the former mice weighs approximately 3 times more than that of the latter. This phenomenon is fairly well reproducible. In fact, we have repeated this experiment more than one hundred times, and the result has never been reversed. In addition, sex-related differences have hardly been observed. Some may suggest that abnormalities of digestive functions are the cause of the enlargement of the stomach; however, for the time being, we have been focusing on feeding abnormalities as the cause of this phenomenon. The reason for this is that the mice that are administered ER stress agents fill their stomach with bedding material and feces in the breeding cage, even when they are not fed.

Discussion & Conclusion

We have primarily been conducting a study on ER stress to date, and have identified and analyzed the function of a molecule that senses ER stress, termed IRE1, in mammals (Iwawaki T. et al, Nat. Cell. Biol.), and developed an ER stress visualization technology in live mice (Iwawaki T. et al, Nat. Med.). Furthermore, recently, we have also been focusing on analyzing the regulation of phosphorylation/dephosphorylation of eIF-2α in pancreatic beta cells (Akai R. et al, Genes Cells.).

Cellular stress responses represented by the regulation of phosphorylation / dephosphorylation of eIF-2α have been studied in detail at the cellular and molecular levels. The mechanisms for regulation of feeding behavior on a full or an empty stomach have also been well understood to the level of being described in physiology textbooks. However, findings regarding molecules that function in cellular stress responses associated with the behaviors of mammals, except the finding of “rejection of nutritionally unbalanced feed,” have not been reported (Hao S. et al, Science and Maurin AC. et al, Cell Metab.). We believe that this interdisciplinary study will allow to link the molecular biology of cellular stress and research on feeding behavior regulation in the future.

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一般の皆様へ

満腹時および空腹時における摂食行動の制御機構は詳細なところまで明確になっている。しかしながらストレスが引き起こすとされる過食や拒食のメカニズムは殆ど分かっていない。私たちはマウスを用いた実験で次の意外なことに気が付いた。細胞の健康状態を悪化させるストレスを与えると過食行動が引き起こされるのである。この現象は「やけ食い」のような心的ストレス性の摂食異常を分子レベルで解明する糸口になるかもしれない。本研究を通じて私たちはストレス応答反応が果たしているであろう摂食行動制御に関する新たな役割を理解しつつある。

Analysis of a signal through ER required for the induction of axon regeneration

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Summary Abstract

The ability of specific neurons to regenerate their axons after injury is governed by cell-intrinsic regeneration pathways. However, the signaling pathways that orchestrate axon regeneration are not well understood. Here we show that DDR-2, an RTK containing a discoidin domain that is activated by collagen, and EMB-9 collagen type IV regulate the regeneration of neurons following axon injury in C. elegans. Further analyses suggest that DDR-2 modulates the SVH-2 - JNK MAP kinase cascade pathway via SHC-1 in the regulation of axon regeneration.
Key Words：axon regeneration, C. elegans, collagen

Introduction

Axon regeneration after nerve injury is a conserved biological process in many animals, including humans (1). The nematode *Caenorhabditis elegans* has recently emerged as a genetically tractable model for studying regenerative responses in neurons (2). Extensive studies over several years using this organism have revealed that a number of intrinsic and extrinsic signal transduction cascades, which are conserved from worms to humans, regulate axon regeneration (3).

Results

We found that DDR-2, a receptor tyrosine kinase (RTK) containing a discoidin domain, is required for efficient axon regeneration in *C. elegans* (4). At 24 hr after axon injury, the frequency of axon regeneration in *ddr-2* deletion mutant animals was reduced. We also monitored severed axons at a later time point (72 hr) after ablation. We observed that the frequency of axon regeneration in *ddr-2* mutant was increased at 72 hr after axon injury to a level, comparable to that of the wild type. Thus, in *ddr-2* mutants, initiation of axon regeneration after laser surgery is delayed. DDR-2 is expressed in several tissues including neurons, and regulates axon regeneration in a cell-autonomous manner. In mammals, DDRs are unique members of the family of RTKs in that they bind to and are activated by triple-helical collagen, the major component of the extracellular matrix (ECM). For this reason, we thought that the basement membrane collagens are likely to be candidates as ligands for DDR-2. In *C. elegans*, three collagens are associated with the basement membrane: the type IV collagens EMB-9 and LET-2 and the type XV/XVIII collagen CLE-1. We found that *emb-9*, but not *cle-1*, is required for the axon regeneration. The *ddr-2*; *emb-9* double mutant showed no further enhancement of axon regeneration defect, and the regeneration defect of *emb-9* could be suppressed by overexpression of DDR-2, indicating that *emb-9* acts upstream of *ddr-2* in axon regeneration. Genetic epistatic analysis also revealed that *ddr-2* acts upstream of *svh-2*, a Met-like growth factor RTK that acts through the JNK MAPK pathway (5). We also executed biochemical analysis using mammalian cultured cells and found that the cytoplasmic domain of DDR-2 can interact with SHC-

1, an adaptor protein required for efficient axon regeneration in *C. elegans* (6). The SHC-1 protein has two phopshotyrosine-binding domains, SH2 and PTB. Interestingly, interaction between DDR-2 and SHC-1 is independent of both the tyrosine kinase activity of DDR-2 and the phosphotyrosine-binding activities of SH2 and PTB domains in SHC-1. Further immunoprecipitation analysis revealed that SHC-1 also associates with the cytoplasmic domain of the tyrosine kinase SVH-2. In contrast to the case of DDR-2, a catalytically inactive mutant form of SVH-2 was unable to associate with SHC-1, suggesting that the interaction between SVH-2 and SHC-1 is dependent on the Tyr-autophosphorylation of SVH-2. We next investigated the functional significance of the SHC-1–SVH-2 interaction in the rescue of the *ddr-2* defect by *shc-1* overexpression. The axon regeneration defect caused by the *ddr-2* mutation was suppressed by overexpression of SHC-1 protein. However, a mutant SHC-1 protein that is unable to interact with SVH-2 failed to suppress the axon regeneration defect in *ddr-2* mutants. These results suggest that the binding of SHC-1 with SVH-2 is important for SVH-2 activity in the axon regeneration pathway.

We further found that a series of ER/Golgi glycosylation enzymes, including a sugar transporter, Galactose transferase and Fucose transferase, are required for efficient regeneration in *C. elegans*. Further analysis revealed that these factors regulate axon regeneration via N-glycosylation of the extracellular domain of DDR-2 protein.

Discussion & Conclusion

In this study we examined the role of DDR-2 in the regeneration of injured axons in *C. elegans*. We found that the *ddr-2* mutation delayed initiation of regeneration in D-type motor neurons after laser surgery. Similarly, the *emb-9* mutation, defective in the type IV collagen, affected the initiation of axon regeneration. Further data indicate that *emb-9* regulates axon regeneration via *ddr-2*.

Our genetic data also suggest that DDR-2, the *C. elegans* Shc adaptor protein SHC-1, and the HGF-like RTK SVH-2 function in the same genetic pathway regulating axon regeneration. When DDR-2 binds to collagen, SHC-1 acts as a bridging molecule linking SVH-2 to the DDR-2 cytoplasmic domain. This triggers SVH-1 growth factor-mediated activation of SVH-2 in a specific region, which in turn leads to the activation of the JNK pathway. Although collagens have been used in the nerve conduit in the treatment of nerve injury in humans (7), the molecular action of the collagen in axon regeneration remained obscure. Thus, our data may provide a cue to understand the role of collagen and downstream signaling in axon regeneration of other organisms including human.

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一般の皆様へ

神経細胞は、軸索という長い突起を介して電気信号を伝達しており、外傷などで軸索が切断されると神経として機能できなくなります。しかし、多くの神経は軸索を再生する能力を潜在的に持っています。今回、モデル動物である線虫 C. エレガンスを用いた解析により、コラーゲンが DDR と呼ばれるコラーゲン受容体を介して切断された軸索再生を促進することを新たに発見し、その分子メカニズムを解明しました。本成果で得られた基礎的知見は、神経再生促進技術の改善・改良の手助けになることが期待されます。

Development of molecular delivery system using cell membrane penetrating DNA nanostructures

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Summary Abstract

We develop a DNA nanostructure having a molecular needle to penetrate lipid membrane for delivering target molecules into the cell. We created the DNA nanostructure to introduce the target molecule to a liposome containing DNA amplification system and successfully initiated the reaction. The system will be employed for molecular delivery for control of the cellular functions.
Key Words：DNA nanostructure, delivery system, lipid membrane, cell function control

Introduction

Development of a new molecular delivery system is a fundamental issue for control of the cellular functions and responses. In this research, we develop basic technology for designing and functionalizing a DNA nanostructure, which can penetrate cell membranes using a nano-sized needle molecule, and applying them for delivery of specific molecules and control of cellular functions. Based on our DNA molecular technology, we designed and constructed DNA nanostructures for expression of various functions depending on the purpose. First, we develop a molecular system that can work in a liposome containing a biochemical reaction system. Then the system will be applied for delivery of target DNA, RNA and biomolecules for control of the cellular functions. By using these functionalized DNA nanostructures, we intend to develop a new molecular system fundamentally different from the conventional molecular delivery approach to the cells.

Results

We designed a cross-shaped DNA nanostructure with a four-helix bundled nano-sized DNA needle which can penetrate cell membranes. We used DNA origami technology for construction of the nanostructure to create a system to control binding and release of target molecules. We first investigated the interaction between the cross-shaped DNA nanostructure and lipid bilayer membrane, and visualized the behavior of the cross-shaped DNA nanostructure and their assembly on the lipid bilayer surface using high-speed atomic force microscopy (AFM).

After investigating the fundamental properties, we examined the interaction between the DNA nanostructure and liposome. First, in order to prove that the needle part penetrates lipid membrane, we prepared a liposome containing DNA amplification system which can be activated by specific DNA strand (trigger). We tested the incorporation of this trigger DNA strand by attaching to the ends of the needle. When the needle part with the trigger molecule penetrates the lipid membrane, the DNA amplification system inside should be initiated to start the reaction. We added the fluorescence-labelled cross-needle DNA nanostructure to the liposome, and observed the attachment of the DNA nanostructure onto the surface of the liposome using a confocal microscope. Then we used the trigger-attached cross-needle DNA nanostructure, and examined the fluorescence-based DNA

amplification in the liposome. Without the trigger strands, the amplification was not observed within the expected period. On the other hand, using the trigger-attached cross-needle DNA nanostructure, we clearly observed the amplification of the DNA inside the liposome. These results indicate that the needles penetrated the lipid membrane and consequently the trigger strand incorporated into the liposome initiated the DNA amplification reaction.

For increasing the efficiency of attachment onto the liposome surface, we incorporated cholesterol molecules to the cross-needle nanostructure for increase of the interaction with the lipid membrane. We also introduced photo-cleavable molecule to rapidly release the trigger strand inside the liposome. These will improve the efficient molecular delivery and reactions, and help creation of a new molecular system that incorporates target molecules into the liposome. In the next step, we will also examine the lattice assembly of the cross-needle structures for more efficient delivery of the target molecules into the liposome.

This cross-needle DNA nanostructure and delivery system will be applied to carry out target molecule delivery to the cells. We plan to examine whether this DNA nanostructure can attach to the cell surface, and confirm whether the needle structure can penetrate the cell membrane and introduce the target molecule into the cytoplasm.

Discussion & Conclusion

We have developed the cross-shaped DNA nanostructure having a four-helix bundled DNA needle, which attaches onto the liposome surface and penetrate the lipid bilayer membrane. Using the liposome containing the DNA amplification system, the system inside the liposome was activated by the addition of the cross-needle DNA nanostructure having the target DNA strand for initiation of the reaction. These results indicate that the target molecule can be incorporated via the needle part, and the molecule delivery and subsequent reaction was controlled using this developed DNA nanostructure. Using our DNA molecular technology, we successfully designed and constructed DNA nanostructures for the initiation of the reaction in the liposome. In addition, the DNA lattice assembly to attach onto the lipid membrane was created, and their formation was visualized by high-speed AFM. For cellular applications, we are planning to adopt the cross-needle DNA nanostructure and molecular system to deliver target DNA, RNA and biomolecules for control of the cell functions and responses such as induction of apoptosis (cell death) and suppression of gene expression.

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一般の皆様へ

本研究では、DNA ナノ構造体の構築技術を利用し、ターゲット分子を細胞内に送り込む分子輸送システムを開発する。DNA 鎖からなる分子針を持つ DNA オリガミ構造体を用いて、脂質二重膜との相互作用を高速原子間力顕微鏡 (AFM) によって観察した。次に、生化学反応系を導入したリポソーム（脂質膜）に、DNA 構造体を結合し、ターゲット分子が脂質膜を貫通し、リポソーム内の生化学反応を開始できることを見出した。これら構築した技術を使って、細胞膜を貫通し、細胞内に分子を送り込む新たな分子システムの構築を検討する。最終的に細胞死の誘導、遺伝子発現の抑制、免疫増強など細胞機能制御や細胞応答を見る。

Studies toward total syntheses of biologically active, bridged polycyclic meroterpenoids

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Summary Abstract

A stereoselective synthesis of C3-dehydrated berkeleytrione has been achieved through a polyene cyclization to construct the [7.3.1.$0^{2,7}$] tricyclic ring system and ring closing metathesis to fashion the six-membered ring as key steps.

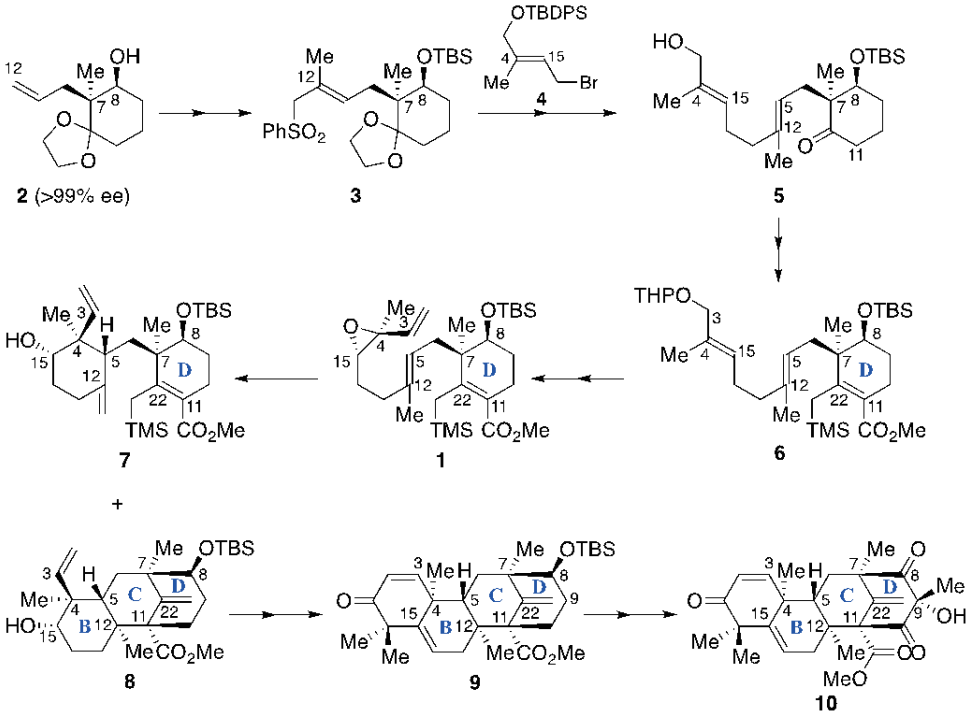
Key Words：meroterpenoids, bridged polycyclic compounds, polyene cyclization

Introduction

Berkeleyones are a family of meroterpenoids isolated from the extremophilic fungus *Penicillium rubrum* by Stierle and co-workers. Berkeleytrione, isolated along with berkeleydione in 2004, is the prominent member of the family and is reported to inhibit matrix metalloproteinase-3 and caspase-1. Having developed a method for the construction of [7.3.1.$0^{2,7}$] tricyclic ring system by a biomimetic polyene cyclization reaction,[1] we addressed the synthesis of berkeleytrione.

Results

Epoxyallylsilane **1** was chosen as the key synthetic intermediate to most likely lead to the completed synthesis, since the siloxy group at C8 would be disposed equatorially in the chair–chair–



chairlike transition state of the crucial polyene cyclization reaction. The synthesis of the cyclization precursor **1** was initiated with known alcohol **2** with >99% ee. A six-step sequence involving TBS protection of the hydroxyl group at C8, ozonolysis, Horner−Wadsworth−Emmons reaction under Masamune conditions, reduction with DIBALH, phenyl sulfide formation and Mo(VI)-catalyzed oxidation provided phenyl sulfone **3**, alkylation of which with allyllic bromide **4** was followed by removal of the phenyl sulfonyl and TBDPS groups and acid hydrolysis of the cyclic acetal to give ketone **5**. After interim protection of the hydroxyl group as a THP ether and installation of an ester functionality at C11 with Mander's reagent, (trimethylsilyl)methyl group was uneventfully introduced by a sequence involving enol triflate formation and Kumada−Tamao−Corriu coupling using (trimethylsilyl) methylmagnesium bromide. Selective liberation of the allylic hydroxyl group in allylsilane **6**, followed by Sharpless asymmetric epoxidation, Dess−Martin oxidation and Wittig olefination completed the synthesis of epoxyallylsilane **1**.

With epoxyallylsilane **1** in hand, we then proceeded to investigate the key cyclization reaction. Although the anticipated reaction proceeded to give desired tricyclic compound **8** upon treatment of epoxyallylsilane **1** with Et_2AlCl in dichloromethane at –78 °C, monocyclized product **7** was obtained as a major product under these conditions. After considerable experimentation, we found that the allylsilane **7** could be cyclized with the aid of a Lewis acid, providing tricyclic compound **8** in acceptable yield. The resultant alcohol was oxidized in preparation for the A ring construction.

While a propargyl group could be introduced at C15, attempts to convert the resultant enyne to the desired tetracyclic compound met with little success. After a survey of nucleophiles and ring construction methods, we found that the six-membered ring could be formed by the ring-closing metathesis of the diene obtained by the addition of an organostannane reagent. The hydroxyl group at C15 could be eliminated upon mesylation, and a two-fold methylation almost completed the functionalization of the A ring.

To functionalize the D ring, deprotection of the C8 TBS ether was followed by oxidation to give the corresponding C8-ketone. After installation of a methyl group at C9 by the enolate alkylation, successive oxidations furnished triketone **10**, which only needs to be hydrated for the total synthesis of berkeleytrione.

Discussion & Conclusion

While introduction of a siloxy group at C8 made the concerted cyclization somewhat difficult to occur, it was found that desired tricyclic compound **8** could be formed by a stepwise approach. It is speculated that monocyclized product **7** adopted the suitable conformation required for the cyclization due to the steric preference of the siloxy group for the equatorial position.

We have also demonstrated that the product **8** can serve as a key intermediate for the stereoselective synthesis of berkeleyone family meroterpenoids. Efforts to convert advanced intermediate **10** to berkeleytrione and its derivatives are in progress and results will be reported in a future.

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一般の皆様へ

バークレーオン類は、米国の銅鉱山跡にできた湖で採取された菌から見つけられた化合物群ですが、バークレージオンが抗がん作用を示すことで知られているなど、創薬のリード化合物として注目を集めています。本研究では、菌がこれらの化合物を作り出す方法を模倣することで、これらの化合物群を化学合成することに挑戦しました。本研究は、当該天然物はもちろんのこと、類縁構造を持つ化合物群の供給に道を拓くものであり、将来的な医薬品開発につながることが期待されます。

Functional significance of chandelier cell in cortical microcircuit

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Summary Abstract

Inhibitory synapse function in the rat frontal cortex are investigated using cell attached recording with GABA uncaging method. The GABA may effectively hyperpolarize the membrane potential of the postsynaptic cell dependent on its excitability, but almost likely it does not induce membrane depolarization in mature layer 5 cortical pyramidal cells.
Key Words：neural network, neocortex, microcircuit, GABA, electron microscopy

Introduction

Cortical microcircuits are very complex and a detailed understanding may be impossible with present techniques. A single neuron receives thousands of synaptic inputs onto their dendrites and somata, and the synaptic inputs are integrated on dendritic trees to initiate spikes at the soma. Cortical inhibitory synapses innervate different sites on target pyramidal cell: soma, proximal dendrite, distal dendrite, spines or axon initial segments. The inhibition is known to counter somatic EPSPs and control spike initiation. However the role of inhibitory inputs on the axon initial segment is in controversial either excitatory or inhibitory. We pursued this question in GABAergic inhibition effect on layer 5 pyramidal cell in rat frontal cortex, which possibly due to GABAergic chandelier/ basket cells.

Results

GFP construct was injected into lateral ventricle of rat embryonic brain using in utero electroporation method to label layer 5 pyramidal cells in the rat frontal cortex. Brain slices of 300 μm thick were obtained from the rat at postnatal 21 - 28 days. Some layer 5 pyramidal cells were successfully showed the GFP. Membrane potential of the GFP labeled layer 5 pyramidal cells was measured using cell attached electrode and ramp current injection (Verheugen JAH, Fricker D, Miles R**,** *J Neuroscience* (1999) **19**:2546-2555). We found the membrane potential of the pyramidal cells was between -85 and -90 mV in control, and was not changed at GABA uncaging. The axon initial segment of the pyramidal cells was uncaged, but no significant difference in the membrane potential was found. This indicates that the GABA effect is probably neither excitatory nor inhibitory at the postsynaptic cell of the resting state. To depolarize the membrane potential, we applied glutamate puff on the pyramidal cell, then GABA uncaging was applied. We found the depolarized membrane potential observed under the glutamate puff condition was hyperpolarized after GABA uncaging dependent on the location. The GABA hyperpolarizing effect by the uncaging was only found in soma or the dendritic tree where the glutamate puff was applied. These results indicate that the GABA inhibition is only effective on neuronal compartments where the membrane potential is depolarized by excitatory signal. We assume the membrane potential of the axon initial segment of the pyramidal cell is probably quite similar as its somatic membrane potential, so the GABA

release on the axon initial segment by the chandelier cell can act as an efficient inhibition at only postsynaptic pyramidal cells in highly active state.

The functional significance of GABA action can be considered thoroughly with morphological evidence of excitatory/inhibitory synapse distribution on the neuron. To understand the synaptic inputs distribution on the layer 5 pyramidal cell, we investigated synapse density on somata and dendrites of neurons stained with nickel-DAB after whole cell recording of slice preparation of the rat frontal cortex using combined three-dimensional reconstructions from light and electron microscopic observations. Somata and dendrites with identified spines and synaptic inputs were reconstructed three-dimensionally (Fig. 1), and synapse distributions were analyzed and quantified. Spine density of the apical dendrite of the pyramidal cells were low in the proximal dendrites and increased gradually to an apical dendritic bifurcation, and decreased gradually to the tips of the tuft dendritic trees. The basal dendritic trees also showed the similar spine distribution at the peak in about 100 μm from the soma. The synapse also showed the similar distribution as the most spine receiving one synaptic input. GABAergic/non-GABAergic synapse distributions on these neurons were analyzed using post-embedding GABA immunohistochemistry with 15 nm colloidal gold labeled secondary antiserum using serial ultrathin sections of large volume electron micrograph data set obtained with automated tape-collecting ultramicrotome (ATUM)/scanning electron microscope (SEM) system. We reconstructed dendritic trees 3 dimensionally from the serial thin sections and found GABAergic terminal contacted on the spine head for about 3 %, on dendritic shaft for about 4% among all synapses. The non-GABAergic terminal innervated mostly spine head (88 %) and only rarely the dendritic shaft (5 %). We estimated that a layer 5 pyramidal cell in rat frontal cortex receives about 20,000 excitatory and 2000 inhibitory synapses.

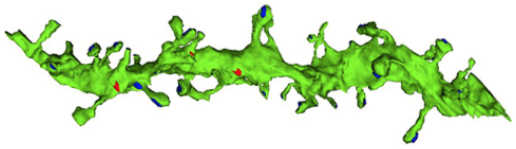

Fig. 1 3D reconstructed pyramidal cell dendrite GABAergic synapses are found on the dendritic shaft (red) and non-GABAergic synapses are on spines (blue).

Discussion & Conclusion

These results indicate that inhibition of the somatic/axon initial segment by GABA release from basket cell/chandelier cell axon terminals can hyperpolarize the membrane potential only when the postsynaptic target cell is in depolarized state due to intense excitatory synaptic inputs. The postsynaptic layer 5 pyramidal cell receives and integrates a huge number of excitatory/inhibitory inputs on its dendritic branches, axon initial segment and soma elaborately. They may effectively hyperpolarize the membrane potential of the postsynaptic cell dependent on its excitability, but almost likely GABA release does not induce membrane depolarization in mature layer 5 cortical pyramidal cells.

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一般の皆様へ

大脳皮質は多くの領域から構成され，それぞれが機能分担をすることで知覚，運動，思考といった我々の知的活動を支えています。大脳皮質の局所神経回路は、多種類の神経細胞と他の領域からの入力線維から構成されており、非常に複雑な情報処理をしていると考えられています。この仕組みを知るためには、皮質内神経回路の構造と機能を明らかにする必要があります。本研究では、シャンデリア細胞やバスケット細胞と呼ばれている大脳皮質の抑制性神経細胞の機能的役割に焦点をあて、この局所神経回路の機能構造を解析しました。これらの神経細胞は、ターゲットの錐体細胞の活動に強力な抑制効果をもたらしているという結果を得ました。

Characterization of T-follicular regulatory cells in human blood and tumor tissues

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Key Words：Immune regulation, Regulatory T-cells (Tregs).

Introduction

Regulatory T-cells (Tregs) are vital for the maintenance of immune homeostasis. T-follicular helper (Tfh) cells are responsible for the generation of high affinity antibody by B-cells. As a result control of these cells is critical to vaccination, antimicrobial immunity and antibody driven autoimmunity such as SLE. Tregs can also undergo a similar pathway of differentiation as Tfh to become T-follicular regulatory cells (Tfr). In mice it has been demonstrated that Tfr are critical for the control of Tfh but Tfr are still poorly understood, particularly in humans. We have made an important discovery of a new population of Foxp3 expressing regulatory T-cells (Tregs) present in human blood and organs.

Results

Naïve regulatory T-cells can be identified by their expression of markers such as CD45RA, CCR7 and lack of CD45RO. The chemokine receptor CXCR5 is expressed by memory and effector T-cells, most notably by T-follicular helper cells. As expected $Foxp3^-$ Naïve T-cells do not express CXCR5. However, we have discovered a novel population of $CXCR5^+CD45RA^+CCR7^+Foxp3^+$ "Naïve" Tregs. These cells make up a significant fraction (around 50%) of CD45RA+ Naïve Tregs in adult human blood. These cells were also found in the liver, spleen and tonsils, but not in tumor samples. Additionally CXCR5+CD45RA+ Tregs are completely absent from cord blood and enriched in the blood of older donors suggesting that this is not a truly naïve population but possibly an early precursor of CXCR5+CD45RA- T-follicular regulatory cells. RNA sequencing reveals that CXCR5+ and CXCR5- Naïve Tregs are extremely similar but that CXCR5+ Naïve Tregs have enhanced expression of the suppressive molecule TIGIT. In vitro suppression assays confirm that CXCR5+ Naïve Tregs have increased suppressive activity in comparison to CXCR5- Naïve Tregs.

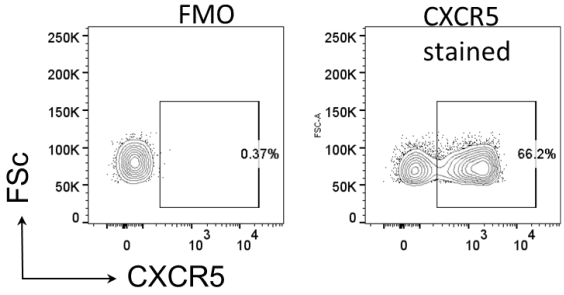

Figure 1: CXCR5 staining of CD45RA+Foxp3+ Tregs from PBMC. Left panel is staining control

Further to this, I also completed work assessing CXCR5+ CD45RA- effector Tfr, described in

human blood. Here I found that these cells termed circulating Tfr (cTfr) gain a transcriptional signature equidistant between circulating Tfh (cTfh) and effector Tregs (eTregs).

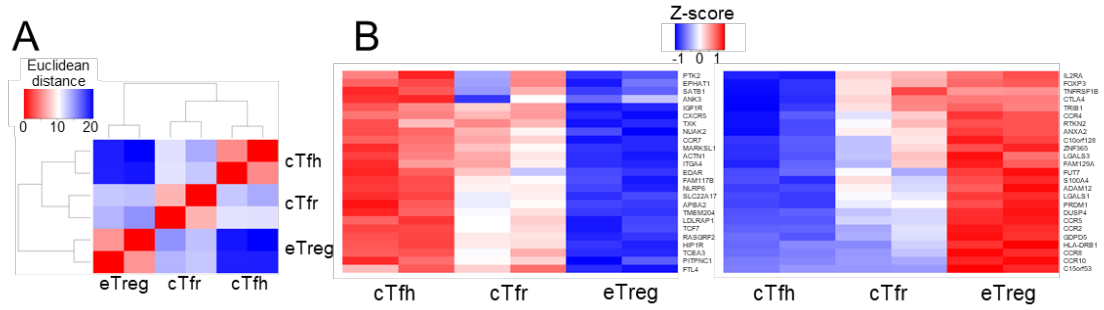

Figure 2: RNA-sequencing of circulating Tfh, Tfr and effector Tregs from human PBMCs. A) Euclidean distance between gene expression profiles. B) Heatmaps of most differentially expressed gene between cTfh and eTregs. cTfr show a mixed expression pattern.

Discussion & Conclusion

This work has yielded two main findings.

1) The discovery of a Novel subset of CXCR5 expressing CD45RA+ regulatory T-cells found in human. This work is ongoing but the data thus far is compelling. We now plan to carry out further experiments to better define the functional role of these cells and determine if they are precursors or activated Tfr cells or another type of memory Treg.
2) The characterization of circulating CXCR5+ CD45RA- Tfr in human blood. These cells have a gene expression pattern

   Characterization of Tfr is likely to be an important step to better defining their role in autoimmunity. This data has been combined with my other work on Murine Tfr cells and has been recently accepted for publication in PNAS (Wing et al. 2017).

   Additionally, it is currently believed that CD45RA+ Tregs are a naïve population and clinical trials to expand Tregs are using these cells on this basis. It now seems possible that a large proportion of these cells are not truly naïve but may instead represent a novel memory population with some naïve characteristics.

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A distinct subpopulation of CD25 negative T-follicular regulatory cells localizes in the germinal centers.

Accepted for publication in PNAS, 2017.

一般の皆様へ

制御性 T 細胞（Treg）は、免疫恒常性を維持する上で重要な役割を果たしている。 Treg のサブセットである Tfr は、抗体反応を制御することに特化しているが、特にヒトにおいてはよく理解されていない。本研究では、ヒト血液中の Tfr の詳細な特徴を明らかにした。この成果は、SLE および関節炎などの自己免疫疾患における役割を理解する上で重要である。 さらに、以前はナイーブな Treg であると考えられていた細胞の大多数が、実際には新しいメモリー Treg 集団である可能性があることを示し、これらの細胞の免疫学的役割およびその制御の理解と応用に重要である。

Conformational plasticity of JRAB regulates transition between mesenchymal and amoeboid invasiveness in cancer cells.

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Summary Abstract

During cancer metastasis and invasion, either MAT (Mesenchymal Amoeboid Transition) or AMT (Amoeboid Mesenchymal Transition) is often observed. The dramatic morphological changes based on actin cytoskeletal dynamics are involved in the switching of mode of cancer cell migration. We recently found that JRAB (Junctional Rab13-binding protein)/MICAL-L2 (molecules interacting with CasL-like 2), which is an effector protein of Rab13 GTPase, spatiotemporally regulates actin cytoskeletal reorganization and also identified that JRAB changes its conformation via Rab13, followed by interplay with several actin-binding proteins. Here we provided a new concept that the conformational plasticity of JRAB plays a pivotal role in the morphological changes leading to the switching of mode of cell migration on the basis of our data derived from a robust approach combining computational biology, bioinformatics, biomechanics, biochemistry, live imaging, and cell biology.

Key Words : cancer metastasis and invasion, morphological change, conformational plasticity of JRAB, actin cytoskeletal reorganization

Introduction

During cancer metastasis and invasion, cancer cells change their morphology to circumvent the effects from the extracellular environment. Dramatic morphological change, such as MAT (Mesenchymal Amoeboid Transition) or AMT (Amoeboid Mesenchymal Transition), is based on actin cytoskeletal dynamics and regulates the switching of the mode of cancer cell migration. Some kinds of cancer cells form cluster and move as a single unit. The morphological change would be occurred in such type of cell migration, termed collective cell migration, especially at the front of the cell cluster. We have identified JRAB (Junctional Rab13-binding protein)/MICAL-L2 (molecules interacting with CasL-like 2) as an effector protein of Rab13, a member of the Rab family small GTPases. Our previous studies have shown that JRAB may spatiotemporally regulate the actin dynamics. In this study, we focused on the conformational plasticity of JRAB in collective cell migration, to explain the mechanism of the switching of mode of cell migration based on the morphological change of the cells located at the leading edge.

Results

We have previously found that JRAB has the intramolecular interaction between its N-terminal and C-terminal regions, and that the binding of Rab13 releases this interaction, resulting in the conformational change; i.e., the closed to the open form, but we have obtained no solid evidence. In this study, we successfully generated a structural model of JRAB and thereby provided the firm evidence for a JRAB conformational change induced by Rab13 using an approach that combines

bioinformatics and biochemistry. We thereby revealed that Rab13 and JRAB-N compete for binding with JRAB-C and that this competition leads to a release of the intramolecular interaction. We also developed a JRAB/MICAL-L2 fluorescence resonance energy transfer (FRET) probe and found using cells expressing this FRET probe that JRAB changes its conformation depending on the position of the cell within the population. Previously we prepared JRAB mutants fixed in a specific conformation (i.e., the open form; JRABΔCC or the closed form; JRABΔCT). Our present results demonstrated that JRABΔCT and JRABΔCC adopt a closed and open form, respectively, based on results from several methods (pull-down assay, ANS analysis, and gel filtration chromatography). In addition, we found that cells expressing JRABΔCT or JRABΔCC exhibit distinctive morphology at the leading edge of each cell group. Formation of thick actin bundles at the free edge is enhanced by JRABΔCT, but inhibited by JRABΔCC. We next examined the detailed structure of cells at the free border using super-resolution microscopy. Super-resolution images revealed the fine meshwork of filamentous actin (F-actin) generated by JRABΔCC. In cells expressing JRABΔCT, several F-actins radiated from the thick bundle and seemed to project onto the growth surface adjacent to the cell. Therefore, we analyzed the structures involved in the cell–matrix adhesion, that is, focal adhesions (FAs). Cell clusters expressing JRABΔCT exhibited prominent staining of long, vinculin-positive structures at the free edge, whereas those expressing JRABΔCC exhibited punctate staining of shorter structures. Long and short vinculin-positive structures indicate mature and immature FAs, respectively. These results suggest that closed JRAB may form mature FAs at the tip of F-actins radiating from the thick F-actin bundle. Moreover, we tried to reveal the relationship between morphological change regulated by conformational plasticity of JRAB and the switching of mode of cell migration. For that, we performed the wound healing assay using the cell groups expressing JRAB mutants and then the obtained results were subjected to computational analysis using optical flow followed by kurtosis and PCA analysis. From these, we found that JRABΔCT and JRABΔCC exhibit different mode of cell migration. Groups of cells expressing JRABΔCT migrated strongly in a fixed direction. By contrast, in groups expressing JRABΔCC, the cells formed ruffles one by one and moved in random directions; former is akin to precision dancing, latter resembles *awa-odori*, like a Rio Carnival in Japan. We also showed by new methods based on biomechanics that JRABΔCT causes excessive rigidity, whereas JRABΔCC causes a loss of directionality due to lack of rigidity. Thus, our a robust approach combining cell biology, live imaging, computational biology, and biomechanics brings a novel concept that the conformational plasticity of a single molecule, JRAB, regulates morphological change of the cells leading to the switching of the mode of cell migration.

Discussion & Conclusion

In this study, we demonstrated that the dynamic morphological change followed by switching of mode of cell migration is regulated through the conformational plasticity of a single molecule, JRAB/MICAL-L2. We do not know the detailed molecular mechanism allowing the morphological change depending on the conformation of JRAB. However, there are several possibilities. First, we found several actin-binding proteins (actinin-1, actinin-4, and filamins) preferentially interacting with the open form of JRAB. Such interplay between them may be the key to solve the question. Second, linkage between JRAB and Rho family protein, which is one of the representative regulatory systems of actin cytoskeletal rearrangement, should not be excluded. Actually, MAT is occurred followed by activation of RhoA signaling pathway, whereas activation of Rac signaling pathway causes AMT. Thus, there may be switching of the activation of Rho family protein under the conformational

change of JRAB; the closed form of JRAB may generate a thick actin bundle with radial F-actins leading to the generation of traction force via activated RhoA, whereas the open form of JRAB may generate a ruffle under the effect of Rac. These possibilities need to be pursued elsewhere. Finally, these results will be applied to the switching of the mode of cell migration in the case of MAT or AMT.

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一般の皆様へ

最近、癌細胞が転移・浸潤していく上で、細胞の形態および運動能の変換が重要な役割を担っていることが明らかになりつつある。したがって、癌の転移・浸潤過程で認められる細胞形態・運動能の変換の分子機構を理解し、その変換を自由に調節することが可能となれば、癌の転移・浸潤に対する新たな治療法の開発や創薬にもつながることが期待される。これまで本分子機構には多くの分子が関与し、それらの分子間相互作用が複雑に絡み合って成立すると考えられていたが、本研究では JRAB というたった1分子の構造変化に依存したアクチン細胞骨格の再編成が細胞の形態・機能の変換を時空間的に制御しているという独創的な成果が得られた。

Development of apoptosis inhibitor targeting mitochondria

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Summary Abstract

Bongkrekic acid is an inhibitor of adenine nucleoside translocator and also known as an inhibitor of apoptosis. In order to develop novel potent apoptosis inhibitor based on our total synthesis of BKA, we developed the stereocontrolled allene-Claisen rearrangement affording multisubstituted 1,3-dienes. Furthermore, we applied to synthesize BKA analogues for the structure-activity relationship.

Key Words：mitochondria, apoptosis, structure-activity relationship

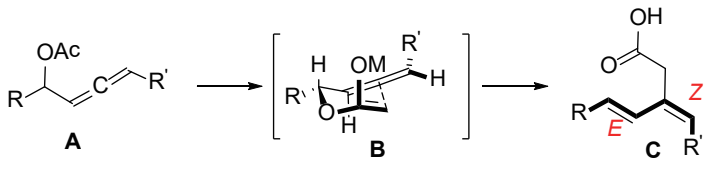
Introduction

Bongkrekic acid (BKA) was discovered as the content responsible for fatal food poisoning from the fermented coconut product. Among the numerous types of bioactivity reported for BKA, it strongly binds to the adenine nucleotide translocator (ANT) residing in the mitochondrial inner membrane, which results in the inhibition of apoptosis. In order to develop apoptosis inhibitor, we examined the synthesis of BKA analogues for the structure-activity relationship based on our total synthesis of BKA.

Results

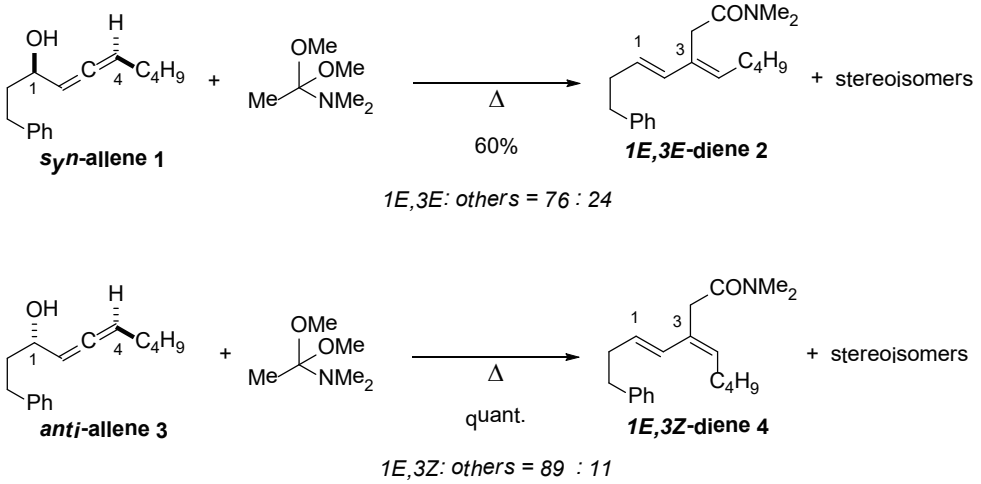
We previously reported the total synthesis of BKA via an efficient convergent strategy based on successive three-component coupling reactions, including a Julia-Kocienski olefination and a Suzuki-Miyaura coupling [1]. However, the final steps to construct the (*2Z,4E*)-3-substituted-2,4-dienoic acid structure of BKA, also known as the 1,3-diene moiety, remained problematic due to the instability and facile isomerization. As such, the development of practical and efficient methods for the stereoselective synthesis of 1,3-dienes is necessary.

The Claisen rearrangement provides a convenient method for the preparation of a wide range of substituted alkenes with reliable positional selectivity and generally high levels of geometrical control. As a number of synthetic approaches to 1,3-dienes have been developed via the Claisen rearrangement, we propose that an allene-Claisen rearrangement approach can be used to construct 1,3-dienes possessing the substitution patterns and functional groups present in BKA in a stereoselective manner. As shown in Scheme 1, starting from allene **A**, the [3,3]-sigmatropic rearrangement of **B** would procced in opposite direction from the allene substituent (R') to preferentially furnish 1,3-diene **C** due to steric repulsion between the R' group and the ester enolate.



Scheme 1 The allene-Claisen rearrangement.

To evaluate the feasibility of the allene-Claisen rearrangement strategy, we examined the Eschenmoser-Claisen rearrangement of both *syn*- and *anti*-allenyl alcohols (**1**, **3**), which were prepared by the known protocol in a stereoselective manner (Scheme 2). When *syn*-allenyl alcohol **1** was heated with *N,N*-dimethylacetamide dimethylacetal, *(1E,3E)*-1,3-dienyl amide **2** was predominantly obtained in 60% yield as a 76:24 mixture of stereoisomers. Furthermore, the reaction of *anti*-allenyl alcohol **3** gave an 89:11 mixture of 1,3-dienes *(1E,3Z)*-1,3-diene **4** and other stereoisomer in quantitative yield. Notably, the *(1E,3Z)*-1,3-diene possessing the same stereochemistry as BKA was obtained from the *anti*-allenyl alcohol in a high yield and a highly

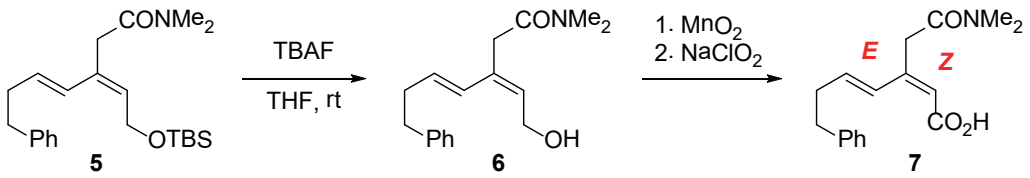


selective manner.

Scheme 2 The stereocontrolled allene-Claisen rearrangement of *syn*- and *anti*- allenyl alcohols.

We assume that the major stereoisomers can be likely produced via the chair-like transition states. In the case of the *syn*-allenyl alcohols, the chair-like transition state, which bears a pseudo-equatorial R-substituent at the C-1 position, is more favorable than other transition state, which bears a pseudo-axial R-substituent due to 1,3-diaxial repulsion. This ultimately results in the preferential formation of (*1E,3E*)-1,3-dienes. Similarly, *anti*-allenyl alcohols proceed through the chair-like transition state, which bears a pseudo-equatorial R-substituent, to selectively afford (*1E,3Z*)-1,3-dienes.

We next examined the feasibility of applying this reaction in the synthesis of BKA analogs (Scheme 3). Initially, TBS ether of 1,3-diene **5** was deprotected by treatment with tetrabutylammonium fluoride (TBAF) to produce dienyl alcohol **6** in quantitative yield. Subsequent oxidation of the primary alcohol of **6** under mild conditions with MnO_2, followed by Pinnick oxidation, gave carboxylic acid **7** in a modest yield without isomerization. As we previously reported that the dienoic acid-containing partial structure of BKA inhibited mitochondrial functions [2], our developed method is expected to provide useful and important insights into the structure-activity relationship of



BKA.

Scheme 3 The synthesis of BKA analogues via allene-Claisen rearrangement.

Discussion & Conclusion

In conclusion, we successfully developed an efficient and stereocontrolled synthetic method for the preparation of multisubstituted 1,3-dienes through a stereospecific allene-Claisen rearrangement. This novel route is expected to address the issues surrounding the preparation of substituted 1,3-dienes, such as their inherently instability under oxidation conditions. In addition, it is noteworthy that *(1E,3Z)*-1,3-dienes possessing the same stereochemistry as BKA were obtained from *anti*-allenes in high stereoselectivity. Further studies will expand this approach to the total synthesis of natural products possessing highly functionalized conjugated dienes. Furthermore, our present work provides important insights into the SAR of BKA and a useful biological tool for the mechanistic investigations of apoptosis.

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一般の皆様へ

アポトーシスは、生理的な細胞死とも呼ばれ、癌、エイズ、アルツハイマー病など多くの疾病に関わっており、アポトーシス阻害剤はこれら難治性疾患の治療薬として期待されています。ボンクレキン酸（BKA）は、ミトコンドリアに作用するアポトーシス阻害剤として知られていますが、非常に高価で大量入手が難しいために詳細な作用機序は未だ十分には解明されていません。本研究では、我々の開発したボンクレキン酸の全合成を足掛かりに、大量供給法の確立および高活性な新規アポトーシス阻害剤の創製を目指しています。

Cortical synaptic plasticity for coexistence of chronic pain and emotions

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Summary Abstract

Chronic pain is an unpleasant sensation and produce synaptic plasticity in the CNS. The anterior cingulate cortex (ACC) is a critical area for nociception and chronic pain. Here, we studied if chronic pain model could produce plastic changes in inhibitory synaptic transmission in the mice ACC. Using whole-cell patch-clamp recording, we found that chronic inflammatory pain model reduced the frequency of spontaneous inhibitory postsynaptic currents. Using western blot analysis, we found that vesicular GABA transporter was reduced in the model. These results suggest that chronic pain model mainly produced GABAergic synaptic plasticity in the presynapse of the ACC.
Key Words：Chronic pain, anterior cingulate cortex, GABA, synaptic plasticity

Introduction

The ACC is a critical cortical area for nociception, chronic pain and emotions such as fear and anxiety. Cortical area including the ACC is composed of excitatory and inhibitory neurons and produce neural network. Chronic pain models show short-term or long-term excitatory plasticity in excitatory neurons in the ACC. However, the synaptic mechanisms of GABAergic transmission for chronic pain are not fully understood, yet.

Results

In this study, we examined three major experiments.

First, we made a major chronic inflammatory pain model and confirmed the mechanical hypersensitivity 2-3 days after the injection.

Next, we performed whole-cell patch-clamp method, and recorded spontaneous and miniature inhibitory postsynaptic currents (sIPSCs & mIPSCs) on pyramidal neurons in layer II/III of the adult mice anterior cingulate cortex (ACC).

Finally, we studied the possible alterations of GABAergic transmission related proteins in the presynapse and postsynapse of the mice ACC. We found that vesicular GABA transporter (vGAT) was reduced in the presynpase of CFA model.

In contrast, $GABA_A$ receptors subunits including $\alpha1$, $\alpha5$, $\beta2$, $\gamma2$ and δ did not change between control and CFA groups.

Chronic inflammatory pain model reduced inhibitory synaptic transmitter release in the ACC

We first made chronic inflammatory pain model using adult mice (male C57BL/6, 8-12 week-old). Complete freund adjuvand (CFA) is a major inflammatory pain model so that we injected the CFA in the left hindpaw (Koga *et al.* 2015, Zhao *et al.* 2006). The 2-3 days after the injection of CFA, we confirmed the hypersensitivity on mechanical stimulation using von Frey filaments in CFA-induced

inflammatory pain model and control group. CFA-induced inflammation mice significantly reduced mechanical thresholds compared with control group.

After the assessment of the mechanical hypersensitivity, we prepared a coronal brain slice for whole-cell patch clamp recording method. We performed whole-cell patch-clamp recording from layer II/III pyramidal neurons and recorded sIPSCs under voltage-clamp mode at 0 mV in the presence of CNQX, an alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid/GluK (AMPA/KA) receptors antagonist, AP-V, a N-methyl-D-aspartic acid (NMDA) receptors antagonist and CGP35845, a γ(gamma)-aminobutyric acid B ($GABA_B$) receptors antagonist in CFA model and control group (Koga *et al.* 2017). We analyzed the averaged frequency and amplitude of sIPSCs between two groups. We performed statistics and found that the averaged frequency of sIPSCs in CFA model was significantly reduced compared with control group. In contrast, averaged amplitudes of sIPSCs were not altered between the two groups. These results suggest that chronic pain model reduced inhibitory synaptic transmitter release in the ACC.

CFA-induced inflammatory pain model inhibited the frequency of mIPSCs

We next recorded mIPSCs in the presence of tetrodotoxin (TTX, 1 μM) to block sodium channels. CFA induced inflammatory pain model reduced the averaged frequency of mIPSCs in the layer II/III pyramidal neurons from the ACC compared to the control group. However, the averaged amplitude of mIPSCs did not change between two groups. Taken together, these results suggest that chronic pain model reduced synaptic transmitter release in the ACC.

Chronic inflammatory pain model decreased the protein expression of vesicular GABA transporter in the ACC

We further studied how the animal model of chronic pain could alter inhibitory synaptic transmissions in the ACC area. Vesicular GABA transporter (vGAT) is expressed in GABAergic neurons, and is responsible for vesicular storage and subsequent exocytosis of these inhibitory amino acids. We tested if chronic pain model could alter the protein expression of vGAT in the ACC area. Interestingly, chronic pain model reduced the expression of vGAT in the ACC.

We examined whether $GABA_A$ receptors subunits including α1, α5, β2, γ2 and δ and GABARAP were changed or not in the ACC of CFA-induced inflammatory pain group. The protein expressions of $GABA_A$ receptors subunits including α1, α5, β2, γ2 and δ and GABARAP did not change between inflammatory and control groups. These results suggest that chronic pain model produced presynaptic plasticity by altering vesicular GABAergic transporter in the ACC.

Discussion & Conclusion

In this study, we recorded transient $GABA_A$ receptors mediated inhibitory synaptic transmission in pyramidal neurons from layer II/III of the ACC. We found that a major type of chronic inflammation model induced by CFA reduced the frequency of both sIPSCs and mIPSCs. Western blotting analysis showed that vGAT was reduced in CFA treated group. On the other hands, $GABA_A$ receptors subunits did not change.

Electrophysiological experiments suggest that chronic inflammatory pain model mice produced synaptic plasticity. Especially, the model reduced the transmitter release of GABA in the ACC. Western blotting analysis showed that chronic pain model decreased the protein expression of

vGATs. Since vGAT express in the presynaptic terminal, chronic pain model may reduce the release of GABA via vGAT. We need further studies how chronic pain model can alter vGAT in the future.

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一般の皆様へ

慢性疼痛は侵害受容と心因性の要因からなり、前帯状回はこれら２つの要因に関わる重要な大脳皮質である。本研究では、前帯状回の抑制性シナプス伝達の可塑性に着目し、慢性疼痛が形成する大脳皮質の局所神経回路を理解することを目的に研究を行った。その結果、慢性疼痛モデルは前帯状回の抑制性シナプス伝達に可塑的な変化を示すことが明らかとなった。特に、GABA の放出が抑制される仕組みとして、GABA をシナプス小胞に貯蔵するトランスポーターである小胞 GABA トランスポーターのタンパク質の発現量が関与する可能性が示された。

Functions and expression of synaptotagmin 13 driving peritoneal metastasis of gastric cancer cells

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Summary Abstract

We identified synaptotagmin 13 overexpressing specifically in gastric cancer tissues with peritoneal metastasis from global expression profiling of 57751 molecules. Inhibition of synaptotagmin 13 led to decreased invasion and migration ability of gastric cancer cells, and formation of peritoneal metastasis in mouse xenograft models. Overexpression of synaptotagmin 13 serves as diagnostic and predictive biomarker for peritoneal metastasis from gastric cancer
Key Words：Gastric cancer, peritoneal metastasis, synaptotagmin 13

Introduction

Advanced gastric cancer frequently recurs because of undetected micrometastases even after curative resection. Peritoneal metastasis has been the most frequent recurrent pattern after gastrectomy and is incurable.

Results

We conducted a recurrence pattern-specific transcriptome analysis in an independent cohort of 16 patients with stage III gastric cancer who underwent curative gastrectomy and adjuvant S-1 for screening candidate molecules specific for peritoneal metastasis of gastric cancer. The effects of siRNA-mediated knockdown on phenotype and fluorouracil sensitivity of gastric cancer cells were evaluated in vitro, and the therapeutic effects of siRNAs were evaluated using a mouse xenograft model.

Synaptotagmin 13 was identified as a candidate biomarker specific to peritoneal metastasis. Inhibition of synaptotagmin 13 expression by gastric cancer cells correlated with decreased invasion, migration.

Intraperitoneal administration of synaptotagmin 13-siRNA inhibited the growth of peritoneal nodules and prolonged survival of mice engrafted with gastric cancer cells. High synaptotagmin 13 levels were significantly and specifically associated with peritoneal metastasis, and served as an independent prognostic marker for peritoneal recurrence-free survival of patients with stage II/III gastric cancer.

Discussion & Conclusion

Synaptotagmin 13 is a promising diagnostic and predictive biomarker for peritoneal metastasis of gastric cancer. Inhibition of SYT13 may represent a key of treatment strategy to overcome uncontrolled peritoneal metastasis from gastric cancer. Since anti-SYT13 treatment is based on quite

different mechanisms of action from existing molecularly targeted therapies, it can open new medical frontiers in the field of gastric cancer treatment, and possibly other malignancies involving peritoneal metastasis.

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一般の皆様へ

57751分子の網羅的遺伝子発現解析から、進行胃癌の転移再発形式の中で最も高頻度かつ難治性である腹膜播種に特異的発現する synaptotagmin 13を発見しました。synaptotagmin 13を阻害することで、胃癌細胞の活動性を低下させ、腹膜播種形成を抑制できることが明らかになりました。　　synaptotagmin 13は、すでに腹膜播種のある胃癌だけでなく、治癒切除術の後、腹膜播種を再発した胃癌でも高発現していることが分かりました。難治性の胃癌腹膜播種の新たな診断、治療への応用を目指しています。

Epigenetic and biological analysis of human cancer-associated fibroblasts

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Summary Abstract

Cancer-associated fibroblasts (CAFs) are composed of heterogeneous populations of cells and it is an important issue to understand the unique roles of the different subpopulations of CAFs. In this study, we evaluated various CAF cell lines by analyzing both their biological tumor-promoting properties and gene expression patterns. Our findings provide gene signatures characterizing functionally active CAFs, which may contribute to developing combinatorial therapies that can target such active CAFs besides neoplastic cells.
Key Words：Cancer-associated fibroblast, microarray, epigenome, lung cancer

Introduction

Tumors are complex tissues composed of carcinoma cells and surrounding stroma including various different types of mesenchymal cells and an extracellular matrix. Carcinoma-associated fibroblasts (CAFs) are frequently observed in the stroma of human carcinomas, and their presence in large numbers is often associated with the development of high-grade malignancies and poor prognoses (1). Although accumulating evidence suggests that it is conceivable to therapeutically target CAFs to promote anti-tumor responses, finding an agent that specifically targets CAFs remains elusive.

Results

CAFs within the tumor stroma are known to acquire activated phenotype and have been shown to stimulate tumor cell proliferation, elevate tumor cell invasion and boost angiogenesis by secreting various growth factors and cytokines (1). However, CAFs are also thought to derive from multiple origins and make up a heterogeneous population of cells (2), it is an important question to understand the unique roles of the different subpopulations of CAFs. In this project, we evaluated various CAF cell lines by analyzing both their biological tumor-promoting properties and gene expression patterns, and sought to identify gene signatures characterizing functionally active CAFs. First, we biologically assessed tumor-promoting activities of human CAFs in vivo. Human CAF cell lines were established from surgically resected tumor tissues of lung cancer patients. We co-injected CAFs with human lung cancer cell line, PC9 cells, into immunodeficient mice subcutaneously. As controls, PC9 cells alone were injected. Tumor growth kinetics over 5 weeks showed that several CAF cell lines significantly accelerated growth of PC9 xenografts to varying degrees, whereas the kinetics of PC9 xenografts were unaffected by some CAF cell lines. CAFs from different cancer patients appear to exhibit different biological effects on tumor growth, suggesting that CAFs are biologically heterogeneous cell populations. Based on their biological tumor growth-promoting abilities, we classified these CAF cell lines into 2 groups: CAF cell lines with functionally tumor-promoting activities (fCAFs) and those without tumor-promoting activities (nCAFs). Mice bearing mixed PC9 cells and fCAFs

also tend to have increased microscopic lung metastases compared to those bearing cancer cells alone or with nCAFs. We next performed comprehensive gene expression analysis of these CAFs to acquire gene signatures characterizing functionally tumor-promoting fCAFs as well as to further gain insights into mechanisms by which activated tumor-promoting fCAFs contribute to cancer microenvironments. Gene expression profiling was carried out using SurePrint G3 Human GE 8x60K ver.3.0 and analyses were done using Genespring GX14.5 and IPA (Ingenuity Pathways Analysis). In these analyses, we identified multiple genes related to cancer progression increased in fCAFs compared to nCAFs, and are now further confirming these data as well as identifying molecular pathways induced in fCAFs. For example, we detected several metalloproteinases dysregulated between fCAF and nCAF groups. Metalloproteinases including MMPs (matrix metalloproteinases) and ADAMs (a disintegrin and metalloproteinases) are known to play a pivotal role in tumor growth and the multistep processes of invasion and metastasis, including proteolytic degradation of ECM, alteration of the cell-cell and cell-ECM interactions, migration and angiogenesis. We have previously shown that dysregulated metalloproteinase activity is involved in the induction of CAF-like activated fibroblast state in fibroblasts (3). We also evaluated the significance of tumor stroma-associated metalloproteinase expression by using publicly available gene expression data sets representing tumor stroma from human breast cancer patients, and found that several metalloproteinases such as ADAM10 were significantly upregulated in human tumor stroma. Thus these findings suggest that funcitionally active CAFs may exhibit a specific gene expression pattern and the gene signature characterizing such active CAFs will contribute to establishing novel therapies targeting tumor stroma.

Discussion & Conclusion

In this study, we evaluated both biological properties and gene expression patterns of CAF cell lines established from human lung cancer patients, and identified multiple genes related to cancer progression increased in functionally active CAF cell lines. Although the mechanisms by which these genes are upregulated in activated tumor-promoting CAFs remain unknown, epigenetic changes, such as global DNA hypomethylation frequently seen in neoplastic cells, may be also involved in this process and further studies are needed. Nevertheless, our study will contribute to classification and precise molecular definition of subtypes of fibroblasts in tumor stroma on the basis of their biological functions, which may offer additional insights into CAF biology and inform clinical opportunities for new cancer therapies.

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一般の皆様へ

がん組織は、がん細胞とそれを支える間質と呼ばれる成分から構成されています。がん間質に出現する活性化した線維芽細胞は「がん関連線維芽細胞（cancer-associated fibroblast：CAF）」と呼ばれ、新たな治療標的の一つとして注目されています。本研究では、網羅的解析手法および生物学的機能解析手法を用いて CAF の層別化を行うとともに、機能的な CAF の細胞特性を明らかにすることにより、CAF を標的とした新規診断法および治療法の開発を目指した基礎的データの取得を行っていきたいと考えています。

Study on the RNA splicing checkpoint mechanism to prevent the accumulation of pre-mRNA

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Key Words：mRNA, splicing, transcription

Introduction

It was reported that an anti-cancer compound, spliceostatin A (SSA) binds to spliceosome to inhibit splicing very potently. Treatment with SSA or another splicing inhibitor pladienolide B also causes transcription elongation defect and dephospholyration of the second serine residues in the C-terminal domain (CTD) of RNA polymerase II (Pol II), which consists of multiple heptad repeat (YSPTSPS) and is important for transcription elongation, suggesting that splicing inhibition causes transcriptional elongation defect. However, the detailed molecular mechanism of transcription elongation defect by splicing inhibition remains unknown.

Results

To reveal the molecular insight of transcription elongation defect caused by splicing inhibition, we performed western blotting after SSA treatment to investigate the effect of splicing inhibition on the protein levels of kinases and phosphatases of Pol II, in addition to activators and repressors of Pol II transcription. However, none of the protein was affected after SSA treatment. In addition, to identify the responsible factor for the transcription elongation defect by splicing inhibition among the kinases and phosphatases of Pol II and activators and repressors of Pol II transcription, we subcloned the genes coding the proteins. We transfected cells with the genes, and treated the transfected cells with SSA to test whether overexpression of these factors suppresses the transcription elongation defect by SSA. However, over expression of any proteins did not rescue the transcription elongation defect by SSA and phosphorylation status of Pol II. In addition, we knocked down these factors using siRNA and observed transcription elongation efficiency. Knocking down of the factors did not affect transcription elongation efficiency. We will try to identify the responsible factor in a future study.

We also performed an immunoprecipitation experiment using an antibody against the largest subunit of Pol II. Several proteins which are related to transcriptional activity were co-immunoprecipitated with Pol II. We will perform another immunoprecipitaion experiment using SSA-treated cells to test whether splicing inhibition affect protein-protein interaction between Pol II and co-immunoprecipitated proteins.

It is well known that splicing and transcription are temporally and spatially coordinated. Therefore, RNA binding proteins, especially splicing factors, might affect transcriptional activity. To investigate the relationship between RNA binding proteins and transcriptional activity, we searched for RNA binding proteins which is able to suppress the transcription elongation defect caused by SSA treatment. As we expected, overexpression of several RNA binding proteins suppressed the transcriptional elongation defect by SSA and some of them increased the basal transcriptional

activity. Since some of these proteins were splicing factors, it is possible that overexpression of these splicing factors rescued the splicing defect caused by SSA, as a consequence, the transcription elongation defect by SSA was suppressed. However, we did not observe any recovery of splicing activity after overexpression of these RNA binding proteins. Therefore, these RNA binding proteins suppressed the transcriptional elongation defect by SSA independent of splicing activity. To understand the molecular mechanism that RNA binding proteins suppress transcriptional elongation defect, we checked whether overexpression of the RNA binding proteins affect phosphorylation level of Pol II, protein levels of Pol II kinases and phosphatases, and protein levels of transcriptional activators and repressors. Out of the RNA binding proteins, several proteins affected phosphorylation status of second serine residues within heptad repeat of Pol II CTD, which are important for transcription elongation, and fifth serine residues of the repeat, which are important for transcription initiation. In addition, protein levels of some transcription factors were affected by the over expression. We will investigate whether these RNA binding proteins affect the protein-protein interaction between Pol II and transcription factors and Pol II kinases and phosphatases. In addition, it will be also studied whether these RNA binding proteins interact with Pol II.

Discussion & Conclusion

We found that some RNA binding proteins affected transcription elongation efficiency. However, these RNA binding proteins did not affect the splicing efficiency. These results suggest that these RNA binding proteins affect the expression level of transcription factors and/or kinases and phosphatases of Pol II. Because RNA binding proteins recognize nascent RNA just after transcription, and numerous RNA binding proteins have physical interaction with transcription factors including Pol II, it is also possible that these RNA binding proteins regulate transcription activity directly.

Considering the results, we propose a model in which the transcription elongation defect after SSA treatment functions as a novel check point mechanism to prevent accumulation of unspliced pre-mRNAs and to protect the integrity of the gene expression when splicing is inhibited.

一般の皆様へ

本研究は、ヒトをはじめとした真核生物の DNA 上にある遺伝情報が、どのようにして正確に読み取られ、タンパク質へと翻訳されるかという、生物学的に非常に本質的な質問の答えを探すものです。本研究の結果から、スプライシングの異常時には異常な pre-mRNA の蓄積を防ぐため、転写伸長を止めてしまうという一種のチェック機構が働いていると考えられます。

Sensing ECM stiffness may trigger the transition from Solid-like to Liquid-like behaviour in collective cell migration of cranial neural crest cells

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Summary Abstract

Unexpectedly we found that cranial neural crest cohorts migrated on the mechano-pattern gel without any other stimulation. The soft/hard boundary may enhance the directional migration. We could call this phenomina as collective durotaxis.

Key Words：Collective durotaxis, photocurable gelatin, neural crest cells

Introduction

In previous research, we found that frog cranial neural crest cells dissociate their cell-cell adhesions intermediately upon going through the in vitro obstacles. The transition from premigratory to migratory neural crest was similar to solid- to liquid-phase transition1. However, we couldn't know if neural crest cells undergo this transition before or after touching the obstacle. Therefore, we started to use new tool having physiologically appropriate rigidness for testing mechano-sensing ability of cranial neural crest cells. We found that mechano-pattern gel triggered collective migration of neural crest cells without any other external signals.

Results

1. Confirmation of the preliminary results

First, we confirmed if the rigidness changed the collective behaviours. The photocurable gelatin alone couldn't accommodated the cranial neural crest cells, therefore, we chemically cross-linked fibronectin to the remaining free-NH2 residues of the photocurable gelatin. We confirmed that the surface of the gels were covered with fibronectin uniformly even when the rigidness between the stripes were very different. Again we confirmed that the soft gel increased the collective cell behaviour, and the hard gel promoted the cell dissociation.

2. Collective Durotaxis

Durotaxis is the behaviour which the cells migrate toward stiff region from softer region on the gradient of the rigidness. The researches of the durotaxis on the single cell migration have been well-established, however, the collective motion on the gradient of the rigidness has not yet been analyzed. We planned to test if neural crest cells dissociated their cell-cell adhesions upon going through the mechano-pattern. Before using the chemokines to induce the directional migration toward vertical direction of the boundaries of stiffness, we performed time-lapse imaging of control without chemokines. However, unexpectedly, neural crest cells started to migrate along the stripes without any other stimulation except the jumping of the stiffness. Moreover, the narrower stripe maintained the collective migration longer as much as in vivo.

3. Sensing the obstacle

When we put microfluidic tunnel to the path of the neural crest cell migration, the neural crest cells squeezed their body to go through the tunnel even narrower than the longer axis of single cells and LPAR2 lacking neural crest cells failed to change their shape.

Therefore, we thought neural crest dissociated to cell adhesion before going through the obstacle. However, when we put neural crest cells lacking LPAR2 gene to mechano-pattern gel, neural crest cells also failed to migrate and stalled at the middle of stripe because cells can not change their positions. Thus, neural crest cells sensed their surrounding circumstance mechanically, and then it might trigger the cell dissociation. Moreover, when we applied chemokine to this system, neural crest cells often ignored the boundaries. It may mean that neural crest cells is parrallely regulated by durotaxis and chemotaxis.

4. The window of the rigidness

Next, we analyzed which the rigidness or the gap of the rigidness controlled cell migration. To test that, we designed two types of the gels: The hard (<200 kPa) and the soft (<10 kPa) ; The hard (>400 kPa) and the soft (100-200kPa). The gap between soft and hard region was similar but the absolute rigidness was different. As the result, the harder gel couldn't promote the collective cell migration, and we also found that the window of the rigidness was very narrow. It might be the rigidness around cranial neural crest cells.

5. Cancer metastasis and the rigidness

We also tested if the rigidness controlled cancer cell migration. We made the sub-cell lines of osteosarcoma cell line, which metastasized into knees, lymphocytes, liver and kidney. The parental cell line (ori) and kidney cell line (kid) were compared. These cell lines didn't show the much differences except N-cadherin expression. However, the cells were put on the mechano-gel (hydrogel), the rigidness where each cell preferred to attach was different. We are going to analyze the correlation between the rigidness and the selectivity of the tissue metastasized.

Discussion & Conclusion

We found a novel system to control the collective cell migration. The local gradient of the rigidness triggered the migration without any other external signals. Our preliminary results suggest that collective durotaxis independently regulated cell migration, however, we haven't clarify the relationships between durotaxis and chemotaxis or the other signals. Moreover, we still need to show in vivo requirement of the collective durotaxis. Otherwise it might be an artifact only happened in vitro system.

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一般の皆様へ

細胞は集団で移動する方がバラバラの細胞が動くよりもはるかに効率が良いことが分かっています。しかし、がん細胞が塊になっていると狭い所で詰まってしまうため生体内でがんが集団移動するとは信じられていませんでした。先の研究でがんに似た神経堤細胞が適度に流動性を持つ集団として移動している事が分かりました。つまり「がんは水の様に器に沿う」のです。次の疑問として器の形を認識して形を変えるのか、元から水の様なのかがありました。本研究助成から、神経堤細胞が器を触って「硬さ」を感じて形を変えていることが分かってきました。この制御方法が分かればがんを閉じ込めることができるようになるでしょう。

Functional analysis of a novel silencing mechanism in higher plants

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Summary Abstract

Unlike conventional transgene silencing models, 35S promoter hypermethylation in gentian occurs irrespective of copy number and genomic location. By using transgenic gentian plants having single copy of some modified 35S promoters, we investigated sequence specificity of DNA methylation of the 35S promoter in gentian. Modified 35S promoter lacking a core (−90) region induced hypermethylation, whereas substitution of two "GAAGA" motifs within the promoter significantly reduced methylation in gentian. A 64-bp fragment of the 35S promoter can induce moderate methylation. These results suggest that the 64-bp region containing the "GAAGA" motif is essential for the 35S methylation in gentian.
Key Words：transgene, silencing, DNA methylation, gentian

Introduction

We have presented a strict cauliflower mosaic virus (CaMV) 35S promoter-specific silencing of transgene associated with DNA methylation in gentian (*Gentiana triflora* × *G. scabra*) [1-3] and lettuce (*Lactuca sativa* L.) [4]. These studies revealed that the 35S-specific silencing occurs regardless of the T-DNA copy number and genomic location, and is species-specific. The silencing phenomenon is distinct from the silencing features previously found in model plants, although the mechanism is unclear. The present study aimed to investigate the mechanism of the 35S promoter silencing in gentian.

Results

1. Hypermethylation of the 35S(Δcore) promoter lacking strong transcriptional activity

To confirm whether the strong transcriptional activity of the 35S promoter is required for the consistent 35S hypermethylation in gentian, a modified 35S promoter lacking core (−90) region, designated as 35S(Δcore), was introduced to gentian. Single copy 35S(Δcore) transgenic gentian lines were selected and methylation status of the introduced 35S(Δcore) region was analyzed by bisulfite genomic sequencing. As a result, hypermethylation at CpG/CpWpG sites on the region was observed in all the lines analyzed. The methylation level of the 35S(Δcore) region is equivalent to that of the unmodified 35S promoter.

2. Gentian nuclear factor binding to the highly methylated regions of the 35S promoter

From the results of the previous studies [2, 3] and the methylation analysis of the 35S(Δcore) region, two regions (−148 to −85 and −298 to −241) within the 35S promoter were highly methylated at CpHpH/CpCpG sites. Moreover, electrophoretic mobility shift assay (EMSA) showed that gentian nuclear factors bound to the −149 to −124 and −275 to −250 sequences, both of which have "GAAGA" and "GTGGAAAA" motifs [2, 3]. To confirm the contribution of these motifs for the

binding, EMSA was performed. Consistent with the previous studies, distinct complexes with gentian nuclear extracts were formed with the −149 to −124 probe. Formation of the complex was competed by excess unlabeled −275 to −250 competitor, but not by −296 to −271 competitor. In addition, substitution of "GTGGAAAA" to "TCTGAAAA" in the −275 to −250 competitor diminished the competition.

3. Substantial methylation reduction by nucleotide substitutions of the 35S promoter

Transgenic gentian plants with a modified 35S promoter designated as 35S(m1), wherein the two "GAAGA" motifs within the promoter were substituted with "GTTCA", were also produced, and methylation of the promoter region was analyzed. Most of the single-copy 35S(m1) lines obtained showed strong transgene expression. The methylation status of the modified 35S promoter region was low level in all the lines at both CpG/CpWpG and CpHpH/CpCpG sites.

4. Methylation induction by the 64-bp fragment of the 35S promoter

The next objective was to determine whether the highly CpHpH/CpCpG methylated region at −148 to −85 (designated as 64-bp) of the 35S promoter can induce methylation alone. Ten single copy transgenic lines were produced and methylation status of the introduced transgene region was analyzed. For the CpG/CpWpG sites, six lines exhibited hypermethylation on the 64-bp region at the same level as that of the unmodified 35S lines. Other four lines showed moderate or somewhat lower methylation levels. On the other hand, CpHpH/CpCpG methylation of the 64-bp region in all ten lines was substantially lower than that in the unmodified 35S lines. A complementary strand of the 153 bp transgene region (consisting 64-bp region, a 39 bp upstream region, and a 50 bp downstream region) was also analyzed to examine CpHpH/CpCpG methylation in detail. Consequently, a distinct methylation peak was found within the 64-bp region (−105 to −88) in all ten lines. Nevertheless, the methylation frequency of the peaks was substantially lower than that found in the unmodified 35S and 35S(Δcore) promoters. Methylation of the outer regions were rare and irregularly dispersed. Transgenic lettuce plants having single copy of the 64-bp construct also showed methylation within the −105 to −88 region.

Discussion & Conclusion

The present study showed that in gentian the 35S methylation status was altered by the modification of the promoter sequence. Hypermethylation of the 35S(Δcore) suggests that basal transcriptional activity is dispensable for the 35S methylation in gentian. EMSA analysis suggests that the −149 to −124 and −275 to −250 regions having "GAAGA" and "GTGGAAAA" motifs are targeted by DNA binding factor(s). Since substitution of the "GTGGAAAA" motif alter the competition of the complex formation, the motif may be required for the binding of the nuclear factor. Transgenic gentians having 35S(m1) showed transgene expression and hypomethylation of the modified promoter. These results suggest that the substituted two "GAAGA" motifs are essential for the methylation. Although the 64-bp region induced methylation in gentian, its activity is weaker than those of the unmodified 35S and 35S(Δcore) promoters. This result suggests that the presence of both the 64-bp and the other highly methylated region is required for a high level of methylation. Overall, a set of the study using modified 35S promoters implies that DNA binding factor(s) binding to the "GAAGA" and "GTGGAAAA" motifs may participate in the 35S methylation machinery.

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一般の皆様へ

遺伝子組換え植物は食料問題や環境問題などの解決に貢献できるものとして、世界中で研究開発が進められています。しかし植物種によっては遺伝子導入が困難なことや、導入した遺伝子が期待通りに働かないことが問題になることがあります。私たちは、組換え植物で広く利用されている35S プロモーターが、園芸植物であるリンドウでは発現抑制が起きることを見出しています。本研究では、この発現抑制を引き起こす配列を明らかにしました。この成果は、組換え植物の発現制御技術の発展に役立つものと考えています。

The origin of animal multicellular system – an approach from genome editing of unicellular holozoans

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Summary Abstract

Unicellular holozoans are the closest unicellular relative to animals. Our recent study revealed that the genomes of unicellular holozoans encode a number of proteins that are involved in multicellular organism-specific mechanisms such as cell-cell adhesion, cell-cell communication, and cell proliferation and differentiation. To understand the function of those genes in unicellular holozoans, we set up the framework of genome editing using the TALEN technique. We made 10 TALEN constructs with the intrinsic promoters, and determined the optimal transformation condition to obtain the maximum number of TALEN-expressing cells, yet the clear footprint of genome editing has not been identified.

Key Words：Evolution of multicellularity, Unicellular holozoans, Genome editing

Introduction

How did the multicellular animals evolve from a single cellular ancestor? To elucidate the origin of multicellularity, we use “unicellular holozoans” as model organisms. Unicellular holozoans are the closest unicellular relatives to animals, presumably maintaining the genetic and cellular features of the direct unicellular ancestor of animals. In former studies we had found that the unicellular holozoans have a wide range of “genetic toolkits” that were previously thought to be multicellular specific, such as intercellular communication genes, developmental transcription factor genes, and cell adhesion genes (Suga et al. 2014). The aim of the study is to understand the functions of such “multicellular genes” owned by unicellular organisms.

Results

We aimed to establish a protocol of genome editing on unicellular holozoans. Although CRISPR/Cas9 system represents the latest trend, we consider TALEN system would be more applicable to our model protists because of its simplicity; just two proteins are required to be expressed in the cell. By a collaboration with Hiroshima University, we have made 10 TALEN constructs against two of our model protists *Capsaspora* and *Creolimax* (Figure 1), both being unicellular holozoans.

We found that the TALEN proteins show some toxicity to the *Capsaspora* cells, regardless of the constructs to be introduced. We therefore first sought for the best amount of the constructs for transformation (i.e. the maximum construct

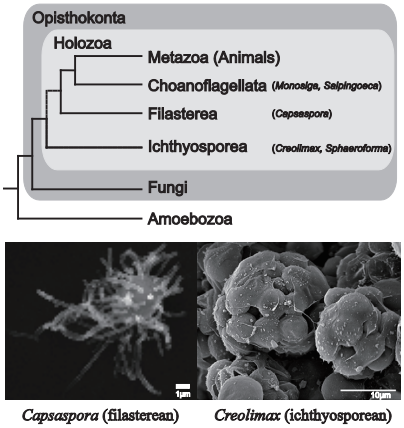

Figure 1 Unicellular holozoans and our model protists Capsaspora and Creolimax

amount that does not drastically impair the survival rate). We also developed the technique to isolate a single transformant using a micropipette or microinjector).

We first performed a pilot experiment, the SSA (single strand annealing) assay, to confirm that TALEN in fact functions in *Capsaspora* and *Creolimax* cells. Unfortunately the conventional CMV promoter did not drive any transcription in the cells. Thus we replaced the CMV promoter in the original SSA constructs (from Addgene) by the elongation factor 1α promoter (for Capsaspora) or the β-tubulin promoter (for *Creolimax*), which are already known to operate in our model organisms. As expected, the new constructs expressed the TALEN proteins and the marker mCherry fluorescent protein. Moreover, the SSA target construct alone did not produce the marker protein EGFP due to the intentionally introduced stop codon. However, when both are introduced into the cell, the EGFP expression was not observed either.

In parallel to the SSA assay, we aimed to knock out the endogenous "multicellularity-related" genes of unicellular holozoans, targeting the Src tyrosine kinases of *Capsaspora* and *Creolimax.* Tyrosine kinases are, in animals, involved in cell-cell communication, cell differentiation, and cell proliferation, which represent essential features of multicellularity, yet the genomes of unicellular holozoans contain many tyrosine kinases including Src (Segawa et al. 2006; Schultheiss et al. 2012, 2014). It remains unclear what are the function of Src proteins in the unicellular context. We therefore introduced three sets of TALEN constructs designed against two *Capsaspora* Src genes and one set of TALEN constructs against a single *Creolimax* Src gene. We successfully established six *Capsaspora* cell lines expressing the marker mCherry protein, indicating the TALEN proteins are also present. From these cell lines the genomic DNA was extracted, and we amplified by PCR the Src gene regions, which were subsequently sequenced. However, we could find any clearly positive evidence supporting the TALEN proteins actually knocked out the target genes. Two possibly positive lines are still under investigation. On the other hand, we did not obtain any TALEN-positive *Creolimax* cell lines due to the low transformation efficiency, which critically went down in the recent years.

For the knock out experiments, controlling ploidy and sexual stage of the organism is essential. In some protists, sexual stages are introduced by a severe environmental condition. To know how unicellular holozoans react against environmental changes, we investigated the protein phosphorylation of the cells under either oxidative or oligotrophic conditions. A western blotting and a following mass-spec analysis revealed two proteins that are specifically downregulated at oxidative condition.

Discussion & Conclusion

The largest question in applying genome editing on unconventional models is at the choice of target gene. Choosing the target in protist models is particularly difficult because the functions of genes are mostly unknown. We chose Src genes because 1) *Capsaspora* has two Src genes with possibly redundant functions, 2) the function of Src has already been studied well in animal models, and 3) tyrosine kinase is one of our most important targets for functional analysis. Until today, we have not obtained any clearly positive results. This could account for the choice of target, i.e. losing even one Src gene may be lethal to *Capsaspora*. On the other hand, it is also possible that the success rate of genome editing is much lower than we expect even if TALEN are expressed in the cell. To obtain the genome-edited cell lines, we need both to 1) try other target genes that seem less lethal such as very redundant gene families and to 2) increase the number of experiments.

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一般の皆様へ

動物のからだはたくさんの細胞からできています。細胞同士は連絡を取り合いながら高度な分業体制をとっており、このことは動物が地球のエコシステム上で優位な地位を占めるに至る一因となったと考えられています。しかし一方で、人類が苦しめられている癌は細胞増殖の制御が狂うことが原因で起きており、多細胞生物にしかありえない病気といえます。我々の研究は、動物に最も近縁な単細胞生物「単細胞ホロゾア」と総称される生物を材料に、動物多細胞性の進化の秘密を探り、多細胞システムが本質的に抱える問題にも迫ろうとするものです。

Regulating microtubule arrays for neuronal dendrite growth and for neuronal regeneration

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Summary Abstract

We describe two previously unknown and independently organized microtubule nucleation centers in growing dendrite branches. We show that at each of these centers microtubule nucleation occurs through a differing molecular mechanism. The microtubule nucleation activities we will describe are critical to promote dendrite outgrowth and branching, we predict they will be mechanism to drive dendrite regeneration after injury.
Key Words：Neuron Growth, Dendrite, Microtubule

Introduction

Neurons must connect through dendrites to receive input from their appropriate partners; this is required for correct circuit wiring and nervous system function. Just small alterations in the differentiation of dendrites can contribute to intellectual disability, which affects 2-3% of population worldwide[1-3]. To understand how these disorders arise it is important to first elucidate the molecular processes of dendrite branching and dendrite pattern formation[4]. Moreover, dendrites are also highly susceptible to damage by trauma, seizure, and stroke. For successful regeneration after injury dendrites must return to a growing state. Therefore, understanding the mechanisms controlling microtubule dynamics in the growing dendrite is critical not only for understanding neuron development, but also for discovering strategies to support neuron regeneration

Results

The microtubule cytoskeleton is critical to provide structure and to exert force in order to create, stabilize, and remodel dendrites during development. Both anterograde and retrograde microtubule polymerization occur within growing dendrites.

We have developed an *in vivo* imaging protocol to follow the development of *Drosophila* pupal sensory neurons from the initial stage of dendrite outgrowth until the branching structure is stabilized[5,6]. From these analyses the critical dendrite outgrowth and branching events that create the major branch structures of the dendrite arbor were identified. Next, in order to understand how microtubule nucleation events are organized with the growing dendrite and how they are used to pattern the dendrite, the spatial organization of microtubule nucleation events during dendrite outgrowth will be mapped. To do this, we will assay EB1::GFP comets[7]. The +Tip protein[8] EB1 concentrates at the growing end of a microtubule. Therefore an EB1::GFP comet marks the end of a polymerizing microtubule, and the appearance of a comet indicates a site of microtubule nucleation[7].

In dendrites the length of microtubules exceeds the diameter of the cell, and therefore microtubules

are necessarily orientated either plus-end-distal and anterograde polymerizing (away from the cell body), or minus-end distal and retrograde polymerizing (towards the cell body)[9]. Our results showed that regulation of the position and polarity of microtubule nucleation events in the growing dendrite arbor is essential for dendrite arbor patterning. Spatial control over microtubule nucleation is used to organize the cellular cytoskeleton. We will show that two spatially separate microtubule organizing centers exist within the terminal region of a developing dendrite. At the tip of the extending dendrite microtubules are nucleated an anterograde direction. Within the shaft of the dendrite is a separate zone where microtubules are nucleated in a retrograde direction.

Abnormalities in dendrite pattern are a central feature in intellectual disability[1,10]. To understand how dendrite patterning is controlled, we carried out a pilot screen to analyze a set of candidate genes centered an on intellectual disability predisposition network[2]. This was done using *in vivo* EB1::GFP live imaging to identify candidates that alter the pattern of microtubule nucleation and polymerization in the growing dendrite. From this pilot screen we identified MyosinVI and Wee Augmin (Wac). MyosinVI is best known as a actin-associated motor; however it has also been show to link to microtubules through recruiting microtubule associated proteins, (in particular the +Tip Clip170), to its cargo binding domain[11]. Furthermore cargo binding induces MyosinVI to dimerize, and in the dimerized form it can act as a non-moving actin filament crosslinker. Wac is associated with the Augmin complex, which nucleates new microtubules from the sides of pre-existing microtubules[12]. Additional factors were identified in this pilot screen. Including an unstudied factor with C2 (a calcium-mediated membrane targeting domain) and DH and PH domains (indicating guanine nucleotide exchange factor (GEF) activity for regulation of the Rho family GTPases). Our present data shows that Myosin VI drives the anterograde directed microtubule nucleation in the developing dendrite tip.

Discussion & Conclusion

We have demonstrated new independent microtubule nucleation centers in the neuron, and show that they control developmental dendrite arbor patterning; furthermore our data begin to that they are regulated by different molecular mechanisms. We predict that MyosinVI localizes microtubule polymerization promoting factors to amplify Augmin-mediated microtubule nucleation. Furthermore, MyosinVI simultaneously bundles actin into tracks that target polymerizing microtubules in the anterograde direction. This is a new paradigm for how neuron shape can be achieved, and whether a similar process also controls shape in other cell types is a key follow up area for study. Importantly, dendrites are highly susceptible to damage, and will next show how these developmental mechanisms are reused for dendrite regeneration and repair after injury.

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一般の皆様へ

神経系が正しく機能するためには、神経細胞は神経樹状突起を介して適切な伝達経路からの入力を受け取る必要がある。よって、神経樹状突起の発達に異常が起こると知的障害および神経発達障害を引き起こす可能性がある。本研究では、神経樹状突起の発育時に起こる【2つの】未知かつ独立して存在する微小管核による中心体形成について明らかにした。我々は、神経系の中心体形成過程において重要な役割を果たすことが知られている微小管核形成は、異なる分子メカニズムを介して起こること示した。また、微小管核形成は神経樹状突起が成長、枝分かれするのを促すことで、神経損傷などが起こった際に神経樹状突起が再生するのに非常に重要な役割を果たすことを示唆した。

A novel network between mature adipocytes and endothelial cells that regulates adipose tissue angiogenesis and homeostasis

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Summary Abstract

Adipose tissue contains highly developed vascular networks and its blood vessel density is closely associated with adipocyte functions. However, detailed molecular mechanisms underlying adipose tissue angiogenesis remain unclear. We searched for genes that is involved in the crosstalk between mature adipocyte and endothelial cells, and identified Nrg4 as one of such genes. We found that Nrg4 is highly expressed in mature adipocyte and it enhanced angiogenic capacity in endothelial cells. Gain and loss of function studies using genetically modified mice revealed that Nrg4-medated adipose tissue angiogenesis play a crucial role in the preservation of metabolic homeostasis, especially in obesity. Our data established Nrg4 as a novel therapeutic target in the treatment and/or prevention of metabolic disorders associated with obesity.
Key Words：Obesity; Adipose tissue; Endothelial cell; Angiogenesis

Introduction

Obesity is epidemic worldwide and its deleterious impact on cardiovascular disease makes it as a life-threatening state. Adipocyte dysfunction is causally implicated in obesity-related metabolic disorders; however molecular mechanisms by which obesity impairs adipocyte functions remain to be elucidated. Insufficient angiogenesis in adipose tissue during obesity causes hypoxia and consequently triggers inflammation and dysfunction in adipose tissue; therefore adipose tissue angiogenesis is a potential pharmacotherapeutic target in the treatment of obesity-related metabolic disorders.

Results

We searched for genes that are involved in the communication between endothelial cells (ECs) and mature adipocytes (MA) by using DNA microarray, and identified Nrg4 as one of such genes. Nrg4 was highly expressed in adipose tissues, predominantly in mature adipocytes. Of note, Nrg4 expression was substantially reduced in adipose tissue of obese mice potentially through inflammatory cytokines and ER-stress. Nrg4 belongs to EGF family of proteins, and recombinant Nrg4 enhanced proliferation and tube-formation in ECs, while it reduced EC apoptosis. In vivo angiogenesis assessed by Matrigel-plug assay also revealed angiogenic function of Nrg4. These results indicate that Nrg4 is a novel angiogenic adipokine. On the other hand, we found that ECs negatively regulated Nrg4 expression in MA. Experiments using inhibitors for various signaling pathway identified a crucial role of JAK signaling in EC-mediated down-regulation of Nrg4 in MA.

To further elucidate the role of Nrg4 in metabolic homeostasis, we generated mice with a targeted deletion of Nrg4. Interestingly, Nrg4-KO mice showed modest but significant increase in body weight and adiposity even while consuming normal chow. Moreover, loss of Nrg4 caused impaired

insulin sensitivity under normal dietary condition. Blood vessel density in brown adipose tissue was modestly reduced and consequently energy expenditure was significantly decreased in Nrg4-KO mice compared to those in WT mice. Blood vessel density in white adipose tissue was also reduced, and adiponectin expression significantly decreased in Nrg4-KO mice. When fed a high fat-diet (HFD), obesity was significantly deteriorated in Nrg4-KO mice. Blood vessel density in brown adipose tissue substantially reduced and energy expenditure significantly decreased in Nrg4-KO mice fed a HFD. Blood vessel density in white adipose tissue also substantially reduced, and hypoxia and chronic inflammation was deteriorated in HFD-fed Nrg4-KO mice. In contrast, targeted activation of Nrg4 in adipocytes improved insulin sensitivity even under normal chow diet, although body weight and adiposity was unchanged. Blood vessel density in white adipose tissue was significantly increased in Nrg4-Tg mice. When fed a HFD, Nrg4-Tg mice showed body weight and adiposity similar to WT mice. Nevertheless, HFD-fed Nrg4-Tg mice exhibited ameliorated insulin resistance and glucose intolerance compared to those in WT mice fed a HFD. Chronic inflammation in white adipose tissue was significantly reduced in HFD-fed Nrg4-Tg mice. To further clarify the role of enhanced adipose tissue angiogenesis in improved metabolic homeostasis in Nrg4-Tg mice, we administered angiogenesis inhibitor SU-5416 that inhibit VEGF-R2 signaling. Blood vessel density in white adipose tissue was similar between WT and Nrg4-Tg mice after the treatment with SU-5416. Of note, administration of SU-5416 abolished the improvements in insulin sensitivity and glucose tolerance observed in Nrg4-Tg mice.

Discussion & Conclusion

Our data collectively indicate that Nrg4 is a novel angiogenic adipokine that regulates adipose tissue angiogenesis, and that Nrg4-mediated angiogenesis is crucially implicated in the maintenance of systemic metabolic homeostasis. Because Nrg4 expression in adipose tissue was down-regulated during obesity, enhancing and/or preserving Nrg4 expression in adipocytes has an attractive therapeutic potential in the treatment of obesity-related metabolic disorders.

一般の皆様へ

肥満は世界的に増加を続けており、その健康被害は甚大です。肥満時には脂肪細胞が病的に肥大し、脂肪組織が機能不全に陥る結果、全身のエネルギー・糖代謝が障害されると考えられていますが、その詳細な分子機構は明らかではありません。脂肪組織は非常に発達した血管網を有しており、その血管密度は脂肪細胞機能と密接に関わっています。私たちは脂肪細胞と血管内皮細胞のコミュニケーションに注目し、Nrg4という分子を発見しました。Nrg4は脂肪細胞から分泌され、血管新生を誘導して脂肪組織の血管密度を高めます。肥満時にはNrg4の発現が減少することが明らかとなり、Nrg4の発現を増やすことで肥満時にも脂肪血管密度を維持し、脂肪組織の機能障害を予防できると考えられます。

Investigation of the mechanism of cell fate determination independent from transcriptional factor

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Department of Hematological Malignancy, Tokai University The Institute of Medical Sciences

Summary Abstract

Trancription factor has been assumed to be critical to determine cell fate.

However, we challenged the canonical notion based on the fact we observed in B cell lineage commitment. Without EBF1, a crucial transcriptional factor, the single miRNA can induce B cell lineage commitment. The mechanistic analysis of the intereting phenomena has been studied in this proposal.

Key Words：Transcription factor, miRNA, EBF1, B cell

Introduction

In the previous study, we found that miR-126 could cause B cell differentiation in MLL-AF4 acute lymphocytic leukemia (ALL) without any upregulation of EBF1, E2A, and Pax5, which are indispensable for B cell differentiation. Moreover, we found that miR-126 could partially rescue EBF deficiency in B cell lineage commitment.

Results

Then we introduced another miRNA, which is also downregulated in MLL-AF4 ALL into EBF1 deficient hematopoietic progenitor cells (HPCs). This miRNA could induce CD19 expression, which is the definitive surface marker of B committed cell. Not only CD19 expression but also B cell related genes such as Pax5, Rag1/2,and lamda5 were upregulated in these cells.

In vivo mouse as well, the miRNA induced CD19 in EBF deficient HPCs.

To investigate why the miRNA causes B cell lineage commitment in EBF1 deficient HPCs, the direct target genes of the miRNA have been studied by use of the comprehensive gene expression profiles and in silico prediction of the miRNA by use of the software. As a results, several target genes were identified and validated by the luciferase reporter assay and overexpression of the miRNA in EBF1 deficient HPCs.

Next, the relationship between EBF1 and the miRNA has been investigated. When overexpressing EBF1 in Baf3 cells, a pro B cell line, no change of the miRNA expression was observed to show that EBF1 does not regulate the expression of the miRNA. The other way around, the miRNA deficient cells showed no change in EBF1 expression, which demonstrated that the EBF1 and the miRNA are independently regulated.

Discussion & Conclusion

Canonical notion of cell fate determination is that the master regulator is transcription factor. Then miRNAs have been assumed to have marginal function, as a fine tuner". However, we observed that only single miRNA can rescue transcription factor deficiency. These results suggest that the canonical notion should be revised and miRNA has more potent function in cell fate determination.

References

Okuyama K, Ikawa T, Gentner B, Hozumi K, Harnprasopwat R, Lu J, Yamashita R, Ha D, Toyoshima T, Chanda B, Kawamata T, Yokoyama K, Wang S, Ando K, Lodish HF, Tojo A, Kawamoto H, Kotani A. MicroRNA-126-mediated control of cell fate in B-cell myeloid progenitors as a potential alternative to transcriptional factors. *Proc Natl Acad Sci U S A.* 2013;110(33):13410-5.

一般の皆様へ

細胞の運命決定においては転写因子が重要な働きをするというのが従来の一般的な考え方でした。私たちは、その考えに沿わない、新しい現象を見出しました。その機序を本研究課題で解析し、細胞運命決定には転写因子のみならず、小分子 RNA という因子も深く関与することを明らかにしました。

III.

Reports from the Recipients of Grants for International Meetings

Report on Research Meeting

170515 Hiroshi Kawamoto Kyoto University

1. Name of Research Meeting / Conference
   KTCC 2017:
   7th International Workshop of Kyoto T Cell Conference

2. Representative
   Organizing committee:

Hiroshi Kawamoto	Kyoto University	Professor
Ichiro Taniuchi	Riken IMS	Group Director
Manami Itoi	Meiji University of Integrative Medicine	Professor
Toshinori Nakayama	Chiba University	Professor
Takehito Sato	Tokai University	Associate Professor
Yosuke Takahama	University of Tokushima	Professor

3. Opening period and Place
   13th to17th March 2017
   Shiran Kaikan, Kyoto University, Kyoto

4. Number of participants / Number of participating countries and areas
   Participants: 295 (domestic 179, oversea 116)
   Presentations: 178 (oral 124, poster 53)
   Participating countries:22
   (Japan, USA, Canada, Australia, China, Taiwan, India, UK, France, Germany, Portugal, Norway, Sweden, Austria, Czech Republic, Netherlands, Belgium, Swizerland, Israel, Singapore, Turkey, Estonia)

5. Total cost
   12 million JPY

6. Main use of subsidy
   Production cost for color posters (A2 size x 500)

7. Result and Impression

   Kyoto T Cell Conference is an international research conference focusing on the development and function of T lymphocytes. This meeting represents as one of four T cell biology meetings held in the world; the other threes being held in USA, Europe, and Australia. These meetings are held in rotation with one meeting per year pace, so the International Workshop of KTCC is held once in four years. This time, the meeting was held as the 7th meeting. Among these four meetings, KTCC is usually the biggest, with more than 200 participants.

   Topics discussed at the meeting included: thymic microenvironments, lymphoid progenitors and lineage commitment, selection and differentiation, recognition and activation, immune response and memory, and immune regulation and diseases, stromal cells for immune function, aging of immune system. Leading scientists in the filed of T cell biology from all over the world

got together. On the first day (March 12th) in the evening, the meeting was started with three keynote lectures: (i) Jonathan Sprent (Garvan Institute, Australia), who talked about the history of discovery of the thymus, (ii) Shigeo Koyasu (Riken, Japan), who talked about the innate lymphoid cells, and (iii) Nagahiro Minato (Kyoto Univesity, Japan), who talked about the aging of immune system. After that, four full days of the meeting followed. During the whole meeting period, all participants shared the presented topics, which included unpublished findings, and enjoyed active discussion on the topics. This meeting especially encourage presenters to show unpublished data, and some of such new data were really exciting. In addition, a special lecture was given by Shimon Sakaguchi (Osaka University, Japan) on March 16th, concerning the biology of regulatory T cells. In conclusion, the meeting was very successful.

We made a full support only for one speaker as a keynote speaker (J. Sprent). Just partial supports were given to 30 core scientists for accommodation, and to 20 students/postdocs for travel based on selection (100,000 JPY for each). Even in such a situation, 116 oversea scientists came to join this meeting. Such a meeting may be very exceptional in Japan.

8. Additional description

We sincerely appreciate the financial support from Novartis. Thank you very much.

Report on Research Meeting

1. Name of Research Meeting / Conference
   CeNeuro2016 & Nagoya BNC Joint Meeting

2. Representative
   Ikue Mori
   Professor, Graduate School of Science, Nagoya University

3. Opening period and Place
   July 27th – 30th, 2016
   Toyoda Auditorium, Nagoya

4. Number of participants / Number of participating countries and areas
   230 participants/ 16 countries and areas

5. Total cost
   14,842,076 JPY

6. Main use of subsidy
   Travel expenses for invited foreign speakers

7. Results and Impression

   The main conference, CeNeuro 2016, was the first International *C. elegans* Neurobiology Meeting held outside of Western countries. Even though several of large and related conferences (e.g. Eastern Asian *C. elegans* meeting, The Allied Genetics Coference) were held around the same time, we still could have as many as 230 participants (138 overseas and 92 domestic) in total. The four-day meeting included 16 invited talks, 50 oral presentations and 113 poster presentations. The number of participants and the size of the venue seemed just appropriate: participants could have intense discussion throughout the entire conference while having enough opportunity for networking with anyone during poster sessions, meals and mixers.

   The conference covered a wide range of topics in *C. elegans* neurobiology, from fundamental studies, such as neural circuit development, to cutting-edge researches including large-scale analysis of neuronal activity underlying behavior. In particular, whole-brain imaging, a field of research which *C. elegans* neurobiology takes the leadership of, caught attention of participants. The recent progress in whole-brain imaging technology, as well as advanced mathematics to deal with big data obtained by it, was introduced and actively discussed.

   After CeNeuro 2016, we had a satellite symposium, Nagoya BNC symposium, which was open to the public. Here, we expanded our discussion to neurobiology of zebrafish and fruit fly, which share the strategy of studying small-scale nervous system with *C. elegans*. The presentations on sensorimotor coordination across animal species shed light on the future direction of this research field towards understanding neuronal network dynamics that integrate external and/or internal information to generate appropriate behavior. We also discussed issues surrounding today's research environment of neuroscience. The proposals by the speakers included multidimentional approach to revise the social systems for publication, funding, carrier development and education.

8. Additional description

We are grateful to the NOVARTIS Foundation (Japan) for its generous financial support. It helped us to lower registration fee, particularly for students, as well as to maximize financial support for students and postdocs.

Report on Research Meeting

September 12, 2016

1. Name of Research Meeting / Conference
   20th International Symposium on Homogeneous Catalysis

2. Representative
   Kyoko Nozaki

3. Opening period and Place
   July 10, 2016 – July 15, 2016; Kyoto Terrsa

4. Number of participants / Number of participating countries and areas
   460 / 27

5. Total cost
   28,869,705 JPY

6. Main use of subsidy
   Travel expenses of invited speakers from abroad

7. Result and Impression
   In this symposium, both leading researchers and young ones in the field of homogeneous catalysis from Japan and other countries in the world got together for 6 days in Kyoto, and actively discussed on this area of research through 22 invited lectures, 32 oral presentations, and 258 poster presentations. Presentations were made not only on bulk homogeneous catalysis such as hydrogenation, oxo-reaction, and oxidation, but also on the synthesis of fine chemicals such as asymmetric catalysis and selective carbon-carbon bond-forming reactions, which are indispensible for the development of pharmaceuticals. The theoretical research was also the topic of interest in this symposium in addition to the experimental research. All the lectures were presented in one lecture hall and enough time was used for the poster presentations, which made it possible for all the participants to interact well with each other through active discussions.

8. Additional description
   Thanks to the financial support from the Novartis Foundation and several other companies and organizations, the symposium was successfully held.

Report on Research Meeting

1. Name of Research Meeting / Conference
   The 4th International Symposium on Salivary Glands in Honor of Niels Stensen

2. Representative
   Masataka Murakami

3. Opening period and Place
   2016.11.30 – 12.2, Okazaki Conference Center, National Institute of Natural Sciences

4. Number of participants / Number of participating countries and areas
   60 participants from 9 countries. America (4), Europe (4), Middle East (1), East Asia (51)

5. Total cost
   JYEN 2,191,565,-

6. Main use of subsidy
   Main use was airfare and accommodation for overseas participants.

7. Result and Impression
   The main purpose of the symposium was to facilitate the clinical application from the basic research in the field of salivary gland. The individual interests of invited speakers were varied but all participants has some idea to apply for clinical application. Andy Wolff impressed the audience that the most of medication influence the salivary secretion and he introduce a stimulator on lingual nerve for clinical use. Jörgen Ekström indicated some anti- psychotic drug effects on salivatory centers. Massimo Castagnola explained an application of mass spectroscopy to analyse human saliva effectively and precisely. Shmuel Muallem proposed possibility for derangement of the ductal CFTR to initiate autoimmune disease and Sjögren Syndrome. The role of P2X7 for carcinogenesis was discussed by Ivana Novak. Matthew Hoffman stressed the important role of intermediate cells such as fibloblast to facillitate the organogenesis in addition to neurogenesis and vascular formation. From the view on Ca^{2+} homeostasis, Indu Ambudkar suggested that uncontrolled Ca^{2+} entry may injure the cells. James Putney reviewed the history of store operated Ca^{2+} entry and expanded the possibility of disease induced by gene modification of Orai and STIM. Masataka Murakami and Akihiko Tanimura stressed the development of in situ and ex vivo studies will be more important and we have to assess the molecular events in situ for application of basic study to clinical procedures.
   The discussion was held friendly after each talk and poster session and Young Japanese researchers could follow the recent results of international pioneers. This is very important and hopeful sign of this field. During the symposium, the organizers discussed for future Stensen symposium and asked the four Japanese scientist to maintein the work of salivary gland and the Stensen Symposium.

8. Additional description

Report on Research Meeting

1. Name of Research Meeting / Conference
   5th International Conference for Food and Environmental Virology (ISFEV2016)

2. Representative
   Hiroyuki Katayama, University of Tokyo (ISFEV2016 Chair)

3. Opening period and Place
   Sep. 13-16, 2016
   Hotel Sakurai (Kusatsu 465-4, Agazuma, Gunma 377-1711)

4. Number of participants / Number of participating countries and areas
   108/21

5. Total cost
   7,581,726 JPY

6. Main use of subsidy
   Participant fee for 9 invited participants

7. Result and Impression

   5th International Conference for Food and Environmental Virology was successfully held at Hotel Sakurai, Kusatsu, Japan from Sep. 13th to 16th, 2016 (Conference Chair: Dr. Hiroyuki Katayama, The University of Tokyo, Japan). In total 108 participants from 21 countries joined this event. The plenary lecture was given by Prof. Joan Jofre, University of Barcelona, and eight key note speeches were given by leading researchers. There were 48 oral presentations and 44 poster presentations. These presentations were evaluated from the viewpoints including novelty and presentation skill, and the 5 presenters were awarded.

   All participants were satisfied by the well-organized conference and enjoyed fruitful discussion in all scientific sessions. Japanese participants also joined the discussion, and learned a lot about the topic of food virology, such as contamination level and detection methods. One of the main topics was norovirus, which causes a lot of infection cases whole through the world through contaminated water and food. The organizing committee invited world-leading researchers for the norovirus study, and organized a special workshop for norovirus. Presenters showed the latest information about norovirus studies, and participants got a lot of suggestions and inspirations for future studies.

   Since this conference was held in a hotel where participants can stay, all participants enjoyed communications even during the time for meals (lunch and dinner). This style has not such common in Japan, while conferences held in western countries frequently employ this style. A difficulty was in that the organizing committee had to take care about not only conference organization but also the hotel reservation, but the satisfaction of participants rewarded the effort.

   This was the first international conference of Food and Environmental Virology in Asia (the first 4 conferences were held in Europe). The organizing committee invited news paper writers to the conference, and they introduced this conference in Yomiuri and Jomo News Paper as the first

conference on this issue in Asia. In total, this conference was so successful and best remembered one for all participants.

8. Additional description
No additional description

Nov. 18, 2016
Daisuke Sano
Organizing Committee Secretary
ISFEV2016

30th Grant Report (FY2016)

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 2016 are as follows.

30th Novartis Research Grant: 35Researchers (JPY 1 mil.), Subtotal JPY 35 mil.
(*Including Kumamoto earthguate diaster：5)
Research Meeting Grant: 5 Meetings (JPY 0.4 mil.), Subtotal JPY 2.0 mil.
Total JPY 3.70 mil.

30th Novartis Research Grant (FY2016)

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	Hirose Tetsuro	Institute for Genetic Medicine, Hokkaido University	Professor	Regulatory function of stress responsive splicing controlled by long non-coding RNA
2	Kikuchi Kiyoshi	University of Tokyo	Associate Professor	How is recombination suppression achieved in the first place between sex chromosomes?
3	Ohta Akane	Konan University	Professor	Identification of novel neural processing mechanism by using single transcriptome analysis
4	Matsushima Ryo	Okayama University, Institute of Plant Science and Resources	Associate Professor	Analysis of starch grain formation using live imaging and computer simulation
5	Takayama Kenichi	Department of Functional Biogerontology, Tokyo Metropolitan Inst	Researcher	Genome-wide analysis of transcriptional and translational regulation by RNA binding protein PSF in prostate cancer
6	Mizuseki Kenji	Osaka City University Graduate School of Medicine	Professor	Selective information routing in hippocampal network
7	Yoshimitsu Takehiko	Okayama University	Professor	Drug discovery from antitumor marine alkaloid
8	Minamino Thru	Niigata University Graduate School Medical and Dental Sciences	Professor	Elucidation of pathogenic mechanism of age-related disease and a stem cell aging controlled by the vascular niche.
9	Iwasaki Yuka	Keio University School of Medicine	Associate Professor	Transposon silencing by small RNA-mediated regulation of the 3D organization of the genome
10	Mutoh Yuichiro	Tokyo University of Science	Associate Professor	Catalytic Synthesis of Spiroketals Using Water as the Oxygen Source Triggered by Rearrangement of Internal Alkynes
11	Shirakawa Hisashi	Kyoto University, Graduate School of Pharmaceutical Sciences	Associate Professor	Molecular analysis for the pathophysiological significance of brain hypoxic response and application for the CNS diseases

#	Name	Institution	Title	Research Project
12	Tago Megumi	Keio University	Associate Professor	Elucidation of the onset mechanism of myeloproliferative neoplasm through the erythropoietin receptor
13	Hirai Maretoshi	Kansai Medical University, Department of Pharmacology	Associate Professor	Induction and visualization of adult cardiomyocytes proliferation through growth factor receptor activation
14	Ohki Rieko	National Cancer Center Research Institute, Division of Rare Canc	Group leader	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
15	Ueda Takeshi	Kindai University	Lecturer	Disease specific regulation of the histone methylation in leukemia development
16	Uchida Hiroaki	The Institute of Medical Science, The University of Tokyo	Senior Assistant Professor	Treatment of metastatic tumors by systemic injection of tumor-retargeted oncolytic herpesviruses
17	Verma Prabhat	Osaka University	Professor	Revealing cell membrane functions through in-vitro analysis by tip-enhanced Raman spectroscopy in liquid
18	Manabe Ichiro	Chiba University	Professor	Elucidation of the immune-nerve-metabolic systems network in cardiac homeostasis and failure
19	Hayashi Yasunori	RIKEN Brain Science Institute	Senior Team Leader	Roles of synaptic plasticity and replay during sleep in memory consolidation
20	Tanaka Minoru	Nagoya University	Professor	Study on the Mechanism of Sex Determination in Germline Stem Cells
21	Yamaguchi Tomoya	Nagoya University School of Medicine	Designated Associate Professor	ROR1-mediated caveolae formation and caveolar endocytosis in lung cancer
22	Matsumura Yoshihiro	The University of Tokyo, RCAST	Associate Professor	Regulation of energy metabolism by environmental cue and epigenome
23	Koshiba Takumi	Kyushu university	Associate Professor	Mitochondrial dynamics and antiviral innate immunity
24	Sasai Noriaki	Nara Institute of Science and Technology	Associate Professor	Analysis of epigenetic regulation toward establishment of a stable culture method of neural progenitor cells
25	Hamamoto Ryuji	National Cancer Center Research Institute	Divisino Chief	Elucidating the regulatory mechanism of histone modification status in colorectal cancer stem cells to maintain stemness and its application for drug development
26	Katayama Yoshio	Kobe University	Junior Associate Professor	Lymphoma progression promoted by the breakdown of the system for convergence of inflammation
27	Furuichi Tatsuya	Co-Department of Veterinary Medical Science Faculty of Agricultu	Professor	Role of CRIM1 as a novel regulator for osteoblast functions revealed using a osteoporotic mutant mouse
28	Fujiki Yukio	Medical Institute of Bioregulation, Kyushu University	Professor	Peroxisome biogenesis disorders: Molecular mechanism of pathogenesis in the cerebellum
29	Okahisa Yoko	Kyoto Institute of Technology	Associate Professor	Optically transparent bamboo-cellulose/collagen nanocomposite having antibacterial action
30	Oinuma Izumi	Graduate School of Life Science, University of Hyogo	Professor	Establishment of therapeutic genome editing techniques applicable for post-mitotic neurons

Novartis Research Grant (Kumamoto Earthguake Diaster frame)

#	Name	Institution	Title	Research Project
1	Nishiyama Koichi	Kumamoto University IRCMS	Associate Professor	Vascular intraluminal pressure-dependent switching machinery of angiogenic mode
2	Nakamura Taishi	Department of Cardiovascular Medicine, Kumamoto University	Associate Professor	Novel therapeutic strategy for heart failure targeting PKG/TORC signaling and its intracellular localization
3	Oshiumi Hiroyuki	Kumamoto University	Professor	Virus-mediated suppression of host innate immune response
4	Mimori Koshi	Kyushu University Beppu Hospital	Professor, MD,PhD	Treg Related Exosomal microRNA Involving to the Spatial and Chronologic Evolution of CRC
5	Ohtsubo Kazuaki	Kumamoto University	Professor	Elucidation of the sialyl-Tn antigen-mediated survival mechanism of hypoxic tumor cells

FY2016 Research Meeting Grant

(JPY 400 thousand x5 = 2.0 million)

#	Meeting	Date (Place)	Institution / Title	Name
1	7th International Symposium of Kyoto T Cell Conference	2017.3.13-17 (Kyoto)	Institute for Frontier Medical Sciences, Kyoto University / Professor	Hiroshi Kawamoto
2	CeNeuro2016 & Nagoya BNC Joint Meeting	2016.7.27-30 (Nagoya)	Nagoya University, Graduate School of Science, Division of Biological Science / Professor	Ikue Mori
3	20th International Symposium on Homogeneous Catalysis	2016.7.10-15 (Kyoto)	The University of Tokyo, Graduate School of Engineering / Professor	Kyoko Nozaki
4	The 4th International Symposium on Salivary Gland in Honor of Niels Stensen	2016.11.30-12.2 (Okazaki)	National Institute for Physiological Society, Department of Cell Physiology / Associate Professor	Masataka Murakami
5	The 5th Conference of International Society for Food and Environmental Virology	2016.9.13-16 (Gunma)	Faculty of Engineering, Hokkaido University / Associate Professor	Daisuke Sano

第30期（2016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。2016年度は、下記の総額3,700万円の助成事業を実施しました。

第30回ノバルティス研究奨励金	35件（1件100万円）	3,500万円
（※内、熊本震災枠	5件）	
研究集会助成	5件（1件 40万円）	200万円
	総額	3,700万円

第30回ノバルティス研究奨励金（2016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	廣瀬　哲郎	北海道大学 遺伝子病制御研究所	教授	Long non-coding RNAによるストレス応答性スプライシングの制御機能の解明
2	菊池　潔	東京大学大学院 農学生命科学研究科	准教授	性染色体組み換え抑制の起源
3	太田　茜	甲南大学	特別研究員	シングルセルトランスクリプトーム解析による神経情報処理の新規機構の同定
4	松島　良	岡山大学 資源植物科学研究所	准教授	ライブイメージングとシミュレーションを用いた澱粉粒の形状決定機構の解明
5	高山　賢一	東京都健康長寿医療センター研究所 老化制御　健康長寿ゲノム探索	研究員	前立腺癌におけるRNA結合タンパク質PSFを介したゲノムワイドでの転写、翻訳制御機構
6	水関　健司	大阪市立大学大学院 医学研究科	教授	海馬における経路特異的な情報処理機構の解明
7	好光　健彦	岡山大学　大学院医歯薬学総合研究科(薬学系)創薬有機化学分野	教授	抗腫瘍活性海洋産アルカロイドをモチーフとする天然物創薬
8	南野　徹	新潟大学	教授	血管ニッチによって制御されるステムセルエイジングと加齢関連疾患発症機序の解明
9	岩崎　由香	慶應義塾大学医学部 分子生物学教室	専任講師	小分子RNAを介したゲノム三次構造制御によるトランスポゾン抑制機構の解明
10	武藤雄一郎	東京理科大学	助教	アルキンの転位を起点とする水を酸素源とするスピロケタールの触媒的合成法の開発
11	白川　久志	京都大学 薬学研究科 生体機能解析学分野	准教授	脳内低酸素応答の病態生理学的意義およびその介入による中枢神経疾患への応用
12	多胡めぐみ	慶應義塾大学	准教授	エリスロポエチン受容体を介した慢性骨髄増殖性腫瘍の発症機序の解明
13	平井　希俊	関西医科大学 薬理学講座	講師	成長因子受容体を介した成熟心筋細胞の増殖誘導とその分子機構の解明

No.	氏　名	所　属	職　位	研究課題
14	大木理恵子	国立がん研究センター研究所	グループリーダー（主任研究員）	希少がんである神経内分泌腫瘍のがん抑制遺伝子PHLDA3の遺伝子診断による新しい予後・治療薬選択の診断法開発～神経内分泌腫瘍患者の個別化医療を目指して～
15	上田　健	近畿大学	講師	病態特異的ヒストンメチル化制御異常の白血病発症における役割
16	内田　宏昭	東京大学医科学研究所	講師	全身投与可能ながん標的化改変ヘルペスウイルスによる転移性悪性腫瘍の治療法開発
17	バルマプラブハット	大阪大学	教授	液中先端増強超解像ラマン顕微鏡による細胞膜のin vitro観察及びその機能解明
18	眞鍋　一郎	千葉大学	教授	免疫－神経－代謝システム間連携による心臓恒常性維持と心不全発症機序の解明
19	林　康紀	国立研究開発法人理化学研究所 脳科学総合研究センター	シニアチームリーダー	睡眠下のシナプス可塑性とリプレー現象の記憶固定化における役割
20	田中　実	名古屋大学	教授	生殖幹細胞の性決定機構の解明
21	山口　知也	名古屋大学大学院 医学系研究科	特任講師	肺腺がんでのROR1によるカベオラ形成とカベオラエンドサイトーシス制御機構の解明
22	松村　欣宏	東京大学 先端科学技術研究センター	助教	環境要因とエピゲノムによるエネルギー代謝制御
23	小柴　琢己	九州大学	准教授	ミトコンドリア・ダイナミクスと抗ウイルス自然免疫
24	笹井　紀明	奈良先端科学技術大学院大学	（独立）准教授	神経前駆細胞の安定的培養法の確立に向けた、エピゲノム的制御機構の解明
25	浜本　隆二	国立がん研究センター研究所	分野長	大腸がん幹細胞の未分化性維持におけるヒストン修飾調節機構の解明と創薬への応用
26	片山　義雄	神戸大学	講師	炎症収束機構の破綻によるリンパ腫進展メカニズム
27	古市　達哉	岩手大学 農学部共同獣医学科	教授	骨粗鬆症マウスを利用した新規骨芽細胞機能調節分子CRIM1の同定と機能解析
28	藤木　幸夫	九州大学 生体防御医学研究所	特任教授	ペルオキシソーム欠損症：小脳における病態発症の分子メカニズムの解明
29	小林（岡久）陽子	京都工芸繊維大学	助教	竹由来抗菌セルロースナノファイバー/コラーゲン透明コンポジットの創製
30	生沼　泉	兵庫県立大学大学院 生命理学研究科	教授	分化後神経細胞への直接的遺伝子治療法確立のための基盤技術開発

ノバルティス研究奨励金 震災枠

No.	氏　名	所　属	職　位	研究課題
1	西山　功一	熊本大学国際先端医学研究機構	特任准教授	血管内腔圧制御による血管新生モードのスイッチング機構
2	中村　太志	熊本大学医学部附属病院 循環器内科	客員助教	PKGのTOR複合体調節機構と細胞内局在化機構を標的とした新たな心不全治療戦略
3	押海　裕之	熊本大学大学院 生命科学研究部免疫学分野	教授	ウイルスが宿主自然免疫応答を抑制する新規メカニズムの解明
4	三森　功士	九州大学病院別府病院　外科	教授	大腸がんの時空間的進展にかかわる制御性T細胞関連のエクソゾーム内包microRNA
5	大坪　和明	熊本大学	教授	sTn糖鎖抗原による低酸素環境下でのがん細胞の生存戦略の解明

2016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。2016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	京都Ｔ細胞会議第７回国際シンポジウム	2017.3.13-17（京都）	京都大学再生医科学研究所・教授	河本　宏
2	2016年線虫神経生物学国際集会・名古屋大学神経回路国際シンポジウム合同集会	2016.7.27-30（名古屋）	名古屋大学大学院 理学研究科　生命理学専攻・教授	森　郁恵
3	第20回均一系触媒国際会議	2016.7.10-15（京都）	東京大学大学院工学系研究科・教授	野崎　京子
4	第４回ニールス・ステンセン記念国際唾液腺シンポジウム	2016.11.30-12.2（岡崎）	生理学研究所細胞器官研究系・准教授	村上　政隆
5	第５回国際食品・環境ウイルス学会	2016.9.13-16（群馬県）	北海道大学大学院工学研究院・准教授	佐野　大輔

30th Financial Report

Balance Sheet

As of March 31, 2017

(Unit：JP Yen)

Account	Amount
I Assets	
1. Current Assets	
Current Assets Total	50,535,116
2. Fixed Assets	
(1) Basic Fund	
Basic Fund Total	1,100,000,000
(2) Specific Assets	
Specific Assets Total	742,194
(3) Other Long - term Assets	
Other Long - term Assets Total	44,950,051
Fixed Assets Total	1,145,692,245
Assets Total	1,196,227,361
II Liabilities	
1. Current Liabilities	
Current Liabilities Total	35,078,870
Liabilities Total	35,078,870
III Equity (Net Assets)	
1. Designated Net Assets	
Designated Net Assets Total	1,000,742,194
(Amount Appropriating to basic Fund)	1,000,000,000
2. General Net Assets	160,406,297
(Amount Appropriating to)	100,000,000
Equity Total (Net Assets)	1,161,148,491
Liabilities & Equity Total	1,196,227,361

Statement of Net Assets

From April 1st, 2016 to March 31, 2017

(Unit：JP Yen)

Account	Amount
I General Net Assets Changes	
1. Ordinary income & Expenditure	
(1) Ordinary income	
Donation	40,054,000
Ordinary Income Total	53,632,169
(2) Ordinaty Expenditure	
Project Expense	46,796,276
Grant Expense	37,000,000
Novartis Research Grant	35,000,000
Research Meeting Grant	2,000,000
Administrative Expense	3,988,467
Ordinary Expenditure Total	50,784,743
Ordinary Balance without Appraisal Profit or Loss	2,847,426
2. Nonrecurring Profit & Loss	
Nonrecurring Balance of Current Period	
General Net Assets Ending Balance	160,406,297
II Designated Net Assets Changes	
Designated Net Assets Change	△ 54,000
Designated Net Assets Ending Balance	1,000,742,194
III Net Assets Balance Ending Balance	1,161,148,491

第30期（2016年度）財務報告

貸借対照表

2017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	50,535,116
2．固定資産	
⑴　基本財産	
基本財産　合計	1,100,000,000
⑵　特定資産	
特定資産　合計	742,194
⑶　その他固定資産	
その他固定資産　合計	44,950,051
固定資産　合計	1,145,692,245
資産合計	1,196,227,361
Ⅱ負債の部	
1．流動負債	
流動負債　合計	35,078,870
負債合計	35,078,870
Ⅲ正味財産の部	
1．指定正味財産	
指定正味財産　合計	1,000,742,194
（うち基本財産への充当額）	1,000,000,000
2．一般正味財産	160,406,297
（うち基本財産への充当額）	100,000,000
正味財産　合計	1,161,148,491
負債及び正味財産合計	1,196,227,361

正味財産増減計算書

2016年4月1日から2017年3月31日まで

（単位：円）

科　　目	金　額
Ⅰ一般正味財産増減の部	
1．経常増減の部	
⑴　経常収益	
受取寄付金	40,054,000
経常収益　計	53,632,169
⑵　経常費用	
事業費	46,796,276
支払助成金	37,000,000
ノバルティス研究奨励金	35,000,000
研究集会助成金	2,000,000
管理費	3,988,467
経常費用　計	50,784,743
当期経常増減額	2,847,426
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	160,406,297
Ⅱ指定正味財産増減の部	
当期指定正味財産増減額	-54,000
指定正味財産期末残高	1,000,742,194
Ⅲ正味財産期末残高	1,161,148,491

List of Board Members

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

Post	Name	Title
Chairman	Akimichi KANEKO	Dean, Professor, MD, Graduate School of Health Science, 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
	Masao TORII	President, Novartis Holding Japan K.K.
Auditor	Tokuzo NAKAJIMA	Certified Public Accountant
	Masanori FUSE	Department Head, Region Finance, Novartis Pharma K.K.

[Board of Councilors] 10 councilors As of Sep 1, 2017

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	Professor, University of Tokyo, Science Department; Team Leader, RIKEN (Institute of Physical & Chemical Research)
	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 Sep 1, 2017

Post	Name	Title
Chairman	Yoshihiro OGAWA	Professor, MD, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University Professor, MD, Graduate School of Medical Sciences, Kyushu University
Member	Motomu KANAI	Professor, Graduate School of Pharmaceutical Sciences, University of Tokyo
	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
	Masafumi TAKIGUCHI	Professor, MD, Center for AIDS Research, Kumamoto University
	Eisuke NISHIDA	Professor, Graduate School of Biostudies, Kyoto University
	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

公益財団法人ノバルティス科学振興財団

役 員 名 簿

2017 年 9 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
理事	金子　章道	畿央大学大学院健康科学研究科長･教授 慶應義塾大学名誉教授	2016年 6月10日	非常勤
	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	2016年 6月10日	非常勤
	遠藤　政夫	山形大学名誉教授	2016年 6月10日	非常勤
	高田　邦昭	群馬県立県民健康科学大学学長	2016年 6月10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	2016年 7月 1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） 経理・財務統括部長	2016年 6月10日	非常勤

評 議 員 名 簿

2017 年 9 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	東京大学大学院理学系研究科教授 理化学研究所光量子工学研究領域チームリーダー	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　美和子	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	ノバルティス 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

2017 年 9 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	小川　佳宏	九州大学大学院医学研究院教授 東京医科歯科大学大学院医歯学総合研究科教授	2014年　6月　6日	非常勤
選考委員	金井　　求	東京大学大学院薬学系研究科教授	2014年　6月　6日	非常勤
	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
	髙濵　洋介	徳島大学先端酵素学研究所教授	2015年　6月26日	非常勤
	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
	西田　栄介	京都大学大学院生命科学研究科教授	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

事 務 局 便 り

ご寄附のお願い

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

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当財団へのご寄付には、下記の税法上の優遇措置が適用されます。

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

法人：支出した寄附金は、通常一般の寄附金の損金算入限度額と同額まで、別枠で損金に算入できます。

ご寄附は、随時受付けております。詳しくは、財団事務局までお問合せ下さい。
（電話：03-6899-2100、E-メール：foundation.japan@novartis.com）

事務局より

本年度もお陰様で、財団年報を発行できることとなりました。これも偏に、助成を受けられた皆様および財団関係者の皆様のご尽力の賜物と心より感謝申し上げます。

1987年９月の財団設立以来、助成件数は総数で約1,700件、総額約20億円となりました。

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。

事務局長　田中　基晴

公益財団法人 ノバルティス科学振興財団
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