（続紙　1　）

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論文題目	Functional Analysis of MTSS1 Regulation of Purkinje Cell Dendritic Development and Actin Dynamics プルキンエ細胞樹状突起発達過程のアクチン動態を制御するMTSS1の機能解析		

（論文内容の要旨）

Dendrite patterning is a critical determinant of neuronal function and connectivity, yet shows high diversity among neuronal types. Thus, the question of how neurons acquire their appropriate morphology is a major interest in the study of neuronal development. Purkinje cells develop large, space-filling dendrites in a single plane with little overlap among the individual dendrites. To achieve this, Purkinje cell dendrites show a characteristic developmental pattern in which contact between dendrites, mediated by numerous small protrusions, signals one or both dendrites to retract. The actin-rich protrusions found on these dendrites function as environmental sensors, and while their dynamic changes in morphology have often been studied in the context of spinogenesis and synaptic plasticity, little work has been done on the contribution of these protrusions to the final morphology of dendritic arbors.

Metastasis-suppressor 1 (MTSS1) is a membrane and actin-scaffolding protein that shows notably high, developmentally-regulated expression in Purkinje cells. Using MTSS1 conditional knockout (cKO) mice, a stereotypical reduction in dendritic arbor size and shape was observed, concomitant with a significant increase in dendritic protrusion length. These morphological phenotypes were similarly observed in Purkinje cells cultured in vitro. Live imaging demonstrated that cKO Purkinje cells underwent frequent branch retraction at the earlier stages of their development, with no reduction in ability to form dendrites, suggesting that the over retraction of dendrites was the main contributor to the MTSS1 knockout dendritic phenotype. This increase in retraction was facilitated by increased contact between the longer dendritic protrusions. To understand what changes in the actin cytoskeleton led to this increase in dendritic protrusion length, several pharmacological inhibitors were added to Purkinje cells with either loss or overexpression of MTSS1. While MTSS1 overexpression upregulated the activity of the branched-actin nucleator ARP2/3, loss of MTSS1 resulted in increased formin-dependent actin regulation. Thus, loss of negative formin regulation in cKO Purkinje cells led to the increased length of dendritic protrusions.

The formin DAAM1 was observed to be highly localized to dendritic protrusions, and could transiently induce longer filopodia in Purkinje cells with induced expression of a constitutively active DAAM1 construct. Through biochemical experiments, the central region of MTSS1 was observed to bind to DAAM1, and expression of this portion was sufficient to rescue the MTSS1 knockout morphological phenotypes observed in Purkinje cells. These results suggested that neither the well-characterized I-BAR nor WH2 domain was necessary for MTSS1 function in Purkinje cells. Using Single-Molecule Speckle Imaging (SiMS), visualization of individual constitutively active DAAM1 dimers demonstrated that in the presence of MTSS1, the DAAM1-dependent elongation of actin was inhibited in two modes. The first was due to a non-specific inhibition of actin elongation due to WH2-dependent sequestration of monomeric actin. The second was a specific induction of DAAM1 pausing during actin elongation, likely due to the specific binding between the central region of MTSS1 and DAAM1. Together, these results suggest that MTSS1 may function as a regulator of DAAM1 that has already been activated, modulating its actin polymerization activity at the tips of dendritic protrusions. This regulation links biological events in distinct hierarchies and spatiotemporal scales: actin dynamics occurring at the molecular, submicron and second scale, and neuronal morphogenesis occurring at the scale of hundreds of microns and several days.

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（論文審査の結果の要旨）

Dendrite patterning is a critical determinant of function and connectivity of neurons in the central nervous system, yet shows high diversity among neuronal types. Thus, the question of how neurons acquire their appropriate morphology is a major interest in the study of neuronal development.

The applicant Kelly Kawabata analyzed the function of the actin-binding protein MTSS1 during proper dendritic branch patterning in cerebellar Purkinje neurons, which form the most elaborate and extensive dendrites of all neurons. She found that MTSS1 was highly expressed in developing dendrites of Purkinje cells and that cell-specific depletion of MTSS1 in a conditional knockout mouse resulted in significant dendritic hypoplasia. Live imaging of growing Purkinje cells in a dissociated culture revealed that the dendritic hypoplasia was caused by excessive retraction of growing branches in MTSS1-deficient Purkinje cells. It is known that the retraction of growing branches is triggered by physical contacts of fine protrusions emanating from the growing dendritic tips. The applicant found that these protrusions were significantly elongated in MTSS1-deficient cells, thereby increasing dendrite reach and contact-dependent retraction.

The applicant then focused on the molecular mechanism of how MTSS1 regulates protrusion morphology. Growing evidence has suggested that the balance of branched and unbranched actin formation is critical in achieving correct protrusion morphology, and is mediated by two major classes of actin nucleators, the ARP2/3 complex and formins, respectively. MTSS1 is an I-BAR domain-containing membrane- and actin-binding protein, which has been demonstrated to be an indirect activator of ARP2/3. The applicant demonstrated that, in addition to activating the ARP2/3 pathway, MTSS1 directly bound to the formin DAAM1, which is present at the tips of dendritic protrusions, and inhibited formin-dependent actin polymerization and steered actin toward ARP2/3-mediated nucleation. She further indicated that overactivation of DAAM1 was a direct cause of the excess elongation of dendritic protrusions in MTSS1-deficient Purkinje cells. Thus, her study demonstrates a link between biological events in distinct hierarchies and spatiotemporal scales: actin dynamics occurring at the molecular, submicron and second scale, and neuronal morphogenesis occurring at the scale of hundreds of microns and several days.

The thesis by Kelly Kawabata indicates not only her excellent competence but also her broad, profound knowledge that is required for life science research. In addition, it contains multiple new findings and conceptual advances that are highly relevant for many fields of life science, including neuroscience, cell biology and developmental biology. The committee concluded that it merits acceptance as a PhD thesis of the Graduate School of Biostudies.

The thesis defense and oral assessment were held on August 8, 2018.

論文内容の要旨及び審査の結果の要旨は、本学学術情報リポジトリに掲載し、公表とする。特許申請、雑誌掲載等の関係により、学位授与後即日公表することに支障がある場合は、以下に公表可能とする日付を記入すること。**（ただし、学位規則第８条の規定により、猶予期間は学位授与日から３ヶ月以内を記入すること。）**

要旨公開可能日：　　年　　月　　日