早稲田大学大学院 先進理工学研究科

博 士 論 文 概 要

論 文 題 目

Functional Analysis of Collapsin Response Mediator Proteins in Dendritic Development of Hippocampal Neurons

海馬神経細胞の樹状突起発達における CRMP の機能解析

申 請 者

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Formation of precise neural network is important for processing flexible information to perform brain functions. Collapsin response mediator proteins (CRMPs) are known to regulate cell migration, axonal growth, and dendritic spine formation. In my thesis, I will present a novel *in vivo* function of CRMPs in dendritic development of hippocampal neurons.

Human possesses high-order cognitive functions such like learning new information, storing it as a memory, and think from what we learned. These functions are processed by neural network. Electrical and chemical signals are sent from axons to dendrites in the neural network. Many studies have focused on revealing axonal development to understand the system of neural network. However, less has been understood for dendritic development. It is known that dendritic morphology is changed in patients who suffer with brain disorders such as Alzheimer's and epilepsy. For instance, reduction of dendritic length, changes in branching patterns, and spine abnormality are observed. These morphological changes lead to functional changes. For instance, they will lead to abnormalities in synaptic integration and plasticity. Molecular mechanisms of many brain disorders are not fully understood and treatment has not been established. Understanding the mechanisms of dendritic development will lead to understand the mechanisms of brain disorders. In future, it may contribute to the treatments for patients.

CRMP2 is one of the CRMP family proteins and is well studied among five members. It mediates Semaphorin 3A (Sema3A) signaling to induce axon growth cone collapse and axon retraction. CRMP2 mediates Sema3A signaling via phosphorylation by Cyclin-dependent kinase 5 (Cdk5)/p35. Previous studies showed that Sema3A-/- cortical neurons showed reduction in both apical and basal dendrites. Moreover, Sema3A-/- mice showed defect in hippocampal pyramidal neurons in CA1 area. In Sema3A-/- mice, many CA1 pyramidal neurons showed apical dendrite bifurcation of proximally to the cell body. Similar defect was reported in p35-/- mice. Cortex-specific Cdk5 knockout mice (CxCdk5KO mice) showed migration defect in cortex and hippocampus. In addition, CxCdk5KO mice showed abnormal dendritic morphology in cortical layer V neurons. These defects suggest that Sema3A signaling regulates dendritic development. However, the molecular mechanisms of Sema3A signaling for dendritic development have not been understood.

The above information strongly indicates that Sema3A signaling regulates dendritic development. It leads me to the speculation that phosphorylation of CRMPs is important for dendritic development. In this thesis, I present a novel function of CRMPs *in vivo*. In addition, I reveal the function and molecular mechanism of CRMPs for dendritic development using neuronal culture system.

This thesis is composed of seven chapters. Each chapter is summarized as follows.

In chapter 1, the background of this thesis is described. The molecular mechanisms of dendritic development are not fully understood. In my thesis, I studied the molecular mechanism of dendritic development. Extracellular cues are important signals for dendritic development. One of the extracellular cues is Sema3A. Sema3A is originally identified as an axon guidance cue. Recent studies showed that Sema3A regulates dendritic development. I have summarized Sema3A function for dendritic development. Studies of Cdk5/p35 showed that it also regulates dendritic development *in vivo*. Therefore, it is presumable that CRMPs mediate Sema3A signaling pathway. I hypothesize that phosphorylation of CRMP2 by Cdk5/p35 is important for dendritic development. In addition, I hypothesize possibility of redundancy among CRMP2 and other CRMP members. Particularly, I speculate the functional redundancy between CRMP2 and CRMP4 because of the following reasons. Firstly, CRMP2 and CRMP4 are phosphorylated at

Ser522 by Cdk5/p35. Secondly, CRMP2 and CRMP4 have high homology and form heterotetoramer. Thirdly, CRMP2 and CRMP4 are expressed highly in the developing brains. For these reasons, I hypothesized that CRMP2 and CRMP4 have functional redundancy.

In chapter 2, materials and methods are described. Methods of generation of CRMP2KI/KI and CRMP4-/- transgenic mice are described in this chapter. CRMP2KI/KI mice were used in order to analyze whether phosphorylation of CRMP2 by Cdk5/p35 regulate dendritic development. CRMP2 is not phosphorylated at Ser522 in CRMP2KI/KI mice, since Ser522 is a phosphorylation site by Cdk5/p35. CRMP4-/- mouse is generated for the first time. To analyze the morphology of neurons easily, I crossed CRMP transgenic mice and GFP-M mice in which GFP is expressed in CA1 pyramidal neurons of hippocampus.

Chapter 3 is the first part of the result section. In chapter 3, expression patterns of CRMP2 and CRMP4 were presented. These expression patterns were confirmed in the forebrain at embryonic stages and at a very early stage of postnatal day. Moreover, the expression patterns were confirmed in cultured neurons.

Chapter 4 is the second part of the result section. In order to examine whether phosphorylation of CRMP2 regulates dendritic development, phenotype of CRMP2KI/KI mice was analyzed. Contrary to my expectation, CRMP2KI/KI mice did not show any severe dendritic defects. However, functional redundancy can be considered between CRMP2 and other CRMP members. Therefore, phenotypes of CRMP4-/- and CRMP2KI/KI;CRMP4-/- mice were analyzed. Interestingly, CRMP4-/- and CRMP2KI/KI;CRMP4-/- mice showed abnormal bifurcation of apical dendrite of CA1 pyramidal neurons proximally to the cell body. CRMP2KI/KI;CRMP4-/- mice showed severe defect compared to CRMP4-/- mice. These results suggest that CRMP2 and CRMP4 regulate apical dendrite bifurcation of CA1 pyramidal neurons. I presented proximal bifurcation phenotype in CRMP4-/- and CRMP2KI/KI;CRMP4-/- mice of CA1 pyramidal neurons *in vivo*. This phenotype was similar to the one observed in Sema3A-/- and p35-/- mice.

Chapter 5 is the last part of the result section. Three major results are contained in this section. Firstly, I presented the function of CRMP2 and CRMP4 that suppress dendritic branches. This was presented by analyzing cultured hippocampal neurons from wild-type, CRMP2KI/KI, CRMP4-/-, and CRMP2KI/KI;CRMP4-/- mice. It suggested that loss of suppression of dendritic branches lead proximal bifurcation *in vivo*. Secondly, I presented that CRMP2 and CRMP4 mediated Sema3A signaling. I confirmed it by analyzing the response of Sema3A to cultured hippocampal neurons from wild-type and transgenic mice. Significant increase in total length and the number of branching points was observed when Sema3A was added to hippocampal neurons from wild-type mice. On the other hand, the response was compromised when Sema3A was added to hippocampal neurons from transgenic mice. Thirdly, I presented the increased binding level between CRMP2 and tubulin in CRMP2KI/KI;CRMP4-/- brain lysates. The increased binding level of CRMP2 and tubulin in CRMP2KI/KI;CRMP4-/- mice suggest that microtubule dynamics was altered. Altered microtubule dynamics might be related to the apical dendrite bifurcation.

In chapter 6, results of my study are discussed. I discuss about the two following points.

Firstly, I discuss about the upstream molecules of CRMP2 and CRMP4. I have presented that Sema3A regulates phosphorylation of CRMP2 and CRMP4 for dendritic development in present study. On the other hand, several studies reported that other molecules also regulate phosphorylation of CRMP2 and CRMP4. For instance, phosphorylation of CRMP2 is regulated by neurotrophins and brain derived neurotrophic factor. Phosphorylation of CRMP4 is regulated by myelin-associated proteins. Therefore, further studies will be necessary to explore upstream molecules that regulate CRMP2 and CRMP4 for dendritic development.

Secondly, I discuss about the downstream molecules of CRMP2 and CRMP4. In my thesis, I presented that binding level of CRMP2 and tubulin was increased in CRMP2KI/KI;CRMP4$^{-/-}$ brain lysates. This indicates the altered cytoskeletal dynamics. Cytoskeleton dynamics such as tubulin polymerization and actin bundling are critical for dendritic development. CRMP4 is known to regulate actin bundling for dendritic outgrowth. Therefore, further studies will be necessary to explore the downstream molecule of CRMP2 and CRMP4 for dendritic development.

Finally, in chapter 7, the conclusions of this research are summarized. In addition, future works are suggested.

Experimental results described in this thesis indicate that CRMP2 and CRMP4 regulate apical dendritic bifurcation *in vivo*. *In vitro* experimental data indicate that proximal bifurcation was lead by loss of suppression of dendritic branching. Moreover, these data indicate that CRMP2 and CRMP4 mediate Sema3A signaling pathway for dendritic development. Therefore, in my thesis I demonstrated a novel function of CRMP2 and CRMP4 in dendritic development.

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