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学位論文題目	Study on the role of protein arginine methyltransferase 1(PRMT1) in the development of the central nervous system (中枢神経系発達過程におけるアルギニンメチル化酵素 PRMT1 の機能解析)

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論文の要旨
Abstract of thesis

< Purpose >

The central nervous system (CNS) development is achieved by proliferation of progenitor cells followed by the transition from a proliferative state to differentiation. Emerging evidence suggests that post-translational modification of proteins is one of the important determinants for cell lineage development in the CNS. Protein arginine methylation catalyzed by protein arginine methyltransferases (PRMTs) is now widely accepted as one of the major post-translational modifications observed in both histone and non-histone proteins. PRMT1, one of the type I PRMTs that catalyze monomethylation and asymmetric dimethylation of proteins, regulates various cellular processes, including transcription, cell death, DNA damage response, and signal transduction. Although PRMT1 has been reported to be expressed at the highest level in the developing CNS tissues of mouse embryos, its physiological functions *in vivo* were poorly characterized due to early embryonic lethality of the conventional knockout mice for *PRMT1*. In the present study, the applicant aimed to investigate *in vivo* functions of PRMT1 during CNS development by generating and

analyzing CNS-specific *PRMT1* conditional knockout mice.

< Results >

-PRMT1 Deletion in the CNS Causes Growth Retardation, Post-natal Lethality, and Behavioral Deficit in Mice

To address the primary function of PRMT1 in the CNS, the applicant produced CNS-specific *PRMT1* knockout mice with the Cre-loxP system. *Prmt1*$^{flox/flox}$ mice were crossed with mice expressing *Nestin-Cre* (*Nes-Cre*) to generate *Prmt1*$^{flox/flox}$*; Nes-Cre* (PRMT1-CKO) mice. *Nestin-Cre* is expressed in neural stem cells that are the origin of the major CNS cells including neuron, astrocyte, and oligodendrocyte. PRMT1-CKO mice were born at the expected Mendelian ratio, however, any CKO pups could not survive after post-natal day 17 (P17). PRMT1-CKO mice exhibited growth retardation and abnormal behavior characterized by ataxic phenotype.

-Loss of PRMT1 in the CNS Results in Dynamic Change in Methyl Arginine Levels in the Brain

To investigate the contribution of PRMT1 to arginine methylation of brain proteins, the applicant analyzed methyl-arginine levels by Western blotting and LC-MS/MS, and obtained the results showing the decrease of asymmetric dimethyl arginine (ADMA) and increase of mono-methyl arginine (MMA) and symmetric dimethyl arginine (SDMA) in the brain of PRMT1-CKO mice. From this data, the applicant concludes that PRMT1 largely contributes to the status of protein arginine methylation on multiple proteins in the brain.

-Severe Hypomyelination and Dramatic Decrease in the Number of Mature Oligodendrocytes in *Prmt1*$^{flox/flox}$*; Nes-Cre* Mice

In order to determine which cell type in the CNS is affected by the deletion of *PRMT1*, the applicant analyzed the levels of major CNS cell marker proteins by Western blotting with the brain lysate. The applicant found remarkable reduction of mature oligodendrocyte proteins including myelin basic protein (MBP), 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase), and myelin-associated glycoprotein (MAG) in the brain of PRMT1-CKO mice without any changes in neuronal and astrocytic marker proteins.

The applicant also found that PRMT1-CKO mice revealed nearly complete loss of MBP positive signals in major brain regions including the white matter tracts in the corpus callosum, striatum, cerebellum, and the spinal cord by immunostaining with MBP antibody. Interestingly, she found by electron microscopic analysis that axon myelination in the corpus callosum and white matter tracts of the spinal cord in P10 PRMT1-CKO pups was impaired. Collectively, the applicant suggested that PRMT1 is critical for the oligodendrocyte lineage among all CNS cell development.

-Number of Oligodendrocyte Lineage Cells Are Reduced in *Prmt1*$^{flox/flox}$*; Nes-Cre* Mice

The applicant also investigated the involvement of PRMT1 in the differentiation of neuronal

stem cells to OPCs and premyelinating oligodendrocytes by co-staining of MBP and the oligodendrocyte lineage marker OLIG2. The applicant found that the number of $OLIG2^{+}MBP^{-}$ cells was significantly reduced in the striatum, in the cerebellum and the spinal cord in PRMT1-CKO mice, suggesting that oligodendrocyte lineage progression is partially suppressed by PRMT1 deletion.

Oligodendrocyte development is mainly promoted by several key transcription factors that belong to the basic-helix-loop-helix (bHLH) protein family, homeodomain proteins, and high-mobility-group (HMG) domain proteins. The applicant found by quantitative RT-PCR that these factors such as *Olig2*, *Olig1*, and *Sox10* were significantly reduced in the brain of P0 and P10 PRMT1-CKO mice, suggesting that PRMT1 positively regulates these transcription factors for cell differentiation.

- PRMT1 Is Expressed in Oligodendrocyte Lineage Cells *In Vitro* and *In Vivo*

Oligodendrocyte lineage progression could be regulated by both cell autonomous and non-autonomous mechanisms. To clarify this issue, the applicant analyzed profiles of PRMT1 expression in oligodendroglia by immunohistochemical and immunocytochemical analyses. Several PRMT1-positive cells were found to be co-labeled with OLIG2 in the cell nucleus in the cerebellar white matter of P4 mice. In addition, OPCs and mature oligodendrocytes in culture were found to express PRMT1. Furthermore, the applicant found that the levels of PRMT1 in differentiating rat OPCs/oligodendrocytes gradually decrease as they differentiate, indicating that PRMT1 is more important in immature stage of cell development rather than mature cell maintenance.

- PRMT1 in Primary OPCs Is Not Involved in the Oligodendrocyte Differentiation

As the data of the *in vivo* studies suggest the possibility that PRMT1 acts as a positive regulator of oligodendrocyte differentiation, the applicant examined whether PRMT1 overexpression stimulates the OPC differentiation into mature oligodendrocyte with primary mouse OPCs by immunocytochemistry for oligodendrocyte stage-specific markers. The applicant found that PRMT1 overexpression did not affect the rate of oligodendrocyte differentiation, indicating that PRMT1 is not essential for oligodendrocyte differentiation.

< Discussion >

In this study, the applicant provided evidence that PRMT1 is essential for CNS development, especially for oligodendrocyte cell lineage progression and myelination. Moreover, results obtained by the applicant in this study suggest the potential role of PRMT1 in stem cell regulation including neural stem cells and earlier stage of oligodendrocyte lineage cells in the context of cell proliferation and differentiation. However, the present study does not totally rule out the possibility for the significances of PRMT1 in the development of other types of cells in the CNS. To address this issue, further analyses are required using cell type-specific conditional knockout mice.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant provided evidence that PRMT1, which catalyzes arginine methylation of proteins, is essential for CNS development, especially for oligodendrocyte cell lineage progression and myelination. This finding gives insight into the molecular mechanisms for oligodendrocyte differentiation, and contributes to the development of new drugs for fatal dysmyelination diseases of unknown etiology including metachromatic leukodystrophy (MLD) and periventricular white matter injuries (PWMIs). Considering that successful oligodendrocyte cell lineage development is also critical for adult demyelinating diseases such as multiple sclerosis (MS), PRMT1 or its brain substrates would be a potential target for regenerative medicine. As the loss of PRMT1 in the CNS shows severe and complex phenotypes such as early postnatal death, PRMT1 could play vital roles in other lineages such as neuron and astrocyte development. Further studies using cell type-specific knockout animals would reveal the precise mechanisms and establish a benchmark for the future research in this field.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 13th January, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.