早稲田大学大学院 先進理工学研究科

博 士 論 文 概 要

A STUDY OF THE FUNCTION OF CDK5 IN OLIGODENDROCYTES DIFFERENTIATION BY USING CONDITIONAL KNOCKOUT STRATEGY

条件付きノックアウト法を用いたオリゴデンドロサイトの分化における Cdk5 の機能に関する研究

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Cyclin-dependent kinases (Cdks) belong to a large family of proline-directed serine-threonine kinases that are activated by the cyclin regulatory subunit and regulate the cell cycle division. Cyclin-dependent kinase 5 (Cdk5) is a member of this kinase family, which is encoded by *Cdk5* gene. Cdk5 presents in most of cell types, however, its highest expression and kinase activity are detected in postmitotic neurons. Thus, Cdk5 is an exceptional member of the Cdks family which does not regulate the cell cycle division but may play a pivotal role in neuronal functions. This property of Cdk5 attracts many neuroscientists to explore its role in central nervous system. Cdk5 substrates are involved in modulating cell morphology and motility. The Cdk5-/- mice showed inverted cerebral cortical layers due to defective neuronal migration. Cdk5 is also known to be involved in axonal elongation. Variety of Cdk5 substrates indicates that Cdk5 may be responsible for not only normal development and physiology but also for pathological states of nervous system. Cdk5 is reported to play a critical role in Alzheimer's disease, amylotropic lateral sclerosis, Parkinson's disease and Huntington's disease.

Oligodendrocytes are the myelinating cells in the vertebrate central nervous system. They function as insulation of axons in the central nervous system. Oligodendrocyte lineage develops during late embryonic and early postnatal stages, most of which process through a series of morphological and protein expression distinct stages before becoming mature oligodendrocytes. There are mainly three waves of oligodendrocyte progenitors generated from different lineages in the telencephalic ventricular zone: the first wave originates in the medial ganglionic eminence (MGE) or in the ventral anterior entopeduncular area (AEP) in the ventral forebrain at embryonic day (E) 11.5 following by a subsequent wave from the lateral and caudal ganglionic eminences (LGE and CGE) at E15. Finally, a third wave arises from the subventricular zone (SVZ) of the postnatal cortex after birth. Once the progenitor cells develop into Oligodendrocyte precursor cells (OPCs), the OPCs proliferate and migrate through the developing white matter until reaching their final destination; finally, the vast majority of them differentiate to myelin-producing oligodendrocyte sheathing axons. Specific markers can be used to identify each developmental stage. For instance, Olig2 is usually used as a marker for precursor cells. PDGFRα can indentify immature oligodendrocyte. MBP, PLP and MAG can detect mature oligodendrocytes.

Myelination is the process that the axon of certain nerve cell is wrapped by a layer of myelin which makes the axon insulated from other axons, prevents the electrical current from leaving the axon in which the impulse is propagated and allows the nerve impulses to travel faster along the myelinated fibers. In human, the complete development of brain does not finish at birth. As a matter of fact, to complete a general development of the brain needs another ten or twelve years after birth. The complete maturation can provide a smooth flow of neural impulses throughout the brain, which allows for information to be integrated across many spatially segregated brain regions. Dysmyelination is a defective phenotype of myelination, also called hypomyelination, in which defective sheaths often arise from genetic mutations affecting the biosynthesis and formation of myelin. One of the representative animal models of dysmyelination is the shiverer mouse. Human diseases where dysmyelination has been implicated include leukodystrophies and schizophrenia.

In this study, I found that the number of oligodendrocyte precursor cells or immature oligodendrocytes did not have significant difference in Emx1-cre mediated Cdk5 conditional knockout (Emx1-cKO) mice compared with control mice. However, the number of mature oligodendrocytes was distinctly reduced in Emx1-cKO mice compared with control group. And I found hypomyelination in Emx1-cKO is due to the impaired

differentiation of oligodendrocytes, rather than to the proliferation or migration of their precursors, and confirmed the *in vivo* role of Cdk5 in oligodendrocyte differentiation.

This dissertation consists of seven chapters, which are summarized as follows.

In chapter 1, the background of this study is described. Cyclin-dependent kinase 5 and its functions are explained in this chapter. I also described what the oligodendrocytes are and how they develop. At the last part of chapter 1, the myelination related nervous system diseases are discussed.

In chapter 2, the purpose of this study is described. Previous work, related studies and the purpose of this study are explained. So far until now, most of the studies have been done to explore the function of Cdk5 and its activator p35 in neurons, but few reports show the role of Cdk5 in glial cells. It was reported that the differentiation of OPCs is accompanied by the increased level of Cdk5 kinase activity. Another study showed that Cdk5 regulates OPC migration and differentiation *in vitro*. They found that suppression of Cdk5 by the specific inhibitor roscovitine or by the retrovirus encoding short-hairpin RNA for Cdk5 impaired the PDGFR-dependent oligodendrocyte precursor cells migration. Moreover, Cdk5 inhibitor roscovitine inhibits oligodendrocyte differentiation. Based on the previous studies, a proposal of what kind of role Cdk5 plays in oligodendrocytes development *in vivo* is discussed in this chapter.

In chapter 3, experimental methods and materials are described. In this chapter, I show the generation of Cdk5 conditional knockout mice. Two types of conditional knockout mice were generated. One of them is Emx1-cKO mice, in which the Cdk5 gene was deleted in neurons, astrocytes and oligodendrocyte -lineage cells. The other one is CaMKII-cre mediated Cdk5 conditional knockout (CaMKII cKO) mice, in which the Cdk5 gene was deleted only in neurons. In addition, the procedures to perform histological analysis, western blot, electron micrograph and immunoprecipitation are also illustrated in this chapter.

In chapter 4, the results of this study are elucidated.

First, I examined the CNPase protein level, a myelin-related protein, in Emx1-cKO mice. I found that the protein level was significantly reduced compared with the control mice. In addition, another conditional knockout mice line (CaMKII cKO mice), in which Cdk5 gene was deleted only in neurons, did not show reduced protein level of CNPase.

Second, because the development of mature oligodendrocytes from oligodendrocyte precursor cells is a complex process, I examined the expression pattern of OPC and immature oligodendrocyte markers (Olig2 and PDGFRα) by performing *in situ* hybridization with the Cdk5-/- mice brain. The result shows that the number of stained cells, however, there was not any significant reduction detected in OPCs and immature oligodendrocytes in the cerebral cortices of Cdk5-/- mutant mice and littermate controls. The different distribution of OPCs is observed, which may be a secondary consequence of the abnormal layer structure of Cdk5-/- mice.

In addition, I performed *in situ* hybridization using markers for the oligodendrocyte precursor cell, immature oligodendrocytes and differentiated oligodendrocyte with the Emx1-cKO and its littermate control mice brain. The results show that the number of MBP, PLP, MAG and CNP labeled mature oligodendrocytes is distinctly reduced in Emx1-cKO mice compared with the control mice at postnatal day (P) 14, in contrast, the Olig2 and PDGFRα labeled oligodendrocyte precursor cells and immature oligodendrocytes in Emx1-cKO mice are the same with the control mice at P 3, P12 and P14, respectively. The results implicates that the oligodendrocyte differentiation in

Emx1-cKO mice was impaired. To further confirm this hypothesis, electron microscopic image is examined which also showed in Emx1-cKO axons were less myelinated in lateral olfactory tract (LOT) and corpus callosum (CC), while the axons of littermate control mice are myelinated by oligodendrocytes.

Furthermore, I injected the mice with BrdU to further examine the proliferation and migration of OPCs, and the results indicate in Emx1-cKO mice, the proliferation and migration of OPCs were no observed abnormal, but the differentiation is severely defected. My study indicates that hypomyelination in Emx1-cKO is due to the impaired differentiation of oligodendrocytes, rather than to the proliferation or migration of their precursors.

Finally, to verify the downstream pathway of Cdk5 in the regulation of the oligodendrocyte differentiation, immunoprecipitation is used to test whether Paxillin is important in the downstream pathway *in vivo*. The result shows that Paxillin plays a less important role in the process of oligodendrocyte differentiation.

In chapter 5, the results of this study are discussed. Previous studies report that the differentiation of primary oligodendrocyte precursor cells is accompanied by the increased level of Cdk5 kinase activity. It has also been reported that Cdk5 is expressed in both precursor cells and oligodendrocytes; however, the activity of Cdk5 is nearly 2-fold higher in oligodendrocytes than in precursor cells. These studies indirectly support our hypothesis that the deletion of Cdk5 in oligodendrocytes may induce more defects than deletion in precursor cells. There are very few reports regarding the function of Cdk5 in oligodendrocytes; this study provides the evidence that Cdk5 also plays a critical role in the differentiation of oligodendrocytes *in vivo*. As reported previously, inverted cortical layers were observed in both Cdk5-/- and Emx1-cKO mice. Whether the defect of oligodendrocyte differentiation is a secondary consequence of abnormal axon trajectory or not needs to be concerned. One study demonstrated that the timing of myelination in *reeler* was similar to that of control mice. Abnormal axonal trajectory and normal myelination were also observed in Shaking rat Kawasaki which shares reelin gene defect with *reeler* mice. Therefore, differential defect of oligodendrocyte in Emx1-cKO mice we described here is not subsequent consequence of abnormal trajectory of axon. Paxllin, one of the substrates of Cdk5 is indentified as an important factor in the process of oligodendrocyte differentiation *in vitro*. However, in this *in vivo* study, Paxillin shows less important in regulating oligodendrocyte differentiation. Because phosphorylated paxillin provides the binding sites for other proteins, or modulates interaction between paxillin and other proteins, paxillin can only be one part of the signal transduction pathway that induces oligodendrocyte differentiation. Previous studies show that lack of Cdk5 can lead to differentiation defect in various cell types and cytoskeletal proteins are substrates of Cdk5. Based on the previous studies, I propose a high hypothesis that Cdk5/p35 complex interacts with cytoskeletal proteins, regulates cytoskeletal reorganization and regulate cell differentiation.

In chapter 6, the conclusion is summarized and future work is described. This study indicates that the lack of Cdk5 leads to hypomyelination in Emx1-cKO mice due to the impaired differentiation of oligodendrocytes, rather than the proliferation or migration of their precursors. This is first report of Cdk5 in oligodendrocyte differentiation *in vivo*.

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