Role of Presenilin-1 in Murine Neural Development

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ABSTRACT: Our previous studies showed that presenilin-1 (PS1) is required for murine neural and skeletal development. Here we report that the reduction in the neural progenitor cells observed in the *PS1*$^{-/-}$ mouse brain is due to premature differentiation of progenitor cells, rather than to increased apoptotic cell death or decreased cell proliferation. In the ventricular zone of *PS1*$^{-/-}$ mice, expression of the Notch1 downstream effector gene *Hes5* is reduced, and expression of the Notch1 ligand *Dll1* is elevated, indicating reduced Notch signaling. These results provide direct evidence that PS1 is involved in the regulation of neurogenesis and Notch signaling during development.

INTRODUCTION

The presenilin-1 gene (*PS1*) is a major gene responsible for familial Alzheimer's disease (FAD), and mutations in *PS1* account for approximately 50% of early-onset FAD cases.[1] Understanding the normal physiological functions of PS1 may shed light on the pathogenic mechanism of FAD-linked PS1 mutations. Studies of the *PS1* homologs in *C. elegans* and *Drosophila* provided the evidence that PS1 interacts with the LIN-12/Notch signaling pathway, which mediates cell-cell interactions that specify cell fate during development. The *PS1* homolog in *C. elegans, sel-12*, facilitates signaling mediated by LIN-12.[2] Furthermore, the wild-type human *PS1* cDNA complements the *sel-12* mutant phenotype, while PS1 containing FAD-linked mutations exhibited reduced ability to rescue *sel-12* mutations.[3,4] Fly mutants lacking both maternal and zygotic PS exhibit a neurogenic phenotype and are virtually indistinguishable from the Notch-null mutant, suggesting that PS function is required for Notch signaling in *Drosophila*.[5,6] PS is required for the proteolytic cleavage of Notch to release its intracellular domain (ICD).[5] This is further supported by studies using truncated Notch1 and primary cell cultures derived from *PS1*$^{-/-}$ mice.[7,8] Levels of the ICD fragment were reduced in cultured *PS1*$^{-/-}$ neurons and fibroblasts, indicating that PS1 facilitates proteolytic release of the ICD.

To characterize the normal physiological role of PS1 in mice, we previously generated mice with a targeted germ-line disruption of the *PS1* gene.[9] *PS1*-null mice exhibited defects in somitogenesis similar to those observed in *Notch1*-null mutant

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mice,[10,11] as well as severe malformation of the axial skeleton and cerebral hemorrhage.[9,12] Furthermore, we showed that lack of PS1 results in a reduction in the neural progenitor population and a subsequent reduction in the neuronal population, indicating a critical role for PS1 in murine neurogenesis.[9] We also observed symmetric, region-specific loss of neural progenitor cells and neurons in the *PS1*$^{-/-}$ brain during the latter stages of neurogenesis, suggesting a neuroprotective role for PS1 during neural development.

Here, we report that the reduction in the neural progenitor population in *PS1*$^{-/-}$ mice is caused by premature differentiation of neural progenitor cells. To investigate the mechanism underlying this neurogenesis defect, we examined the expression of genes in the Notch signaling pathway. We find a reduction in the level of *Hes5* transcripts as well as an increase in the level of *Dll1* transcripts in the *PS1*$^{-/-}$ brain, indicating that absence of PS1 function leads to reduced Notch signaling during neural development. Taken together, our findings provide the evidence that PS1 controls the cell fate decision between neural progenitor cells and postmitotic neurons and regulates Notch signaling during neural development.

RESULTS

Beginning at embryonic day 12.5 (E12.5), it becomes evident that the ventricular zone in the *PS1*$^{-/-}$ brain is thinner than in the control, reflecting a reduction in the population of neural progenitor cells.[9] Three mechanisms could contribute to the reduction in the progenitor population: premature differentiation of progenitor cells, decreased proliferation, or increased apoptotic cell death. To distinguish among these possibilities, we compared the *PS1*$^{-/-}$ and control brains between E10.5 and E14.5 for differences in neuronal differentiation, cell proliferation, and survival.

Premature Differentiation of Neural Progenitor Cells in PS1$^{-/-}$ *Mice*

To assess neuronal differentiation in the *PS1*$^{-/-}$ and control brains, we performed immunostaining for microtubule-associated protein 2 (MAP2), a marker specific for postmitotic neurons.[14] Comparable transverse sections of the *PS1*$^{-/-}$ and littermate control brains were compared. In the *PS1*$^{-/-}$ brain at E10.5, increases in the number of MAP2-immunoreactive neurons are evident in the diencephalic neuroepithelium. At E11.5, increases in MAP2 immunoreactivity in the *PS1*$^{-/-}$ brain relative to the control are found in the anterior telencephalon, and more substantially in the posterior telencephalon. In the diencephalon of the *PS1*$^{-/-}$ brain, the MAP2-immunoreactive neurons encompass many cell layers and have largely replaced the MAP2-negative neural progenitor cells in the ventricular zone.

In summary, markedly increased numbers of postmitotic neurons accumulate in the telencephalon and diencephalon of the *PS1*$^{-/-}$ brain during early neural development, accompanied by a progressive reduction in size of the ventricular zone. These observations indicate that neural progenitor cells differentiate into postmitotic neurons prematurely in the absence of PS1, resulting in early depletion of the neural progenitor population.

Although premature neuronal differentiation provides an explanation for the reduction of progenitor cells in the *PS1*$^{-/-}$ brain, it remained possible that a decrease

in cell proliferation and/or an increase in apoptotic cell death could be contributing factors as well. Cell proliferation rate was measured by the percentage of progenitor cells in S-phase during a short pulse labeling with bromodeoxyuridine (BrdU). We observed no significant differences in the BrdU-labeling patterns in the *PS1*$^{-/-}$ and littermate control brains at E10.5. The ratio of BrdU-labeled cells to the total number of progenitor cells in the ventricular zone of the telencephalon and diencephalon is similar in the *PS1*$^{-/-}$ and control brains, indicating that lack of PS1 does not lead to a reduction in the proliferation rate.

To determine whether increased apoptotic cell death might contribute to the reduction in the neural progenitor population in the *PS1*$^{-/-}$ brain, we assessed the number of apoptotic cells labeled by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) assay in the *PS1*$^{-/-}$ and control brains at E11.5 and E12.5. Quantitative comparison of the number of apoptotic cells in the *PS1*$^{-/-}$ and control brains revealed no significant differences, indicating that PS1 is not involved in the regulation of apoptosis during early neurogenesis.

Reduced Notch Signaling in PS1$^{-/-}$ *Mice*

To understand the mechanism underlying the premature neuronal differentiation observed in the *PS1*$^{-/-}$ brain, we examined the expression of genes in the Notch signaling pathway, which is known to be involved in cell fate determination in *Drosophila*.[15] The role of Notch receptors in murine neurogenesis is poorly understood. Mice lacking Notch1 or Notch2 function die at approximately E9 or E11, respectively, before cortical neurogenesis begins.[10,11,16] However, excess cells expressing proneural transcription factors were identified in the midbrain and hindbrain of *Notch1*$^{-/-}$ mice.[17] The basic helix-loop-helix transcription factors Hes1 and Hes5, which are downstream effectors of the Notch signaling pathway, have been shown to regulate murine neuronal differentiation.[18–21] We therefore first examined the levels of *Hes1* and *Hes5* transcripts to determine whether their expression is affected in *PS1*$^{-/-}$ mice.

In situ hybridization analysis revealed that at E11.5 the level of *Hes5* transcripts in *PS1*$^{-/-}$ mice is reduced in the anterior telencephalon, ganglionic eminence, and diencephalon, while expression of *Hes1* is unchanged. Consistent with previous studies,[22] *Hes5* expression is localized to the ventricular zone within each brain region. Northern analysis of poly(A)+ RNA derived from the *PS1*$^{-/-}$ and control brains also showed reduced levels of *Hes5* transcripts in the *PS1*$^{-/-}$ brain. These results indicate that lack of PS1 function leads to a reduction in *Hes5* expression, providing *in vivo* evidence for an involvement of PS1 in the Notch signaling pathway during neurogenesis and an explanation for the premature differentiation of neural progenitor cells observed in the *PS1*$^{-/-}$ brain.

It has been postulated in *Drosophila* that Notch and its ligand Delta are linked by a regulatory negative feedback loop under the transcriptional control of the *Enhancer-of-split* and *achaete-scute* complex gene products.[23–25] In addition, expression of *Dll1* is upregulated in the *Notch1*$^{-/-}$ embryo.[17] To determine whether reduced Notch signaling in *PS1*$^{-/-}$ mice leads to upregulation of *Dll1* expression, we examined the levels of *Dll1* transcripts by *in situ* hybridization and Northern analyses. *In situ* hybridization analysis revealed that *Dll1* expression is localized in isolated cells in the ventricular zone, consistent with previous reports.[26,27] At E11.5, the number of

Dll1-expressing cells is increased in the telencephalon and more substantially in the diencephalon of the *PS1*$^{-/-}$ brain. Quantitative comparison of *Dll1*-expressing cells in the telencephalon revealed a 40% increase in the density of *Dll1*-expressing cells in the *PS1*$^{-/-}$ neuroepithelium. Northern analysis also showed a marked increase in the level of *Dll1* transcripts, providing further support for the downregulation of Notch signaling in *PS1*$^{-/-}$ mice.

To determine whether PS1 regulates Notch signaling at the level of transcription, translation, and/or posttranslational maturation and activation, we examined the *PS1*$^{-/-}$ and control brains for differences in the levels of *Notch1* expression. *In situ* hybridization and Northern analyses of the *PS1*$^{-/-}$ and littermate control brains revealed no significant differences in the level of *Notch1* transcripts. Immunohistochemical analysis of comparable sections of the *PS1*$^{-/-}$ and control brains using a polyclonal antiserum[28] raised against the ICD of mouse Notch 1 also showed no differences in the intensity of Notch1 immunoreactivity. These results support a role for PS1 in the regulation of Notch1 posttranslational activation.

DISCUSSION

Our previous characterization of *PS1*$^{-/-}$ mice documented specific defects in central nervous system (CNS) development, revealing a function for PS1 in the mammalian brain.[9] Here we characterize the mechanisms underlying the progressive reduction in neural progenitor population that we observed in the *PS1*$^{-/-}$ brain. During early neurogenesis, very few progenitor cells in the ventricular zone exit the cell cycle to differentiate into postmitotic neurons, while the vast majority of progenitor cells remain in the cell cycle after mitosis, resulting in a steady expansion of the progenitor population. Here we have shown that lack of PS1 function leads to an increase in the number of differentiated postmitotic neurons at the expense of neural progenitor cells, indicating that PS1 regulates the cell fate decision between neural progenitor cells and postmitotic neurons in the developing brain. The premature differentiation of neural progenitor cells results in depletion of the neural progenitor population, providing an explanation for the progressive reduction in neural progenitor cells observed in the *PS1*$^{-/-}$ brain at E12.5, E14.5, and E16.5, particularly in the posterior portion of the brain.[9]

To understand further the mechanism by which PS1 controls cell fate decisions during neural development, we examined the expression of genes involved in the Notch signaling pathway, *Notch1*, *Dll1*, and the Notch downstream effector genes *Hes1* and *Hes5*. Previous studies have suggested a connection between Notch signaling and the regulation of neuronal differentiation. Premature neuronal differentiation has been observed at E10.5 in mutant mice lacking Hes1, Hes5, or both.[18,20] Here we have shown that expression of *Hes5* is downregulated in the *PS1*$^{-/-}$ brain, indicating that Notch signaling in the developing brain is reduced in the absence of PS1. Furthermore, analysis of the proteolytic processing of truncated Notch1 proteins in cultured cells derived from *PS1*$^{-/-}$ mice has shown that PS1 is required for efficient release of the Notch1 ICD.[7,8] The present study supports such a role for PS1 in the regulation of Notch signaling, and further provides evidence that the reduced Notch processing observed in *PS1*$^{-/-}$ cells is functionally significant in cell fate determination.

Lack of PS1 function was previously reported to result in reduced transcription of *Notch1* and *Dll1* in the presomitic mesoderm of the *PS1*$^{-/-}$ embryo, suggesting a role for PS1 in the regulation of *Notch1* and *Dll1* at the transcriptional level.[12] However, we detected unaltered levels of *Notch1* transcripts and elevated levels of *Dll1* transcripts in the embryonic brain and presomitic mesoderm of *PS1*$^{-/-}$ mice by Northern analysis. These results indicate that regulation of Notch signaling does not differ in the CNS and paraxial mesoderm, and that the lateral inhibition feedback mechanism first postulated in *Drosophila* is conserved in mice.

Finally, our results indicate a difference in the consequences of reduced Notch signaling during neurogenesis in *Drosophila* and mice. In *Drosophila*, loss of function mutations in Notch leads to excessive neuronal production at the expense of epidermis.[15] Our findings show that Notch1 regulates a cell fate choice between neural progenitor cells and terminally differentiated neurons early in mammalian neurogenesis, promoting regeneration of neural precursor cells at the expense of differentiation of postmitotic neurons. The early depletion of progenitor cells in the *PS1*$^{-/-}$ brain ultimately leads to an overall reduction in the postmitotic neuronal population.

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