Perturbed Neurogenesis in the Adult Hippocampus Associated with Presenilin-1 A246E Mutation

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In addition to its well-established role in γ -secretase cleavage, presenilin (PS) also plays a role in regulating the stability of cytosolic β -catenin, a protein involved in Wnt signaling. Several familial Alzheimer's disease-associated PS1 mutations have been shown to increase the stability of the signaling pool of β -catenin, correlating with enhanced cell proliferation. Accordingly, we hypothesized that in the setting of PS1 mutations, abnormal activation of Wnt/ β -catenin signaling leads to increased cell division. We tested this hypothesis by examining whether there is evidence of increased neurogenesis in the hippocampus of adult transgenic mice that overexpress the PS1 A246E mutation. In PS1/PS2-deficient fibroblasts, expression of PS1 A246E Familial AD mutation failed to restore the rapid turnover of β -catenin compared with wild-type PS1. We then examined whether the same mutation enhanced neurogenesis *in vivo* in adult hippocampus of PS1-deficient mice when restored by wild-type human PS1 (PS1^{-/-}WT) or A246E PS1 mutation (PS1^{-/-}AE). The PS1 A246E mutation stimulated the proliferation of progenitor cells in the dentate gyrus of adult mice, as assessed by 5-bromo-2-deoxyuridine incorporation, but did not influence their survival or differentiation. These observations suggest that the PS1 A246E mutation influences cell growth putatively via abnormal β -catenin signaling *in vivo*. (Am J Pathol 2005, 167:151–159)

Alzheimer's disease (AD) is characterized by selective damage of brain regions important for memory, including the hippocampus. The major histopathological hallmarks are neurofibrillary tangles and extracellular deposits of β -amyloid protein (A β) in senile plaques. Mutations in the presenilin 1 (PS1) gene are associated with early onset of most Familial AD (FAD).¹ PS1 FAD mutations are be-

lieved to contribute to AD pathogenesis by increasing the production of the amyloidogenic A β 42 peptide.^{2,3} PS1 and the closely related PS2 are polytopic membrane proteins located primarily in the endoplasmic reticulum and the Golgi apparatus but can also be found in the plasma membrane. PS1 is expressed in all tissues, including neurons in the brain and also neural progenitor cells in the hippocampus.^{4,5}

Presenilins (PS1 and PS2) are obligatory members of the γ -secretase complex required for the regulated intramembrane proteolysis of an expanding family of substrates, including APP and Notch.⁶ Absence of presenilins results in drastic reduction in A β generation and Notch signaling,⁷ such that loss of Notch signaling is believed to underlie the perinatal mortality in mice lacking PS1. Presenilins may function as an aspartyl protease within the catalytic core of the γ -secretase complex consisting of nicastrin, Aph-1, and Pen-2 to cleave APP and other substrates. After cleavage, the intracellular domain is released from the membrane and, in some cases, enters the nucleus to activate nuclear signaling.

Presenilins are multifunctional molecules that exhibit other physiological roles beyond γ -secretase cleavage. One of these activities of PS1 is in the regulation of β -catenin turnover. β -Catenin is a key modulator of the canonical Wnt signaling pathway such that multiple mechanisms have been developed to tightly regulate the level of free cytosolic β -catenin levels. Specifically, rapid degradation of β -catenin is effected by two independent mechanisms through axin- and PS1-mediated pathways. In both pathways, axin and PS1 independently facilitate the paired phosphorylation of β -catenin by a priming kinase and glycogen synthase kinase GSK-3 β that are necessary for proteasomal degradation. Accumulation of

Supported in part by National Institutes of Health grants NS28121 (to E.H.K.), AA IIRG-02-3892 (to S.S.), and AG18440 (to E.M.).

Accepted for publication March 18, 2005.

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β -catenin levels secondary to mutations in axin, β -catenin, and adenomatous polyposis coli (APC) genes are associated with various tumors in animals and in man. In rodents, it has been documented that in the absence of PS1, there is an increase in the levels of cytosolic β -catenin; and this, in turn, is associated with increased cellular proliferation. Specifically, in embryos of PS1-deficient animals, there is hyperplasia of neuronal progenitor cells in the spinal cord.⁸ In adult PS1-deficient animals that have been rescued from perinatal lethality by the expression of PS1 driven by the neuron-specific Thy-1 promoter, epidermal hyperplasia and tumorigenesis in skin were consistently observed.⁹ These hyperproliferative changes have been postulated to be secondary to the dysregulation of β -catenin degradation. In brain, there is evidence that β -catenin levels are elevated in animals expressing a FAD-associated PS1 mutation.¹⁰ This finding is consistent with the concept that several FAD PS1 mutations behave as a partial loss-of-function phenotype with respect to β -catenin degradation.

The aim of this study is to test whether the cellular proliferation phenotype is present *in vivo* in brains of adult PS1-deficient animals rescued by the expression of a FAD-associated PS1 mutation compared with wild-type PS1. Specifically, we examined the proliferation of undifferentiated neural progenitor cells in the dentate gyrus of the hippocampus that continue to divide during adulthood. These progenitor cells have been shown to differentiate into neurons and form functional synaptic connections.¹¹ As a result these newly derived neurons may impact memory and cognition in the adult brain. Neural progenitor cells have the capacity to self-renew and to differentiate into neurons and astrocytes. Thus, they offer the opportunity to examine the proliferation and survival of progenitor cells in the setting of PS1 mutation that alters β -catenin turnover and signaling. By 5-bromo-2-deoxyuridine (BrdU) incorporation, we found that in the adult hippocampi of PS1 null mice rescued by the expression of FAD mutant A246E PS1 (PS1^{-/-}AE), there is increased cell proliferation compared with PS1 null mice rescued by wild-type human PS1. This is in accord with the delayed turnover of β -catenin in cells expressing this PS1 mutation. Interestingly, this increase in proliferation is not sustained, indicating that this increased signal for cell growth does not result in prolonged survival.

Materials and Methods

Animals and BrdU Labeling Protocol Design

Littermates of adult wild-type control animals (PS1^{+/+}), PS1^{+/-}, PS1^{-/-} rescued with wild-type human PS1 (PS1^{-/-}WT; line 17-2), and PS1^{-/-} rescued with mutant human PS1 A246E (PS1^{-/-}A246E; line 16-3) were used in this study. These animals were generated in the C57Bl/6J $\times$ B6SJL/F1 as previously described¹² and were maintained in the hybrid background. A minimum of five animals of 12 weeks of age were studied in each group. In a preliminary study, BrdU was given at 50, 100, 150, and 200 μ g/g body weight, and the best labeling was

observed at 150 and 200 μ g/g. Consequently, all experimental mice received two shots (intraperitoneally) of BrdU, 4 hours apart at 150 μ g/g. Animals were sacrificed with an overdose of halothane either 24 hours after the first BrdU injection (cell proliferation study) or 4 weeks later (cell survival and differentiation studies). The cell cycle in dentate gyrus is estimated to be 12 to 14 hours.^{13,14} As a result, this injection protocol avoids re-labeling the same population of progenitor cells.

Immunofluorescence Staining

For immunostaining, brains were removed, fixed overnight in 4% paraformaldehyde, and cryoprotected in 30% sucrose. Coronal sections (40 μ m thick) were sequentially collected in a cryoprotectant solution containing 25% glycerol, 25% ethylene glycol, and 0.05 mol/L phosphate buffer and stored at -20°C until used. BrdU staining or double labeling for BrdU and NeuN or PS1 were pretreated with 50% formamide in 2 $\times$ standard saline citrate for 2 hours at 65°C , rinsed in 2 $\times$ standard saline citrate, incubated in 2 N HCl for 30 minutes at 37°C , and neutralized in 0.1 mol/L borate buffer. Primary and secondary antibodies were diluted in the blocking buffer (0.1% Triton X-100 and 3% goat serum). The primary antibodies used were rat anti-BrdU (Accurate, Westbury, NY), mouse anti-BrdU (Chemicon, Temecula, CA), mouse anti-NeuN (Chemicon), and rat anti-PS1 [24-4B5].¹⁵

BrdU Quantification and Neuronal Phenotyping

For quantification of BrdU-labeled cells, every sixth section was stained and quantified under a fluorescent microscope at $\times 40$. All BrdU-positive cells present in the subgranular zone of the dentate gyrus were counted. Results from both hemispheres were totaled and multiplied by six to obtain an estimation of the total number of BrdU-positive cells for each brain. Neuronal phenotype was determined in animals sacrificed 4 weeks after BrdU injections by performing BrdU-NeuN double labeling on every sixth section. Twenty to 30 BrdU-positive cells per animal were checked for NeuN expression using a laser scanning confocal microscope. All quantifications were performed with the observer blinded to the genotype and were repeated twice.

Immunoblotting for PS1 in the Mice Brains

For immunoblotting analysis, brains were removed, and one-half of the forebrain was homogenized in 1% Nonidet P-40 lysis buffer containing 50 mmol/L Tris, 150 mmol/L NaCl, 0.02% NaN₃, and complete protease inhibitors cocktail (Roche, Indianapolis, IN). The homogenates were centrifuged 15 minutes at 14,000 rpm, and the supernatants were collected. Proteins (20 μ g) were loaded in each lane for separation in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. PS1 N- and C-termini fragments were detected

with human-specific mouse anti-PS1 (PSN2) and rabbit PS1 polyclonal antibody (490), respectively.

Cell Lines and Transfections/Cell Lysis and Homogenization/ β -Catenin Turnover Assay

Immortalized $PS^{+/+}$ and $PS^{-/-}$ mouse embryonic fibroblasts (genetically deficient in both $PS1$ and $PS2$) have been described previously.⁸ Wild-type and mutant $PS1$ constructs were subcloned into retroviral vectors (pLPCX; Clontech, Mountain View, CA) as reported before. To introduce wild-type or mutant $PS1$ constructs into the fibroblasts, retroviral supernatants from 293GP transduced packaging cells were added to $PS^{-/-}$ cells in the presence of 10 μ g/ml polybrene. Transduced cells were then selected with 3 μ g/ml puromycin. Resistant cells were pooled and analyzed without further clonal selection. β -Catenin degradation was monitored by treating confluent cultures with 25 μ g/ml cycloheximide for the indicated times as described,⁸ and CHAPS lysates were subjected to immunoblotting for phospho-45-specific β -catenin antibody (Cell Signaling, Beverly, MA).

Statistical Analysis

All statistical analyses were performed with GraphPad Prism. One-way analysis of variance was performed for all comparisons of quantitative data followed by Newman-Keuls multiple comparison post hoc test, when appropriate.

Results

Presenilin 1 A246E Mutant Impairs β -Catenin Turnover

We previously showed that the loss of $PS1$ resulted in hyperplasia and tumorigenesis in skin of adult mice as well as hyperproliferation of progenitor cells in the developing spinal cord resembling that of ectopic Wnt-1 overexpression. Both of these phenotypes are accompanied by increased β -catenin stability hypothesized to be secondary to impairment of the paired phosphorylation of β -catenin.^{8,9,16} To test our hypothesis that neuronal progenitor cells in the adult hippocampus show more proliferation in the $PS1^{-/-}$ A246E mice compared with $PS1^{-/-}$ WT mice, we must first establish whether this $PS1$ mutation behaves as a partial loss-of-function mutant with respect to β -catenin turnover. Our prediction is that if the $PS1$ A246E mutation retarded β -catenin turnover, then there should be an increase in proliferation of neuronal progenitors in the adult hippocampus because activation of the canonical Wnt/ β -catenin signaling pathway is known to stimulate proliferation of progenitor cells in the developing brain.^{17,18} Accordingly, we examined presenilin-deficient fibroblasts replaced with either wild-type $PS1$ or different FAD-associated $PS1$ mutants, including the A246E mutation. We assessed the levels of serine 45 (S45)-phosphorylated β -catenin, a species that accumu-

Figure 1. Defective turnover of β -catenin in cells genetically deficient in mouse presenilins and rescued by hPS1-bearing FAD-linked mutations. **A:** Confluent cultures of $PS^{-/-}$ cells and $PS^{-/-}$ cells reconstituted with either wild-type $PS1$ or FAD mutations were treated with 25 μ mol/L cycloheximide (CHX) for the indicated time periods and immunoblotted for P-45 β -catenin. Immunoblot exposures were adjusted to show similar signal at time 0. **B:** The graph represents the relative turnover in P-45 β -catenin from three independent experiments. **Error bars** represent SEM.

lates in presenilin deficiency condition because of the impairment of the subsequent phosphorylation events on residues 41, 37, and 33.⁸ As expected, expression of wild-type $PS1$ in $PS^{-/-}$ cells markedly stimulated the turnover of S45-phosphorylated β -catenin, whereas FAD mutations (A246E, M146L, and $\Delta X9$) were all less active (Figure 1). In particular, the A246E and M146L mutations dramatically reduced the turnover of S45 β -catenin compared with wild-type $PS1$ and did not differ substantially from parental $PS^{-/-}$ cells. Expression of the $PS1$ M146L mutation in brain was previously shown to increase β -catenin levels.¹⁰ Thus, these results strongly indicated that these $PS1$ mutations, including A246E, retard β -catenin turnover, leading to increased stability of cytosolic β -catenin and presumably downstream nuclear signaling events.

Mutant Presenilin 1 Stimulates the Proliferation of Subgranular Progenitor Cells in the Dentate Gyrus

Having shown that β -catenin degradation is impaired in cells expressing $PS1$ A246E mutation compared with wild-type $PS1$, we next examined brains of $PS1^{-/-}$ mice

Figure 2. Increased cell proliferation in the adult dentate gyrus of mice genetically deficient in mouse PS1 and transgenic for PS1A246E. The numbers of BrdU-positive cells are similar in PS1^{+/+} and PS1^{-/-}WT animals but significantly lower than PS1^{-/-}AE and PS1^{+/-} animals. Results shown are means of total numbers of BrdU-positive cells ($\pm$ SEM) for each genotype studied ($n = 5$ to 7 mice per group). Statistical significance was evaluated using analysis of variance followed by Newman-Keuls post hoc multiple comparison test.

rescued by either PS1 A246E or wild-type PS1. BrdU incorporation examined the day after the injections provides an estimate of the ongoing proliferation rate in the dentate gyrus by marking newly divided progenitor cells. As expected, these cells were observed in the subgranular zone of the dentate gyrus. Clusters of dividing cells, which are typical of vigorous mitotic activity, were often seen in the PS1 mutant line. The BrdU immunoreactivity was intense and generally stained the whole nuclei. The numbers of BrdU-positive cells were quantitated in four different groups of animals. As predicted, PS1^{-/-}AE mice had significantly more BrdU-positive nuclei in the hippocampus compared with the PS1^{+/+} control group (+44%, $P < 0.01$) or the PS1^{-/-}WT animals (+27%, $P < 0.05$) (Figure 2). This difference was strengthened by the fact that there was no difference between PS1^{-/-}WT mice and the PS1^{+/+} controls ($P > 0.05$). Unexpectedly, PS1^{+/-} showed more BrdU-positive cells than did wild-type PS1^{+/+} animals (+26%, $P < 0.05$) and showed BrdU labeling comparable with PS1^{-/-}AE animals ($P > 0.05$).

Because a partial reduction in PS1, as observed in PS1^{+/-} mice versus PS1^{+/+} (Figure 3A), can induce more cell proliferation in the dentate gyrus, we wanted to ascertain that the enhanced cell proliferation observed in the PS1^{-/-}AE group was due solely to the A246E mutation and not to a difference in the expression of hPS1 protein in this transgenic line. Using two different antibodies that recognize the N- and C-terminal endoproteolytic fragments of PS1, respectively, hPS1 protein level was approximately 40% higher in the PS1^{-/-}A246E mutant line compared with the PS1^{-/-}WT line ($n = 3$) (Figure 3B). Therefore, the increased BrdU labeling observed in PS1^{-/-}AE is not due to lower level of hPS1 expression in this transgenic line but is likely secondary to aberrant activity of the A246E mutation.

Figure 3. Expression of PS1 transgenic protein in PS1 wild-type and PS1 A246E mice. Immunoblots of brain homogenates reacted with the 490 antibody (human and mouse PS1 C-terminal fragment) and PSN2 antibody (human-specific PS1 N-terminal fragment). **A:** The level of endogenous mouse PS1 C-terminal fragment is reduced in PS1^{+/-} brain compared with PS1^{+/+}. **B:** The expression level of both N- and C-terminal PS1 fragments is approximately 40% higher in PS1^{-/-}A246E mice compared with PS1 wild-type mice ($n = 3$ mice per genotype).

To ascertain that mutant PS1 has a direct effect on cell proliferation in our model, the neural progenitors undergoing cell division must express the human PS1 transgene. Therefore, the sections were double labeled for BrdU and hPS1 in mice sacrificed the day after BrdU injections (Figure 4). hPS1 immunostaining was diffusely distributed in the granule cell layer of the dentate gyrus, as well as along the subgranular zone, where the progenitor cells are located (Figure 4, A and B). Within the perykarya, the staining tended to be punctate in nature (Figure 4C, red for PS1). Immunostaining was also present in the nucleus, a localization that appeared after the harsh pretreatment conditions required for the BrdU labeling, an observation that has been previously reported.⁴ Laser scanning confocal microscopy showed that all BrdU-positive cells were stained for hPS1 (Figure 4C), a finding consistent with the known distribution of the human thy1 promoter in neurons,¹⁹ and whose promoter activity now extends to neural progenitor cells where PS1 is known to be present.⁴

Influence of Presenilin 1 on Cell Survival and Cell Death in the Dentate Gyrus

After exiting the cell cycle, most of the nascent cells will die, and the surviving cells will differentiate into neurons and glia. Therefore, the number of BrdU-positive cells was assessed 4 weeks after BrdU injections to determine the survival of these newly derived cells. Typically, the surviving cells started to migrate toward the granular cell layer, but a few cells were still observed in the subgranular zone. At this time, most of the BrdU-positive nuclei showed a round regular shape with punctate staining

Figure 4. Expression of human PS1 transgenic protein in progenitor cells. Immunostaining of human PS1 using a human-specific rat anti-PS1 monoclonal antibody 24-4B5 was performed the day after BrdU injections. **A:** Specific human PS1 immunostaining is seen diffusely in the dentate gyrus of a PS1^{-/-}AE mouse (tg, transgenic; **top panel**) but not in a control PS1^{+/+} nontransgenic animal (non-tg; **bottom panel**). gcl, granular cell layer of the dentate gyrus. Scale bar = 100 μ m. **B:** PS1 (**top panel**, red) is expressed in the subgranular zone (sgz) where dividing progenitor cells are located (**bottom panel**, green for BrdU). **Arrowheads**, BrdU-positive cells. Note that due to the thickness of the section (40 μ m), only a few BrdU cells are in the plane of focus. Pictures in **B** are a close-up view of the rectangle highlighted in **A**. Scale bar = 25 μ m. **C:** In higher magnification, representative confocal laser scanning microscopy images showing colocalization of a neuron immunolabeled for PS1 and BrdU. The **bottom panel** [BrdU] represents an overexposure of the BrdU photomicrograph shown in the second panel to demonstrate location of BrdU-negative cells (**asterisks**). Systematic assessment of double-labeled images by confocal laser scanning microscopy study confirmed that all of the BrdU-positive cells were immunopositive for human PS1. Scale bar = 10 μ m.

pattern characteristic of postmitotic cells rather than the diffuse homogenous staining seen earlier. Compared with the short term study, approximately 25% of the original number of BrdU-labeled cells remained, with the PS1^{-/-}AE group showing a slight but insignificant trend for more BrdU-positive cells (Figure 5). The remaining three groups all showed comparable numbers of BrdU-labeled cells. Only one-fourth of the original BrdU-labeled cells persisted; this indicated that about 75% of the newly generated cells did not survive after 4 weeks. Finally, because the numbers of BrdU-positive cells are approximately the same for all four groups, more BrdU cells were eliminated in the PS1^{-/-}AE and PS1^{+/-} animals. In line with this notion, we consistently detected more TUNEL and activated caspase-3-positive cells in the brains of PS1^{+/-} and PS1^{-/-}AE animals (data not shown). However, the number positively stained cells was low (average number of TUNEL-positive cells per animal was 97 in the PS1^{+/-} and PS1^{-/-}AE groups, $n = 5$, and 10 in the control animals, when present, $n = 3$), and in some of the animals, especially in the control

groups, we could not detect any positively stained cells. As a result, formal statistical comparison was not done.

Presenilin 1 Modification Does Not Alter the Rate of Neuronal Differentiation of the Progenitor Cells in the Dentate Gyrus

It has been demonstrated that newly derived neurons generated in the dentate gyrus in adult hippocampus survive and establish functional connections.¹¹ Differentiation of the surviving BrdU-positive cells in neurons was determined by double labeling for BrdU and NeuN, a marker of neuronal differentiation (Figure 6A). In brains of animals sacrificed 4 weeks after BrdU injections, BrdU and NeuN double-positive cells were scored. From this analysis, we found comparable percentages of neuronal differentiation in all four groups in that NeuN staining was positive in about 80% of the BrdU-positive cells (Figure 6B; Table 1). This indicated that there was no difference in the rate of neuronal differentiation or in number of

Figure 5. Survival of BrdU cells 4 weeks after injections is comparable between the different experimental groups. The total number of BrdU-positive cells (means of total $\pm$ SEM) were similar among all four genotypes ($P > 0.05$, $n = 5$ to 10 animals per group).

newly born neurons between any of the genotypes studied.

Discussion

In this study, we tested the hypothesis that PS1 A246E FAD mutation aberrantly stimulates the proliferation of neuronal progenitor cells in brains of adult animals. The rationale is that certain PS1 mutations show partial loss-of-function with respect to β -catenin degradation and, consequently, should demonstrate enhanced basal activity related to β -catenin/Wnt signaling pathway. If this reduced turnover of β -catenin persists in brain, then there might be physiological evidence of this perturbation *in vivo*. Because increased cell proliferation is one phenotype of enhanced Wnt/ β -catenin signaling, we tested this hypothesis in adult hippocampus in which ongoing neurogenesis persists in adulthood. In this study, we showed that expression of the PS1 A246E mutation in fibroblasts did demonstrate partial loss-of-function of the stepwise phosphorylation of β -catenin, resulting in accumulation of S45-phosphorylated β -catenin species. Furthermore, in transgenic mice overexpressing human PS1 A246E mutation in a PS1 null background as well as in PS1^{+/-} animals, there was more proliferation of neural progenitor cells in the hippocampus compared with mice expressing wild-type human PS1 and control animals. Interestingly, this stimulatory effect was transient as the increased progenitor cells were no longer in evidence after 4 weeks, implying that there was greater death of newly divided progenitor cells in the A246E transgenic mice. However, the percentage of cells that showed neuronal differentiation was unchanged between the different groups.

In the canonical Wnt pathway, activation of downstream signaling pathways via β -catenin is critical in many developmental systems. In adults, a causal role of β -catenin mutations, as well as in the proteins that regulate β -catenin degradation such as axin and APC, has been established in various human cancers.^{20,21} In this model, stabilization of cytosolic β -catenin leads to abnormal activation of nuclear targets, such as *c-myc* and cyclin D1, to promote cell division. In this context, increased cell proliferation in PS1-deficient mouse embryonic fibroblasts was correlated with increased levels of

Figure 6. Neuronal differentiation of progenitor cells is unchanged by PS1 mutation. Double immunofluorescent labeling of BrdU and NeuN was performed on brain sections 4 weeks after BrdU injections to confirm neuronal differentiation. **A:** NeuN-positive cells (red) decorate the granule cell layer of the dentate gyrus. Newly differentiated neurons are double-labeled for BrdU (green) and NeuN (red) (top panels). **Bottom panels:** An example of a BrdU-positive cell that did not show NeuN immunostaining and presumably failed to differentiate in a neuron. Scale bar = 10 μ m. **B:** Neuronal differentiation of progenitor cells was similar between the four experimental groups. The percent BrdU cells that are double-labeled for NeuN was scored from the animals illustrated in **A** ($P > 0.05$, $n = 4$ to 6 per group). No statistical differences were found between any of the studied genotypes.

cytosolic β -catenin.¹⁶ Subsequent studies suggested that PS1 serves as a scaffold to facilitate the sequential phosphorylation of β -catenin that is necessary for its rapid degradation.⁸ Interestingly, several FAD-associated PS1 mutations were only able to partially restore β -catenin to normal levels. One PS1 mutation, M146L, was correlated with increased β -catenin levels in brains of transgenic animals.¹⁰ Here, we confirmed that the PS1 A246E mutation, like the M146L and Δ X9 mutations, was unable to restore the turnover of the pool of S45-phosphorylated β -catenin species in cultured fibroblasts. Consequently, this elevation of S45-phosphorylated β -catenin would be predicted to lead to elevated β -catenin in the cytosol and subsequently to increased cyclin D1 transcription and accelerated cell proliferation.

In the skin of mice lacking PS1 expression in the periphery and in the spinal cord of PS1-deficient embryos, a hyperproliferation phenotype was observed, presumably secondary to increased β -catenin stability.⁹ Consistent with this model, we postulate that the increased proliferation observed in the dentate gyrus of PS1^{-/-}AE mice is a consequence of β -catenin stabilization in the progenitor cells because of a partial loss-of-activity of this mutation. Our findings are consistent with a recent study demonstrating that expression of abnormally stabilized β -catenin species by deleting the N-terminal regulatory domain

Table 1. Calculated Net Neurogenesis

Genotype	PS1 ^{+/+}	PS1 ^{+/-}	PS1 ^{-/-} WT	PS1 ^{-/-} AE
Calculated no. of newborn neurons	649 (67)	578 (151)	695 (115)	992 (86)

Total number of newborn neurons was calculated by multiplying the number of BrdU-positive cells by the percentage of BrdU-PS1 double-labeled cells for each individual animal. Data are presented as average ($\pm$ SEM). No statistically differences were found between any of the groups studied.

resulted in massive proliferation of neuronal progenitors, resulting in enlarged brains. This phenotype is a direct consequence of stabilizing β -catenin to drive the progenitor cells to re-enter the cell cycle, thus expanding the precursors population and later the number of neurons in the cortex.^{17,22} Stabilizing β -catenin by lithium treatment to inhibit GSK-3 β activity also resulted in increased hippocampal neurogenesis.²³ Conversely, embryos deficient in LEF-1, one of the nuclear cofactors that mediate Wnt signaling, lack granule cells in the dentate gyrus and show a decrease in BrdU incorporation in the hippocampus, further linking proliferation of neuronal progenitors in the dentate gyrus with Wnt/ β -catenin signaling.¹⁸ Interestingly, in our study, stimulation of the Wnt/ β -catenin pathway by mutant PS1 perturbed only cell division but did not result in greater number of newly divided daughter cells. Four weeks after BrdU injections, the number of surviving BrdU-positive cells, now predominantly differentiated into neurons, were comparable in all experimental groups. These findings indicated that stimulation of proliferation of progenitor cells by mutant PS1 did not influence their subsequent differentiation into neurons. Furthermore, more BrdU-positive cells were eliminated in the PS1^{-/-}AE and the PS1^{+/-} animals, suggesting compensatory mechanisms that regulate survival and maturation of these cells that are likely distinct from β -catenin/PS1 pathway. These observations are consistent with the concept that genetic controls on proliferation, cell death, and survival are to some extent independent,¹⁴ and therefore, β -catenin/PS1 regulation is only one of the factors that can impact cell proliferation.

Although we have interpreted our results in light of the activity of presenilin on β -catenin turnover, the contribution of presenilins to γ -secretase activity cannot be dismissed. PS1-dependent γ -secretase activity generates the active form of Notch, NICD, among a burgeoning list of membrane protein substrates.^{7,24,25} Impaired Notch signaling is the likely explanation of altered neurogenesis in PS1 null embryos.²⁶⁻³⁰ Specifically, there is depletion of neural stem cells in brain, probably due to reduced renewal of embryonic neural stem cells as well as increased neuronal and astroglial differentiation of progenitor cells.²⁷ However, although several FAD-associated PS1 mutations behave as a partial loss-of-function mutant in cultured cells and poorly rescue Notch-dependent signaling *in vivo* in *Caenorhabditis elegans*, this lack of Notch activity was not evident in PS1 null mice rescued by transgenic animals expressing either wild-type or PS1 mutants.^{12,31-33} Therefore, on first approximation, the hyperproliferation of hippocampal progenitors cells in PS1^{-/-}A246E mice may be independent of Notch deficiency. However, the consequences of Notch activation are complex. For example, Notch activation has been

associated with a number of oncogenic conditions,³⁴ but yet in embryonic brain, notch activation has been shown to induce quiescence in telencephalic precursors.³⁵ Nonetheless, although not absolute, we interpret our findings to be more consistent with enhanced Wnt/ β -catenin rather than perturbed Notch signaling. But clearly, this is a complex situation that is likely not simply due to perturbed activity of one or two linear signaling pathways.

Several studies have previously documented contrasting effects of PS1 on adult neurogenesis in the context of PS1 mutations. Overexpression of either wild-type or the PS1 P117L mutation driven by the neuron-specific enolase promoter (NSE) had no effect on hippocampal neurogenesis.^{36,37} However, wild-type, but not mutant PS1, promoted the survival and differentiation of progenitor cells into granule cells. Although the reduced ability of mutant PS1 to promote survival of progenitor cells is reminiscent of our finding in PS1 A246E animals, the studies are not directly comparable. Significantly, NSE promoter-driven transgene is not active in the hippocampal progenitor cells. Therefore, the reported phenotype in NSE-driven wild-type and mutant PS1 is indirect and different from the effects we observed. In another study, impaired hippocampus-dependent associative learning was associated with reduced adult neurogenesis in the PS1 M146V knock-in model.³⁸ However, this reduction was only detected when the PS1 mutant mice within a PS1 null background were compared with PS1^{+/-} animals. Because we and others²⁷ have shown increased BrdU uptake in hippocampal progenitor in PS1^{+/-} animals compared with control (PS1^{+/+}) animals, it is unclear whether neurogenesis in the PS1 M146V mutant animals is impaired when compared with wild-type animals. Furthermore, the PS1 FAD-associated mutations that have been evaluated in transgenic mice are different, and it may be that their effects on β -catenin stability are not the same.

As mentioned above, increased cell proliferation in adult PS1^{+/-} brains has been described previously.²⁷ Hitoshi et al²⁷ reported that in brains of adult PS1^{+/-} animals, there was increased numbers of neural progenitors. Because neural progenitor cells are generated after asymmetric division of the neural stem cells, it was proposed that enhanced proliferation of hippocampal progenitor cells in PS1^{+/-} animals was a compensatory response to the low numbers of neural stem cells observed in these mice. Consequently, proliferation of the neuronal progenitor cells may be inversely correlated to the amount of PS1 in brain. In this context, because both the PS1^{+/-} and PS1^{-/-}AE animals displayed a similar hyperproliferating phenotype, we hypothesize that the A246E mutation represents a partial loss-of-function for

PS1 with respect to its effect on cell proliferation in the adult dentate gyrus.

In summary, our results demonstrated that there is enhanced proliferation of neuronal progenitor cells in the dentate gyrus of adult mice transgenic for PS1 A246E mutation in a PS1 null background and that the same mutation aberrantly stabilizes β -catenin in cultured cells. These findings suggest that, in addition to the well established effect on γ -secretase activity to increase the production of the amyloidogenic A β 42 species, PS1 FAD-associated mutations may contribute to AD pathogenesis by affecting the regulation of adult neurogenesis in the hippocampus. This would be consistent with the view that there is perturbed neurogenesis and cell cycle control in brains of individuals with sporadic Alzheimer's disease.^{39–41} In various APP transgenic mice, both increased and decreased neurogenesis have been described.^{42–46} This issue is not addressed in this study because there is no over-production of A β in our model. Furthermore, after ischemia or brain trauma, neurogenesis is increased in the subventricular zone and the hippocampus.^{47,48} Therefore it is possible that a number of factors contribute to the regulation of neurogenesis in the adult brain, some of which may play a role in hippocampal function in AD.

Acknowledgments

We thank Dr. Hui Zheng for providing the PS1 transgenic animals. We thank Drs. Rusty Gage, Henriette van Praag, and Linda Kitabayashi for helpful discussions and technical advice. We also thank Drs. Hiroshi Mori and Allan Levey for gift of antibodies.

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