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Evidence that γ-secretase mediates oxidative stress-induced β-secretase expression in Alzheimer's disease

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Abstract

β-Secretase (BACE1), an enzyme responsible for the production of amyloid β-peptide (Aβ), is increased by oxidative stress and is elevated in the brains of patients with sporadic Alzheimer's disease (AD). Here, we show that oxidative stress fails to induce BACE1 expression in presenilin-1 (γ-secretase)-deficient cells and in normal cells treated with γ-secretase inhibitors. Oxidative stress-induced β-secretase activity and sAPPβ levels were suppressed by γ-secretase inhibitors. Levels of γ- and β-secretase activities were greater in brain tissue samples from AD patients compared to non-demented control subjects, and the elevated BACE1 level in the brains of 3xTgAD mice was reduced by treatment with a γ-secretase inhibitor. Our findings suggest that γ-secretase mediates oxidative stress-induced expression of BACE1 resulting in excessive Aβ production in AD.
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Keywords: β-Secretase; Alzheimer's disease; Oxidative stress; γ-Secretase

1. Introduction

Sequential proteolytic cleavages of the β-amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase generate the amyloid β-peptide (Aβ) believed responsible for damage and death of neurons that occurs in Alzheimer's disease (AD) (Mattson, 2004). The causes of Aβ accumulation in sporadic AD remain unclear, but may include age-related increases in oxidative stress (Mattson, 2004).

The amount of lipid peroxidation is increased in AD patients and is correlated with the clinical severity of the disease (Pratico et al., 2000). In the Tg2576 mice, increased lipid peroxidation is localized in brain regions involved with Aβ plaques and precedes the onset of Aβ plaque deposition (Pratico et al., 2001). Moreover, several risk factors for AD are known to increase cellular oxidative stress in the brain including aging and ApoE4 genotype (Barja, 2004; Montine et al., 1997). These observations suggest that oxidative stress

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is integral to AD pathogenesis, although it remains unclear whether oxidative stress is a cause or consequence of AD neuropathology.

It has been reported that BACE1 expression and activity are elevated in the postmortem brain regions affected by AD (Holsinger et al., 2002; Li et al., 2004) and in mouse models of AD (Zhao et al., 2007), suggesting a role for BACE1 in AD. BACE1 expression is also increased in conditions known to involve oxidative stress including ischemia (Zhao et al., 2007), hypoxia (Sun et al., 2006) and in cultured neuronal cells exposed to hydrogen peroxide or the lipid peroxidation product 4-hydroxy-2,3-nonenal (HNE) (Tamagno et al., 2002, 2008). In addition, we recently found that ischemia and hypoxia increase γ-secretase activity (Arumugam et al., 2006). In the present study, we provide evidence for previously unknown links between oxidative stress γ-secretase activity and BACE1 expression in the pathogenesis of AD.

2. Materials and methods

2.1. Human subjects and brain specimens

Inferior parietal lobule and cerebellum specimens from the brains of 10 patients with AD and 10 control subjects that had been enrolled in the University of Kentucky Alzheimer's Disease Center Autopsy Program were used for this study. All patients with AD met both clinical diagnostic criteria and neuropathological diagnostic criteria of AD (NIARI, 1997). The control subjects had no history or neuropathological signs of a brain disorder. At autopsy, tissue specimens were rapidly removed and frozen, and were stored at −80 °C.

2.2. Animals and treatment with γ-secretase inhibitor

Seven month-old PS1 mutant knock-in mice (PS1KI) (Guo et al., 1996), triple-transgenic AD mice (3xTgAD; swAPP, PS1-M146V, tau-P301L) (Oddo et al., 2003), and wildtype mice of the same genetic backgrounds were maintained in our animal facility under pathogen-free conditions on a 12 h light/12 h dark cycle with continuous access to food and water. Mice were treated with the γ-secretase inhibitor DAPT (100 mg/kg, subcutaneously), or vehicle every day for 7 days and the mice were then euthanized and brains removed for processing. All procedures were approved by the NIA Animal Care and Use Committee.

2.3. Cell culture and experimental treatments

Human neuroblastoma SH-SY5Y cells and immortalized mouse embryonic fibroblasts (MEF) from wildtype, $PS1^{-/-}$, $PS2^{-/-}$, $PS1^{-/-}/PS2^{-/-}$ mice (generous gifts from B. De Strooper, Leuven, Belgium) and $BACE1^{-/-}$ mice were cultured in Dulbecco's minimum Eagle's medium (DMEM) (GIBCO, Carlsbad, CA) supplemented 10% with fetal bovine serum (FBS) (GIBCO) and 1% penicillin/streptomycin (GIBCO). Primary dissociated cell cultures of hippocampal and cortical neurons were prepared from 18-day-old rat embryos by using methods described previously (Mattson et al., 1995). Experiments were performed in cells that had been in culture for 6–8 days. The atmosphere consisted of 6% CO_2/94% room air and was maintained near saturation with water. 4-Hydroxy-2,3-nonenal (HNE; Cayman Chemical, Ann Arbor, MI) was prepared as a 1000× stock in ethanol. γ-Secretase inhibitors including L685,458, DAPT, compound E, WPE-III-31C, pepstatin A and γ-secretase inhibitor IV were purchased from Calbiochem (Cambridge, MA).

2.4. Immunoblots

Total cellular lysates were obtained by washing the cells in ice-cold PBS and resuspending the cell pellets in cell lysis buffer. Tissue protein was extracted using T-PER (Pierce, Rockford, IL) tissue protein extraction buffer with protease inhibitor cocktail (Sigma, St. Louis, MO). Protein concentration was determined using a BCA protein assay kit (Pierce). Fifty micrograms of protein was separated by SDS-PAGE (4–12%) and transferred to PVDF membranes. The membranes were blocked in 5% nonfat milk for 1 h at room temperature. Membranes were then probed with antibody specific for BACE1 (a generous gift from H. Cai, Bethesda, USA) (Cai et al., 2001), or the commercially available anti-BACE1 (ProSci, Poway, CA), BACE2 (Calbiochem), PS1 (Chemicon, Temecula, CA), Nicastrin (Sigma), ADMAM10 (Chemicon), TACE (Chemicon), APP (Zymed), sAPPβ (COVANCE), APP-CTFs (Sigma) and β-actin (Sigma) antibodies. Membranes were then washed and incubated with secondary antibodies for 1 h at room temperature. Protein bands were visualized using a chemiluminescence detection kit (Amersham Biosciences, Buckinghamshire, UK).

2.5. Luciferase assay

The pB1P-A plasmid contains the 2.1 kb 5′-UTR from −1944 to +292 bp of the human BACE1 gene upstream of the luciferase reporter gene (Christensen et al., 2004). The luciferase assay was performed according to the manufacturer's protocol (Promega, Madison, WI). Transfected cells were harvested and lysed in 200 μL of 1× Reporter Lysis Buffer for Dual Luciferase Assay (Promega). Twenty microliters of lysate was mixed with the firefly luciferase assay reagent II and the luminescent signal was measured using a luminometer. Stop and Glo® Reagent (100 μL) was added to the same tube. The *Renilla* (sea pansy) luciferase vector pCMV-Rluc (Promega) was cotransfected to normalize the transfection efficiency. The firefly luciferase activity was normalized according to *Renilla* luciferase activity and expressed as relative luciferase units (RLU) to reflect the promoter activity. The empty vector pGL3-basic was used as the negative control and pGL3 promoter as the positive control.

2.6. Fluorometric detection of activities of β- and γ-secretases

The activities of β- and γ-secretases present in human brain specimens were determined using fluorescent transfer peptides consisting of APP amino acid sequences containing the cleavage sites of BACE or γ-secretase (R&D Systems, Minneapolis, MN). The method is based on the secretase-dependent cleavage of a secretase-specific peptide conjugated to the fluorescent reporter molecules EDANS and DABCYL, which results in the release of a fluorescent signal that was detected using a fluorescent microplate reader with excitation wavelength of 355 nm and emission at 510 nm. The level of secretase enzymatic activity is proportional to the fluorometric reaction, and the data are expressed as fold increase in fluorescence over that of background controls (reactions in the absence of tissue or cell lysate).

2.7. Quantification of mRNA using real time reverse transcription-PCR

Total RNA was isolated from primary cultured rat neurons using QIAshredder and RNeasy kits (Qiagen Inc., Hilden, Germany). The isolated RNA (2 μg) and oligo(dT) primers were utilized to synthesize single-stranded cDNA using a reverse transcription kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The PCR was set up using SYBR Green Master Mix (Applied Biosystems, Foster City, CA) along with 1 μl of cDNA and the gene-specific primers at a final concentration of 0.3 μM. Thermal cycling was carried out on an Applied Biosystems GeneAmp 7700 sequence detector, and SYBR green dye intensity was analyzed using GeneAmp 7700 SDS software. The rat BACE1 and 18 S primers were designed with the Primer3 software and were as follows: BACE1 forward 5′-CATTGCCATCACTGAAT-3′, reverse 5′-CAGTGCCTCAGTCTGGTTGA-3′; 18 S forward 5′-TCATCAGACCCCAGAAAAGG-3′, reverse 5′-GATTCGGCAGGTGAGTTATT-3′.

2.8. siRNA experiments

SH-SY5Y cells were transfected with 1 nM of PS1, Nicastrin, or scrambled siRNAs (Santa Cruz, Santa Cruz, CA). Each siRNA is a pool of 3 target-specific 20–25 nt siRNAs designed to knock-down gene expression. For transfections, Amaxa nucleofection technology was used (Amaxa, Gaithersburg, MD). Cells were resuspended in the nucleofector V solution, available as part of the Amaxa cell optimization kit, and were electroporated. Briefly, 5×10^6 cells were aliquoted in 100 μl with 10 μl of siRNA and were transferred to a cuvette and nucleofected with the Amaxa Nucleofector device. Cells were immediately transferred into wells containing 37 °C pre-warmed culture medium in 6-well plates. All experiments were performed in triplicate. At days 1–2 after electroporation, the expression level of PS1, Nicastrin, BACE1 and β-actin were analyzed by immunoblotting.

2.9. Aβ quantitative assay

Secreted Aβ42 levels in the cell culture media were quantitatively measured using colorimetric ELISA kits (BioSource) according to the protocol provided by the manufacturer. Culture medium was collected and cleared by centrifugation ($14{,}000 \times g$ for 10 min at 4 °C), and a 100 μl sample of the medium was used for Aβ measurement.

2.10. Statistical analysis

Statistical differences were analyzed using one-way ANOVA tests. Multiple comparisons were performed using a *post hoc* Dunnett's *t*-test.

3. Results

3.1. γ-Secretase activity affects BACE1 expression

Because the activities of both β-secretase and γ-secretase are required to produce Aβ, we first determined whether there is a relationship between β-secretase and γ-secretase activities in AD brains. We observed a positive correlation between β- and γ-secretase activities in rapidly autopsied (postmortem interval: 2.98 ± 0.89 h) samples from the inferior parietal cortex of AD and age-matched control subjects (Fig. 1A). However, there was not a significant correlation between β-secretase activity and γ-secretase activity in parietal lobe within the AD patient group or the non-dementia control group, suggesting that the correlation in the pooled sample data may be due to the fact that AD patients, on average, have higher activities of both β- and γ-secretases compared to non-dementia controls. In samples from cerebellum, a brain region with little or no amyloid pathology, we found no significant correlation between β- and γ-secretase activities in the samples from AD and age-matched control subjects (Fig. 1A). This finding prompted us to determine if γ-secretase activity affects BACE1 expression. We tested this hypothesis by determining the effect of several potent γ-secretase inhibitors on BACE1 levels in culture neurons. First, we checked BACE1 levels in wildtype and BACE1$^{-/-}$ MEFs after treatment with HNE, a lipid peroxidation product previously implicated in the pathogenesis of AD (Keller et al., 1997; Mark et al., 1997; Mattson, 2004). Expression of BACE1 was increased by HNE in wildtype MEF, in agreement with previous results (Fig. 1B) (Tamagno et al., 2002). Also, BACE1 was not detected in BACE1$^{-/-}$ MEFs, demonstrating that anti-BACE1 antibody successfully recognized BACE1 protein. BACE1 levels were increased and primary cultured cortical neurons treated with lipid peroxidation product 4-hydroxy-2,3-nonenal (HNE) (Fig. 1B and C). The HNE-mediated BACE1 induction was suppressed by treatment with several γ-secretase inhibitors (ComE, L685, 31C and PepA), but not by a β-secretase inhibitor (BI4) (Fig. 1C). These results were confirmed in primary cultured

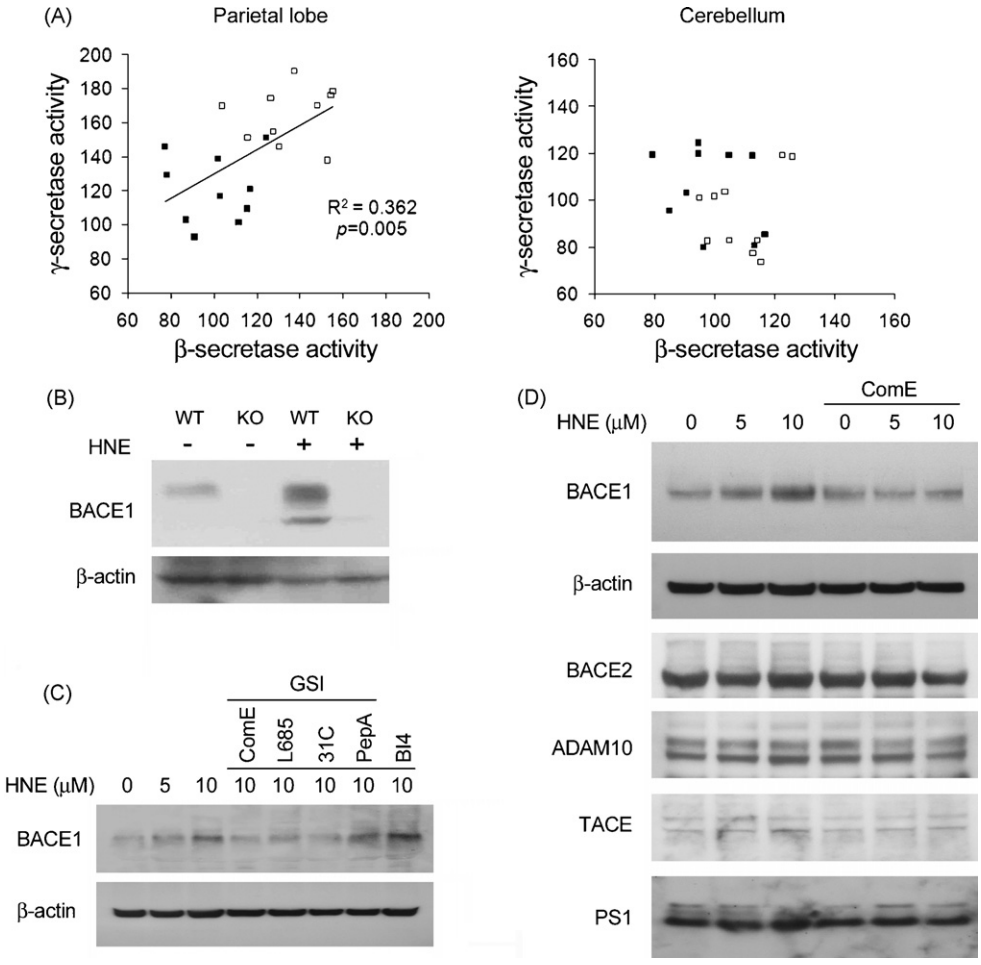

Fig. 1. Evidence that γ-secretase induces an increase in β-secretase levels in AD. (A) Linear regression analysis of (-secretase and γ-secretase activities in inferior parietal lobule and cerebellum from clinically diagnosed and neuropathologically confirmed AD (□) and non-demented control subjects (■). (B) Wildtype and BACE $^{-/-}$ MEFs were exposed to HNE (15 μM) for 2 h. BACE1 levels were examined by immunoblot analysis. (C and D) Effects of γ-secretase inhibitors (GSI) on HNE-mediated induction of BACE1. Primary cultured rat cortical (C) and hippocampal (D) neurons were pretreated with the indicated inhibitors (compound E, 10 nM; L685,458, 2 μM; WPE-III-31C, 2 μM; pepstatin A, 2 μM; (-secretase Inhibitor IV, 2 μM) for 2 h, and then cells were exposed to HNE for 16 h. BACE1, BACE2, PS1, ADAM10, TACE, and β-actin levels were evaluated by immunoblot analysis.

hippocampal neurons where HNE enhanced the expression of BACE1 in a γ-secretase-dependent manner (Fig. 1D). However, levels of two putative α-secretases, ADAM10 and TACE, were not changed by γ-secretase inhibition or by exposure to HNE (Fig. 1D), supporting specific effects of HNE and γ-secretase on BACE1 levels. Indeed, the levels of the BACE1 homolog BACE2 and the catalytic component of γ-secretase presenilin-1 (PS1) were not changed by HNE and γ-secretase inhibitors (Fig. 1D).

3.2. γ-Secretase inhibitors reduce β-secretase activity and the production of sAPPβ

We next performed experiments to determine the effects of HNE or secretase inhibitors on the production of APP, sAPPβ, Aβ42, and APP C-terminal fragments (APP-CTFs) (Fig. 2). First, we determined whether the lipid peroxidation product HNE induces β-secretase, γ-secretase activities, and Aβ42 production. Indeed, HNE enhanced the production of Aβ42 and the activities of β-secretase and γ-secretase, and these effects of HNE were blocked by inhibitors of the respective secretases (Fig. 2A and B). However, γ-secretase inhibitors also suppressed β-secretase activity (Fig. 2A). Consistent with our finding that HNE-mediated BACE1 induction was suppressed by γ-secretase inhibitors (Fig. 1), the production of sAPPβ was significantly reduced by γ-secretase inhibitors, whereas the levels of full-length APP and APP-CTFs were not affected by HNE (Fig. 2C). All of these results support the hypothesis that γ-secretase induces BACE1 production and activity under conditions of oxidative stress.

3.3. PS1/γ-secretase is required for oxidative stress-mediated BACE1 induction

We next examined the oxidative stress-mediated induction of BACE1 in MEF from wildtype, PS1$^{-/-}$, PS2$^{-/-}$ and PS1$^{-/-}$/PS2$^{-/-}$ mice. Hydrogen peroxide and HNE induced

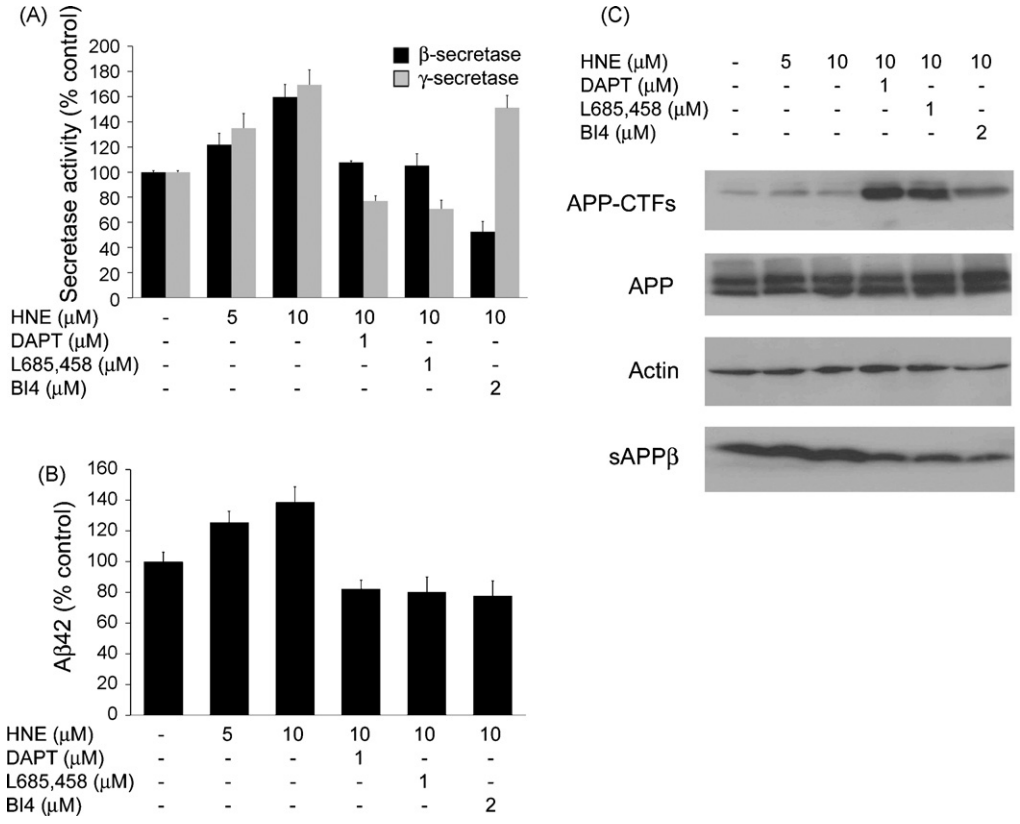

Fig. 2. Oxidative stress-mediated β-secretase activity and sAPPβ levels are reduced by γ-secretase inhibitors. (A) The lipid peroxidation product HNE enhances β- and γ-secretase activities. SH-SY5Y cells were pre-incubated with the β-secretase inhibitor BI4 or the γ-secretase inhibitors DAPT and L-685,458 for 1 h before treatment with HNE. Cells were collected after 24 h of incubation with 5 μM or 10 μM HNE. The cell lysates were tested for β- and γ-secretase activities by addition of secretase-specific peptide substrate conjugated to the reporter molecules EDANS and DABCYL. (B) HNE treatment increased the amounts of secreted Aβ42. After treatment of SH-SY5Y cells stably expressing mutant (Swedish) APP for 24 h, medium was harvested and analyzed by a sandwich ELISA for specific quantification of secreted Aβ42. (C) γ-secretase inhibitors suppress HNE-induced sAPPβ levels. After treatment of SH-SY5Y cells stably expressing wildtype APP for 24 h, cells were harvested and analyzed by Western blots for APP, APP-CTFs and actin. Culture media were also analyzed by Western blot for sAPPβ.

the expression of BACE1 in the wildtype MEF, but not in PS1$^{-/-}$ MEF (Fig. 3A). Oxidative stress-induced BACE1 expression was completely abolished in the PS1$^{-/-}$/PS2$^{-/-}$ MEF (Fig. 3B), and partially reduced in the PS2$^{-/-}$ MEF, compared to wildtype MEF (Fig. 3C). We confirmed that γ-secretase inhibition could suppress oxidative stress-mediated induction of BACE1 in the wildtype MEF (Fig. 3B). In agreement with the results obtained in PS1 null cells, we found that down-regulation of endogenous γ-secretase using siRNAs targeting PS1 and Nicastrin resulted in reduced-BACE1 expression and *BACE1* promoter activity (Fig. 3D and E). These results indicate that PS1/γ-secretase is required for the increased production of BACE1 in response to oxidative stress.

3.4. γ-Secretase activity modulates BACE1 expression at the transcriptional level

To examine whether transcription of BACE1 can be affected by γ-secretase activity, quantitative PCR was performed. Using real time PCR, we found that levels of BACE1 mRNA are increased in response to HNE, and that this effect is blocked by γ-secretase inhibition (Fig. 4A). The promoter activity of BACE1 was also increased by HNE in a γ-secretase-dependent manner (Fig. 4B). The effects of PS1 reconstitution on BACE1 expression were evaluated by transient expression of several familial AD-causing PS1 mutants (PS1-M146V and PS1-ΔE9) in the PS1$^{-/-}$/PS2$^{-/-}$ MEF. We found increased *BACE1* promoter activity following transfection with wildtype PS1, PS1-M146L and PS1-ΔE9 (Fig. 4C). In the presence of HNE, wildtype PS1 and all of the FAD PS1 mutants showed similar levels of BACE1 promoter activities (Fig. 4C). We consistently observed that the loss-of-function mutations PS1-D257A and PS1-D385A, in which the aspartate residue essential for endoproteolysis and γ-secretase activity is replaced with an alanine residue, did not affect the level of *BACE1* promoter activity (Fig. 4C).

The γ-secretase mediated cleavage of APP leads to the formation of Aβ and an intracellular APP domain called AICD. We therefore, examined whether AICD overexpression could modulate *BACE1* in PS-deficient cells. Transfection of AICD increased the transactivation of BACE1 promoters (Fig. 4C), and led to a dose-dependent increase in BACE1 levels in

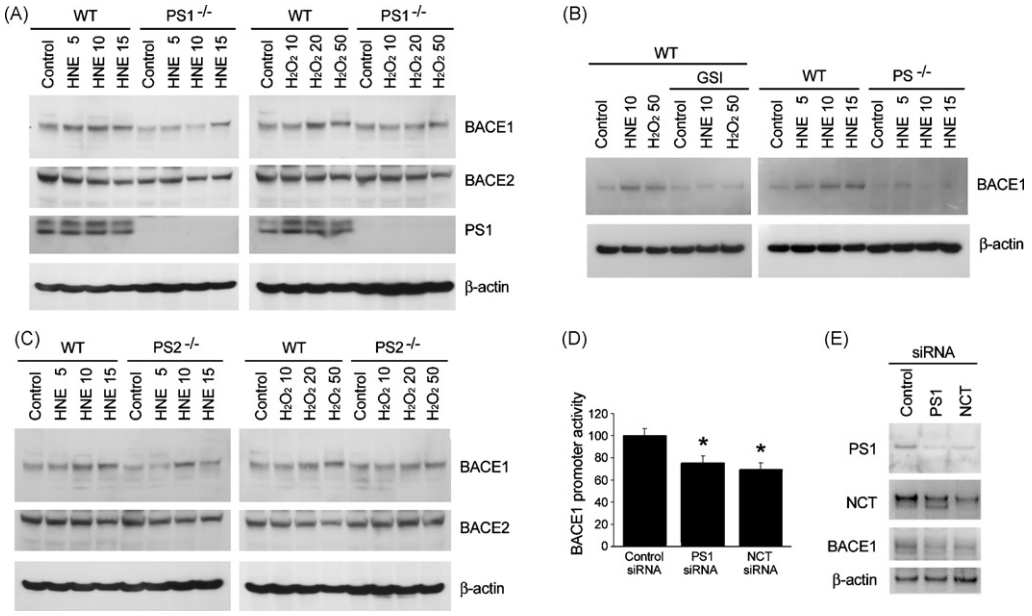

Fig. 3. Oxidative stress-induced BACE1 expression is reduced in presenilin-deficient cells. (A and B) Wildtype, $PS1^{-/-}$ (A) and $PS1^{-/-}/PS2^{-/-}$ ($PS^{-/-}$) MEFs (B) were treated with HNE (5, 10, and 15 μM) or H_2O_2 (10, 20, and 50 μM) for 3 h. Some wildtype cells were also co-treated with GSI (2 μM L685,458). BACE1, BACE2, PS1, and β-actin protein levels were measured by immunoblot analysis. (C) Oxidative stress-induced BACE1 expression is partially reduced in PS2 deficient cells. Wildtype, $PS1^{-/-}$ and $PS1^{-/-}/PS2^{-/-}$ MEFs were treated with HNE (5, 10, and 15 μM) or H_2O_2 (10, 20, and 50 μM) for 3 h. BACE1, BACE2, PS1, and β-actin expression were evaluated by immunoblot analysis. (D and E) SH-SY5Y cells were transfected with BACE1 promoter construct pB1P-A, in combination with PS1 or Nicastrin siRNAs. At 36 h, cells were harvested and analyzed for BACE1 promoter activity by dual-luciferase assay (D) or were used to examine levels of PS1, Nicastrin, BACE1, and β-actin by immunoblotting (E). Values are the mean and S.D. of three independent experiments. $^*P < 0.01$ compared to control.

the Western blot analysis (Fig. 4D). Cells expressing PS1 mutants exhibit a significant increase of the BACE1 promoter activity in the absence of HNE; however, no significant increase is observed after HNE is applied. By contrast, when we transfected AICD the addition of HNE induced a further increase in BACE1 promoter activity. These data suggest the possibility that oxidative stress and AICD production may saturate BACE1 promoter activity in cells overexpressing mutant PS1.

3.5. γ-Secretase inhibitor reduces BACE1 expression in the brains of AD model mice

To determine if BACE1 expression is regulated by γ-secretase *in vivo*, we measured BACE1 levels in cerebral cortical tissue samples from PS1 mutant knock-in mice bearing the PS1 M146L mutation (PS1KI mice), from triple-transgenic AD (3xTgAD) mice (mice expressing APP, PS1 and tau mutations), and from age matched wildtype control mice. We found that the level of BACE1 was significantly higher in the cortex of PS1KI and 3xTgAD mice compared to wildtype mice (Fig. 5). The expression of BACE1 was significantly reduced by treatment with a γ-secretase inhibitor compared to vehicle-treated control littermates (Fig. 5). These results suggest that PS1 positively regulates BACE1 expression in vivo in a γ-secretase activity-dependent manner.

4. Discussion

Our data suggest that, by generating AICD, γ-secretase induces transcription of the *BACE1* gene resulting in increased production of BACE1, increased β-secretase cleavage of APP and increased production of Aβ. In addition, Aβ may itself increase γ-secretase activity by inducing membrane-associated oxidative stress and HNE production (Mark et al., 1997; Mattson, 2004). AICD mediates transcriptional up-regulation of BACE1 expression in response to membrane-associated oxidative stress, a mechanism consistent with evidence suggesting that AICD can translocate to the nucleus and regulate gene transcription (Kimberly et al., 2001; von Rotz et al., 2004).

Apart from BACE1, other genes including tetraspanin KAI1/CD82, APP, glycogen synthase kinase-3β (GSK-3β) and Neprilysin have been suggested to be regulated by AICD (von Rotz et al., 2004; Baek et al., 2002; Kim et al., 2003; Pardossi-Piquard et al., 2005), although regulation of some of these genes is still controversial (Hebert et al., 2006; Chen and Selkoe, 2007). We showed that overexpression of AICD increases BACE1 transcription and protein levels. We also found that selective inhibition of γ-secretase blocked oxidative stress-mediated BACE1 expression, but did not affect BACE1 expression in the absence of oxidative stress. These observations suggest, not surprisingly, that BACE1 expression is likely controlled by multiple transcription factors.

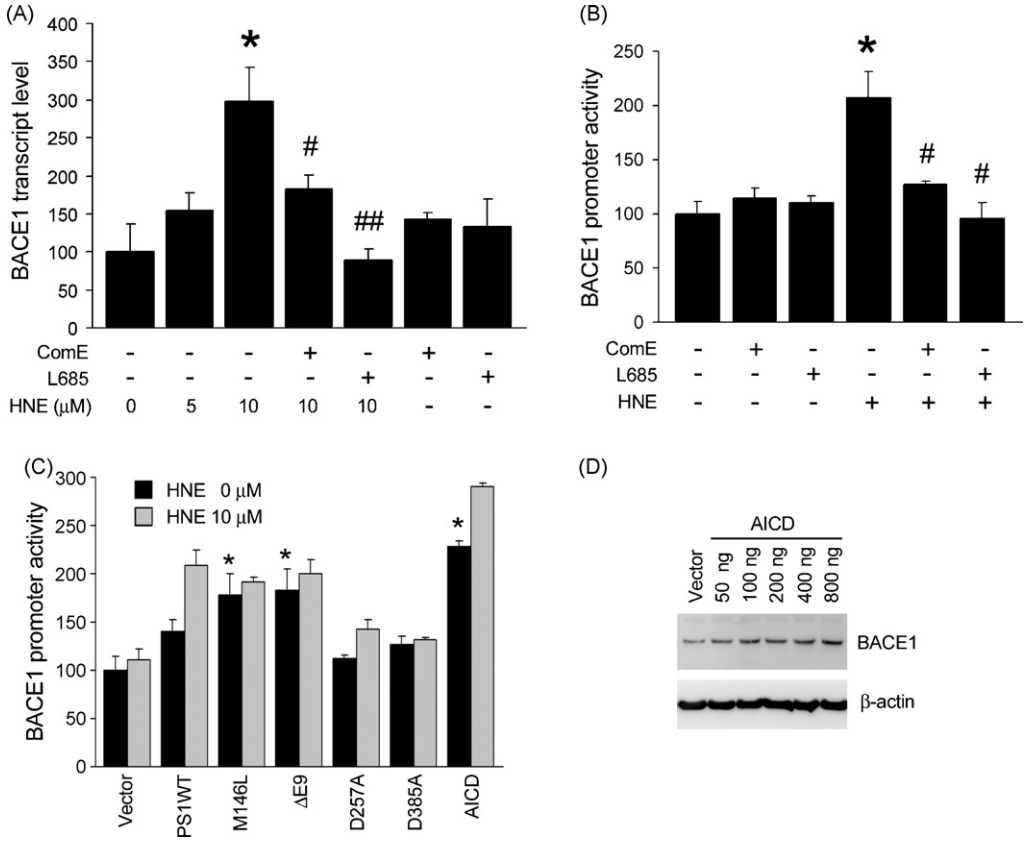

Fig. 4. γ-Secretase proteins regulate BACE1 promoter activity. (A) Quantitative real time PCR analysis was performed on RNA samples from rat cortical neurons that had been exposed for 3 h to the indicated treatments. γ-Secretase inhibitors (compound E, 10 nM; L685,458, 2 μM) were added for 2 h before HNE exposure. Values are the means and S.D. of three independent experiments. (B) HNE increases human BACE1 promoter activity, an effect suppressed by γ-secretase inhibitors. SH-SY5Y cell were transfected with BACE1 promoter constructs pB1P-A or pGL3-Basic (negative control) in association with renilla luciferase vector pCMT-Rluc to verify transfection efficiency. Twenty four hours after transfection, cells were treated for 3 h with HNE in the presence of the indicated γ-secretase inhibitors (compound E, 10 nM; L685,458, 2 μM). BACE1 transcriptional activity was measured using a dual-luciferase assay. Values are the mean and S.D. of three independent experiments. $^{*}P < 0.01$ compared to control, $^{\#}P < 0.01$ and $^{\#\#}P < 0.001$ compared to the 10 μM HNE treated samples. (C) BACE1 promoter activity is increased by PS1, FAD PS1 mutations and AICD in PS-deficient cells. $PS^{-/-}$ fibroblasts were transiently co-transfected with pB1P-A and PS1, PS1-M146L, PS1-ΔE9, PS1-D257A, PS1-D385A, or AICD. After 24 h incubation, BACE1 promoter activity was analyzed. Results were obtained from four independent experiments, each performed in triplicate (mean and S.D.). $^{*}P < 0.01$ compared to vector transfected control. (D) BACE1 protein levels are increased by AICD expression. $PS^{-/-}$ fibroblasts were transiently transfected with increasing amounts of AICD cDNA, and then BACE1 and β-actin levels were measured by immunoblot analysis.

In agreement with the idea that γ-secretase activity is needed for oxidative stress-mediated BACE1 induction, we found that BACE1 protein levels and *BACE1* promoter activity are decreased under basal conditions when PS1 or Nicastrin levels are decreased using RNA interference methods. On the other hand, treatment of neurons with γ-secretase inhibitors has no effect on the basal BACE1 mRNA level or BACE1 promoter activity, indicating that γ-secretase activity is required for the BACE1 induction by oxidative stress while its basal expression is independent of γ-secretase activity. It has been reported that γ-secretase activity is not directly required for several functions of PS1 including protein trafficking, Wnt-mediated signaling, PI3K/Akt and GSK-3β signaling and calcium regulation (Parks and Curtis, 2007). However, the ability of γ-secretase inhibitors to block the effects of oxidative stress on BACE1 expression suggests an important role for γ-secretase activity in the amyloidogenic effect of oxidative stress.

Free radical accumulation and peroxidative membrane damage similar to that observed in AD brains has been shown to increase Aβ generation in cell culture and animal models and may also mediate synaptic and cognitive dysfunction (Mattson, 2004). Previous studies have shown BACE1 protein levels and enzyme activities are elevated in brain regions affected by AD (Holsinger et al., 2002; Li et al., 2004), suggesting that abnormal BACE1 activity contributes significantly to AD pathogenesis. Our findings suggest a mechanism for the induction and amplification of Aβ production in AD. We found that levels of β-secretase and γ-secretase activities were greater in postmortem brain tissue from AD patients compared to control subjects. Oxidative stress and membrane lipid peroxidation, which are known to be increased during brain aging and more so in AD (Mattson, 2004), increased β-secretase expression which was blocked in PS-deficient cells, and prevented by γ-secretase inhibitors. We also showed that γ-secretase inhibitors suppress the expression of BACE1 in

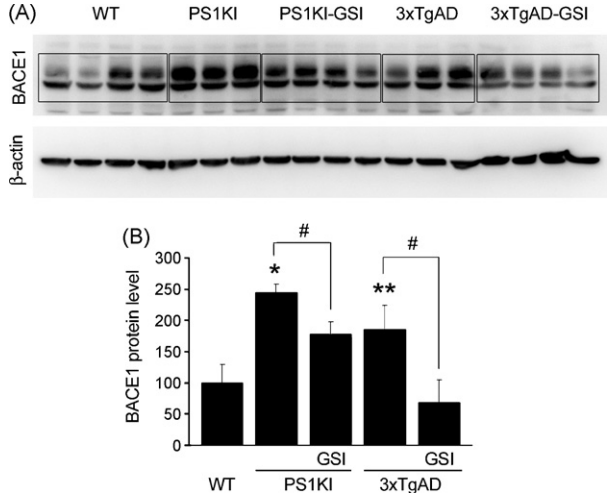

Fig. 5. γ-Secretase inhibitor reduces BACE1 expression in the brains of AD model mice. PS1KI and 3xTgAD mice were injected with γ-secretase inhibitor (GSI) or vehicle every day for 7 days. (A) Cerebral cortex tissue was homogenized and BACE1 and β-actin levels were examined by immunoblots. (B) Quantitative analysis showed increased levels of BACE1 in PS1KI and 3xTgAD mice compared to wildtype mice, and a significant reduction following γ-secretase inhibitor treatment. Values are the means and S.D. (5 mice/group). $^{*}P<0.001$ and $^{**}P<0.05$ compared to wildtype. $^{\#}P<0.01$.

the 3xTgAD mouse model of AD. Interestingly, we found that ischemia/hypoxia (Arumugam et al., 2006) and oxidative increased γ-secretase activity further supporting the idea that induction of BACE1 by oxidative stress is mediated by γ-secretase.

It has been well established that oxidative damage to lipids and proteins increases in the brain during aging and more so in AD. Lipid peroxidation and the accumulation of HNE have been localized to sites of Aβ deposition and neuronal degeneration in the brains of AD patients (Montine et al., 1998; Butterfield et al., 2002). Cell culture studies have shown that during the process of aggregation Aβ can induce membrane lipid peroxidation and the accumulation of HNE in neurons, and that HNE may contribute to the neurotoxic effects of Aβ (Keller et al., 1997). The present findings suggest that lipid peroxidation and HNE enhance amyloidogenic processing of APP, resulting in increased production of Aβ. Whereas increased production of Aβ appears to be the factor that initiates disease in familial AD cases caused by APP or PS mutations, our findings suggest that age-related increases in oxidative stress may be responsible, at least in part, for the increased production of Aβ in sporadic late-onset AD. The available data therefore suggest the involvement of a feed-forward pathogenic mechanism of AD in which lipid peroxidation increases Aβ production which, in turn, enhances lipid peroxidation.

Conflicts of interest

The authors declare that they have no actual or potential conflicts of interest to disclose. Appropriate approval and procedures were used concerning human subjects and animals.

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