Alzheimer’s disease (AD), the most common type of dementia in the aged population, can be divided into familial AD (FAD) and sporadic AD (SAD). The pathology of FAD is identical to SAD characterized by deposition of Aβ and hyperphosphorylated tau inclusion in neurofibrillary tangles (Grundke-Iqbal et al. 1986; Ogomori et al. 1989). Most early onset FAD cases are associated with mutations in presenilin-1 (PS1). Mutations in PS1 genes account for the vast majority of FAD (Rogaev et al. 1995; Sherrington et al. 1995). The presenilins are polytopic proteins with 6–8 transmembrane domains that are found in high molecular weight complexes together with nicastrin, pen-2, and aph-1. These four proteins are required by γ-secretase-involved intramembrane proteolysis of various substrates, including the amyloid precursor protein (Periz and Fortini 2004). Aβ is the proteolytic product of amyloid precursor protein, and autosomal dominant mutations in the gene of PS1 increase production of Aβ and may cause FAD (Oakley et al. 2006). Although the amyloid hypothesis is the leading model for AD pathogenesis, accumulating data suggest that PS1 mutation also contributes to tau hyperphosphorylation and neurofibrillary tangles (NFTs) formation. This is especially plausible in light of the recent observations that three
separate PS1 mutations are associated with frontotemporal dementia, a neurodegenerative disorder characterized by tauopathy and without Aβ pathology (Raux et al. 2000; Tang-Wai et al. 2002; Dermaut et al. 2004). This suggests that PS1 mutations play a role in tau hyperphosphorylation (Feng et al. 2004; Saura et al. 2004).

In our previous study, we found a novel missense mutation of PS1 (Val97Leu mutation, V97L) in members of a Chinese family that had had early onset AD (Jia et al. 2005). To explore the pathological function of the mutation, we generated human V97L mutant PS1 transgenic mice and found they had spatial memory deficit and tau hyperphosphorylation at 10 months of age. The memory deficit was significantly correlated with tau phosphorylation level in the hippocampus of the mice. Furthermore, the tau phosphorylation level also increased in human V97L mutant PS1 transfected N2a cells at multiple epitopes as a result of glycogen synthase kinase-3 (GSK-3) activation and N-cadherin-associated phosphoinositol-3 kinase (PI3K) and Akt signaling inhibition.

Materials and methods

Antibodies, chemicals and generation of transgenic mice

The detailed information for the antibodies used in this work is listed in Table 1. 3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (SB-216763, GS3 specific inhibitor) were purchased from Sigma Chemical Corp. (St Louis, MO, USA).

To elucidate the role of PS1 V97L mutation in the pathogenesis of Alzheimer’s disease, we generated wt and V97L mutant PS1 transgenic mice as follows. The pcDNA3.1/Zeo (+) plasmid with human full-length wild-type (wt) PS1 cDNA was a kind gift from Prof. Xia at the Department of Neurology, Harvard Medical School. We generated relevant V97L mutant PS1 plasmid described previously (Fang et al. 2006). In the present study, we cut down the cDNA of full-length wt and V97L mutant PS1 from these two plasmids by HindIII and XhoI enzymes, and ligated them into analogous sites of pCEP4-platelet-derived growth factor (PDGF) expression vector to create PDGF-PS1wt and PDGF-PS1V97L transgenes. The correctness of PDGF-PS1wt and PDGF-PS1V97L transgenes were confirmed by sequencing across modified regions. PDGF promoter was chosen because it has been successfully used to target the expression of other human proteins to neurons in transgenic models of neurodegenerative disease (Games et al. 1995). After linearization by Tth111 I, PDGF promoter-controlled target transgenes were microinjected into zygotes of C57BL/6J mice, with ICR mice as pseudopregnancy acceptor, to generate wt and V97L mutant PS1 transgenic mice (TE2000U, Microinjection). For each construct, several transgenic founders were generated, and their offspring were screened for cerebral transgene expression. Transgenic expresers were maintained by crossing heterozygous transgenic mice with non-transgenic C57BL/6J mice. Genotype was identified by polymerase chain reaction methods using primers: For 5’-GAA CGG CAG GAG CAC AAC-3’ and Rev 5’-ACA GTA ATG TAG TCC ACA GCA ACG-3’. Non-transgenic litters served as controls. The protocol of this study was approved by the Institute Ethical Committee.

Cell culture and transient transfection

The neuro2a (N2a) cells were cultured in a 1 : 1 mixture of Dulbecco’s modified Eagle’s medium and OPTI-MEM supplemented with 15% fetal bovine serum. The cells were maintained at 37°C in 5% CO2. The pcDNA3.1/Zeo (+) plasmid with human full-length wt PS1 cDNA was a kind gift from Prof. Xia at the Department of Neurology, Harvard Medical School. We generated relevant V97L mutant PS1 plasmid described previously (Fang et al. 2006). In the present study, we respectively fused human full-length PS1wt and PS1V97L plasmids with enhanced green fluorescent

Table 1 Antibodies employed in the study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specific</th>
<th>Type</th>
<th>WB</th>
<th>ICC/Co-IP</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHF-1</td>
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<td>1 : 1000</td>
<td>1 : 200</td>
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<tr>
<td>pT231</td>
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<tr>
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<tr>
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<td>1 : 50</td>
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<td>pAb</td>
<td>1 : 1000</td>
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<td>Abcam</td>
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</table>

WB, western blotting; ICC, immunocytochemistry; Co-IP, co-immunoprecipitation; mAb, monoclonal antibody; pAb, polyclonal antibody.
Morris water maze test

The male and female transgenic mice of 10 months old were divided into three groups: non-transgenic littermates group (Con group), wt PS1 transgenic group (PS1 group) and V97L mutant PS1 transgenic group (V97L group). All the mice were housed in groups of two per cage with free access to food (standard rodent) and water in experimental animal center of Capital Medical University, Beijing, China, and were maintained on 12 light : 12 dark cycle (lights on at 06:00 AM, and off at 06:00 PM) so that the behavioral tests were performed during their active hours. Housing and testing were carried out at stable temperature (23–25℃) and humidity. Morris water maze was used to test spatial memory (Chisti et al. 2001). In brief, the mice were trained to find a submerged platform placed in the center of one quadrant by using a stationary array of cues outside the pool tub. When mice did not find the hidden platform, they were guided to the platform and allowed to stay on it for 15 s. Acquisition training lasted for 5 days, with four trials per day from different quadrant. Escape latency (the time spent when the mice found the hidden platform) was recorded to evaluate spatial learning. After the learning trial, the platform was removed from the pool and each mouse was subjected to a 60-s swimming ‘probe trial’ from day 7 to day 11 (one trial per day). Time in target quadrant (s), platform crossings and swim speed (cm/s) were recorded using on-line video tracking system (camera, TOTA-450III, Japan) of type DNS-2 (Hirano 1986). For immunocytochemistry, the sections were incubated with PIP2 substrate in kinase reaction buffer for 2 h at 23–25℃. The generation of PIP3 product was determined by competitive ELISA.

Western blot and co-immunoprecipitation

Western blot analysis was performed as described previously (Wang et al. 2008). In brief, the hippocampal extracts and cell lysates with equal amount of total protein were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and the proteins were transferred to polyvinylidene difluoride membranes, and probed with PHF-1, pS199/202, pT231, pS396, pS214, pS422, DM1A, pS9-GSK-3β, total GSK-3β, pS473-Akt, and total Akt antibodies. Immunoreactive materials were detected using enhanced chemiluminescence method according to the manufacturer’s instructions. The blots were scanned and the sum optical density was quantitatively analyzed by Kodak Digital Science 1D software (Eastman Kodak Company, New Haven, CT, USA). The levels of the phosphorylated tau, pS9-GSK-3β and pS473-Akt were normalized against the levels of β-tubulin (DM1A), total GSK-3β and total Akt respectively.

For co-immunoprecipitation, cells were lysed in lysis buffer (50 mM Tris–HCl, pH 7.4, 250 mM NaCl, 5 mM EDTA, 0.1% Triton X-100) containing 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 mg/mL leupeptin, 0.5 mg/mL aprotinin and 0.1 mM Na3VO4 for 30min on ice. Cell lysates were clarified by centrifugation at 10 000 g for 10 min at 4℃. The supernatants (0.5 mg) were incubated with PI3K p85 and N-cadherin antibodies at 4℃ overnight. Twenty microliters of a 1 : 1 suspension of protein G-Sepharose beads were added to the mixture and incubated for 2 h at 4℃ with gentle rotation. The beads were collected by centrifugation and washed extensively with cell lysis buffer. The bound proteins were dissociated by boiling the beads in 2x Laemnlli sample buffer and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Precipitated proteins were probed using PI3K p85 and N-cadherin antibodies by western blot method.

PI3K activity assay

PI3K activity was measured as enzymatic production of phosphatidylinositol-3,4,5-trisphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2) substrate by means of a PI3K ELISA kit purchased from Echelon Biosciences (Salt lake city, UT, USA) as previously described (Hajishengallis et al. 2005). Briefly, PI3K was immunoprecipitated from N2a cell lysates using anti-PI3K p85 antibody and protein A-agarose beads, and the bead-bound enzyme was subsequently incubated with PIP2 substrate in kinase reaction buffer for 2 h at 23–25℃. The generation of PIP3 product was determined by competitive ELISA.

Modified Bielschowsky’s silver staining and immunocytochemistry staining

The mice brains were fixed in situ for 20 min by transcardial perfusion with Zamboni’s solution containing 2% paraformaldehyde, 15% saturation picric acid, and 24 mM NaH2PO4/126 mM Na2HPO4, pH 7.2, pre-incubated at 4℃. Then, the brains were sliced coronally and the sections containing hippocampal and frontal formation were fixed in the same solution for another 12 h at 4℃. Twenty-micrometer-thick sections were made for modified Bielschowsky’s silver staining as described previously (Yamamoto and Hirano 1986). For immunocytochemistry, the sections were incubated for 24 h at 4℃ with primary antibodies of PHF-1 and pS199/202 and pT231. The slices were developed with HistostainTM-SP kits (ZEMED, South San Francisco, CA, USA) and visualized with 3,3’-diaminobenzidine.

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). The one-way ANOVA procedure followed by LSD’s post hoc tests was used to determine the different means among groups (p < 0.05). Pearson’s test was applied for the partial correlation analyses (p < 0.05).

Results

V97L mutant PS1 induces impairment in spatial learning and memory retention in mice

Although the novel V97L mutation of PS1 had been found in our previous study (Jia et al. 2005), no research reported whether V97L mutant PS1 was responsible for the memory loss in AD patients. Now, we microinjected linearized PDGF-PS1wt and PDGF-PS1V97L transgenes (Fig. 1b) into zygotes of C57BL/6j mice and generated wt and V97L mutant PS1 transgenic mice expressing PS1 protein.
Then, we assessed the spatial learning and memory of the transgenic mice by Morris water maze test. All the male and female mice were put in the pool to learn finding the hidden platform. In the first day of training, no mice could find the platform rapidly. After trained for five consecutive days, both non-transgenic and wt PS1 transgenic mice could find the platform within 15 s, but V97L mutant PS1 transgenic mice need about 25 s on average to complete the searching work (Fig. 2a). At days 7–11, we removed the platform and carried out the memory retention study. We found the swimming time in target quadrant of V97L group is obviously reduced at day 7 compared with that of PS1 group (Fig. 2b). The mice in V97L group also have less platform crossings than that of PS1 group at days 7 and 9 (Fig. 2c). During the memory retention test, we did not find any swimming speed difference among three groups (Fig. 2d). No significant alterations in escape latency, time in target quadrant and platform crossings were detected between Con and PS1 group throughout the behavioral test (Fig. 2a–c).

**V97L mutant PS1 increased tau phosphorylation level in transgenic mice**

To explore whether V97L mutation PS1 could regulate tau phosphorylation level in transgenic mice, we measured the tau phosphorylation level in the hippocampus of wt and V97L mutant PS1 transgenic mice at 10 months of age. We found that expression of wt PS1 in hippocampus of mice decreased the tau phosphorylation level probed by PHF-1, pS199/202, pT231 and pS396 antibodies, but the tau phosphorylation level prominently increased in V97L mutant PS1 transgenic mice (Fig. 3). A similar trend of the alterations in tau phosphorylation level was also observed in CA3 and CA4, but not in CA1, regions of hippocampus by immunocytochemistry (Fig. 4a and b). The phosphorylation level of tau probed by pS214 and pS422 remained unchanged among all groups (Fig. 3). By modified Bielschowsky’s silver staining, we observed enhanced staining of black granules in CA3 and CA4 region of hippocampus in V97L group, and this kind of argyrophilic substances accumulated mainly in neurites. The distribution pattern of the black granules is in agreement with the immunocytochemistry results of PHF-1 (Fig. 4a). We found tangle-like structure and wizened degenerative neurons in frontal cortex of V97L mutant PS1 transgenic mice (Fig. 4c).

**Correlation analysis of memory deficit with tau phosphorylation level**

We analyzed the correlation of memory deficit of the transgenic mice with relevant tau phosphorylation level, and found that time in target quadrant is significantly correlated with phosphorylation level of tau at PHF-1, pS199/202, pT231 and pS396 epitopes, but not at pS214 and pS422 epitopes (Fig. 5).

**V97L mutant PS1 increased tau phosphorylation level in GSK-3-dependent manner in N2a cell**

After 48 h transfection of wt and V97L mutant PS1 plasmids (Fig. 1a), green fluorescent protein expressed extensively in N2a cells, which indicated wt and V97L mutation PS1 well expressed (Fig. 6a, PS1 and V97L), whereas no green fluorescent was found in the untreated cells (Fig. 6a, Vector). As tau hyperphosphorylation is an early event of AD, therefore, we measured the alteration of tau phosphorylation in the N2a cells after transfection. We found that tau phosphorylation level of tau at PHF-1, pS199/202, pT231 and pS396 epitopes, but not at pS214 and pS422 epitopes (Fig. 5).
Whereas the V97L mutation PS1 loosed its dephosphorylation function of tau and increased tau phosphorylation level obviously at the same epitopes compared with Con group (Fig. 6b and c). As these tau phosphorylation epitopes are GSK-3 specific sites, therefore, we simultaneously added 7 μM SB-216763 (Liu et al. 2008), specific inhibitor of GSK-3, to V97L mutant PS1 transfected cells. The hyperphosphorylation of tau was counteracted at relevant epitopes. We also studied other non-GSK-3-related tau phosphorylation sites, such as pS214 and pS422. No alterations of tau phosphorylation level at these two epitopes were found in all groups (Fig. 6b and c).

V97L mutant PS1 activated GSK-3 through PI3K/Akt signaling pathway

The preceding results show that GSK-3 involves in tau phosphorylation alteration induced by wt and V97L mutant PS1 transfection in N2a cell. Generally speaking, PI3K/Akt signaling pathway is crucial to regulation of GSK-3 activity, therefore, we measured activity of these serial kinases to find how GSK-3 activity is influenced. It has been proved that GSK-3 is inhibited by phosphorylation at Ser9 and Akt is activated by phosphorylation at Ser473 respectively, so we assess GSK-3 and Akt activity by their relevant phosphorylation levels here (Liu et al. 2003; Sarbassov et al. 2005). We found that the levels of pS9-GSK-3β (inactive form) and pS473-Akt (active form) both increased in PS1 group and decreased in V97L group significantly. However, total GSK-3β and Akt remained unchanged (Fig. 7a and b). Next, we measured production amounts of PIP3 as PI3K activity estimation. PI3K activity was dramatically enhanced in PS1 group compared with Vector group, but declined when transfected with V97L mutant PS1 compared with PS1 group (Fig. 7c). It has been reported that N-cadherin/PI3K association involved in PI3K activation (Baki et al. 2004). In the present study, we detected that expression of wt PS1 actually promoted association between N-cadherin and PI3K in N2a cell, but V97L mutant PS1 expression abolished the association promoting action of wt PS1 (Fig. 7d).

Discussion

Alzheimer’s disease is clinically characterized by progressive memory loss. Although a great number of studies have tried to find out how PS1 mutations result in memory deficit, no overt mutant phenotype was found in single PS1 mutation transgenic mice in earlier studies (Duff et al. 1996; Guo et al. 1999; Holcomb et al. 1999). Whereas we demonstrated that single V97L mutant PS1 transgenic mice exhibited higher escape latency compared with wt PS1 transgenic mice at day 5 during learning stage of Morris water maze test. When the mice were put into the pool for probe trial, the V97L mutant PS1 transgenic mice exhibited shorter time staying in platform quadrant and less platform crossings. All the memory evaluation indexes indicated that the spatial memory of V97L mutant mice was impaired at 10 months of age. Actually presenilin protein is essential to memory maintenance as the PS1/PS2-knock-out mice had completely lost their spatial learning ability when examined with a water-maze task (Chen et al. 2008). In the same way, a
number of tentative researches have also acquired similar results of memory impairment induced by PS1 mutation recently. The transgenic mice expressing PS1 with FAD mutation of L286V or M146V showed synapsis-related neurodegeneration in recent reports (Wang et al. 2009; Kunimoto et al. 2010). Furthermore, PS1 M146V knock-in mice exhibited hippocampus-dependent memory impairments in probe trial of water maze and in a contextual fear conditioning paradigm (Wang et al. 2004; Sun et al. 2005). In contrast, L235P mutant PS1 did not affect the acquisition of a spatial memory in the water maze (Huang et al. 2003). These results suggested that the effect of PS1 mutations on memory is dependent on mutant locus and V97L mutation of PS1 is an effective mutant locus which is harmful to learning and memory.

However, the effect only existed at day 8 or day 9 and we did not observe persistent spatial memory loss since then. Especially from day 8, the performance of wt PS1 mice declined substantially, which may resulted in reduction of disparity with that of V97L mutant mice. An possible explanation might be that once these mice were exposed to platform removal, they already experienced an extinction test. Thus, less time in target quadrant on day 8 for wt PS1 mice might be explained as a new learning experience, not simply loss of memory. We also took notice that all mice swim almost half as fast from day 8. Low velocity of swimming may be attributed to looking about and making no move of mice after platform removing, and then affect parameters of spatial memory. In general, we may obtain lasting and marked results if we extend the observation age of mice (more than 12 months of age) and/or prolong the learning days, such as 12 days (Sun et al. 2005).

It has been reported that PS1 mutant FAD cases demonstrate an accelerated development of neurofibrillar pathology with significantly higher percentage of NFTs compared with SAD cases (Schmidt et al. 1989; Woodhouse et al. 2009). These initial findings suggest that there is a close relationship between PS1 mutation and tau hyperphosphorylation/NFTs. Recently, the researchers have become more focused on the role of PS1 in regulation of tau phosphorylation. The loss of PS1 increased tau phosphorylation and NFTs-like structures in PS1 knock-out cells and mice (Kang et al. 2005; Chen et al. 2008). Wt PS1 suppresses phosphorylation of tau, nevertheless PS1 mutations, including ΔE9, M146L, A246E and E280A, promote tau hyperphosphorylation at pS202 and pS396 epitopes in fibroblasts (Baki et al. 2004). Furthermore, PS1 Δ213T and A246E mutant transgenic mice exhibited tau hyperphosphorylation at GSK-3-dependent pS199 and pS396/404 epitopes (Tanemura et al. 2006; Dewachter et al. 2008). In this work, we found the novel V97L mutant PS1 could increase tau phosphorylation level in transgenic mice and N2a cells. More GSK-3-dependent phosphorylation epitopes of tau were found in our models, including PHF-1, pS199/202, pT231 and pS396. Whereas wt PS1 decreased tau phosphorylation level in our experiment. The results supplemented and enriched previous research contents and confirmed the regulatory function of wt and V97L mutant PS1 to tau phosphorylation. It should be noticed that tau protein was hyperphosphorylated in hippocampus of V97L mutant transgenic mice of 10 months old. The pathological alteration is present more earlier in our transgenic mice than that in PS1 Δ213T mutation knock-in mice of 14 months old (Tanemura et al. 2006), suggesting relatively early pathological impairments present in our mouse model. We also measured other tau phosphorylation epitopes, such as pS214 and pS422, which could be associated with the activation of cyclic AMP-dependent protein kinase and extracellular signal-regulated kinase1/2, but no alterations were found. Therefore, it suggested that PS1 V97L mutation is specific for tau phosphorylation at multiple GSK-3-dependent epitopes.
The hyperphosphorylated tau is the main component of NFTs (Singer et al. 2002), which is significantly correlated with the memory impairment in AD patients (Riley et al. 2002; Bussiere et al. 2003). Although both tau hyperphosphorylation and cognitive deficit were separately observed in some AD models with specific single PS1 mutation as described above, nobody had previously reported the correlation between tau hyperphosphorylation and memory deficit in those cases. We studied this point here and found that phosphorylation of tau at PHF-1, pS199/202, pT231, and pS396 epitopes was significantly correlated with the memory impairment in transgenic mice, but not pS214 and pS422 epitopes. The results suggested that spatial memory deficit of mice with PS1 V97L mutation is, at least partially, caused by GSK-3-dependent tau hyperphosphorylation. New content has been added to the mechanism how mutant PS1 destroyed memory. We also found that the increased tau was prominently detected in the CA3 and

![Fig. 4](image-url)  

(a) The alterations of phosphorylated tau distribution and silver staining pattern in hippocampus of transgenic mice. The hippocampal slices of the mice were prepared immediately after the behavioral test. The immunocytochemistry and Bielschowsky’s silver staining were carried out as described in Materials and methods section. V97L mutant PS1 transgenic mice exhibited enhanced silver staining and phosphorylated tau staining at PHF-1, pS199/202 and pT231 epitopes in CA3 and CA4 region of hippocampus compared with wt PS1 transgenic mice. The phosphorylated tau probed by PHF-1 antibody distributed in neurites of neurons, which is in the similar distribution manner with argentophilic structures of silver staining. The phosphorylated tau at pS199/202 epitope was only found in neuronal body, whereas the phosphorylated tau at pT231 epitope spread all over the neuron. In contrast, the tau phosphorylation level at CA1 region was unchanged. (bar = 50 μm). (c) We also separated the frontal cortex (FC) from V97L mutant PS1 transgenic mice and carried out the immunocytochemistry and silver staining. The results demonstrated the tangle-like structure and wizened degenerative neurons (arrows, bar = 20 μm) (n = 3–6/group).

The hyperphosphorylated tau is the main component of NFTs (Singer et al. 2002), which is significantly correlated with the memory impairment in AD patients (Riley et al. 2002; Bussiere et al. 2003). Although both tau hyperphosphorylation and cognitive deficit were separately observed in some AD models with specific single PS1 mutation as described above, nobody had previously reported the correlation between tau hyperphosphorylation and memory deficit in those cases. We studied this point here and found that phosphorylation of tau at PHF-1, pS199/202, pT231, and pS396 epitopes was significantly correlated with the memory impairment in transgenic mice, but not pS214 and pS422 epitopes. The results suggested that spatial memory deficit of mice with PS1 V97L mutation is, at least partially, caused by GSK-3-dependent tau hyperphosphorylation. New content has been added to the mechanism how mutant PS1 destroyed memory. We also found that the increased tau was prominently detected in the CA3 and

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CA4, but not in the CA1, regions of hippocampus by immunocytochemistry. The distribution of PHF-1 staining is mainly in the neuronal processes, which is in agreement with the silver staining pattern. It supports the idea that silver staining could act as NFTs marker well (Yamamoto and Hirano 1986). In contrast, the staining of pS199/202 is distributed in neuron bodies and the staining of pT231 extends to the whole neurons. It seems that hyperphosphorylated tau is toxic to neurons independent on their distribution. Additionally, we found the tangle-like structure and wizened degenerative neurons in frontal cortex of V97L mutant PS1 transgenic mice, which proved that V97L mutant PS1 has an actual function resulting in tangle formation and neuron degeneration.

GSK-3 is an enzyme with diverse functions in intracellular signaling, and deregulation of GSK-3 activity has been postulated to play an important role in AD (Russ et al. 2001). GSK-3β has been identified as a crucial tau kinase that can actively phosphorylate tau at most of the abnormal sites seen in the AD brains (Plattner et al. 2006). PI3K is known to

![Graphs showing correlation between tau phosphorylation and time in target quadrant.](image)

**Fig. 5** After recording the time in target quadrant in probing test at the day 7, we killed part of mice and analyzed relevant tau phosphorylation level in hippocampus. Here, we showed the correlation analyzing results of tau phosphorylation level at different epitopes with the time in target quadrant in transgenic mice (n = 8/group).
stimulate Akt activity, which in turn inhibits GSK-3 activity by phosphorylating GSK-3β at Ser9 epitope (Grimes and Jope 2001). It has been hypothesized more than a decade ago that PS1 is involved in tau hyperphosphorylation by inhibiting GSK-3β and mutations in PS1 reverse this function (Takashima et al. 1998). Recently, serial reports have shown that both wt and mutant PS1 do regulate PI3K/Akt/GSK-3 signaling activity resulting in multiple pathological impairments including tau hyperphosphorylation. It has been proved that the loss of PS1 inhibits PI3K/Akt signaling function.
in PS1 knock-out cells (Kang et al. 2005). Studies in vitro show that wt PS1 prevents neuronal degeneration and apoptosis by promoting PI3K signaling, but FAD mutant PS1 fails in the promotion of PI3K (Baki et al. 2008). Additionally, M146V mutant PS1 impaired kinesin-based axonal transport of vesicles and mitochondria through GSK-3 activation in cultured cells (Pigino et al. 2003). It was also reported in vivo that A246E mutant PS1 transgenic mice exhibited increased tau phosphorylation by activating GSK-3β through decreasing phosphorylation on Ser-9 (Dewachter et al. 2008). In general, although the previous studies suggest that FAD mutant PS1 prevents activation of PI3K/Akt and results in activation of GSK-3 in multiple models, no integrated analysis of the PI3K/Akt/GSK-3 signaling is reported in a particular PS1 mutation model. We measured activity of all these three enzymes in N2a cells expressing V97L mutant PS1 and found that PI3K and Akt were inhibited and GSK-3 was activated, which convinced us of the involvement of the whole PI3K/Akt/GSK-3 signaling in regulation of wt or V97L mutant PS1 on tau phosphorylation.

It has been reported that PS1 binds cadherins and promotes cadherin–cadherin cell–cell adhesion interactions (Baki et al. 2001). As a key pre-requisite, cadherin–cadherin interactions initiate a cascade of signaling events that result in increased cadherin/PI3K association and activation of PI3K/Akt signaling (Tran et al. 2002; Yap and Kovacs 2003). The results strongly suggested a potential relationship between PS1 and PI3K activation, which seemed to be mediated by N-cadherin. To explore how V97L mutant PS1 inhibits the PI3K activity, we immunoprecipitated PI3K p85 and N-cadherin and evaluated association level between them. It turned out that expression of wt PS1 increased the level of PI3K/N-cadherin associating complex, while V97L mutant PS1 impaired the ability of wt PS1 to promote the association. Baki et al. (2004) have also shown similar result that the association of PI3K p85 with N-cadherin is severely reduced in PS1 knock-out cells and E123T mutant PS1 knock-in mice. Taking account of the results that multiple mutations of PS1, including ΔE9, M146L, A246E and E280A, all can intervene PI3K/Akt/GSK-3 signaling, PS1 mutations seem to have a common pattern of action on this signaling pathway.

In conclusion, we are here reporting, for the first time, that a single point FAD-linked PS1 mutation is sufficient to cause deficits in brain functions. In our newly created transgenic mice, we found that the expression of V97L mutant PS1 proteins caused spatial memory deficits, which were significantly correlated with tau hyperphosphorylation. Furthermore, in our in vitro studies, we found that the tau hyperphosphorylation caused by the over-expression of the mutant PS1 protein depends upon GSK-3 activity and is likely to be mediated through the PI3K/Akt/GSK-3 intracellular pathway.

Acknowledgements

This work was supported by National Key Technology R&D Program in the Eleventh Five-year Plan Period (2006BAI02B01), the key project of the National Natural Science Foundation of China (30830045), the National Basic Research 973 Program (2006CB500700), the National High Technology Research and Development Program 863 (2006AA02A408), the National Natural Science foundation of China (81000472), Beijing Natural Science Foundation (7102071), the key project of Science and Technology Plan of Beijing Municipal Education Commission (KZ200910025005).

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