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The γ-Secretase Modulator CHF5074 Reduces the Accumulation of Native Hyperphosphorylated Tau in a Transgenic Mouse Model of Alzheimer's Disease

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Abstract The relationship between β-amyloid (Aβ) and tau is not fully understood, though it is proposed that in the pathogenesis of Alzheimer's disease (AD) Aβ accumulation precedes and promotes tau hyperphosphorylation via activation of glycogen synthase kinase-3beta (GSK-3β). Both events contribute to learning and memory impairments. Modulation of γ-secretase activity has proved to reduce the Aβ burden and cognitive deficits in mouse models of AD, but its ability in reducing the tau pathology remains elusive. Chronic treatments with two γ-secretase modulators, ibuprofen and CHF5074, disclosed higher activity of CHF5074 in ameliorating brain plaque deposition and spatial memory deficits in transgenic mice expressing human amyloid precursor protein (hAPP) with Swedish and London mutations (APP_{SL} mice). The aim of our study was to investigate in APP_{SL} mice the effect of the two compounds on the accumulation of native hyperphosphorylated tau as well as on the GSK-3β signaling. CHF5074 was more effective than ibuprofen in reducing tau pathology, though both compounds decreased the GSK-3β level and increased the GSK-3β inhibitory phosphorylation near to the non-Tg values. The inhibition of GSK-3β appeared to be secondary to the reduction of Aβ generation as, differently from LiCl, CHF5074 reproduced its effect in hAPP-overexpressing neuroglioma cells, but not in wild-type primary neurons. Our data show that the novel γ-secretase modulator CHF5074 can fully reverse β-amyloid-associated tau pathology, thus representing a promising therapeutic agent for AD.

Keywords Alzheimer · Tau · Human APP transgenic mice · Ibuprofen · CHF5074

Abbreviations

Aβ	β-Amyloid
AD	Alzheimer's disease
$hAPP_{SL}$	Human amyloid precursor protein with Swedish and London mutations
COX	Cyclooxygenase
CHF5074	1-(3′,4′-dichloro-2-fluoro[1,1′-biphenyl]-4-yl)-cyclopropanecarboxylic acid
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
GSK-3β	Glycogen synthase kinase-3beta
LiCl	Lithium chloride

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NFTs	Neurofibrillary tangles
PHF-1	Paired helical filament-1
Ser	Serine
Swe	Swedish mutation
Tg	Transgenic
Thr	Threonine

Introduction

The accumulation of insoluble β-amyloid (Aβ) plaques and the presence of neurofibrillary tangles (NFTs) are the histopathological hallmarks of Alzheimer's disease (AD) (Selkoe 2001). While the amyloid plaques consist of fibrils of Aβ peptide generated by the cleavage of amyloid precursor protein (APP), the NFTs are composed primarily of hyperphosphorylated forms of the microtubule-associated protein tau. APP is initially cleaved by β-secretase to generate a carboxy-terminal fragment (CTFβ) that is subsequently cleaved by γ-secretase to form Aβ. Modulation of γ-secretase appears a good strategy to decrease Aβ accumulation in brain. This can be realized by 1-(3′,4′-dichloro-2-fluoro[1,1′-biphenyl]-4-yl)-cyclopropanecarboxylic acid (CHF5074), a Notch-sparing γ-secretase modulator devoid of anti-cyclooxygenase (COX) activity (Imbimbo et al. 2007). Treatment with CHF5074 reduced the brain plaque burden and attenuated memory deficit in different APP transgenic mouse models of AD (Imbimbo et al. 2007, 2009, 2010). Reduction of brain plaques was also described with another γ-secretase modulator, ibuprofen (Lim et al. 2000; Yan et al. 2003; Heneka et al. 2005). In a 6-month oral treatment study, ibuprofen appeared to be less effective than CHF5074 in lowering Aβ accumulation and cognitive deficits of hAPP transgenic APP_{SL} mice (Hutter-Paier et al. 2004; Schilling et al. 2008), though either drugs equally reduced the microglial reactivity (Imbimbo et al. 2009).

It is proposed that in the pathogenesis of AD, Aβ accumulation precedes tau hyperphosphorylation and both processes contribute to the cognitive decline (Ballatore et al. 2007), but the efficacy of γ-secretase modulators in modifying tau pathology is still poorly investigated. Hyperphosphorylation and accumulation of tau has been linked extensively to neurodegeneration (Lee et al. 2001), and it has been shown that phosphorylation at serine (Ser) and threonine (Thr) sites are critical for neurotoxicity (Jackson et al. 2002; Nishimura et al. 2004; Dias-Santagata et al. 2007; Fulga et al. 2007; Steinhilb et al. 2007). Among the kinases catalyzing tau phosphorylation, glycogen synthase kinase-3β (GSK-3β) is mostly involved (Gong et al. 2005; Liu et al. 2006). It has been shown that GSK-3β induces the hyperphosphorylation of tau in vitro and in cell-based models of neurodegeneration, implicating GSK-3β as a crucial tau kinase in the formation of NFTs in vivo. Consistent with this, GSK-3β transgenic mice display tau hyperphosphorylation and neurodegeneration (Lucas et al. 2001). Furthermore, in the brains of AD patients and APP transgenic mice, the altered localization of GSK-3β is associated with the NFTs formation (Baum et al. 1996; Pei et al. 1997, 1999; Lucas et al. 2001; Ishizawa et al. 2003). In early stages, GSK-3β accumulates with tau in the cytoplasm of pre-tangle neurons (Pei et al. 1997) and in neuritic plaques (Shiurba et al. 1996), whereas in mature NFTs the colocalization with GSK-3β is reduced (Baum et al. 1996; Harr et al. 1996; Shiurba et al. 1996).

In this context, the main objective of our study was to investigate whether the γ-secretase modulators CHF5074 and ibuprofen might act as potentially disease-modifying drugs by limiting the pathological changes of native tau and GSK-3β in APP_{SL} mice.

Materials and Methods

Animals and Treatments

Transgenic Mice All animal care and handling were performed according to the European guidelines. hAPP transgenic (Tg) mice expressing human APP_{751} with the Swedish (K670N/M671L) and London (V717I) mutations (APP_{SL} mice) were treated as previously described (Imbimbo et al. 2009). Briefly, Tg mice were treated with CHF5074 (375 ppm in the diet) or ibuprofen (375 ppm in the diet) or standard diet from 6 to 12 months of age. Non-transgenic (non-Tg) control mice received standard diet.

Primary Cultures of Mouse Cortical Neurons C57BL/6 mice were purchased from Charles River, Italy. Fifteen-day embryonic mice were harvested with caesarean section from anesthetized pregnant dams and cortical neurons were isolated as previously described (Sarnico et al. 2009). Briefly, cells were plated at a density of 1.0×10^5 cells/cm^2 in 21-cm^2 culture dishes (Nunc; VWR, West Chester, PA, USA) in Neurobasal medium (Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 2% B27 (Invitrogen), 0.5 mM L-glutamine (Euroclone, UK), and 50 U/ml penicillin/streptomycin (Euroclone). Three days after plating, 50% of the medium was changed with fresh medium and, subsequently, 50% of the medium was changed twice a week. At 11 days in vitro, neurons were incubated in Neurobasal medium, containing 0.4% B27 supplement with 3 μM CHF5074, 500 μM ibuprofen, and 5 mM lithium chloride (LiCl), all dissolved in dimethyl sulfoxide (0.1% final concentration), or vehicle (control) for 30 min, 5 or

18 h. At the end of incubation, total proteins were extracted for western blot analyses.

H4swe Cells H4swe human neuroglioma cells, expressing hAPP with the Swedish mutation, were seeded at a density of 1.25×10^5 cells/cm^2 in 8-cm^2 culture dishes (Nunc) in Opti-MEM culture medium (Invitrogen) containing 10% fetal bovine serum and allowed to grow to confluence for 24 h in 5% CO_2, 95% air in a humidified atmosphere. Cells were incubated in Opti-MEM without serum for 18 h with CHF5074 3 μM, LiCl 5 mM or vehicle (control). At the end of treatments, total proteins were extracted for western blot analysis.

Western Blot Analyses

Hemispheres of Tg mice were homogenized in TBS buffer containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Mouse cortical neurons and H4swe cells were harvested in 100 μl of lysis buffer (pH 6.9) containing 1 mM methyl sulfonyl fluoride, 1 μg/ml leupeptin, 5 μg/ml aprotinin, 1 μg/ml pepstatin, 1 mM sodium orthovanadate, and 10 mM sodium fluoride. The suspension was sonicated for 30 s at full power and centrifuged at 11,000×*g* for 20 min at 4°C. Brain lysates (50 μg proteins/sample) and cell extracts (40 μg proteins/sample) were suspended in the sample loading buffer (62.5 mM Tris–HCl, 1% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.02% bromophenol blue, pH 7.5), resolved by 4–12% SDS/polyacrylamide gel, and proteins were transferred electrophoretically onto nitrocellulose membrane. Membranes were incubated overnight at 4°C with anti-GAPDH mouse antibody (1:3,000, MAB374; Chemicon-Millipore, Billerica, MA, USA) together with one of the following primary antibodies: PHF-1 mouse antibody (1:100; gifted by P. Davies), which recognizes phosphorylated tau at the Ser$^{396/404}$ residues; CP13 mouse antibody (1:50, gifted by P. Davies), which recognizes phosphorylated tau at the Ser^{202} residue; Tau46 mouse antibody (1:3,000; Cell Signaling, Danvers, MA, USA), which recognizes total tau; anti-βIII-tubulin rabbit antibody (1:2,000; Sigma-Aldrich) anti-phospho-GSK-3β(Ser9) rabbit antibody (1:1,000; Cell Signaling), which identify the inactivated kinase; anti-GSK-3β rabbit antibody (1:1,000; Cell Signaling). The immunoreaction was revealed by 1-h incubation at 37°C with secondary antibodies coupled to horseradish peroxidase (1:1,500; Santa Cruz Biotechnology, CA, USA) and chemoluminescence detection using ECL Western blotting reagents (GE Healthcare, UK). Quantification of immunoblots was performed by densitometric scanning of exposed films using GelPro Analyzer software (Media Cybernetics, Bethesda, MO, USA). To compare data from the numerous animals in each group, brain extracts (2–3 per group) were loaded in any single gel so that all the samples were distributed in five separate membranes. Each protein band was normalized to the relative GAPDH signal detected simultaneously by the blot development.

Immunohistochemical Detection of Phospho-tau

Right brain hemispheres were sagittally cut (10-μm sections) using a cryotome (CM3050S; Leica Microsystems, Wetzlar, Germany). To unmask the antigen sites, the slides were boiled for 20 s in citrate buffer, pH 6 (containing 8.0 mM sodium citrate and 2.0 mM citric acid) if Tau46 antibody was used, or incubated in 70% formic acid for 10 min at room temperature in case of PHF-1 antibody. To inhibit the endogenous peroxidase, the slides were incubated in a buffer containing 0.1% igepal, 20% methanol, and 3% hydrogen peroxide for 15 min at room temperature. Tau labeling was performed by immunohistochemistry using the following primary antibodies: PHF-1 mouse antibody (1:50; gifted by P. Davies) and Tau46 mouse antibody (1:500, #4019; Cell Signaling) incubated for 24 h at 4°C. Then, slides were treated with biotinylated anti-mouse immunoglobulins (1:200; Dako, Milan, Italy) for 1 h at room temperature, incubated with ABC complex/HRP (Dako) for 45 min and with 0.5% cobalt chloride in Tris–HCl 50 mM, pH 7.4, for 10 min. Peroxidase labeling was detected by incubation with PBS containing 0.025% 3,3″-diaminobenzidine (DAB) (Sigma-Aldrich) and hydrogen peroxide. For all procedures except the final DAB reaction, PBS was used as washing buffer.

$A\beta_{42}$ Measurement in Cell Culture Medium

$A\beta_{42}$ measurement was performed following the manufacturer's suggested protocols. In brief, $A\beta_{42}$ levels (picograms per liter) were quantified using a sandwich enzyme-linked immunosorbent assay (Invitrogen). $A\beta_{42}$ levels were normalized to the protein concentrations from the cell lysates.

Statistical Analysis

Data were analyzed with two-way analysis of variance with "genotype" (non-transgenic and Tg2576) and "treatment" (standard diet, diet with ibuprofen, diet with CHF5074) as fixed factors and mouse as random factor. Post hoc comparisons were directed versus the non-transgenic or the Tg2576 control group. Two-tailed p values were calculated. Calculations were done with the statistical software SigmaStat™ (version 3.5; SPSS, Chicago, IL, USA). Results were generally presented as mean ± standard error of the mean (SEM).

Results

Effect of CHF5074 and Ibuprofen Treatment on Brain Content of Tau and Phospho-tau

The effects produced in vivo by CHF5074 and ibuprofen on tau phosphorylation were evaluated in brain extracts of a large number of Tg mice treated for 6 months with the drugs in the diet or with standard diet (control group). To detect hyperphosphorylated tau, two antibodies were used, PHF-1 and CP13, that respectively recognize the tau forms phosphorylated at the $Ser^{396/404}$ and Ser^{202} residues (Weaver et al. 2000). Western blot analyses showed a significant increase in hyperphosphorylated tau detected by PHF-1 antibody in Tg mice when compared to wild-type animals (non-Tg group). The levels of hyperphosphorylated tau fell down to the wild-type value in the CHF5074- as well as in the ibuprofen-treated groups. The CP13 immunoreactivity showed a trend to increase in Tg mice; it sloped down in mice treated with CHF5074 and it significantly decreased in mice treated with ibuprofen (Fig. 1a). Concomitantly, a significant increase in total tau was detected in Tg mice when compared to the wild-type group, in line with previous evidence showing accumulation of phosphorylated tau in APP Tg mice (Samura et al. 2006; Iqbal et al. 2009). CHF5074 and, with lower efficacy, ibuprofen treatment significantly reduced the level of total tau protein too, suggesting that both drugs were reducing accumulation of phosphorylated tau rather than tau phosphorylation. Results from densitometry analysis of tau levels have been expressed as ratio of PHF-1, CP13, or Tau46 to relative GAPDH levels (Fig. 1b–d). No change was observed in the content of βIII tubulin, ruling out a possible general effect of the two drugs on microtubule proteins.

The accumulation of hyperphosphorylated tau to form NFT-like cell inclusions was also visualized by immunohistochemical analysis in brain sections from control APP_{SL} Tg mice and Tg mice treated with CHF5074 or ibuprofen. Figure 2 shows representative images from retrosplenial granular cortex. The PHF-1 and Tau46 immunoreactivity was evident in Tg mice cortices but decreased in sections of mice treated with CHF5074 or ibuprofen (Fig. 2), confirming the results obtained by western blot analysis.

CHF5074 and Ibuprofen Decrease Cerebral GSK-3β Level and Increase GSK-3β Inhibitory Phosphorylation

GSK-3β kinase can phosphorylate tau at specific sites including the $Ser^{202,396,404}$ recognized by PHF-1 ($Ser^{396,404}$) and CP13 (Ser^{202}) antibodies. To investigate whether the effects of CHF5074 and ibuprofen on tau

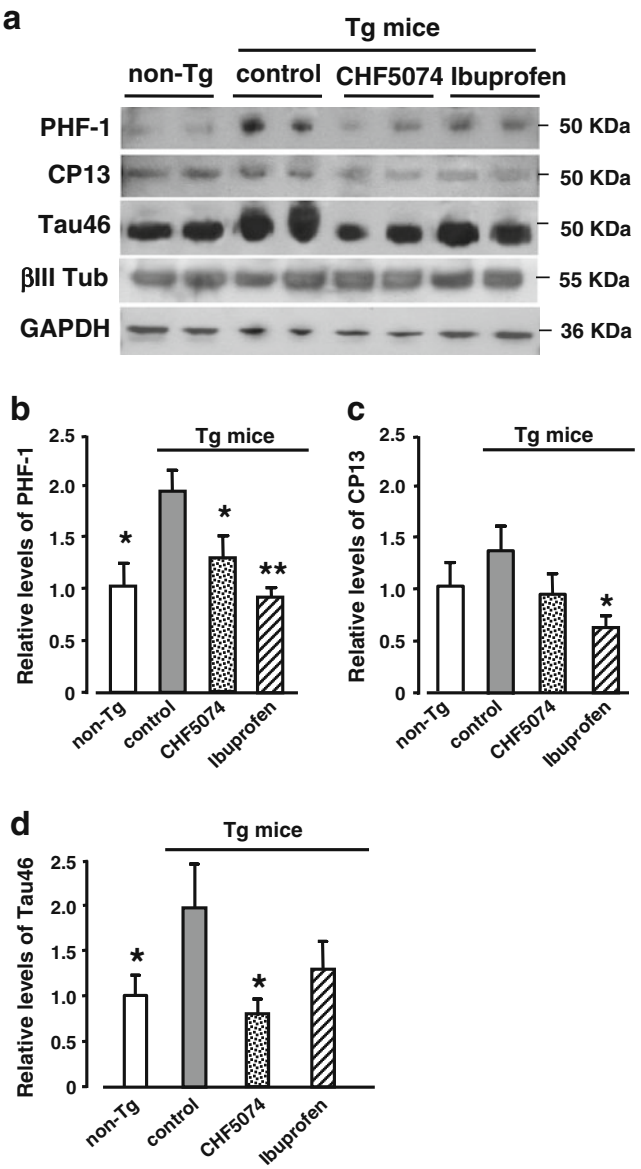

Fig. 1 Effects of CHF5074 and ibuprofen treatments on tau and phospho-tau. **a** Picture from a representative western blot analysis performed using PHF-1, CP13, Tau46, βIII tubulin and anti-GAPDH antibodies in brain extracts from non-Tg mice and Tg mice treated with standard diet or diet with CHF5074 and ibuprofen for 6 months. The content of phospho-tau ($pSer^{396/404}$) detected by PHF-1 antibody increased in control Tg mice compared to non-Tg group. Either CHF5074 or ibuprofen treatments reduced the level of PHF-1-positive tau in Tg mice. Phospho-tau ($pSer^{202}$) detected by CP13 antibody showed a trend to increase in Tg mice. It dropped slightly in Tg mice treated with CHF5074, while it significantly decreased in mice treated with ibuprofen. CHF5074, but not ibuprofen treatment, significantly reduced the level of total tau. No change of βIII tubulin immunoreactivity was observed in the diverse groups. **b**, **c**, **d** Data from densitometry analyses of PHF-1, CP13, and Tau46 immunoblots, expressed as relative levels to non-Tg value, are the ratio of PHF-1, CP13, and Tau46 to GAPDH. For each antigen determination, blots were obtained by simultaneous exposure of membranes to primary antibody and anti-GAPDH antibody. *Bars* are means ± SEM of 10–14 animals for each group; $*p<0.05$, $**p<0.01$ vs. Tg control value. PHF-1, CP13, and Tau46 values relative to CHF5074 and ibuprofen treatments were not statistically different from the non-Tg groups

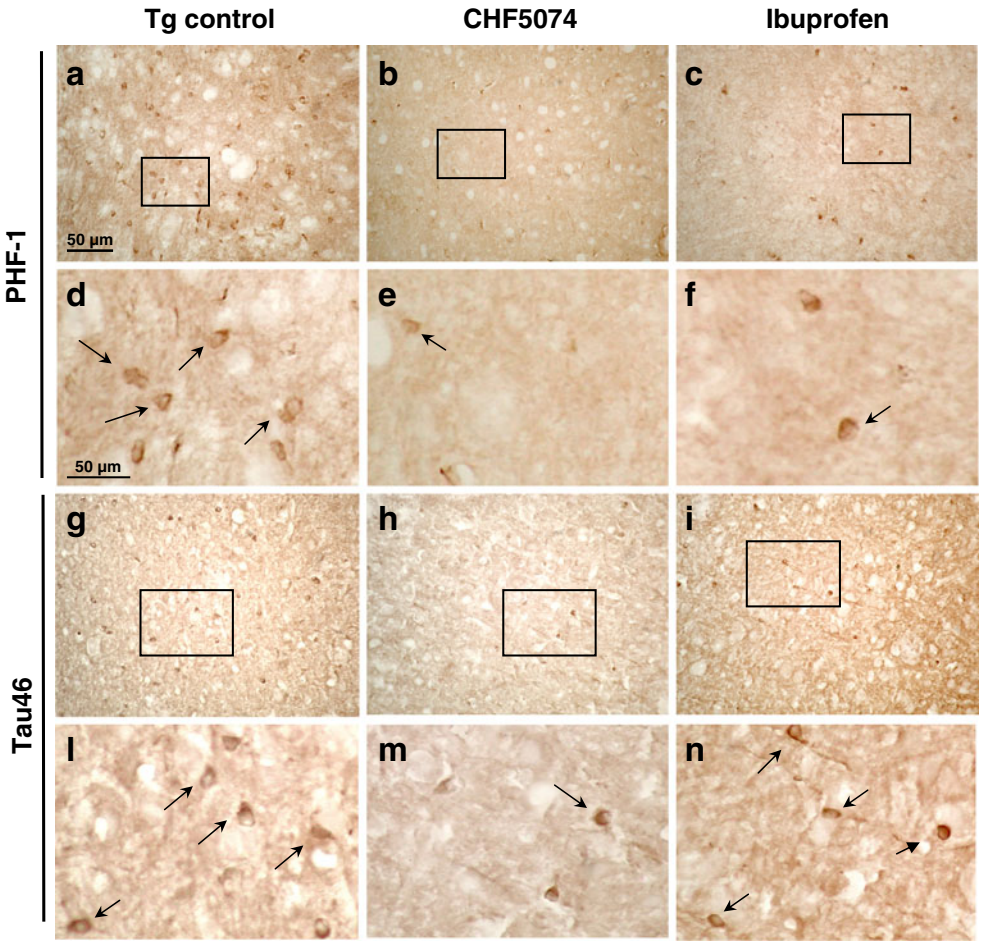

Fig. 2 Immunohistochemical detection of hyperphosphorylated and total tau in cortical sections of Tg mice. Analysis was performed using anti-hyperphosphorylated (PHF-1) and total tau (Tau46) antibodies in slices from right brain hemispheres of Tg mice. Pictures show that positive inclusions (*arrows*) to PHF-1 (**a**, **b**, **c** and relative inset magnification **d**, **e**, **f**) were reduced in sections from animals treated with CHF5074 and ibuprofen. The Tau46 positive neurons (**g**, **h**, **i** and relative inset magnifications **l**, **m**, **n**) appeared markedly reduced in CHF5074-treated group, but not in the ibuprofen group. *Bar*=50 μm

phosphorylation were associated with different expression and activation of this kinase, western blot analyses were carried out using antibodies against either the inactive ($pSer^9$) GSK-3β form or total GSK-3β. Only a slight increase of cerebral amounts of total GSK-3β was found in Tg mice compared to the non-Tg, while a significant (~50%) reduction of the kinase level was measured in brain extracts of mice treated with ibuprofen and CHF5074 (Fig. 3a, b). The ratio of p-GSK-3β and total GSK-3β was significantly lower in Tg mice when compared to wild-type mice, suggesting that GSK-3β is in a prevalent active form in $hAPP_{SL}$ mice. The treatment with CHF5074 and ibuprofen increased the ratio near to the non-Tg values, though the increased levels did not reach statistical significance when compared to the Tg group (Fig. 3c).

To verify whether the γ-secretase modulators might increase the inhibitory phosphorylation of GSK-3β independently of changes in $A\beta_{42}$ production, we exposed primary cortical neurons from wild-type mice to 3 μM CHF5074 and 500 μM ibuprofen for diverse times, starting from 30 min to 18 h. Cortical cultures, which release hardly detectable amounts of $A\beta_{42}$ (Fig. 4c), were treated with the GSK-3β inhibitor LiCl at 5 mM concentration as a positive control. The concentrations of CHF5074 and ibuprofen were chosen on the basis of their activity in lowering $A\beta_{42}$ generation in hAPP cells (Weggen et al. 2001; Imbimbo et al. 2007) and the cerebral level of CHF5074 (3±0.6 μM) detected in mice chronically treated with the compound (Imbimbo et al. 2009). Contrary to LiCl, CHF5074 as well as ibuprofen did not increase the p-GSK-3β/GSK-3β ratio at any time tested (Fig. 4a, b). Ibuprofen even decreased the p-GSK-3β/GSK-3β ratio at 18 h in cortical cultures.

When tested in H4-APPswe cells expressing hAPP with the Swedish mutation and releasing high amounts of $A\beta_{42}$ (Fig. 4f), either CHF5074 or LiCl increased the inhibitory phosphorylation of GSK-3β as indicated by the elevated p-GSK-3β/GSK-3β ratio (Fig. 4d, e). Cell treatment with CHF5074 also reduced the level of total GSK-3β. The CHF5074 effect on GSK-3β correlated with the inhibition of $A\beta_{42}$ release in the culture medium after 18-h incubation (Fig. 4f). Taken together, these results suggest that the inhibitory phosphorylation of GSK-3β induced by the γ-secretase modulation may be an event secondary to the reduction of endogenous Aβ generation.

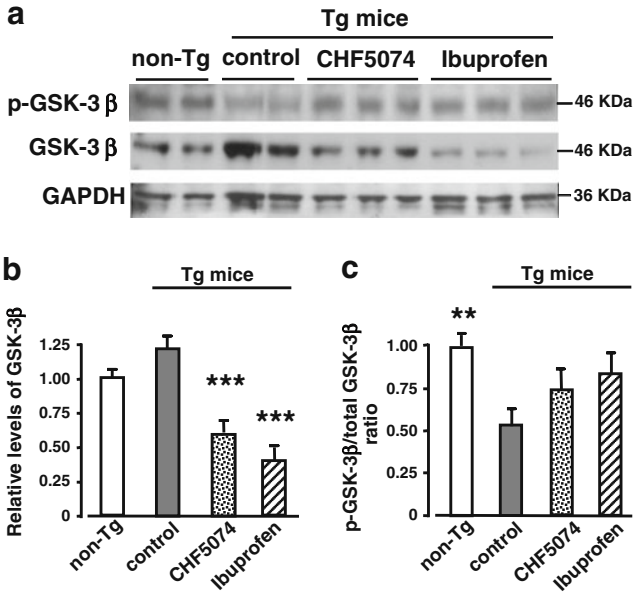

Fig. 3 Effects of CHF5074 and ibuprofen treatments on GSK-3β and phospho-GSK-3β levels. **a** Picture from a representative western blot analysis performed using anti-phospho-GSK-3β(pSer^{9}) and anti-GSK-3β antibodies in brain extracts from non-Tg mice or Tg mice treated with standard diet or diet with CHF5074 and ibuprofen. The amount of total GSK-3β did not change in Tg mice compared to non-Tg group, while it significantly reduced in mice treated with ibuprofen and CHF5074. **b** Data from densitometry analysis of GSK-3β immunoblots, expressed as relative levels to non-Tg value, are the ratio of GSK-3β to relative GAPDH. Blots were obtained by simultaneous exposure of membranes to anti-GSK-3β and anti-GAPDH antibodies. *Bars* are the means ± SEM of 9–10 animals per group; ***$p<0.001$ vs. Tg control value as well as vs. non-Tg value. **c** Ratio of p-GSK-3β to relative GSK-3β; **$p<0.01$ vs. Tg control value; Tg, but not the CHF5074 and ibuprofen groups, were significantly different from the non-Tg group

Discussion

This study provides the first evidence that chronic treatment with γ-secretase modulators reduces the hyperphosphorylation and pathological accumulation of native tau as well as the level of activated GSK-3β in the brain of APP Tg mice. We previously reported that chronic treatment of APP_{SL} mice with CHF5074 reduces the Aβ burden without modifying the generation of APP carboxy-terminal fragments, reduces the number of plaque-associated microglia, and attenuates spatial memory deficit (Imbimbo et al. 2009). Similar effects on Aβ plaque accumulation and microglial immunoreactivity were observed with ibuprofen (Lim et al. 2000; Yan et al. 2003; Heneka et al. 2005; Morihara et al. 2005), though in APP_{SL} mice ibuprofen showed to be less effective than CHF5074 (Imbimbo et al. 2009). Together with Aβ deposition, accumulation of intracellular hyperphosphorylated tau represents the neuropathological hallmark of AD (Selkoe 2001). A large body of evidence indicates that Aβ peptides, the primary constituents of neuritic plaques, may initiate the process of neurodegeneration in AD brains (Clippingdale et al. 2001; Casas et al. 2004; Oakley et al. 2006; Smith et al. 2006). Aβ deposits promote hyperphosphorylation, accumulation, and aggregation of tau in a conformationally altered and argyrophilic form (Samura et al. 2006) in HEK293 cells overexpressing APP (Rank et al. 2002), in transgenic mice expressing double mutated hAPP form (Bellucci et al. 2007), in double transgenic mice expressing mutant hAPP(swe) and presenilin-1 (Samura et al. 2006), and in triple transgenic mice expressing the hAPP(swe), presenilin-1 and tau (Oddo et al. 2003a, b). Song and colleagues (2008) observed that phosphorylation of tau increases in cortical neurons as early as 3 h of $A\beta_{42}$ treatment, evoking an AD-like accumulation of phosphorylated tau. The abnormal phosphorylation of tau promotes its misfolding, decreases the degradation, and induces the self-assembly into tangles of paired helical filaments which alter the microtubule stability and normal neuronal functions (Iqbal et al. 2009). In addition, cytosolic abnormally hyperphosphorylated tau sequesters normal tau and two other neuronal microtubule-associated proteins (MAPs), MAP1A/MAP1B and MAP2, further contributing to microtubule disassembly and neuronal damage (Alonso et al. 2008; Iqbal et al. 2008). Neurofibrillary degeneration appears to be required for the clinical expression of dementia as β-amyloidosis in the absence of neurofibrillary degeneration is not associated with the development of cognitive symptoms (Alafuzoff et al. 1987; Arriagada et al. 1992; Dickson et al. 1992). In line with this evidence, tau reduction without changing Aβ plaque deposition prevents learning and memory deficits and protects mice against excitotoxicity (Roberson et al. 2007). On the other hand, in mice expressing a human tau variant under the control of an inducible promoter (tet-off), after the suppression of tau expression, memory function recovers and neuron number stabilizes although NFTs continue to form, suggesting that accumulation of hyperphosphorylated tau, and not its aggregation, is required for cognitive decline and behavioral impairment (Santacruz et al. 2005). Thus, although it is supposed to occur as a consequence of Aβ pathology (Wilcock et al. 2010), the tau hyperphosphorylation may be considered per se a useful therapeutic target for slowing AD progression (Churcher 2006; Roberson et al. 2007).

In this study, we investigated the effects produced by chronic administration of the γ-secretase modulators, CHF5074 and ibuprofen (Imbimbo et al. 2009), on native tau. By checking tau phosphorylation, we found that ibuprofen treatment produced a significant reduction of phosphorylated tau recognized by both the PHF-1 and CP13 antibodies, which respectively identify the late (pSer$^{396/404}$) and the early (pSer^{202}) forms of phosphory-

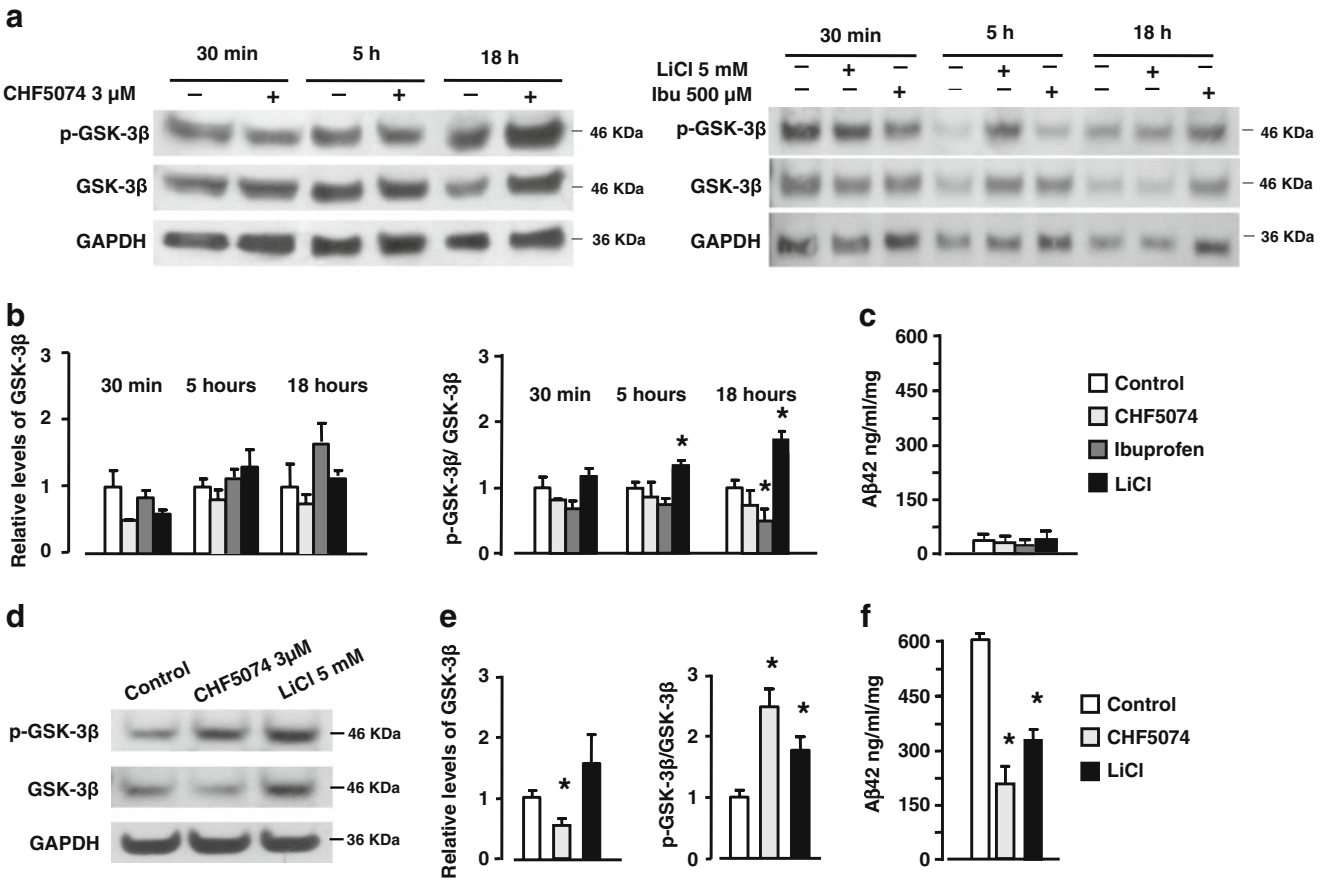

Fig. 4 Western blot analysis of GSK-3β phosphorylation in neuronal cultures exposed to 3 μM CHF5074, 500 μM ibuprofen, or 5 mM LiCl. **a** Immunoblot, representative of three different experiments, shows the p-GSK-3β and GSK-3β levels in extracts of cortical neurons exposed to the various treatments for 30 min, 5 h, and 18 h. **b** Densitometry analysis of immunoblots, expressed as relative levels to control value, reveals no change of GSK-3β or p-GSK-3β/GSK-3β ratio in neurons exposed to CHF5074 or ibuprofen. Neurons treated with LiCl for 5 or 18 h displayed a significant increase in p-GSK-3β/GSK-3β ratio (*p<0.05 vs. corresponding control value). **c** Aβ42 released in the medium of cortical neurons exposed for 18 h to 3 μM CHF5074, 500 μM ibuprofen, or 5 mM LiCl; *p<0.01 vs. corresponding control value. **d** Representative immunoblot of GSK-3β and p-GSK-3β in H4-APPswe cells exposed to CHF5074 and LiCl for 18 h. Similar results were obtained in two additional experiments. **e** Data from densitometry analysis, expressed as relative levels to control value, show the means of GSK-3β and p-GSK-3β/GSK-3β ratios of three separate experiments. *Bars* are the means ± SEM (*p< 0.05 vs. control value). **f** Aβ42 released in the medium of H4-APPswe cells exposed for 18 h to CHF5074 3 μM or LiCl 5 mM; *p<0.01 vs. corresponding control value

lated tau (Klein et al. 2004; Liu et al. 2006). This is in line with previous evidence showing that ibuprofen reduces hyperphosphorylated tau in 3xTg-AD mice expressing hAPP(swe), presenilin-1(M146V), and tau(P301L) (Mckee et al. 2008). The effect of CHF5074 was more evident on PHF-1- than on CP13-positive phospho-tau. In particular, CHF5074 and to a lesser extent ibuprofen limited the accumulation of tau protein, as suggested by the parallel decrease in total tau level. This evidence suggests that the γ-secretase modulators can reduce the accumulation of phosphorylated tau and not simply tau phosphorylation.

It is recognized that GSK-3β acts as a major kinase responsible for tau hyperphosphorylation and subsequent resistance to degradation (Iqbal et al. 2009) in AD pathology (Gong et al. 2005). GSK-3β is constitutively active and is a substrate for other kinases capable of phospho-regulating its activity through both inhibition and activation (Grimes and Jope 2001). In the case of deactivation, signaling through phosphoinositide 3-kinase (PI3K) and subsequent activation of the Ser-Thr kinase Akt inhibits GSK-3β activity via Ser^9 phosphorylation. A body of evidence suggests that Aβ promotes tau phosphorylation through activation of GSK-3 (Takashima et al. 1996, 1998; Tomidokoro et al. 2001; Hoshi et al. 2003; Song et al. 2008). The GSK-3β activation mediated by its Ser^9dephosphorylation (Wang et al. 2006; Song et al. 2008) has been demonstrated after exogenous application of Aβ peptide as well as during intracellular accumulation of endogenous Aβ in cell-based models of AD. In line with previous

evidence (Rockenstein et al. 2007; Bitner et al. 2009), we found that the level of total GSK-3β was not modified in APP_{SL} mice when compared to wild-type littermates, while its phosphorylated form significantly reduced, suggesting a sustained activation of the kinase in AD mice. This effect was more evident in our 12-month-old mice than in 3-month-old APP_{SL} mice previously investigated by Rockenstein et al. (2007). The chronic treatment with CHF5074 or ibuprofen inhibited the GSK-3β activity by increasing the pGSK-3β/GSK-3β ratio near to the non-Tg group and by reducing the total protein level. Likewise the γ-secretase modulators, caffeine was recently found to decrease active and total GSK-3β level, in addition to Aβ burden and cognitive impairment in AD mice (Arendash et al. 2009). It remains to be ascertained whether the diminished levels of GSK-3β are associated with decreased protein synthesis or increased degradation. We checked the possibility that the γ-secretase modulators might indeed regulate the GSK-3β level and activation independently of their anti-amyloidogenic effect. However, this hypothesis was denied by experiments on primary cortical neurons from wild-type mice which showed that, contrary to the positive control LiCl, neither brief (30 min) nor long-time (18 h) applications of CHF5074 and ibuprofen could decrease the level of GSK-3β or enhance its phosphorylation. Ibuprofen even reduced the p-GSK-3β/GSK-3β ratio at 18 h. It can be inferred that ibuprofen neuroprotective activity reported in pure cortical neurons (Iwata et al. 2010) is associated with signaling pathway other than the GSK-3β inhibition as, possibly, the activation of the peroxisome proliferator-activated receptor-γ pathway (Dill et al. 2010). The capability of CHF5074 to reduce GSK-3β and increase the GSK-3β inhibitory phosphorylation was restored in H4swe cells overexpressing hAPP. Thus, while possible direct effects elicited in vivo by CHF5074 metabolites on GSK-3β cannot be excluded by our study, it is conceivable that reduction of kinase activity and tau phosphorylation is the resultant of decreased generation of endogenous Aβ overproduction.

The central role of Aβ in driving tau pathology has recently been underlined by studies showing that in APP mice expressing native or transgenic tau as well as in AD patients, the pathological accumulation of hyperphosphorylated tau develops concomitantly with Aβ accumulation in hippocampal synaptic terminals, linking these intraneuronal pathological signs to the synaptic alteration and cognitive dysfunction of AD (Guo et al. 2006; Takahashi et al. 2008). Moreover, decreasing Aβ in APP Tg mice by immunotherapy significantly lowers tau pathology and reverses memory deficits (Wilcock et al. 2010). In this context, the higher efficacy of CHF5074 compared to ibuprofen in reducing the cognitive deficits in APP_{SL} mice may be associated with its higher efficacy in reducing Aβ generation (Imbimbo et al. 2009) and tau dysregulation, besides its capability to conserve the COX-2-mediated memory retrieval (Murray and O'Connor 2003; Sharifzadeh et al. 2006). Consistent with this evidence, our data support Aβ as therapeutic target for the disease-modifying treatment and propose the γ-secretase modulator, CHF5074, as a suitable tool to afford this approach by avoiding heavy collateral effects associated with the immunotherapy (von Bernhardi 2010).

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References

Alafuzoff I, Iqbal K, Friden H, Adolfsson R, Winblad B (1987) Histopathological criteria for progressive dementia disorders: clinical–pathological correlation and classification by multivariate data analysis. Acta Neuropathol 74:209–225

Alonso AC, Li B, Grundke-Iqbal I, Iqbal K (2008) Mechanism of tau-induced neurodegeneration in Alzheimer disease and related tauopathies. Curr Alzheimer Res 5:375–384

Arendash GW, Mori T, Cao C, Mamcarz M, Runfeldt M, Dickson A, Rezai-Zadeh K, Tane J, Citron BA, Lin X, Echeverria V, Potter H (2009) Caffeine reverses cognitive impairment and decreases brain amyloid-beta levels in aged Alzheimer's disease mice. J Alzheimers Dis 17:661–680

Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 42:631–639

Ballatore C, Lee VM, Trojanowski JQ (2007) Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci 8:663–672

Baum L, Hansen L, Masliah E, Saitoh T (1996) Glycogen synthase kinase 3 alteration in Alzheimer disease is related to neurofibrillary tangle formation. Mol Chem Neuropathol 29:253–261

Bellucci A, Rosi MC, Grossi C, Fiorentini A, Luccarini I, Casamenti F (2007) Abnormal processing of tau in the brain of aged TgCRND8 mice. Neurobiol Dis 27:328–338

Bitner RS, Nikkel AL, Markosyan S, Otte S, Puttfarcken P, Gopalakrishnan M (2009) Selective alpha7 nicotinic acetylcholine receptor activation regulates glycogen synthase kinase3beta and decreases tau phosphorylation in vivo. Brain Res 1265:65–74

Casas C, Sergeant N, Itier JM, Blanchard V, Wirths O, van der Kolk N, Vingtdeux V, van de Steeg E, Ret G, Canton T, Drobecq H, Clark A, Bonici B, Delacourte A, Benavides J, Schmitz C, Tremp G, Bayer TA, Benoit P, Pradier L (2004) Massive CA1/2 neuronal loss with intraneuronal and N-terminal truncated Abeta42 accumulation in a novel Alzheimer transgenic model. Am J Pathol 165:1289–1300

Churcher I (2006) Tau therapeutic strategies for the treatment of Alzheimer's disease. Curr Top Med Chem 6:579–595

Clippingdale AB, Wade JD, Barrow CJ (2001) The amyloid-β peptide and its role in Alzheimer's disease. J Pept Sci 7:227–249

Dias-Santagata D, Fulga TA, Duttaroy A, Feany MB (2007) Oxidative stress mediates tau-induced neurodegeneration in *Drosophila*. J Clin Invest 117:236–245

Dickson DW, Crystal HA, Mattiace LA, Masur DM, Blau AD, Davies P, Yen SH, Aronson MK (1992) Identification of normal and

pathological aging in prospectively studied nondemented elderly humans. Neurobiol Aging 13:179–189

Dill J, Patel AR, Yang XL, Bachoo R, Powell CM, Li S (2010) A molecular mechanism for ibuprofen-mediated RhoA inhibition in neurons. J Neurosci 30(3):963–972

Fulga TA, Elson-Schwab I, Khurana V, Steinhilb ML, Spires TL, Hyman BT, Feany MB (2007) Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration in vivo. Nat Cell Biol 9:139–148

Gong CX, Liu F, Grundke-Iqbal I, Iqbal K (2005) Post-translational modifications of tau protein in Alzheimer's disease. J Neural Transm 112:813–838

Grimes CA, Jope RS (2001) The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Prog Neurobiol 65:391–426

Guo JP, Arai T, Miklossy J, McGeer PL (2006) Abeta and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer's disease. Proc Natl Acad Sci USA 103:1953–1958

Harr SD, Hollister RD, Hyman BT (1996) Glycogen synthase kinase 3 alpha and 3 beta do not colocalize with neurofibrillary tangles. Neurobiol Aging 17:343–348

Heneka MT, Sastre M, Dumitrescu-Ozimek L, Hanke A, Dewachter I, Kuiperi C, O'Banion K, Klockgether T, Van Leuven F, Landreth GE (2005) Acute treatment with the PPARg agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1-42 levels in APPV717I transgenic mice. Brain 128:1442–1453

Hoshi M, Sato M, Matsumoto S, Noguchi A, Yasutake K, Yoshida N, Sato K (2003) Spherical aggregates of beta-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3beta. Proc Natl Acad Sci USA 100:6370–6375

Hutter-Paier B, Huttunen HJ, Puglielli L, Eckman CB, Kim DY, Hofmeister A, Moir RD, Domnitz SB, Frosch MP, Windisch M, Kovacs DM (2004) The ACAT inhibitor CP-113,818 markedly reduces amyloid pathology in a mouse model of Alzheimer's disease. Neuron 44:227–238

Imbimbo BP, Del Giudice E, Colavito D, D'Arrigo A, Dalle Carbonare M, Villetti G, Facchinetti F, Volta R, Pietrini V, Baroc MF, Serneels L, De Strooper B, Leon A (2007) 1-(3′,4′-Dichloro-2-fluoro[1,1′-biphenyl]-4-yl)-cyclopropanecarboxylic acid (CHF5074), a novel gamma-secretase modulator, reduces brain beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease without causing peripheral toxicity. J Pharmacol Exp Ther 323:822–830

Imbimbo BP, Hutter-Paier B, Villetti G, Facchinetti F, Cenacchi V, Volta R, Lanzillotta A, Pizzi M, Windisch M (2009) CHF5074, a novel gamma-secretase modulator, attenuates brain beta-amyloid pathology and learning deficit in a mouse model of Alzheimer's disease. Br J Pharmacol 156:982–993

Imbimbo BP, Giardino L, Sivilia S, Giuliani A, Gusciglio M, Pietrini V, Del Giudice E, D'Arrigo A, Leon A, Villetti G, Calzà L (2010) CHF5074, a novel gamma-secretase modulator, restores hippocampal neurogenesis potential and reverses contextual memory deficit in a transgenic mouse model of Alzheimer's disease. J Alzheimers Dis 20:159–173

Iqbal K, Alonso Adel C, Grundke-Iqbal I (2008) Cytosolic abnormally hyperphosphorylated tau but not paired helical filaments sequester normal MAPs and inhibit microtubule assembly. J Alzheimers Dis 14:365–370

Iqbal K, Liu F, Gong CX, Alonso Adel C, Grundke-Iqbal I (2009) Mechanisms of tau-induced neurodegeneration. Acta Neuropathol 118:53–69

Ishizawa T, Sahara N, Ishiguro K, Kersh J, McGowan E, Lewis J, Hutton M, Dickson DW, Yen SH (2003) Co-localization of glycogen synthase kinase-3 with neurofibrillary tangles and granulovacuolar degeneration in transgenic mice. Am J Pathol 163:1057–1067

Iwata Y, Nicole O, Zurakowski D, Okamura T, Jonas RA (2010) J Thorac Cardiovasc Surg 139(2):489–493

Jackson GR, Wiedau-Pazos M, Sang TK, Wagle N, Brown CA, Massachi S, Geschwind DH (2002) Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in *Drosophila*. Neuron 34:509–519

Klein RL, Lin WL, Dickson DW, Lewis J, Hutton M, Duff K, Meyer EM, King MA (2004) Rapid neurofibrillary tangle formation after localized gene transfer of mutated tau. Am J Pathol 164:347–353

Lee VM, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. Annu Rev Neurosci 24:1121–1159

Lim GP, Yang F, Chu T, Chen P, Beech W, Teter B, Tran T, Ubeda O, Ashe KH, Frautschy SA, Cole GM (2000) Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci 20:5709–5714

Liu F, Liang Z, Shi J, Yin D, El-Akkad E, Grundke-Iqbal I, Iqbal K, Gong CX (2006) PKA modulates GSK-3beta- and cdk5-catalyzed phosphorylation of tau in site- and kinase-specific manners. FEBS Lett 580:6269–6274

Lucas JJ, Hernandez F, Gomez-Ramos P, Moran MA, Hen R, Avila J (2001) Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. EMBO J 20:27–33

McKee AC, Carreras I, Hossain L, Ryu H, Klein WL, Oddo S, LaFerla FM, Jenkins BG, Kowall NW, Dedeoglu A (2008) Ibuprofen reduces Abeta, hyperphosphorylated tau and memory deficits in Alzheimer mice. Brain Res 1207:225–236

Morihara T, Teter B, Yang F, Lim GP, Boudinot S, Boudinot FD, Boudinot FD, Frautschy SA, Cole GM (2005) Ibuprofen suppresses interleukin-1b induction of proamyloidogenic a1-antichymotrypsin to ameliorate beta-amyloid (Ab) pathology in Alzheimer's models. Neuropsychopharmacology 30:1111–1120

Murray HJ, O'Connor JJ (2003) A role for COX-2 and p38 mitogen activated protein kinase in long-term depression in the rat dentate gyrus in vitro. Neuropharmacology 44:374–380

Nishimura I, Yang Y, Lu B (2004) PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in *Drosophila*. Cell 116:671–682

Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R (2006) Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. J Neurosci 26:10129–10140

Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM (2003a) Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging 24:1063–1070

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003b) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39:409–421

Pei JJ, Tanaka T, Tung YC, Braak E, Iqbal K, Grundke-Iqbal I (1997) Distribution, levels, and activity of glycogen synthase kinase-3 in the Alzheimer disease brain. J Neuropathol Exp Neurol 56:70–78

Pei JJ, Braak E, Braak H, Grundke-Iqbal I, Iqbal K, Winblad B, Cowburn RF (1999) Distribution of active glycogen synthase kinase 3beta (GSK-3beta) in brains staged for Alzheimer disease neurofibrillary changes. J Neuropathol Exp Neurol 58:1010–1019

Rank KB, Pauley AM, Bhattacharya K, Wang Z, Evans DB, Fleck TJ, Johnston JA, Sharma SK (2002) Direct interaction of soluble human recombinant tau protein with Aβ1-42 results in tau

aggregation and hyperphosphorylation by tau protein kinase II. FEBS Lett 514:263–268

Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucki L (2007) Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science 316:750–754

Rockenstein E, Torrance M, Adame A, Mante M, Bar-on P, Rose JB, Crews L, Masliah E (2007) Neuroprotective effects of regulators of the glycogen synthase kinase-3beta signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. J Neurosci 27:1981–1991

Samura E, Shoji M, Kawarabayashi T, Sasaki A, Matsubara E, Murakami T, Wuhua X, Tamura S, Ikeda M, Ishiguro K, Saido TC, Westaway D, St George Hyslop P, Harigaya Y, Abe K (2006) Enhanced accumulation of tau in doubly transgenic mice expressing mutant βAPP and presenilin-1. Brain Res 1094:192–199

Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kuskowski M, Hyman B, Hutton M, Ashe KH (2005) Tau suppression in a neurodegenerative mouse model improves memory function. Science 309:476–481

Sarnico I, Lanzillotta A, Boroni F, Benarese M, Alghisi M, Schwaninger M, Inta I, Battistin L, Spano PF, Pizzi M (2009) NF-kappaB p50/RelA and c-Rel-containing dimers: opposite regulators of neuron vulnerability to ischaemia. J Neurochem 108:475–485

Schilling S, Zeitschel U, Hoffmann T, Heiser U, Francke M, Kehlen A, Holzer M, Hutter-Paier B, Prokesch M, Windisch M, Jagla W, Schlenzig D, Lindner C, Rudolph T, Reuter G, Cynis H, Montag D, Demuth HU, Rossner S (2008) Glutaminyl cyclase inhibition attenuates pyroglutamate Abeta and Alzheimer's disease-like pathology. Nat Med 14:1106–1111

Selkoe DJ (2001) Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. J Alzheimers Dis 3:75–80

Sharifzadeh M, Tavasoli M, Soodi M, Mohammadi-Eraghi S, Ghahremani MH, Roghani A (2006) A time course analysis of cyclooxygenase-2 suggests a role in spatial memory retrieval in rats. Neurosci Res 54:171–179

Shiurba RA, Ishiguro K, Takahashi M, Sato K, Spooner ET, Mercken M, Yoshida R, Wheelock TR, Yanagawa H, Imahori K, Nixon RA (1996) Immunocytochemistry of tau phosphoserine 413 and tau protein kinase I in Alzheimer pathology. Brain Res 737:119–132

Smith WW, Gorospe M, Kusiak JW (2006) Signaling mechanisms underlying Abeta toxicity: potential therapeutic targets for Alzheimer's disease. CNS Neurol Disord Drug Targets 5:355–361

Song MS, Rauw G, Baker GB, Kar S (2008) Memantine protects rat cortical cultured neurons against beta-amyloid-induced toxicity by attenuating tau phosphorylation. Eur J Neurosci 28:1989–2002

Steinhilb ML, Dias-Santagata D, Fulga TA, Felch DL, Feany MB (2007) Tau phosphorylation sites work in concert to promote neurotoxicity in vivo. Mol Biol Cell 18:5060–5068

Takahashi RH, Capetillo-Zarate E, Lin MT, Milner TA, Gouras GK (2008) Co-occurrence of Alzheimer's disease beta-amyloid and tau pathologies at synapses. Neurobiol Aging 31:1145–1152

Takashima A, Noguchi K, Michel G, Mercken M, Hoshi M, Ishiguro K, Imahori K (1996) Exposure of rat hippocampal neurons to amyloid beta peptide (25–35) induces the inactivation of phosphatidyl inositol-3 kinase and the activation of tau protein kinase I/glycogen synthase kinase-3 beta. Neurosci Lett 203:33–36

Takashima A, Honda T, Yasutake K, Michel G, Murayama O, Murayama M, Ishiguro K, Yamaguchi H (1998) Activation of tau protein kinase I/glycogen synthase kinase-3beta by amyloid beta peptide (25–35) enhances phosphorylation of tau in hippocampal neurons. Neurosci Res 31:317–323

Tomidokoro Y, Ishiguro K, Harigaya Y, Matsubara E, Ikeda M, Park JM, Yasutake K, Kawarabayashi T, Okamoto K, Shoji M (2001) Aβ amyloidosis induces the initial stage of tau accumulation in APPswe mice. Neurosci Lett 299:169–172

von Bernhardi R (2010) Immunotherapy in Alzheimer's disease: where do we stand? Where should we go? J Alzheimers Dis 19:405–421

Wang ZF, Li HL, Li XC, Zhang Q, Tian Q, Wang Q, Xu H, Wang JZ (2006) Effects of endogenous beta-amyloid overproduction on tau phosphorylation in cell culture. J Neurochem 98:1167–1175

Weaver CL, Espinoza M, Kress Y, Davies P (2000) Conformational change as one of the earliest alterations of tau in Alzheimer's disease. Neurobiol Aging 21:719–727

Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, Findlay KA, Smith TE, Murphy MP, Bulter T, Kang DE, Marquez-Sterling N, Golde TE, Koo EH (2001) A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature 414:212–216

Wilcock DM, Gharkholonarehe N, Van Nostrand WE, Davis J, Vitek MP, Colton CA (2010) Amyloid reduction by amyloid-beta vaccination also reduces mouse tau pathology and protects from neuron loss in two mouse models of Alzheimer's disease. J Neurosci 30:1197–1198

Yan Q, Zhang J, Liu H, Babu-Khan S, Vassar R, Biere AL, Citron M, Landreth G (2003) Anti-inflammatory drug therapy alters b-amyloid processing and deposition in an animal model of Alzheimer's disease. J Neurosci 23:7504–7509