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# PTEN, a negative regulator of PI3 kinase signalling, alters tau phosphorylation in cells by mechanisms independent of GSK-3

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**Abstract Deregulation of PTEN/Akt signalling has been recently implicated in the pathogenesis of Alzheimer's disease (AD), but the effects on the molecular processes underlying AD pathology have not yet been fully described. Here we report that overexpression of PTEN reduces tau phosphorylation in CHO cells. This effect was abrogated by mutant PTEN constructs with either a catalytically inactive point mutation (C124S) or with only inactive lipid phosphatase activity (G129E), suggesting an indirect, lipid phosphatase-dependent process. The predominant effects of PTEN on tau appeared to be mediated by reducing ERK1/2 activity, but were independent of Akt, GSK-3, JNK and the tau phosphatases PP1 and PP2A. Our studies provide evidence for an effect of PTEN on the phosphorylation of tau in AD pathogenesis, and provide some insight into the mechanisms through which deregulation of PTEN may contribute towards the progression of tauopathy.**


*Keywords:* Phosphatase and tensin homolog deleted on chromosome 1; Akt; glycogen synthase kinase 3; Alzheimer's disease; Tau phosphorylation

## 1. Introduction

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a tumour suppressor gene [1–3] encoding a dual specificity phosphatase that dephosphorylates both lipid and protein substrates [4]. As a lipid phosphatase PTEN dephosphorylates the inositol ring of the second messenger phosphatidylinositol (3,4,5)-triphosphate ($PIP_3$), thus inhibiting cell survival responses to PI3-kinase and Akt [5–7]. PTEN has been shown to modulate cell migration, growth, survival and apoptosis [[8] for review] and as such its role as a tumour suppressor is well-established [9]. More recent studies have shown that PTEN is expressed in neurons in human [10], mouse [11] and rat brain [12] and suggest a functional role for PTEN in central nervous system (CNS) development. PTEN is an important regulator of proliferation in neural stem cells [13] and regulates neuronal migration [14], differentiation [11], soma size [15,16] and apoptosis [12].

Our particular interest in PTEN stems from studies suggesting that Akt is a vital promoter of neuronal survival in Alzheimer's disease (AD). PI3-kinase/Akt activation prevents amyloid beta (Aβ)-induced neurotoxicity in cells [17] and in mouse models of AD [18]. Akt inhibits the enzyme glycogen synthase kinase 3 (GSK-3), which is increasingly thought to play a pivotal role in the regulation of both tau phosphorylation [19–22] and Aβ production [23–25]. PTEN increases GSK-3 activity by inhibiting Akt in cells [26,27] and would, therefore, be predicted to promote tau and Aβ pathologies via this signalling mechanism.

The gene encoding PTEN locates to the region of chromosome 10 linked to late-onset AD [28], further suggesting a functional role for PTEN in disease development. Several studies have reported increases in Akt activity in AD brain [29,30], and more recent observations suggest that this may be due to reductions in PTEN levels [31]. These findings suggest a potentially complex effect of PTEN signalling on the molecular processes underlying AD pathology. We aimed, therefore, to further investigate the effect of PTEN on tau phosphorylation using cell culture models and to establish the signalling mechanisms via which the relationship between PTEN and tau may be mediated.

## 2. Materials and methods

### 2.1. Tau and PTEN plasmids

For expression in mammalian cells the cDNA coding for wild-type tau 2N4R was subcloned into the pcDNA3.1/V5-His-TOPO vector (Invitrogen, Paisley, UK), yielding a construct with C-terminal V5 and His tags. PTEN cDNA was cloned and inserted into the pcDNA3.1+ vector (Invitrogen) and also into the EGFP-C2 vector (Clontech). Inactive variants of PTEN were generated using the quick-change site directed mutagenesis kit according to the manufacturers guidelines (Stratagene, La Jolla, USA), to produce a mutant with a catalytically inactive point mutation (C124S) or a mutant with only inactive lipid phosphatase activity (G129E). C124S-PTEN was derived from WT-PTEN using the mutagenic oligonucleotide

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*Abbreviations:* PTEN, phosphatase and tensin homolog deleted on chromosome 1; GSK-3, glycogen synthase kinase 3; AD, Alzheimer's disease; MAP kinase, mitogen activated protein kinase; ERK, extracellular regulated MAP kinase; JNK, c-jun N-terminal kinase; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; $PIP_3$, phosphatidylinositol (3,4,5)-triphosphate; Aβ, amyloid beta; CHO, Chinese hamster ovary; Akt, protein kinase B

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5′-GCAGCAATTCACAGTAAAGCT-3′. G129E-PTEN was derived from WT-PTEN using the mutagenic oligonucleotide 5′-GCA ATT CAC TGT AAG GCT GGA AAG GAA CGG ACT GG-3′. All resulting cDNAs were sequenced for verification.

### 2.2. Cell culture and transfection

Chinese Hamster Ovary (CHO) cells were cultured in Ham-F12 medium supplemented with 10% foetal calf serum, penicillin/streptomycin (50 U/mL) and L-glutamine (2 mM) and maintained at 37 °C in a humidified, 5% $CO_2$ atmosphere. CHO cells were transiently transfected with tau and PTEN cDNA plasmids using lipofectamine transfection reagent according to the manufacturer's instructions (Invitrogen). Cells were then cultured for 24 h in complete medium prior to cell lysis in 2× Laemmli sample buffer containing β-mercaptoethanol and boiling for 5 min. In some experiments cells were treated 24 h after transfection with 100 nM okadaic acid in complete culture medium for 1, 3 or 5 h.

Primary mouse cortical neurons were cultured in Neurobasal medium containing B27 supplement 2% (v/v), L-glutamine (2 mM) and penicillin/streptomycin (50 U/mL). For transfection of primary neurons, cells were cultured on cover slips in 12-well plates (500 000 cells/well) for 6 days before transfecting with GFP-tagged PTEN cDNA using lipofectamine 2000 transfection reagent. Cells were cultured for a further 24 h before analysis of endogenous tau phosphorylation by immunofluorescence.

### 2.3. Western blotting

Proteins were separated on 10% sodium dodecyl sulfate–polyacrylamide (SDS–PAGE) gels at 150 V for 1 h. Gels were then transferred to Immobilon-P membranes (Millipore), incubated in a blocking solution containing 3% milk proteins in TBST for 1 h at room temperature, then probed with primary antibodies overnight at 4 °C. The blots were then washed three times with TBST and incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG or sheep anti-mouse IgG secondary antibodies (1:2000 dilution; Amersham Biosciences, UK Ltd.). Blots were developed using the enhanced chemiluminescence method (ECL; Amersham Biosciences). The relative immunoreactivities of proteins were analysed using a GS710 scanning densitometer and quantified using Quantity One (BioRad, UK) software.

### 2.4. Immunofluorescence

Transfected cortical neurons were washed twice in ice-cold PBS, fixed in 4% paraformaldehyde (PFA) in PBS for 20 min, then permeabilised in 0.1% Triton X-100 in PBS for 5 min. Cells were washed three times in PBS, then incubated in blocking solution, PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween-20, for 1 h at room temperature to reduce non-specific binding. Cells were incubated with primary antibodies in blocking solution for 1 h, then washed three times in PBS before being incubated for 1 h with blocking solution containing secondary antibodies linked to Texas Red. Cells were washed four times with PBS, and nucleic acids stained with Hoescht in the final wash. Coverslips were mounted onto slides using mounting fluid (Dako).

Immunofluorescence was visualised and captured using a Zeiss LSM510 meta confocal microscope with pinhole settings at ~1 airy unit for all images. Images were processed using LSM5 image examiner (Zeiss) and were assembled using Adobe Photoshop (Adobe, USA).

### 2.5. Antibodies

The anti-tau polyclonal antibody (DAKO, UK) is phosphorylation independent and recognises all tau isoforms. The monoclonal antibody PHF-1 recognises tau phosphorylated at epitope Ser396/404 and was kindly provided by Dr. P. Davies (Albert Einstein College of Medicine, NY, USA). AT270 (Insight Biotechnology, UK) recognises tau phosphorylated at residue Thr181. pS422, pS214, and pT212 tau antibodies were purchased from Biosource, UK. Monoclonal anti-GSK3α/β antibody (Stressgen) recognises total GSK3-α/β. Phospho-GSK3α/β Ser21/9, Akt, phospho-Akt Ser473, ERK1/2, phospho-ERK1/2 Thr202/Tyr204, c-jun N-terminal kinase (JNK), phospho-JNK T183/Y185 and PTEN antibodies were purchased from Cell Signaling Technology (Beverley, MA). PP1 (E-9) and PP2A (C-20) catalytic domain antibodies were purchased from Santa Cruz Biotechnology.

## 3. Results

### 3.1. PTEN reduces tau phosphorylation indirectly, by mechanisms requiring its lipid phosphatase activity

Phosphorylation independent antibodies recognise multiple bands of tau in non-neuronal cells transfected with cDNA coding for a single tau isoform indicating that endogenous kinases at least partially phosphorylated tau in these cells. We first examined the effects of PTEN on tau phosphorylation in CHO cells co-transfected with wild type (WT) PTEN and tau (2N4R) (Fig. 1). Overexpression of WT-PTEN resulted in a small reduction in total tau protein levels (Fig. 1A) compared with cells transfected with tau and vector. Phosphorylation of tau was measured using phospho-epitope specific antibodies and normalised relative to the total tau immunoreactivity for each sample. WT-PTEN significantly reduced phosphorylation of tau at several epitopes including S396/404, T181 and S422 (Fig. 1B–D) in cells co-transfected with tau and PTEN compared with those transfected with tau and vector. Phosphorylation at the T212 site (Fig. 1E), however, was only moderately reduced and the S214 site was unaffected by PTEN overexpression (Fig. 1F).

PTEN is a dual specificity phosphatase and can dephosphorylate both lipids [5] and serine, threonine and tyrosine protein residues [32]. To examine whether PTEN protein phosphatase activity reduces tau phosphorylation directly, cells were co-transfected with tau and lipid and protein phosphatase negative mutants of PTEN. Mutation of the PTEN active-site cysteine C124S completely disrupts phosphatase activity against lipid and protein substrates whereas the G129E mutation, found in the germ line of patients suffering from Cowden disease, lacks lipid phosphatase activity but retains protein phosphatase activity [33]. Both the C124S and G129E mutants abolished the reduction in tau expression and in tau phosphorylation seen with WT-PTEN (Fig. 1A–F). As both mutants lack lipid phosphatase activity but the G129E mutant retains protein phosphatase activity we conclude that the PTEN mediated reduction in tau phosphorylation and expression is via its lipid phosphatase activity. We confirmed that the expression of PTEN mutants and WT-PTEN was similar (Fig. 1A). These results suggest that PTEN regulates the phosphorylation of tau indirectly either by decreasing activity of tau kinases or increasing activity of tau phosphatase enzymes.

### 3.2. PTEN reduces tau phosphorylation independently of GSK-3 activity, but possibly via inhibition of Akt and ERK1/2

Glycogen synthase kinase-3 (GSK-3) is well established to play a major role in the phosphorylation of tau protein. The activities of the two mammalian GSK3 isoforms, GSK3α and GSK3β, are regulated by phosphorylation at serine 21 in GSK-3α and Ser9 in GSK-3β, resulting in enzyme inhibition [34]. Akt inhibits GSK-3 activity by enhancing phosphorylation at the Ser9 residue, and so PTEN would be predicted to increase GSK-3 activity by inhibiting Akt. To confirm this hypothesis, the activities of endogenous kinases were analysed in CHO cells transfected with WT-PTEN, by Western blotting using activity-dependent phospho-specific antibodies.

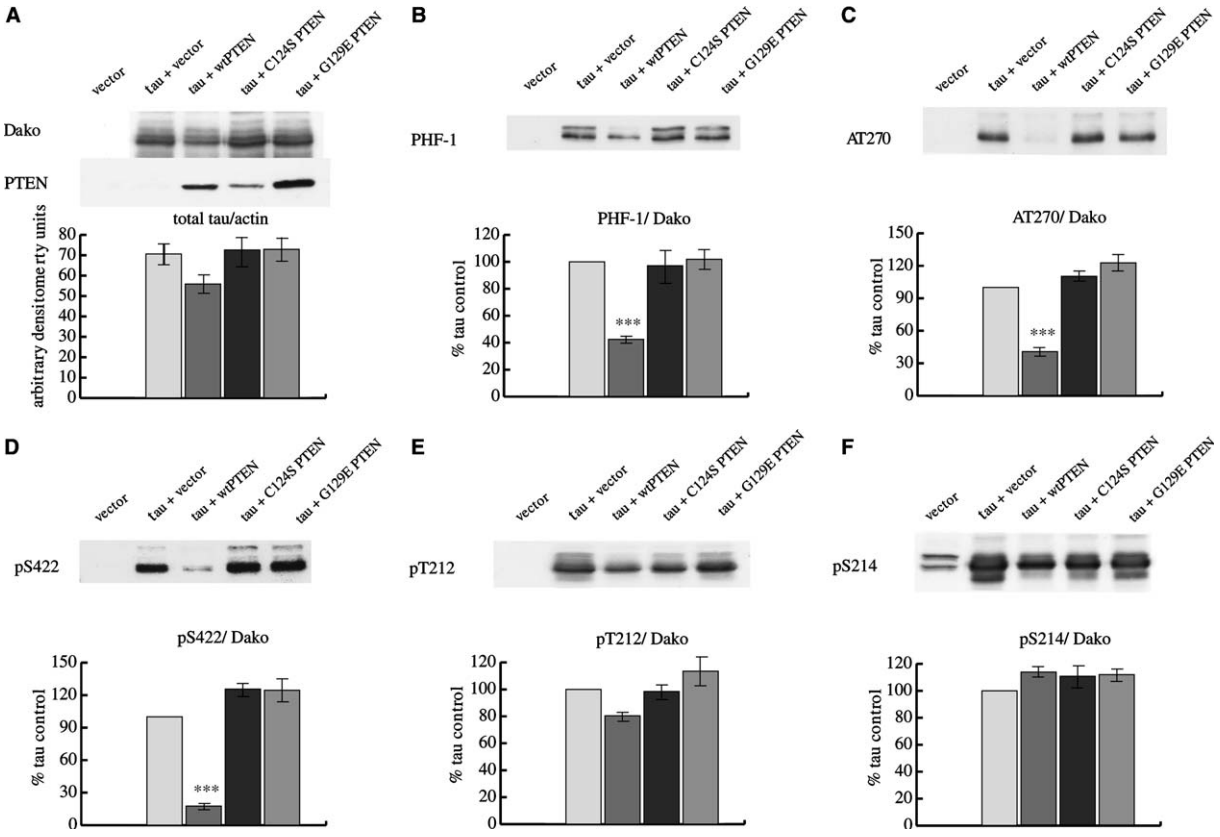


Fig. 1. Effects of PTEN on tau phosphorylation in CHO cells. Cells were transfected with pcDNA3.1 vector, tau and vector or co-transfected with tau and WT, catalytically inactive C124S or lipid phosphatase negative G129E PTENs. (A) Total tau protein levels were normalised to actin and are presented as arbitrary densitometry units. Tau phosphorylation was examined at several epitopes using phospho-specific antibodies (B) PHF-1 (S396/404), (C) AT270 (T181), (D) pS422, (E) pT212 and (F) pS214. Phospho-tau levels were normalised to total tau protein for each sample, then expressed as a percentage of the phosphorylation in samples transfected with tau plus vector. Statistical analysis was performed by ANOVA followed by Tukey's post hoc comparison, $^{***}P< 0.001$.

Phosphorylation of Akt at S473 was reduced (Fig. 2A) in CHO cells overexpressing WT-PTEN compared with cells transfected with vector alone, suggesting an inhibition of Akt activity as predicted. A reduction in phosphorylation of GSK-3β at S9 was also observed, indicating that the PTEN-induced inhibition of Akt resulted in a moderate increase in GSK-3β activity (Fig. 2B). PTEN, therefore, reduced tau phosphorylation at known GSK-3β sites (S396/404, T181; Fig. 1B and C) despite increasing GSK-3β activity. This result suggests that PTEN regulates an independent pathway that overrides the effects of GSK-3β on tau.

Recent studies have shown that tau contains an optimal consensus site for Akt phosphorylation at S214 [35,36], and also phosphorylates T212 in vitro. Reduced Akt activity could possibly explain the modest reduction in T212 phosphorylation observed in WT-PTEN overexpressing cells. The optimal Akt phosphorylation site S214 was unaltered by WT-PTEN, however, suggesting that the predominant effects of PTEN on tau phosphorylation are not mediated directly by Akt. Akt regulates several downstream signalling pathways independent of GSK-3 and so inhibition of this enzyme could still potentially mediate the effects of PTEN on tau via some indirect mechanism.

The mitogen-activated protein (MAP) kinase family, including JNK, p38 and ERK1/2, have also been implicated as potential tau kinases in vitro [37] in animal models [38–40] and in Alzheimer's and related tauopathies [41–44]. Studies have also shown that PTEN inhibits activity of the extracellular regulated mitogen activated protein (MAP) kinase (ERK1/2), but not JNK [45] via its protein phosphatase activity. In agreement with these findings phosphorylation of ERK1/2 at T202/Y204 was moderately reduced by WT-PTEN overexpression (Fig. 2C) suggesting kinase inhibition as predicted, but the phosphorylation of JNK at T183/Y185 was unaltered. WT-PTEN reduced tau phosphorylation most significantly at the S422 epitope (Fig. 1D), which has been suggested to be preferentially phosphorylated by MAP kinases [37]. This suggests that ERK1/2 could play a significant role in mediating the effects of PTEN on tau phosphorylation in this system.

### 3.3. PTEN does not inhibit tau phosphorylation by increasing activity of protein phosphatases

Protein phosphatases 1 and 2A (PP1, PP2A) have been implicated in the dephosphorylation of tau in vitro and in vivo [[46] for review]. To examine the effects of PTEN on phosphatase activity, PP1 and PP2A catalytic domain levels were measured by Western blotting (Fig. 3). Both PP1 and PP2A catalytic domain immunoreactivities were unaltered in cells overexpressing WT-PTEN compared with those transfected with vector alone (Figs. 3A and B). Furthermore, treatment of WT-PTEN and tau co-transfected CHO cells with 100 nM okadaic acid ($IC_{50}$

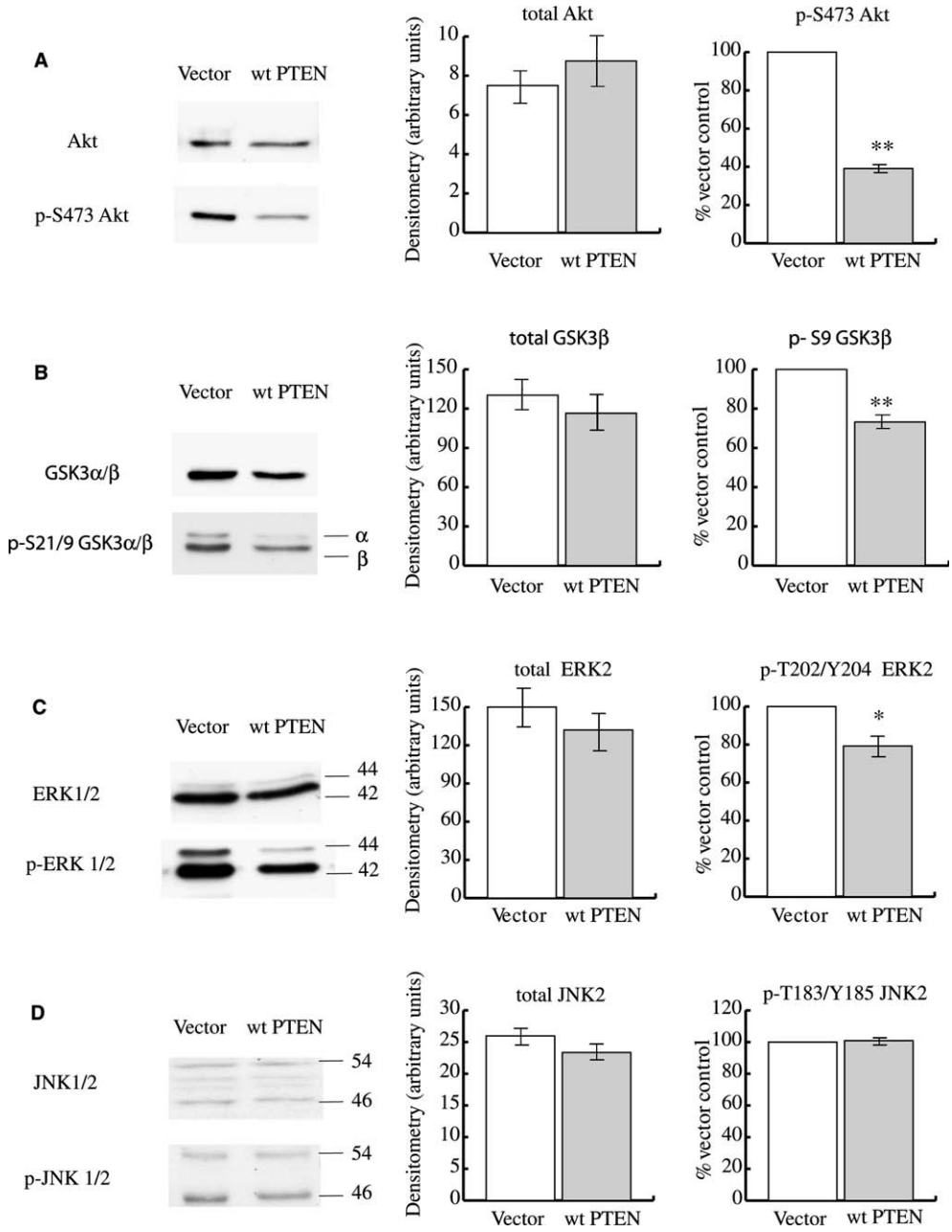


Fig. 2. Effects of WT-PTEN on activity of potential tau kinases. Cells were transfected with pcDNA3.1 vector or WT-PTEN alone. Activities of the endogenous kinases were then analysed by Western blotting using activity-dependent phospho-specific antibodies. (A) pS473 Akt activation-dependent phosphorylation, (B) pS9 inhibitory GSK-3 phosphorylation, (C) pT202/Y204 ERK1/2 activation-dependent phosphorylation and (D) pT183/Y185 JNK activation-dependent phosphorylation. Total protein levels were normalised to actin and are presented as arbitrary densitometry units. Phospho-protein immunoreactivity was normalised to total protein reactivity for each kinase and is presented as a percentage of the phosphorylation in samples transfected with vector alone. Data were analysed by Student's $t$ test, $^{**}P < 0.01$, $^{*}P < 0.05$.

PP2A: 0.1 nM; $IC_{50}$ PP1: 10–15 nM) did not reverse the effects of PTEN on tau (Fig. 3C). These results suggest that PTEN does not reduce tau phosphorylation by increasing the activities of other potential tau phosphatases in CHO cells.

### 3.4. WT-PTEN reduces tau phosphorylation at S422 in primary cortical neurons

To further examine the functional relevance of the effects of PTEN on tau in AD, we measured tau phosphorylation by immunofluorescence in primary mouse neurons transfected with WT and mutant GFP-tagged PTEN constructs. Tau expression and phosphorylation were assessed by examining the fluorescent intensity in cells expressing GFP-tagged PTEN compared with cells not expressing the transfected plasmids. As the S422 epitope was most-affected by PTEN in CHO cells we continued to study this site in neuronal cells. Total tau expression was unaltered by overexpression of WT, C124S or G129E PTEN (Figs. 4A–F). WT-PTEN, however, reduced S422 phosphorylation in cell bodies and processes compared with untransfected cells (Figs. 4G and J). In agreement with our studies in non-neuronal cells the effects of PTEN on S422 tau phosphorylation were abolished using C124S (Figs.

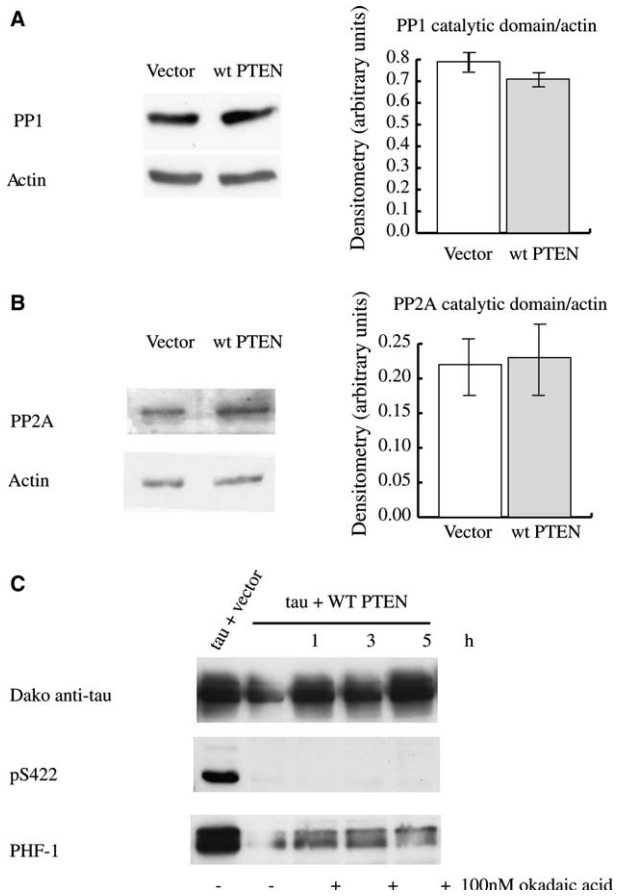


Fig. 3. PTEN does not reduce tau phosphorylation by upregulating activity of potential tau phosphatases. (A and B) CHO cells were transfected with pcDNA3.1 vector or WT-PTEN and catalytic domain levels of protein phosphatases analysed by Western blotting. Total protein levels were normalised to actin and are presented as arbitrary densitometry units. (C) Cells were transfected with vector or WT-PTEN, then treated with 100nM okadaic acid for 1, 3 and 5 h. Samples were then analysed for tau phosphorylation by Western blotting.

4H and K) or G129E (Figs. 4I and L) mutant PTEN constructs. These results suggest that our observations in CHO cells are relevant to the neuronal setting.

## 4. Discussion

Our hypothesis that PTEN may alter tau phosphorylation is derived from studies showing that activation of the PI3-kinase/Akt signalling pathway can reduce tau phosphorylation in cells [47] and animal models of AD [48]. We predicted that PTEN overexpression would hyperphosphorylate tau by reducing Akt activity and subsequently increasing the activity of GSK-3, a well-established tau kinase. We confirmed that overexpression of WT-PTEN leads to a reduction in Akt activity and increased GSK3-β activity in CHO cells. However, contrary to our expectations we observed a reduction in tau phosphorylation at several sites. We postulated that PTEN, via its ability to dephosphorylate protein residues, could possibly act directly as a tau phosphatase. However, the protein phosphatase activity present in the G129E mutant PTEN, without the presence of lipid phosphatase activity, was not sufficient to mediate the effects of PTEN on tau. This suggests an indirect mechanism that is dependent on the lipid phosphatase activity of PTEN.

PP2A has been shown to override the effects of GSK-3 on tau in starvation-induced mouse models of tau hyperphosphorylation [49]. Further analysis, however, revealed that the effects of PTEN on tau phosphorylation were not due to changes in activity of key tau phosphatases. The most significant PTEN-induced reduction in tau phosphorylation was observed at the S422 epitope, which is preferentially phosphorylated by MAP-kinases [37]. Consistent with this result PTEN reduced activity of the extracellular regulated MAP-kinase ERK1/2, but not JNK, in accordance with published results [45]. ERK1/2 may, therefore, play a significant role in mediating the effects of PTEN on tau phosphorylation. Published data, however, suggests that the effects of PTEN on ERK1/2 are mediated by the protein phosphatase activity of PTEN acting on upstream regulators of MAP-kinase, including the insulin receptor substrate (IRS) [50]. Our observations that the effects of PTEN on tau are predominantly lipid phosphatase-dependent, raises the possibility that PTEN may alter other pathways downstream of PIP3 to regulate tau phosphorylation in addition to MAP kinase inhibition.

PIP3 substrates include serine/threonine kinases (Akt and PDK1), guanine nucleotide binding proteins (Rac and Rho) and protein tyrosine kinases of the Tec family (TEC and Brunton's tyrosine kinase (BTK)). Of these Akt is best established to be involved in the phosphorylation of tau. Recent studies have shown that tau contains an optimal consensus site for Akt phosphorylation at S214 [35,36], which together with T212 comprises the AT100 epitope that is specific to paired helical filaments (PHFs) found in AD and other tauopathies [51]. We found that PTEN altered phosphorylation of these epitopes modestly in respect to the other sites analysed, suggesting that the predominant effects of PTEN on tau phosphorylation are not mediated directly by Akt. Akt has several downstream substrates other than GSK-3 including the forkhead family of transcription factors (FKHR), $p27^{Kip1}$, Bad and the mammalian target of rapamycin (mTOR). Alterations in mTOR/p70S6 kinase signalling have been described in AD [52–54], and have recently been shown to alter tau phosphorylation in cells [55]. However we found no change in pS2448 mTOR phosphorylation in WT-PTEN overexpressing CHO cells (data not shown).

More recently, [56] have described a novel cross-talk between GSK-3 and ERK1/2 signalling pathways, whereby inhibition of GSK-3 leads to increased ERK1/2 phosphorylation and activity via protein kinase Cδ (PKCδ). It is tempting, therefore, to speculate that the increased activity of GSK-3 in cells transfected with PTEN could result in reduced ERK1/2 activity via similar mechanisms. Further studies are, however, required to unravel the precise pathways connecting PTEN and ERK1/2 in the context of tau phosphorylation.

Griffin et al. [31] provided the first evidence for aberrant PTEN signalling in AD. It is possible that the reductions in PTEN and increases in Akt activity seen in AD brain are indicative of a survival response to neuronal injury. Indeed, inactivation and downregulation of PTEN levels observed following ischemic insults in neurons and mouse models of transient focal ischemia [57,58] have been suggested to promote neuronal survival during toxic insults. On the other hand, a potentially protective role for PTEN in neurodegenerative disease has

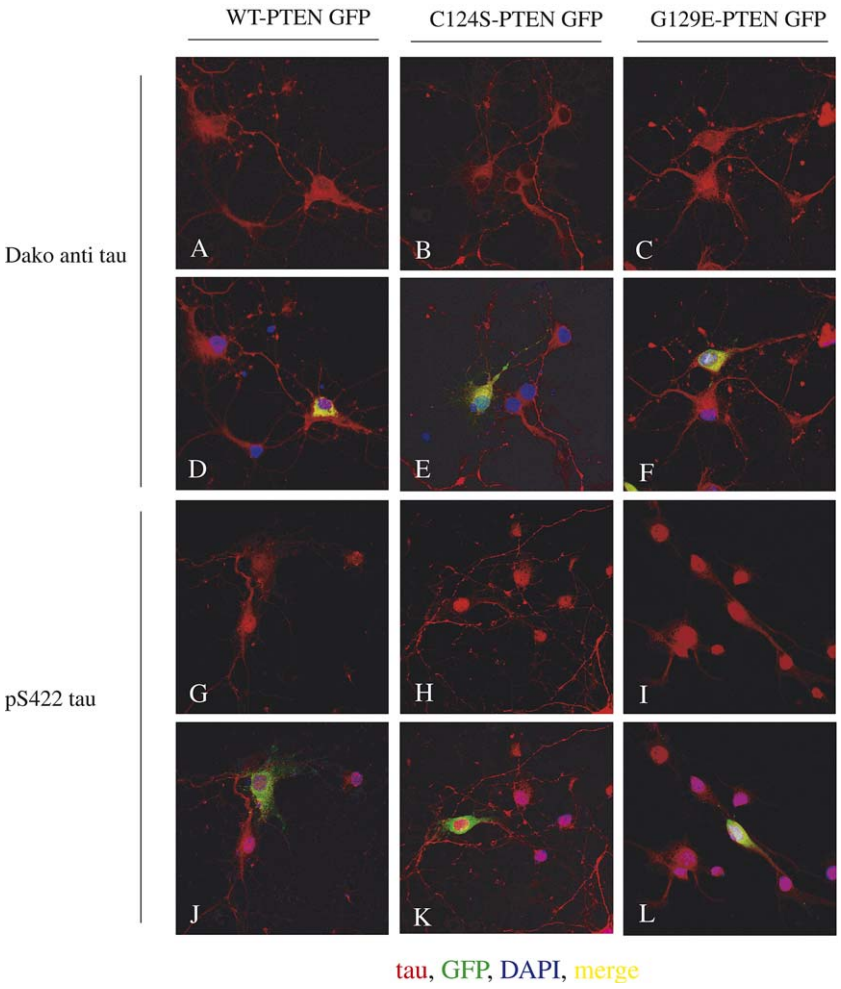


Fig. 4. Effects of PTEN on tau phosphorylation in primary neurons. Tau expression and phosphorylation were assessed by examining immunostaining intensity in cells expressing GFP-PTEN constructs (green) compared with neighbouring cells not expressing the transfected plasmids. (A–F) Total tau levels were measured using the Dako anti-tau antibody (A–C tau only, D–F merged). (G–L) Tau phosphorylation at residue S422 was measured using a pS422 specific antibody (G–I phospho-tau only, J–L merged).

been suggested by the observation that loss of PTEN is also associated with a number of other neurological diseases. Germline mutations in PTEN have been found in several familial tumour syndromes, including Cowden's disease, which are associated with neural defects [59]. More recently, mutations in PTEN-induced putative kinase 1 (PINK1) have been associated with early-onset Parkinson's disease (EOPD) [60] and abrogate the protective effects of PINK-1 against neuronal apoptosis [61].

Our results show that PTEN can actually prevent tau phosphorylation in cells, contrary to its expected effects on GSK-3, and agree with the suggestion that reduced PTEN levels and increased Akt activity may promote the development of neurofibrillary changes in AD [31]. The precise role of PTEN in Alzheimer's disease, however, requires further investigation in light of more recent studies showing that PTEN activity is increased in AD brain [62]. Nonetheless, our studies highlight PTEN as a potential new target for the prevention of AD and other tauopathies.

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## References

[1] Li, D.M. and Sun, H. (1997) TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res. 57, 2124–2129.
[2] Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., Bigner, S.H., Giovanella, B.C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M.H. and Parsons, R. (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275, 1943–1947.
[3] Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D.H. and Tavtigian, S.V. (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet. 15, 356–362.
[4] Leslie, N.R. and Downes, C.P. (2004) PTEN function: how normal cells control it and tumour cells lose it. Biochem. J. 382, 1–11.

[5] Maehama, T. and Dixon, J.E. (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J. Biol. Chem. 273, 13375–13378.
[6] Stambolic, V., Suzuki, A., de la Pompa, J.L., Brothers, G.M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J.M., Siderovski, D.P. and Mak, T.W. (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95, 29–39.
[7] Wu, X., Senechal, K., Neshat, M.S., Whang, Y.E. and Sawyers, C.L. (1998) The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. PNAS 95, 15587–15591.
[8] Sulis, M.L. and Parsons, R. (2003) PTEN: from pathology to biology. Trends Cell Biol. 13, 478–483.
[9] Di Cristofano, A., Pesce, B., Cordon-Cardo, C. and Pandolfi, P.P. (1998) Pten is essential for embryonic development and tumour suppression. Nat. Genet. 19, 348–355.
[10] Sano, T., Lin, H., Chen, X., Langford, L.A., Koul, D., Bondy, M.L., Hess, K.R., Myers, J.N., Hong, Y.K., Yung, W.K. and Steck, P.A. (1999) Differential expression of MMAC/PTEN in glioblastoma multiforme: relationship to localization and prognosis. Cancer Res. 59, 1820–1824.
[11] Lachyankar, M.B., Sultana, N., Schonhoff, C.M., Mitra, P., Poluha, W., Lambert, S., Quesenberry, P.J., Litofsky, N.S., Recht, L.D., Nabi, R., Miller, S.J., Ohta, S., Neel, B.G. and Ross, A.H. (2000) A role for nuclear PTEN in neuronal differentiation. J. Neurosci. 20, 1404–1413.
[12] Kyrylenko, S., Roschier, M., Korhonen, P. and Salminen, A. (1999) Regulation of PTEN expression in neuronal apoptosis. Brain Res. Mol. Brain Res. 73, 198–202.
[13] Groszer, M., Erickson, R., Scripture-Adams, D.D., Lesche, R., Trumpp, A., Zack, J.A., Kornblum, H.I., Liu, X. and Wu, H. (2001) Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science 294, 2186–2189.
[14] Marino, S., Krimpenfort, P., Leung, C., van der Korput, H.A., Trapman, J., Camenisch, I., Berns, A. and Brandner, S. (2002) PTEN is essential for cell migration but not for fate determination and tumourigenesis in the cerebellum. Development 129, 3513–3522.
[15] Backman, S.A., Stambolic, V., Suzuki, A., Haight, J., Elia, A., Pretorius, J., Tsao, M.S., Shannon, P., Bolon, B., Ivy, G.O. and Mak, T.W. (2001) Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. Nat. Genet. 29, 396–403.
[16] Kwon, C.H., Zhu, X., Zhang, J., Knoop, L.L., Tharp, R., Smeyne, R.J., Eberhart, C.G., Burger, P.C. and Baker, S.J. (2001) Pten regulates neuronal soma size: a mouse model of Lhermitte–Duclos disease. Nat. Genet. 29, 404–411.
[17] Wei, W., Wang, X. and Kusiak, J.W. (2002) Signaling events in amyloid beta -peptide-induced neuronal death and insulin-like growth factor I protection. J. Biol. Chem. 277, 17649–17656.
[18] Stein, T.D. and Johnson, J.A. (2002) Lack of neurodegeneration in transgenic mice overexpressing mutant amyloid precursor protein is associated with increased levels of transthyretin and the activation of cell survival pathways. J. Neurosci. 22, 7380–7388.
[19] Hanger, D.P., Hughes, K., Woodgett, J.R., Brion, J.P. and Anderton, B.H. (1992) Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. Neurosci. Lett. 147, 58–62.
[20] Mandelkow, E.M., Drewes, G., Biernat, J., Gustke, N., Van Lint, J., Vandenheede, J.R. and Mandelkow, E. (1992) Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau. FEBS Lett. 314, 315–321.
[21] Lovestone, S., Reynolds, C.H., Latimer, D., Davis, D.R., Anderton, B.H., Gallo, J.M., Hanger, D., Mulot, S., Marquardt, B. and Stabel, S., et al. (1994) Alzheimer's disease-like phosphorylation of the microtubule-associated protein tau by glycogen synthase kinase-3 in transfected mammalian cells. Curr. Biol. 4, 1077–1086.
[22] Brownlees, J., Irving, N.G., Brion, J.P., Gibb, B.J., Wagner, U., Woodgett, J. and Miller, C.C. (1997) Tau phosphorylation in transgenic mice expressing glycogen synthase kinase-3beta transgenes. Neuroreport 8, 3251–3255.
[23] Phiel, C.J., Wilson, C.A., Lee, V.M. and Klein, P.S. (2003) GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature 423, 435–439.
[24] Ryder, J., Su, Y., Liu, F., Li, B., Zhou, Y. and Ni, B. (2003) Divergent roles of GSK3 and CDK5 in APP processing. Biochem. Biophys. Res. Commun. 312, 922–929.
[25] Sun, X., Sato, S., Murayama, O., Murayama, M., Park, J.M., Yamaguchi, H. and Takashima, A. (2002) Lithium inhibits amyloid secretion in COS7 cells transfected with amyloid precursor protein C100. Neurosci. Lett. 321, 61–64.
[26] Persad, S., Troussard, A.A., McPhee, T.R., Mulholland, D.J. and Dedhar, S. (2001) Tumor suppressor PTEN inhibits nuclear accumulation of beta-catenin and T cell/lymphoid enhancer factor 1-mediated transcriptional activation. J. Cell. Biol. 153, 1161–1174.
[27] Sharma, M., Chuang, W.W. and Sun, Z. (2002) Phosphatidylinositol 3-kinase/Akt stimulates androgen pathway through GSK3beta inhibition and nuclear beta-catenin accumulation. J. Biol. Chem. 277, 30935–30941.
[28] Myers, A., Holmans, P., Marshall, H., Kwon, J., Meyer, D., Ramic, D., Shears, S., Booth, J., DeVrieze, F.W., Crook, R., Hamshere, M., Abraham, R., Tunstall, N., Rice, F., Carty, S., Lillystone, S., Kehoe, P., Rudrasingham, V., Jones, L., Lovestone, S., Perez-Tur, J., Williams, J., Owen, M.J., Hardy, J. and Goate, A.M. (2000) Susceptibility locus for Alzheimer's disease on chromosome 10. Science 290, 2304–2305.
[29] Pei, J.J., Khatoon, S., An, W.L., Nordlinder, M., Tanaka, T., Braak, H., Tsujio, I., Takeda, M., Alafuzoff, I., Winblad, B., Cowburn, R.F., Grundke-Iqbal, I. and Iqbal, K. (2003) Role of protein kinase B in Alzheimer's neurofibrillary pathology. Acta Neuropathol. (Berl) 105, 381–392.
[30] Rickle, A., Bogdanovic, N., Volkman, I., Winblad, B., Ravid, R. and Cowburn, R.F. (2004) Akt activity in Alzheimer's disease and other neurodegenerative disorders. Neuroreport 15, 955–959.
[31] Griffin, R.J., Moloney, A., Kelliher, M., Johnston, J.A., Ravid, R., Dockery, P., O'Connor, R. and O'Neill, C. (2005) Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer's disease pathology. J. Neurochem. 93, 105–117.
[32] Myers, M.P., Stolarov, J.P., Eng, C., Li, J., Wang, S.I., Wigler, M.H., Parsons, R. and Tonks, N.K. (1997) P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc. Natl. Acad. Sci. USA 94, 9052–9057.
[33] Myers, M.P., Pass, I., Batty, I.H., Van der Kaay, J., Stolarov, J.P., Hemmings, B.A., Wigler, M.H., Downes, C.P. and Tonks, N.K. (1998) The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc. Natl. Acad. Sci. USA 95, 13513–13518.
[34] Doble, B.W. and Woodgett, J.R. (2003) GSK-3: tricks of the trade for a multi-tasking kinase. J. Cell Sci. 116, 1175–1186.
[35] Ksiezak-Reding, H., Pyo, H.K., Feinstein, B. and Pasinetti, G.M. (2003) Akt/PKB kinase phosphorylates separately Thr212 and Ser214 of tau protein in vitro. Biochim. Biophys. Acta 1639, 159–168.
[36] Kyoung Pyo, H., Lovati, E., Pasinetti, G.M. and Ksiezak-Reding, H. (2004) Phosphorylation of tau at THR212 and SER214 in human neuronal and glial cultures: the role of AKT. Neuroscience 127, 649–658.
[37] Reynolds, C.H., Betts, J.C., Blackstock, W.P., Nebreda, A.R. and Anderton, B.H. (2000) Phosphorylation sites on tau identified by nanoelectrospray mass spectrometry: differences in vitro between the mitogen-activated protein kinases ERK2, c-Jun N-terminal kinase and P38, and glycogen synthase kinase-3beta. J. Neurochem. 74, 1587–1595.
[38] Puig, B., Gomez-Isla, T., Ribe, E., Cuadrado, M., Torrejon-Escribano, B., Dalfo, E. and Ferrer, I. (2004) Expression of stress-activated kinases c-Jun N-terminal kinase (SAPK/JNK-P) and p38 kinase (p38-P), and tau hyperphosphorylation in neurites surrounding betaA plaques in APP Tg2576 mice. Neuropathol. Appl. Neurobiol. 30, 491–502.
[39] Echeverria, V., Ducatenzeiler, A., Dowd, E., Janne, J., Grant, S.M., Szyf, M., Wandosell, F., Avila, J., Grimm, H. and Dunnett, S.B. (2004) Altered mitogen-activated protein kinase signaling,

tau hyperphosphorylation and mild spatial learning dysfunction in transgenic rats expressing the [beta]-amyloid peptide intracellularly in hippocampal and cortical neurons. Neuroscience 129, 583–592.

[40] Lambourne, S.L., Sellers, L.A., Bush, T.G., Choudhury, S.K., Emson, P.C., Suh, Y.H. and Wilkinson, L.S. (2005) Increased tau phosphorylation on mitogen-activated protein kinase consensus sites and cognitive decline in transgenic models for Alzheimer's disease and FTDP-17: evidence for distinct molecular processes underlying tau abnormalities. Mol. Cell Biol. 25, 278–293.

[41] Perry, G., Roder, H., Nunomura, A., Takeda, A., Friedlich, A.L., Zhu, X., Raina, A.K., Holbrook, N., Siedlak, S.L., Harris, P.L. and Smith, M.A. (1999) Activation of neuronal extracellular receptor kinase (ERK) in Alzheimer disease links oxidative stress to abnormal phosphorylation. Neuroreport 10, 2411–2415.

[42] Ferrer, I., Blanco, R., Carmona, M. and Puig, B. (2001) Phosphorylated mitogen-activated protein kinase (MAPK/ERK-P), protein kinase of 38 kDa (p38-P), stress-activated protein kinase (SAPK/JNK-P), and calcium/calmodulin-dependent kinase II (CaM kinase II) are differentially expressed in tau deposits in neurons and glial cells in tauopathies. J. Neural. Transm. 108, 1397–1415.

[43] Ferrer, I., Blanco, R., Carmona, M., Ribera, R., Goutan, E., Puig, B., Rey, M.J., Cardozo, A., Vinals, F. and Ribalta, T. (2001) Phosphorylated map kinase (ERK1, ERK2) expression is associated with early tau deposition in neurones and glial cells, but not with increased nuclear DNA vulnerability and cell death, in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. Brain Pathol. 11, 144–158.

[44] Pei, J.J., Braak, H., An, W.L., Winblad, B., Cowburn, R.F., Iqbal, K. and Grundke-Iqbal, I. (2002) Up-regulation of mitogen-activated protein kinases ERK1/2 and MEK1/2 is associated with the progression of neurofibrillary degeneration in Alzheimer's disease. Brain Res. Mol. Brain Res. 109, 45–55.

[45] Weng, L.P., Brown, J.L., Baker, K.M., Ostrowski, M.C. and Eng, C. (2002) PTEN blocks insulin-mediated ETS-2 phosphorylation through MAP kinase, independently of the phosphoinositide 3-kinase pathway. Hum. Mol. Genet. 11, 1687–1696.

[46] Gong, C.X., Liu, F., Grundke-Iqbal, I. and Iqbal, K. (2005) Post-translational modifications of tau protein in Alzheimer's disease. J. Neural. Transm. 112, 813–838.

[47] Hong, M. and Lee, V.M. (1997) Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. J. Biol. Chem. 272, 19547–19553.

[48] Baki, L., Shioi, J., Wen, P., Shao, Z., Schwarzman, A., Gama-Sosa, M., Neve, R. and Robakis, N.K. (2004) PS1 activates PI3K thus inhibiting GSK-3 activity and tau overphosphorylation: effects of FAD mutations. Embo J. 23, 2586–2596.

[49] Planel, E., Yasutake, K., Fujita, S.C. and Ishiguro, K. (2001) Inhibition of protein phosphatase 2A overrides tau protein kinase I/glycogen synthase kinase 3 beta and cyclin-dependent kinase 5 inhibition and results in tau hyperphosphorylation in the hippocampus of starved mouse. J. Biol. Chem. 276, 34298–34306.

[50] Weng, L.P., Smith, W.M., Brown, J.L. and Eng, C. (2001) PTEN inhibits insulin-stimulated MEK/MAPK activation and cell growth by blocking IRS-1 phosphorylation and IRS-1/Grb-2/Sos complex formation in a breast cancer model. Hum. Mol. Genet. 10, 605–616.

[51] Matsuo, E.S., Shin, R.W., Billingsley, M.L., Van deVoorde, A., O'Connor, M., Trojanowski, J.Q. and Lee, V.M. (1994) Biopsy-derived adult human brain tau is phosphorylated at many of the same sites as Alzheimer's disease paired helical filament tau. Neuron 13, 989–1002.

[52] Lafay-Chebassier, C., Paccalin, M., Page, G., Barc-Pain, S., Perault-Pochat, M.C., Gil, R., Pradier, L. and Hugon, J. (2005) mTOR/p70S6k signalling alteration by Abeta exposure as well as in APP-PS1 transgenic models and in patients with Alzheimer's disease. J. Neurochem. 94, 215–225.

[53] Li, X., Alafuzoff, I., Soininen, H., Winblad, B. and Pei, J.J. (2005) Levels of mTOR and its downstream targets 4E-BP1, eEF2, and eEF2 kinase in relationships with tau in Alzheimer's disease brain. Febs J. 272, 4211–4220.

[54] An, W.L., Cowburn, R.F., Li, L., Braak, H., Alafuzoff, I., Iqbal, K., Iqbal, I.G., Winblad, B. and Pei, J.J. (2003) Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. Am. J. Pathol. 163, 591–607.

[55] Pei, J.J., An, W.L., Zhou, X.W., Nishimura, T., Norberg, J., Benedikz, E., Gotz, J. and Winblad, B. (2006) P70 S6 kinase mediates tau phosphorylation and synthesis. FEBS Lett. 580, 107–114.

[56] Wang, Q., Zhou, Y., Wang, X. and Evers, B.M. (2006) Glycogen synthase kinase-3 is a negative regulator of extracellular signal-regulated kinase. Oncogene 25, 43–50.

[57] Ning, K., Pei, L., Liao, M., Liu, B., Zhang, Y., Jiang, W., Mielke, J.G., Li, L., Chen, Y., El-Hayek, Y.H., Fehlings, M.G., Zhang, X., Liu, F., Eubanks, J. and Wan, Q. (2004) Dual neuroprotective signaling mediated by downregulating two distinct phosphatase activities of PTEN. J. Neurosci. 24, 4052–4060.

[58] Choi, J.S., Park, H.J., Kim, H.Y., Kim, S.Y., Lee, J.E., Choi, Y.S., Chun, M.H., Chung, J.W. and Lee, M.Y. (2005) Phosphorylation of PTEN and Akt in astrocytes of the rat hippocampus following transient forebrain ischemia. Cell Tissue Res. 319, 359–366.

[59] Padberg, G.W., Schot, J.D., Vielvoye, G.J., Bots, G.T. and de Beer, F.C. (1991) Lhermitte-Duclos disease and Cowden disease: a single phakomatosis. Ann. Neurol. 29, 517–523.

[60] Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M.M., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A.R., Healy, D.G., Albanese, A., Nussbaum, R., Gonzalez-Maldonado, R., Deller, T., Salvi, S., Cortelli, P., Gilks, W.P., Latchman, D.S., Harvey, R.J., Dallapiccola, B., Auburger, G. and Wood, N.W. (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304, 1158–1160.

[61] Petit, A., Kawarai, T., Paitel, E., Sanjo, N., Maj, M., Scheid, M., Chen, F., Gu, Y., Hasegawa, H., Salehi-Rad, S., Wang, L., Rogaeva, E., Fraser, P., Robinson, B., St George-Hyslop, P. and Tandon, A. (2005) Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. J. Biol. Chem. 280, 34025–34032.

[62] Rickle, A., Bogdanovic, N., Volkmann, I., Zhou, X., Pei, J.J., Winblad, B. and Cowburn, R.F. (2006) PTEN levels in Alzheimer's disease medial temporal cortex. Neurochem. Int. 48, 114–123.