Neurosci Bull April 1, 2014, 30(2): 359–366. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1415-y


·Review·

# Tau hyperphosphorylation induces apoptotic escape and triggers neurodegeneration in Alzheimer's disease

Jian-Zhi Wang, Zhi-Hao Wang, Qing Tian

*Department of Pathology and Pathophysiology, Key Laboratory of Ministry of Education of China for Neurological Disorders, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

Corresponding author: Jian-Zhi Wang. E-mail: wangjz@mails.tjmu.edu.cn



Since abnormal post-translational modifications or gene mutations of tau have been detected in over twenty neurodegenerative disorders, tau has attracted widespread interest as a target protein. Among its various post-translational modifications, phosphorylation is the most extensively studied. It is recognized that tau hyperphosphorylation is the root cause of neurodegeneration in Alzheimer's disease (AD); however, it is not clear how it causes neurodegeneration. Based on the findings that tau hyperphosphorylation leads to the escape of neurons from acute apoptosis and simultaneously impairs the function of neurons, we have proposed that the nature of AD neurodegeneration is the consequence of aborted apoptosis induced by tau phosphorylation. Therefore, proper manipulation of tau hyperphosphorylation could be promising for arresting AD neurodegeneration. In this review, the neuroprotective and neurodegenerative effects of tau hyperphosphorylation and our thoughts regarding their relationship are presented.

**Keywords:** Alzheimer's disease; microtubule-associated protein tau; hyperphosphorylation; apoptosis; neurodegeneration

## Mechanisms Underlying Tau Hyperphosphorylation in Alzheimer's disease

Tau accounts for >80% of the microtubule-associated proteins. The major biological function of tau is to promote microtubule assembly and maintain the stability of microtubules, the track of axonal transport. In Alzheimer's disease (AD), tau is abnormally hyperphosphorylated and aggregated into paired helical filaments (PHFs)/ neurofibrillary tangles in neurons[1, 2]. Activation of protein kinases and/or inhibition of protein phosphatases are the direct cause of tau hyperphosphorylation. Many kinases and phosphatases are reportedly involved in AD-like tau hyperphosphorylation; among them, glycogen synthase kinase-3β (GSK-3β) and protein phosphatase-2A (PP2A) are the most involved[3-5].

GSK-3β phosphorylates tau at multiple AD-associated sites[6, 7]. Activation of GSK-3β inhibits long-term potentiation (LTP) through mechanisms involving the presynaptic release of neurotransmitter, as well as causing tau hyperphosphorylation and spatial memory deficit. Conversely, spatiotemporal inhibition of GSK-3β to ~70% of the normal control level potentiates LTP, attenuates tau hyperphosphorylation, and improves memory[8-13]. Many factors can cause tau hyperphosphorylation by activating GSK-3β, such as peroxide nitrite, advanced glycation end-products, endoplasmic reticulum (ER) stress, β-amyloid (Aβ), and proteasome dysfunction[14-18]. Priming phosphorylation of tau by protein kinase A makes it a better substrate for GSK-3β, then tau can be hyperphosphorylated by basal activity of GSK-3β[19], suggesting a synergistic effect of kinases and/or phosphatases on tau hyperphosphorylation that deserves further clarification. Interestingly, tau hyperphosphorylation by GSK-3β seems to be required for neurogenesis in the hippocampal dentate gyrus but not

in the sub-ventricular zone of rat brain[20].

The activity of protein phosphatases is decreased in the AD brain[21]. Several phosphatases such as PP2A, PP2B, and PP1 can dephosphorylate abnormally hyperphosphorylated tau isolated from AD brains; among these, PP2A is the most effective[22-24]. *In vitro*, PP2A can dephosphorylate abnormal tau at multiple sites and thus restore its biological activity[5]. Inactivation of PP2A by okadaic acid, homocysteine, or zinc induces tau hyperphosphorylation/accumulation and axoplasmic transport deficits[25-27]. PP2A activity is regulated *in vivo* by a constitutive protein inhibitor called inhibitor 2 of PP2A (I2PP2A) that is increased in the AD brain, and phosphorylation of I2PP2A at serine-9 results in its cytoplasmic accumulation, as seen in the AD brain[28]. Conversely, betaine and nicotinamide mononucleotide adenylyltransferase 2 can activate PP2A and attenuate tau hyperphosphorylation[29, 30]. In the astrocytes of tg2567 mice, a widely-used amyloidogenic model of AD, PP2A is activated. This stimulates the migration of astrocytes towards amyloid plaques by inhibiting p38 MAPK, indicating that the PP2A deficit in the AD brain may cause Aβ accumulation by hindering astrocyte migration[31].

GSK-3β activation can inhibit PP2A by upregulating protein tyrosine phosphatase 1B (PTP1B), which phosphorylates PP2A at tyrosine-307[32, 33], suggesting that activation of GSK-3β and inhibition of PP2A may form a vicious cycle that promotes tau hyperphosphorylation in the AD brain. Tau is also highly glycosylated through N-linked glycosidic bonds in the AD brain[34], while a negative correlation between tau O-glycosylation and phosphorylation has been reported in a rat model of starvation[35]. A study also showed that prion protein can also inhibit tau-mediated microtubule formation[36].

## Evidence Supporting the Toxic Effects of Tau Hyperphosphorylation

Since the formation of neurofibrillary tangles from hyperphosphorylated tau is a hallmark of several neurodegenerative disorders including AD, corticobasal degeneration (CBD), Down syndrome, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Pick disease, post-encephalitic parkinsonism, progressive supranuclear palsy (PSP), and Niemann-Pick type C disease, tau hyperphosphorylation has been considered detrimental to neurons. The following evidence supports the idea that tau hyperphosphorylation is toxic to neurons.

### *Tau Hyperphosphorylation Disrupts Microtubule Dynamics*

Homeostatic microtubule assembly and disassembly is essential for normal cell morphology, functions, and viability, while microtubule dynamics is regulated by tau phosphorylation. The abnormally hyperphosphorylated tau isolated from AD brains and *in vitro* hyperphosphorylated recombinant human tau are no longer competent to promote microtubule assembly or bind to microtubules[37, 38], and dephosphorylation dissociates tau from PHFs and restores its biological activity[22]. Hyperphosphorylation of tau not only diminishes its biological activity, but also causes it to gain toxic functions. For instance, hyperphosphorylated tau detached from microtubules sequesters normal tau and other high molecular-weight microtubule-associated proteins, thus disrupting microtubule dynamics and causing somatodendritic accumulation of tau proteins. Hyperphosphorylation promotes tau aggregation that can block the intracellular trafficking of neurotrophins and other functional proteins, and cause axonopathy[39-41]. Moreover, transgenic mice that overexpress the 4R human tau isoform develop axonal degeneration in specific neurons of the brain and spinal cord[42].

### *Tau Hyperphosphorylation Promotes Tangle Formation*

Normal tau binds to tubulin and promotes its assembly into microtubules, while the abnormally hyperphosphorylated form binds to normal tau and disrupts microtubules[36]. In *Drosophila*, only the simultaneous expression of human tau and GSK-3 or cyclin-dependent kinase-5 (Cdk-5), but not human tau alone, stimulates the formation of neurofibrillary inclusions, while reducing tau phosphorylation decreases tau aggregation in P301L mice treated with LiCl, an inhibitor of GSK-3[43-45]. *In vitro* study has also shown that phosphorylation of all six recombinant human brain isoforms of tau promotes their self-assembly into tangles of PHFs[39] but the contribution of each isoform to tangle formation is not known. In addition, it has recently been reported that tau meditates the synaptic toxicity of Aβ, but the mechanisms are not clear.

### *Tau Hyperphosphorylation Inhibits Proteases*

Polyubiquitylated tau proteins are present in tangles and proteasome activity is decreased in the AD brain[46]. Inhibition of proteasomes by lactacystin induces phosphorylation and accumulation of tau[47]. The extensive tau hyperphosphorylation induced by inhibition of PP2A or activation of GSK-3 inhibits proteasomes in HEK293 cells stably expressing human $tau_{441}$, while moderate tau phosphorylation stimulates proteasome activity[48]. Incubation of proteasomes with PHF-tau isolated from AD brains inhibits their activity[49], providing direct evidence for the inhibition of proteasomes by tau. It seems that tau hyperphosphorylation and proteasome inhibition can form a vicious cycle, though the causality needs to be clearly established.

## The "Neuroprotective" Role of Tau Hyperphosphorylation

Although it is widely believed that tau hyperphosphorylation induces neuron loss, evidence that directly links wild-type tau proteins with cell death is still lacking. Instead, some recent studies suggest that tau hyperphosphorylation and/or accumulation may be neuroprotective, especially when cells are exposed to an acute insult.

### *Evidence Supporting the Protective Role of Tau Hyperphosphorylation*

By quantitative analysis of neuron loss and neurofibrillary tangle formation as a function of disease duration, it was found that CA1 hippocampal neurons can survive with neurofibrillary tangles for ~20 years[50]. In a transgenic mouse model expressing human mutant P301L tau (rTg4510), neuron loss occurs before neurofibrillary lesions appear in the dentate gyrus and, conversely, neurofibrillary pathology appears without major cell loss in the striatum[51]. In P301S mice, tau hyperphosphorylation is significant but no apoptosis is detectable[52]. Tau hyperphosphorylation occurs during hibernation, a model of neuroprotection[53, 54].

With ageing or evolution of AD, neurons in the brain are constantly exposed to an environment with enriched pro-apoptotic factors, such as oxidative stress and Aβ[55]. However, as noted above, there is surprisingly little evidence for the completion of apoptotic cell death in the AD brain, implying that certain mechanism may allow neurons to escape from apoptosis. Since abnormally hyperphosphorylated tau is the major protein component of the tangles in degenerating neurons of the AD brain at autopsy, researchers believe that tau hyperphosphorylation plays a crucial role in neurodegeneration, but how this happens is not clear.

### *Mechanisms Underlying the Neuroprotection of Tau Hyperphosphorylation*

To understand how the abnormal tau hyperphosphorylation causes neurodegeneration, we expressed exogenous tau proteins in a human embryonic kidney cell line that does not express endogenous tau (HEK293) and a mouse neuroblastoma cell line that expresses very low levels of endogenous tau (N2a) and established lines with stable expression of tau or the vector. We treated these cells and primary hippocampal neurons with different types of pro-apoptotic factors, including staurosporine, camptothecin, hydrogen peroxide, ER stress, Aβ, death-associated protein kinase-1, and GSK-3β. We found that tau hyperphosphorylation induced by these factors is accompanied by unexpectedly reduced apoptosis compared with controls[56-59], while tau dephosphorylation promotes apoptosis through failed dephosphorylation of Bcl-2[60].

As a cytoskeleton protein, how is tau phosphorylation and/or accumulation involved in cell viability or apoptotic arrest? Our data showed that tau hyperphosphorylation inhibits the phosphorylation of β-catenin through substrate competition, and the nuclear translocation of unphosphorylated β-catenin primes the expression of survival signals (the nuclear pathway). Tau hyperphosphorylation preserves Bcl-2 and suppresses the release of cytochrome-*c* from mitochondria into the cytosolic fraction, while also inhibiting Bax expression and caspase-9/3 activity; tau hyperphosphorylation mediated by GSK-3β and Cdk-5 inhibits p53 phosphorylation that may be linked to mitochondrial damage (the mitochondrial pathway). Mitochondrial dysfunction and cellular metabolic deficiency have also been reported in AD patients[61]. Tau hyperphosphorylation upregulates the unfolded protein response in the ER, including elevation of phosphorylated PERK, eIF2, and IRE1 with increased cleavage of ATF6 and ATF4 (the ER pathway)[57-59]. Furthermore, tau hyperphosphorylation induced by knockdown of the

endogenous protein inhibitor of PP2A, namely inhibitor-2 of PP2A, is concomitant with the upregulation of p53 and Akt[56]. Since p53 and Akt serve as pro- and anti-apoptotic factors, respectively, we speculate that the anti-apoptotic effects of Akt may overwhelm the pro-apoptotic role of p53, causing cells to abort apoptosis (Akt/p53 signaling). Some of the currently verified molecular mechanisms underlying the apoptotic arrest associated with tau hyperphosphorylation are summarized in Fig. 1. Further studies are needed to reveal how intracellular tau accumulation affects the expression and/or activity-dependent modification of cell factors regulating viability as noted in this review.

### *Nature of Tau Hyperphosphorylation-associated Neurodegeneration*

Based on these findings and the fact that apoptosis is not the major mechanism of cell loss in the AD brain, we have proposed that hyperphosphorylation of tau may play a dual role in leading neurons to abort acute apoptosis and simultaneously enter chronic degeneration[2]. We believe that tau hyperphosphorylation-associated apoptotic arrest is the first step of neurodegeneration in the AD brain; in other words, the apoptotic arrest associated with tau hyperphosphorylation triggers neurodegeneration. Our thoughts are as follows: during the evolution of AD, all neurons experience pro-apoptotic attack (such as by Aβ and oxidative stress); those without tau hyperphosphorylation die from acute apoptosis (as may be the case for the massive loss of cholinergic neurons in the early stage of AD), while those with tau hyperphosphorylation survive (apoptotic escape); however, surviving neurons bearing hyperphosphorylated and accumulated tau proteins are

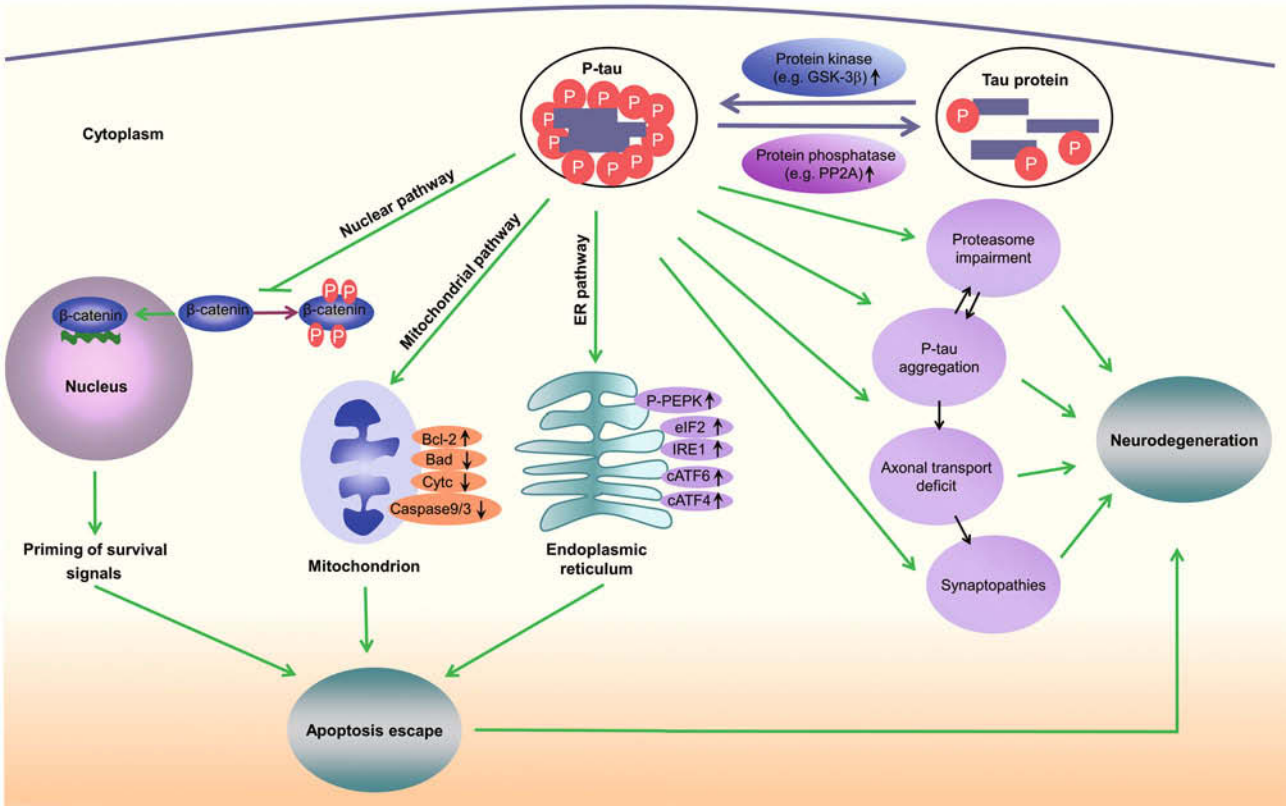


**Fig. 1. Mechanisms underlying apoptotic escape and neurodegeneration associated with tau hyperphosphorylation. Tau hyperphosphorylation renders cells more resistant to apoptosis by rescuing nuclear β-catenin (the nuclear pathway); preserving Bcl-2 and inhibiting Bax, Cytc, and caspases (the mitochondrial pathway); and upregulating the unfolded protein response system (the ER pathway). Simultaneously, tau hyperphosphorylation results in its pathological actions, inhibiting proteasome activity, damaging axonal transport, and causing synaptopathies, which eventually lead to chronic neurodegeneration.**

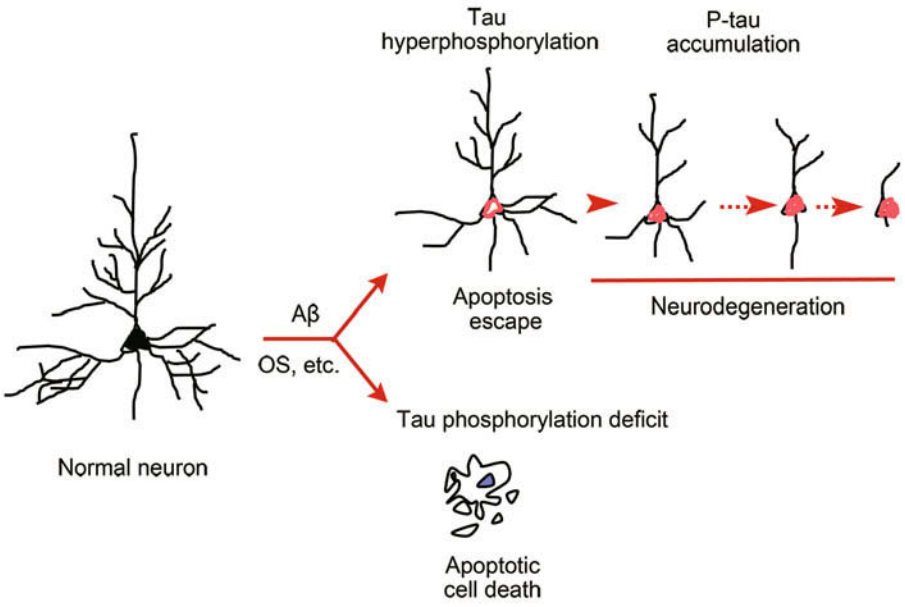


**Fig. 2. Cartoon showing the fate of neurons during AD evolution and the dual role of tau hyperphosphorylation in anti-apoptosis and degeneration. Neurons under oxidative stress (OS) or Aβ stimulation undergo apoptosis when they lack tau hyperphosphorylation. Neurons with tau hyperphosphorylation escape from acute apoptosis, but persistent tau hyperphosphorylation/accumulation leads to chronic neurodegeneration.**

"sick" and destined for chronic neurodegeneration (Fig. 2). Therefore, tau hyperphosphorylation/tangle formation allows neurons to escape from acute apoptosis and survive to the end-stage as seen in the AD brain at autopsy. Our findings reveal the nature of neurodegeneration. Since there is currently no specific molecular marker to measure neurodegeneration, researchers have used markers of apoptosis or necrosis to detect it. Clearly, neurodegeneration is different from apoptosis, rather, it may be a unique and precisely-regulated form of chronic cell death that we have termed "neurodegenerasis"[2].

### *How Should We Evaluate Tau Hyperphosphorylation?*

As mature neurons in the adult brain are rarely replenished, apoptotic escape induced by tau phosphorylation may be one of the mechanisms that evolved to allow neurons to survive apoptotic attack and so avoid rapid and massive neuron loss, while the "sick" neurons can wait for a chance to self-repair.

However, "sick" neurons loaded with hyperphosphorylated tau proteins are no longer able to perform normal physiological functions, such as microtubule assembly and axonal transport. Moreover, the extended survival time of these "sick" neurons by apoptotic escape makes them less resistant to environmental/metabolic insults and also allows tangles to evolve from the hyperphosphorylated tau, which leads to slow but progressive retrograde degeneration. In addition, these "sick" neurons may "infect" neighboring neurons and cause transmissible degeneration through currently unknown mechanisms. Furthermore, since "sick" neurons may not be recognized by the "scavengers" of the brain, these dysfunctional neurons can occupy the limited space and so prohibit neurogenesis, which can normally compensate for neuron loss. Therefore, proper modulation of tau phosphorylation at different stages in the development of AD offers promising opportunities to prevent massive apoptotic neuron loss and to at least slow neurodegeneration.

Received date: 2013-11-18; Accepted date: 2014-01-27

## REFERENCES

[1] Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A

component of Alzheimer paired helical filaments. J Biol Chem 1986, 261: 6084–6089.

[2] Hu YK, Wang X, Li L, Du YH, Ye HT, Li CY. MicroRNA-98 induces an Alzheimer's disease-like disturbance by targeting insulin-like growth factor 1. Neurosci Bull 2013, 29: 745–751.

[3] Wang JZ, Liu F. Microtubule-associated protein tau in development, degeneration and protection of neurons. Prog Neurobiol 2008, 85: 148–175.

[4] Tian Q, Wang J. Role of serine/threonine protein phosphatase in Alzheimer's disease. Neurosignals 2002, 11: 262–269.

[5] Wang JZ, Xia YY, Grundke-Iqbal I, Iqbal K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. J Alzheimers Dis 2013, 33 Suppl 1: S123–139.

[6] Wang JZ, Grundke-Iqbal I, Iqbal K. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. Eur J Neurosci 2007, 25: 59–68.

[7] Sun XJ, Zhao L, Zhao N, Pan XL, Fei GQ, Jin LR*, et al.* Benfotiamine prevents increased beta-amyloid production in HEK cells induced by high glucose. Neurosci Bull 2012, 28: 561–566.

[8] Zhu LQ, Wang SH, Liu D, Yin YY, Tian Q, Wang XC*, et al.* Activation of glycogen synthase kinase-3 inhibits long-term potentiation with synapse-associated impairments. J Neurosci 2007, 27: 12211–12220.

[9] Zhu LQ, Liu D, Hu J, Cheng J, Wang SH, Wang Q*, et al.* GSK-3 beta inhibits presynaptic vesicle exocytosis by phosphorylating P/Q-type calcium channel and interrupting SNARE complex formation. J Neurosci 2010, 30: 3624–3633.

[10] Liu SJ, Zhang AH, Li HL, Wang Q, Deng HM, Netzer WJ*, et al.* Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads to hyperphosphorylation of tau and impairment of spatial memory. J Neurochem 2003, 87: 1333–1344.

[11] Xiong YS, Wang DL, Tan L, Wang X, Chen LM, Gong CX*, et al.* Inhibition of glycogen synthase kinase-3 reverses tau hyperphosphorylation induced by pin1 down-regulation. CNS Neurol Disord Drug Targets 2013, 12: 436–443.

[12] Yin J, Liu YH, Xu YF, Zhang YJ, Chen JG, Shu BH*, et al.* Melatonin arrests peroxynitrite-induced tau hyperphosphorylation and the overactivation of protein kinases in rat brain. J Pineal Res 2006, 41: 124–129.

[13] Peng CX, Hu J, Liu D, Hong XP, Wu YY, Zhu LQ*, et al.* Disease-modified glycogen synthase kinase-3beta intervention by melatonin arrests the pathology and memory deficits in an Alzheimer's animal model. Neurobiol Aging 2013, 34: 1555–1563.

[14] Zhang YJ, Xu YF, Liu YH, Yin J, Wang JZ. Nitric oxide induces tau hyperphosphorylation via glycogen synthase kinase-3beta activation. FEBS Lett 2005, 579: 6230–6236.

[15] Li XH, Du LL, Cheng XS, Jiang X, Zhang Y, Lv BL*, et al.* Glycation exacerbates the neuronal toxicity of beta-amyloid. Cell Death Dis 2013, 4: e673.

[16] Li XH, Lv BL, Xie JZ, Liu J, Zhou XW, Wang JZ. AGEs induce Alzheimer-like tau pathology and memory deficit via RAGE-mediated GSK-3 activation. Neurobiol Aging 2012, 33: 1400–1410.

[17] Liu YH, Wei W, Yin J, Liu GP, Wang Q, Cao FY*, et al.* Proteasome inhibition increases tau accumulation independent of phosphorylation. Neurobiol Aging 2009, 30: 1949–1961.

[18] Wang ZF, Li HL, Li XC, Zhang Q, Tian Q, Wang Q*, et al.* Effects of endogenous beta-amyloid overproduction on tau phosphorylation in cell culture. J Neurochem 2006, 98: 1167–1175.

[19] Liu SJ, Zhang JY, Li HL, Fang ZY, Wang Q, Deng HM*, et al.* Tau becomes a more favorable substrate for GSK-3 when it is prephosphorylated by PKA in rat brain. J Biol Chem 2004, 279: 50078–50088.

[20] Hong XP, Peng CX, Wei W, Tian Q, Liu YH, Yao XQ*, et al.* Essential role of tau phosphorylation in adult hippocampal neurogenesis. Hippocampus 2010, 20: 1339–1349.

[21] Gong CX, Shaikh S, Wang JZ, Zaidi T, Grundke-Iqbal I, Iqbal K. Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain. J Neurochem 1995, 65: 732–738.

[22] Wang JZ, Gong CX, Zaidi T, Grundke-Iqbal I, Iqbal K. Dephosphorylation of Alzheimer paired helical filaments by protein phosphatase-2A and -2B. J Biol Chem 1995, 270: 4854–4860.

[23] Wang JZ, Grundke-Iqbal I, Iqbal K. Restoration of biological activity of Alzheimer abnormally phosphorylated tau by dephosphorylation with protein phosphatase-2A, -2B and -1. Brain Res Mol Brain Res 1996, 38: 200–208.

[24] Gong CX, Lidsky T, Wegiel J, Zuck L, Grundke-Iqbal I, Iqbal K. Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease. J Biol Chem 2000, 275: 5535–5544.

[25] Sun L, Liu SY, Zhou XW, Wang XC, Liu R, Wang Q*, et al.* Inhibition of protein phosphatase 2A- and protein phosphatase 1-induced tau hyperphosphorylation and impairment of spatial memory retention in rats. Neuroscience 2003, 118: 1175–1182.

[26] Yang Y, Yang XF, Wang YP, Tian Q, Wang XC, Li HL*, et al.* Inhibition of protein phosphatases induces transport deficits and axonopathy. J Neurochem 2007, 102: 878–886.

[27] Xiong Y, Jing XP, Zhou XW, Wang XL, Yang Y, Sun XY*, et al.* Zinc induces protein phosphatase 2A inactivation and tau hyperphosphorylation through Src dependent PP2A (tyrosine

307) phosphorylation. Neurobiol Aging 2013, 34: 745–756.

[28] Yu G, Yan T, Feng Y, Liu X, Xia Y, Luo H, *et al*. Ser9 phosphorylation causes cytoplasmic detention of I2PP2A/SET in Alzheimer disease. Neurobiol Aging 2013, 34: 1748–1758.

[29] Chai GS, Jiang X, Ni ZF, Ma ZW, Xie AJ, Cheng XS, *et al.* Betaine attenuates Alzheimer-like pathological changes and memory deficits induced by homocysteine. J Neurochem 2013, 124: 388–396.

[30] Cheng XS, Zhao KP, Jiang X, Du LL, Li XH, Ma ZW, *et al.* Nmnat2 attenuates Tau phosphorylation through activation of PP2A. J Alzheimers Dis 2013, 36: 185–195.

[31] Liu XP, Zheng HY, Qu M, Zhang Y, Cao FY, Wang Q, *et al.* Upregulation of astrocytes protein phosphatase-2A stimulates astrocytes migration via inhibiting p38 MAPK in tg2576 mice. Glia 2012, 60: 1279–1288.

[32] Yao XQ, Zhang XX, Yin YY, Liu B, Luo DJ, Liu D, *et al*. Glycogen synthase kinase-3beta regulates Tyr307 phosphorylation of protein phosphatase-2A via protein tyrosine phosphatase 1B but not Src. Biochem J 2011, 437: 335–344.

[33] Liu GP, Zhang Y, Yao XQ, Zhang CE, Fang J, Wang Q, *et al.* Activation of glycogen synthase kinase-3 inhibits protein phosphatase-2A and the underlying mechanisms. Neurobiol Aging 2008, 29: 1348–1358.

[34] Wang JZ, Grundke-Iqbal I, Iqbal K. Glycosylation of microtubule-associated protein tau: an abnormal posttranslational modification in Alzheimer's disease. Nat Med 1996, 2: 871–875.

[35] Li X, Lu F, Wang JZ, Gong CX. Concurrent alterations of O-GlcNAcylation and phosphorylation of tau in mouse brains during fasting. Eur J Neurosci 2006, 23: 2078–2086.

[36] Han J, Wang XF, Yao HI, Gao C, Li F, Zhang BY, *et al.* Prion protein inhibits tau-mediated microtubule formation. Neurosci Bull. 2005,21(6): 398–403.

[37] Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K. Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. Proc Natl Acad Sci U S A 1994, 91: 5562–5566.

[38] Wang JZ, Wu Q, Smith A, Grundke-Iqbal I, Iqbal K. Tau is phosphorylated by GSK-3 at several sites found in Alzheimer disease and its biological activity markedly inhibited only after it is prephosphorylated by A-kinase. FEBS Lett 1998, 436: 28–34.

[39] Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci U S A 2001, 98: 6923–6928.

[40] Salehi A, Delcroix JD, Mobley WC. Traffic at the intersection of neurotrophic factor signaling and neurodegeneration. Trends Neurosci 2003, 26: 73–80.

[41] Xiao AW, He J, Wang Q, Luo Y, Sun Y, Zhou YP, *et al.* The origin and development of plaques and phosphorylated tau are associated with axonopathy in Alzheimer's disease. Neurosci Bull 2011, 27: 287–299.

[42] Spittaels K, Van den Haute C, Van Dorpe J, Bruynseels K, Vandezande K, Laenen I, *et al.* Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein. Am J Pathol 1999, 155: 2153–2165.

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

[44] Perez M, Hernandez F, Lim F, Diaz-Nido J, Avila J. Chronic lithium treatment decreases mutant tau protein aggregation in a transgenic mouse model. J Alzheimers Dis 2003, 5: 301–308.

[45] Noble W, Planel E, Zehr C, Olm V, Meyerson J, Suleman F, *et al.* Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. Proc Natl Acad Sci U S A 2005, 102: 6990–6995.

[46] Mori H, Kondo J, Ihara Y. Ubiquitin is a component of paired helical filaments in Alzheimer's disease. Science 1987, 235: 1641–1644.

[47] Ren QG, Liao XM, Wang ZF, Qu ZS, Wang JZ. The involvement of glycogen synthase kinase-3 and protein phosphatase-2A in lactacystin-induced tau accumulation. FEBS Lett 2006, 580: 2503–2511.

[48] Ren QG, Liao XM, Chen XQ, Liu GP, Wang JZ. Effects of tau phosphorylation on proteasome activity. FEBS Lett 2007, 581: 1521–1528.

[49] Keck S, Nitsch R, Grune T, Ullrich O. Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. J Neurochem 2003, 85: 115–122.

[50] Morsch R, Simon W, Coleman PD. Neurons may live for decades with neurofibrillary tangles. J Neuropathol Exp Neurol 1999, 58: 188–197.

[51] Spires TL, Orne JD, SantaCruz K, Pitstick R, Carlson GA, Ashe KH, *et al.* Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse model of tauopathy. Am J Pathol 2006, 168: 1598–1607.

[52] Allen B, Ingram E, Takao M, Smith MJ, Jakes R, Virdee K, *et al*. Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. J Neurosci 2002, 22: 9340–9351.

[53] Arendt T, Stieler J, Strijkstra AM, Hut RA, Rudiger J, Van der Zee EA, *et al*. Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. J Neurosci 2003,

23: 6972–6981.

[54] Zhou F, Zhu X, Castellani RJ, Stimmelmayr R, Perry G, Smith MA*, et al*. Hibernation, a model of neuroprotection. Am J Pathol 2001, 158: 2145–2151.

[55] Zhang DL, Chen YQ, Jiang X, Ji TT, Mei B. Oxidative damage increased in presenilin1/presenilin2 conditional double knockout mice. Neurosci Bull 2009, 25: 131–137.

[56] Liu GP, Wei W, Zhou X, Zhang Y, Shi HH, Yin J*, et al.* I(2)(PP2A) regulates p53 and Akt correlatively and leads the neurons to abort apoptosis. Neurobiol Aging 2012, 33: 254–264.

[57] Li HL, Wang HH, Liu SJ, Deng YQ, Zhang YJ, Tian Q*, et al*. Phosphorylation of tau antagonizes apoptosis by stabilizing beta-catenin, a mechanism involved in Alzheimer's neurodegeneration. Proc Natl Acad Sci U S A 2007, 104: 3591–3596.

[58] Wang ZF, Yin J, Zhang Y, Zhu LQ, Tian Q, Wang XC*, et al.* Overexpression of tau proteins antagonizes amyloid-beta-potentiated apoptosis through mitochondria-caspase-3 pathway in N2a cells. J Alzheimers Dis 2010, 20: 145–157.

[59] Wang HH, Li HL, Liu R, Zhang Y, Liao K, Wang Q*, et al.* Tau overexpression inhibits cell apoptosis with the mechanisms involving multiple viability-related factors. J Alzheimers Dis 2010, 21: 167–179.

[60] Liu XA, Liao K, Liu R, Wang HH, Zhang Y, Zhang Q*, et al.* Tau dephosphorylation potentiates apoptosis by mechanisms involving a failed dephosphorylation/activation of Bcl-2. J Alzheimers Dis 2010, 19: 953–962.

[61] Gu XM, Huang HC, Jiang ZF. Mitochondrial dysfunction and cellular metabolic deficiency in Alzheimer's disease. Neurosci Bull 2012, 28: 631–640.