STATE OF THE ART

Post-translational modifications of tau protein

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Abstract

Microtubule-associated protein tau is a phosphoprotein whose expression and phosphorylation is developmentally regulated. Whereas in adult mammalian brain several isoforms are produced from a single gene by alternative splicing, in fetal brain only a single isoform exists, corresponding to the smallest of the tau isoforms. Main physiological function of tau is the promotion of assembly and stabilization of microtubular network, which is essential for normal axonal transport of vesicles within the neuron. In human, tau protein undergoes several posttranslational modifications: such as phosphorylation, truncation, nitration, glycation, glycosylation, ubiquitination and polyaminations. When these modifications are disturbed, they play a serious role during the pathogenesis of Alzheimer's disease (AD). Hyperphosphorylation and truncation as the early events in AD pathogenesis, play significant role in the formation of neurofibrillary pathology. Phosphorylated tau has reduced capability in binding to microtubules and hyperphosphorylation together with truncation contributes to the formation of pathological tau filaments. This leads to destabilization of microtubular network and subsequent impairment of microtubule associated axonal transport.
Since many data suggest that sporadic AD is the "disease of posttranslational modifications" of tau protein, more detailed investigation of tau protein modifications is urgently needed in order to understand pathogenesis of sporadic Alzheimer's disease *(Fig. 1, Ref. 86)*.
Key words: Alzheimer's disease, phosphorylation, tau truncation, post-translational modifications.

Tau is a major neuronal microtubule associated protein (MAP). The main biological function of tau is promoting of assembly and stability of microtubules (MT). During normal development, tau protein undergoes several posttranslational modifications: such as phosphorylation, truncation, nitration, glycation, glycosylation, ubiquitination and polyamination. To the most important posttranslational modifications of tau belong truncation and phosphorylation. Phosphorylated tau has reduced capability in binding to MT and hyperphosphorylation contributes to the formation of pathological tau filaments, as observed in Alzheimer's disease (Alonso et al, 1994, 2001; Gorath et al, 2001). Tau in adult brain normally contains 2–3 moles of phosphates per mole of tau, but hyperphosphorylated tau from AD brain contains 5 to 9 mol of phosphate group per mol of the protein (Kopke et al, 1993). In vitro studies also indicate that the abnormal glycosylation modulates phosphorylation of tau with PKA, GSK-3ß and cdk5 and inhibits dephosphorylation of tau with PP2A and PP5 (Liu et al, 2002). Glycosylation and glycation, both probably help to maintain and stabilize neurofibrillary tangles (NFT) (Ledesma et al, 1996). Major constituent of the NFT are Paired Helical Filaments (PHFs), which consist from hyperphosphorylated tau and are present in the brains of AD patients (Kosik, 1992; Iqbal et al, 1989; Grundke-Iqbal et al, 1986). Tau in NFTs is hyperphosphorylated at specific sites and exhibits an abnormal conformation (Grundke-Iqbal et al, 1986; Weaver et al, 2000). The nitrative damage to tau may be a pathological process that occurs dynamically at several steps in the sequence of events leading to the formation of glial and neu-

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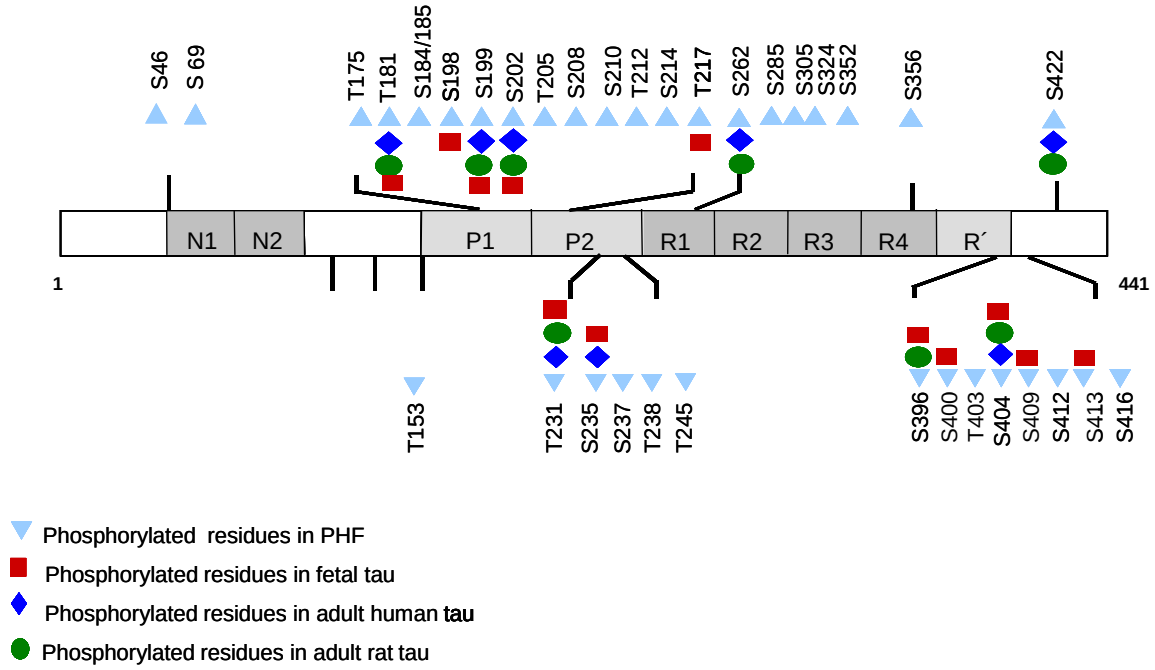

Fig. 1. Tau protein is phosphosphorylated in embryonic and early postnatal period much more than in adulthood.

ronal filamentous tau inclusions in selected regions of the diseased brain (Horiguchi et al, 2003). A critical role of tau protein in the pathogenesis of AD was definitely recognized in genetic studies. The discovery of mutations in the tau gene that promote familial FTDP-17 indicates that tau protein alone could be a sole cause of the neurodegenerative disorders, including AD (Avila, 2006; Spillantini et al, 1998).

Tau phosphorylation

Tau protein is phosphosphorylated in embryonic and early postnatal period much more than in adulthood (Fig. 1) (Goedert et al, 1993; Brion et al, 1993; Wagner et al, 1996). Physiological pattern of tau protein phosphorylation is therefore represented by extensive phosphorylation of single tau isoform during embryonal development that is followed by its disappearance after early postnatal period. Simultaneously the tau protein isoforms appear in this ontogenetic period. Tau phosphorylation controls MT dynamics during normal neurite growth and maturation. Tau in adulthood is phosphorylated in very low extent in healthy person. However during development of AD, phosphorylation of tau is increasing rapidly. The abnormal hyperphosphorylation of tau could be the result of upregulation of tau kinases or downregulation of tau phosphatases. In addition to the activities of the tau kinases and phosphatases, the phosphorylation of tau is also regulated by its conformational state (Iqbal et al, 2005). In PHFs there are all six isoforms assembled and thirty-one phosphorylation sites have been identified here (Iqbal et al, 1989; Morishima-Kawashima et al, 1995; Goedert et al, 1989).

Tau phosphorylation might have a developmental-specific role and take place early in ontogenesis and hyperphosphorylation of tau in Alzheimer's disease is obviously a pathogenic mechanism. Therefore thorough investigation of tau protein phosphorylation and its regulation in neurons is important for understanding of neurofibrillary degeneration in AD.

Phosphorylation sites on tau protein

On the longest brain tau isoform (441 amino-acids) there are seventy-nine putative Ser or Thr phosphorylation sites. Using phosphorylation-dependent monoclonal antibodies against tau, mass spectrometry and sequencing of tau, at least thirty phosphorylation sites have been described, including Thr39, Ser46Pro, Thr50Pro, Thr69Pro, Thr153Pro, Thr175Pro, Thr181Pro, Ser184, Ser185, Ser195, Ser198, Ser199Pro, Ser202Pro, Thr205Pro, Ser208, Ser210, Thr212Pro, Ser214, Thr217Pro, Thr231Pro, Ser235Pro, Ser237, Ser238, Ser245, Ser258, Ser262, Ser285, Ser293, Ser305, Ser320, Ser324, Ser352, Ser356, Thr377, Ser396Pro, Ser400, Thr403, Ser404Pro, Ser409, Ser412, Ser413, Ser416 and Ser422Pro (Hasegawa et al, 1992; Morishima-Kawashima et al, 1995) (Tab. 1). All of these sites are localized outside the microtubule-binding domains with the exception of Ser262 (R1), Ser285 (R1-R2 inter-repeat), Ser305 (R2-R3 inter-repeat), Ser324 (R3), Ser352 (R4) and Ser356 (R4). A large number of the abnormally hyperphosphorylated sites in tau are proline-directed, i.e. serine/treonine followed by proline (Ser-Pro and Thr-Pro) (Seubert et al, 1995; Roder et al, 1997; Iqbal et al, 2005).

Tau kinases

Most of the kinases involved in tau phosphorylation belong to the proline-directed protein kinases (PDPK), which include mitogen activated protein kinase (MAPK), glycogen synthase kinase 3 (GSK-3), tau-tubulin kinase and cyclin-dependent kinases including cdc2 and cdk5 (Goedert et al, 1997; Vulliet et al, 1992; Hanger et al, 1992).

To the non-PDPK belong cAMP-dependent protein kinase (PKA), calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase C, casein kinase I (CK-1), and casein kinase-II (CK-2) (Sengupta et al, 1997; Singh et al, 1996 a).

Proline-directed protein kinases

Glycogen synthase kinase-3ß (GSK-3ß) was originally identified as a kinase that phosphorylates and inactivates glycogen synthase, which catalyses a regulatory step in insulin-mediated glycogen synthesis. Insulin induces a phosphorylation dependent down-regulation of GSK-3, which leads to the activation of glycogen synthase and therefore increased glycogen synthesis (Woodgett, 1991). The GSK-3 has two isoforms (and), which are encoded by different genes. GSK-3α and GSK-3β have been shown to induce cellular phosphorylation of tau in such a way that it resembles fetal/PHF tau (Lovestone et al, 1994; Sperber et al, 1995). Glycogen synthase kinase-3β, a predominant tau kinase, accumulates in the cytoplasm of pretangle neurons (Ishiguro et al, 1993). GSK-3α can phosphorylate sites, which are in prolin-rich regions of a protein, or sites where a Ser or Thr is prephosphorylated by another protein kinase (Dajani et al, 2001; Grimes and Jope, 2001). It was found that after prephosphorylation of tau by cdk5 the intensity of phosphorylation in several Alzheimer-like epitopes is greatly increased by GSK-3 (Sengupta et al, 1997).

One of the more prominent sites in tau protein is Thr231, which is phosphorylated by GSK-3β (Goedert et al, 1994). Phosphorylation of Thr231, which is increased after prephosphorylation of Ser235, plays a significant role in regulating tau,s ability to bind and stabilize microtubules, because it changes tau conformation (Jicha et al, 1997; Daly et al, 2000). It is likely that increased phosphorylation of tau at Thr231 may be an early event in the pathogenic processes of AD (Cho and Johnson, 2003). Also epitope Ser396 is phosphorylated after prephosphorylation of Ser400 (Li and Paudel, 2006).

Cyclin-dependent kinase 5 (Cdk5) has been characterized as a proline-directed Ser/Thr protein kinase that contributes to phosphorylation of human tau on Ser202, Thr205, Thr212, Thr217, Ser235, Ser396 and Ser404. These are the sites on tau that are hyperphosphorylated in AD brains (Maccioni et al, 2001; Tsai et al, 2004). Cdk5, which is active in post mitotic neurons, has been implicated in cytoskeleton assembly and its organization during axonal growth (Maccioni et al, 2001). Therefore, cdk5 appears as one of the important kinases phosphorylating tau in neuronal cells. The cdk5/p35 system participates in normal development of neuronal cells and the axonal growth. Cdk5 and its neuron-specific activator p35 are essential molecules for neuronal migration and for the laminar configuration of the cerebral cortex. Disruption of the p35 gene is not harmful, since the activity could be compensated by activator p39 (Tsai et al, 2004). Conversion of p35 to p25 causes prolonged activation and miss-localization of cdk5 and the hyperphosphorylation of tau. In vitro studies indicate that kinase activity of cdk5 in phosphorylation of tau is significantly higher in the presence of p25 as opposed to p35 (Maccioni et al, 2001). Cdk5 is deregulated in AD brains and may contribute to the pathogenesis of AD.

Non-proline directed protein kinase

Cyclic-AMP-dependent kinase (PKA, A-kinase, protein kinase A) is a ubiquitous serine/ threonine protein kinase present in a variety of tissues. The intracellular cAMP level regulates responsiveness of cell by altering the interaction between the catalytic C and regulatory R subunits of PKA. The inactive PKA holoenzyme is activated when cAMP binds to the regulatory subunits, which induces the release of two active catalytic subunits. PKA phosphorylates tau at Ser214, Ser217, Ser396/404, and at Ser416. PKA and MARK kinases have also been shown to phosphorylate tau at Ser262 (Drewes et al, 1997).

Ca2+/calmodulin-dependent protein kinase II (CaMKII) regulates important neuronal functions, including neurotransmitter synthesis and neurotransmitter release, modulation of ion channel activity, synaptic plasticity and gene expression (Kennedy et al, 1981). CaMKII phosphorylates tau at Ser262, Ser356, Ser409 and Ser416 and some of these sites are phosphorylated in brains of patients with Alzheimer's disease (Singh et al, 1996b; Steiner et al, 1990). For instance, if Ser262 and Ser356 (present at tubulin-binding region of tau) are phosphorylated, the ability of tau to bind to microtubules and promote MT assembly is reduced (Singh et al, 1996b).

Tau phosphatases

Several studies have shown that three major protein phosphatases (PP), PP1, PP2A and PP2B (calcineurin), but not PP2C can dephosphorylate tau in vitro (Gong et al, 1994).

Phosphatase 2A (PP2A) is a heterotrimer, composed of the structural A (65kDa) and catalytic C (36 kDa) subunits forming the constitutive core of the enzyme, which associates with regulatory B subunits (Janssens and Goris, 2001). PP2A is localized on microtubules and binds directly to tau (Sontag et al, 1999). Decreased activity of PP2A was found in AD brain (Gong et al, 1993). Nevertheless, PP2A does not dephosphorylate all the phosphorylation sites of tau, and down regulation of PP2A in brain could not produce PHFs (Kins et al, 2001). It seems that PP2A is the major phosphatase that regulates phosphorylation of tau in the brain (Goedert et al, 1995; Gong et al, 1994). Hyperphosphorylation of tau at the same sites as in AD was observed after inhibition of PP2A activity by okadaic acid in cultured cells and in metabolically active rat brain slices. The hyperphosphorylation was not only the result of direct decrease in dephosphorylation,

but it was associated also with promoting the activities of CaMKII, PKA and MAP kinases (Bennecib et al, 2001).

Phosphatase 2B (PP2B, calcineurin) is a heterodimeric protein with a catalytic subunit A (60kDa) and a regulatory subunit B (19kDa) that binds Ca^{2+} (the protein is under the direct control of Ca^{2+}). PP2B is closely associated with developing of microtubules and microfilaments. An inhibition of PP2B affects axonal determination and could cause a hyperphosphorylation of tau (Ferreira et al, 1993). PP2A and PP2B can dephosphorylate serine-proline and threonine-proline sites including Ser262, which is phospho-site specific to AD and is known as important site for binding tau protein to microtubules (Harris et al, 1993).

Protein phosphatase 5 (PP5) contains a C-terminal catalytic domain structurally related to the PP1/PP2A/PP2B family, and an N-terminal regulatory domain consisting of three tetratricopeptide repeats that usually mediate protein-protein interaction (Chinkers, 2001). PP5 is ubiquitously expressed in all types of mammalian tissue, with a relatively high level in the brain (Bahl et al, 2001). PP5 dephosphorylates tau protein, which is localized in the neuronal cytoplasm in human brain and appears to be associated with MT (Gong et al, 2004). It was found, that levels of unsaturated fatty acids are decreased in the brain regions, which are affected with neurofibrillary tangles (Bahl et al, 2001). Since the unsaturated fatty acids stimulate PP5 (Prasad et al, 1998) it is not excluded that the hyperphosphorylation of tau in these regions is at least partially a result of lowered PP5 activity due to the decreased level of fatty acids (Chen et al, 1994).

Tau glycosylation

Glycosylation is one of the most common co-/ posttranslational modifications of proteins, through which oligosacharides covalently attach to the side chain of polypeptides under enzymatic catalysis. According to the nature of glycosidic bonds, two types of glycosylation are classically defined: O-linked (in which sugars are linked to the hydroxyl groups of serine or threonine residues) and N-linked (in which sugars are linked to the amide group of the asparagines side chain of proteins) (Gong et al, 2005).

O-glycosylation is a dynamic and abundant post-translational modification that is characterized by the addition of an O-linked N-acetylglucosamine (O-GlcNac) residue on Ser or Thr in the proximity of Pro residues. The number of O-GlcN-acylated sites on tau proteins is lower than the number of phosphorylation sites (Sergeant et al, 2005). Site specific or stoichiometric changes in O-GlcN-acylation may modulate tau function. Phosphorylation and O-GlcN-acylation may have opposite effects. O-GlcN-acylation of tau proteins and other microtubule-associated proteins suggest a role for O-GlcNac in mediating their interactions with tubulin (Buee et al, 2000). N-glycosylation normally occurs in the rough endoplasmic reticulum and Golgi apparatus and is generally believed to be restricted to membrane-bound and secreted proteins (Gong et al, 2004).

Aberrant glycosylation of tau may precede abnormal hyperphosphorylation in AD brain raised the intriguing possibility that former might promote the latter. The effect of glycosylation on subsequent phosphorylation of tau is site-specific, and the influence at each phosphorylation site is different. Aberrant glycosylation facilitates hyperphosphorylation of tau both by promoting phosphorylation and by inhibiting dephosphorylation of tau (Gong et al, 2005).

Deglycosylation of PHF tangles by endoglycosidase F/Nglycosidase F converts them into bundles of straight filaments, similar to those generated by the interaction of normal tau and abnormally hyperphosphorylated tau. Dephosphorylation of deglycosylated PHF tangles results in increased tau release (Wang et al, 1996).

Ubiquitination

Ubiquitin-proteasome system participates in the defense against unfolded proteins and provides an effective protein quality control system that is essential for cellular functions and survival. The accumulation of misfolded proteins triggers stress responses that lead to the induction of heat shock proteins (HSPs) (Richter-Landsberg and Goldbaum, 2003). HSPs help to target nonrepairable proteins to the ubiquitin proteasomal pathway, i.e., misfolded proteins are ubiquitinated and degraded by the proteasomal machinery (Schwartz and Ciechanover, 1999). Proteins after association with ubiquitin, which is a 76-amino acid protein, are being degraded in an ATP-dependent manner (Avila et al, 2004).

PHF-tau, but not normal tau or abnormally hyperphosphorylated tau is modified with polyubiquitins (Morishima and Ihara, 1994; Iqbal and Grundke-Iqbal, 1991). Although PHF-tau is highly ubiquitinated, it is apparently not degraded, but is deposited as NFTs in AD brain. It is still not understood why ubiquitinated PHF-tails are not degraded and cleared in AD brain (Keller et al, 2000).

Tau glycation

PHF isolated from AD brains are glycated, which refers to non-enzymatic linkage of glucose or other reducing sugars to the amino side chain of polypeptide, whereas no glycan is detected in normal tau (Munch et al, 2002). Glycation like ubiquitination are late events. Glycation normally leads to subsequent oxidation, dehydration, condensation, and finally formation of heterogenous products called advanced glycation end products (Yan et al, 1995).

Tau nitration

Protein tyrosine nitration is a posttranslational modification, which plays a role in physiological processes, including signal transduction. Nitrated tau has been found in the neurofibrillary tangles of AD, however, how nitrated tau may contribute to AD pathology is not understand. Two biomarkers of nitration injury (3-nitrotyrosine and dityrosine) were elevated consistently in the hippocampus, neocortical regions and cerebrospinal fluid (CSF)

of AD patients (Hensley et al, 1998). Some studies have demonstrated that tyrosine nitration of tau significantly inhibits microtubule-binding activity compared with normal tau. Also nitration of tau may disrupt cytoskeleton of the cells (Zhang et al, 2005; Blanchard-Fillion et al, 2001; Souza et al, 2000).

Tau polyamination

Tissue transglutaminase is upregulated in Alzheimer's disease brain and is localized to neurofibrillary tangles with the tau protein. Tau is an *in vitro* substrate of tissue transglutaminase, being cross-linked and/or polyaminated. Tissue transglutaminase can incorporate polyamines into tau both in vitro and *in situ*. Polyamination of tau does not affect its microtubule binding, but makes tau less susceptible to the degradation by the calcium-activated protease calpain (Tucholski et al, 1999). Tissue transglutaminase also catalyzes the linkage between glutamine residues and primary amines of lysine residues of proteins leading to an insoluble and protease-resistant high molecular weight complexes (Appelt and Balin, 1997).

Tau truncation

Truncation of tau has been observed in PHFs, with microtubule-binding domains as a predominant core structure of these filaments (Wischik et al, 1988; Novak et al, 1989, 1994; Mena et al, 1996). Truncation of a large portion of tau at C-terminus has also been proposed to lead to polymerization and resultant filament accumulation in AD. Extensive studies of pronase-digested filaments isolated from AD brains and immunochemical studies of SDS-soluble PHF preparations have demonstrated that tau and specific proteolytic fragments of tau compose the PHF (Novak et al, 1991; Novak et al, 1993). PHF-tau is resistant to proteolysis, because some of the potential proteolytic sites are buried in the core of filaments and are inaccessible to proteases (Novak, 1994). The monoclonal antibody, MN 423, raised against the core of PHF, recognizes peptides of tau terminating at E391; addition or removal of a single amino acid in the C terminus totally abolishes this immunoreactivity (Novak et al, 1989, 1993). Tau truncated at E391 exists in NFTs and also in abnormal neurites that are present in the brains of patients with AD (Novak et al, 1993). Studies have indicated that tau truncated at E391 is toxic to cultured cells, suggesting that this could also be the case in NFTs of AD. With help of moAb MN 423 it was discovered that tau truncated at E391 showed weaker potency in promoting of microtubule assembly than longest isoform of human tau with four internal repeats. This finding shows that truncation effectively disables tau protein and makes it available for some form of assembly into PHFs that leads to major pathological changes in neuronal cells (Novak, 1993,1994; Zilka et al, 2006).

Tau cleaved by caspase at D421 was toxic and after cleavage a discrete peptide (AK 422-441) was released. Tau truncated at D421 exerts stronger MT-assembly potency than full-length tau molecule in in vitro assay (Binder et al, 2005; Berry et al, 2003; Fasulo et al, 2000). As these data indicate that the C terminus of tau serves to inhibit tau filament formation, it was reasoned that site-specific phosphorylation in this region of tau molecule would also stimulate assembly by altering the conformation of this putative regulatory region of tau (Binder et al, 2005).

Both the amino- and C-terminal regions flanking the microtubule binding domains of tau are inhibitory to self-assembly into filaments. Thus, neutralisation of these inhibitory domains by abnormal hyperphosphorylation, or partially by truncation might result in the formation of neurofibrillary tangles (Alonso et al, 2001).

It has been shown that truncated tau proteins are conformationally different from normal healthy tau (Vechterova et al, 2003; Skrabana et al, 2004). Subpopulations of these structurally different tau species promoted abnormal microtubule assembly in vitro suggesting toxic gain of function. Neuronal expression of the most active human truncated tau protein in rats led to the development of the neurofibrillary degeneration of Alzheimer's type. Biochemical analysis of neurofibrillary changes revealed that massive sarcosyl insoluble tau complexes consisted of human Alzheimer's tau and endogenous rat tau in ratio 1:1 including characteristic Alzheimer's disease (AD)-specific proteins (A68). These results represent first insight into the possible causative role of truncated tau in AD neurofibrillary degeneration in vivo (Zilka et al, 2006).

Conclusion

Tau protein undergoes several posttranslational modifications, which play different roles during the pathological process leading to Alzheimer's disease. Some of the modifications, such as hyperphosphorylation, truncation and glycosylation, occur at early stage of tau pathology. Hyperphosphorylation and truncation, but not glycosylation, inhibit biological activity of tau such as promotion of microtubule assembly and stabilization of microtubules. On the other hand glycosylation of tau stabilizes the structure of neurofibrillary tangles. The fact that assembled tau in tauopathies is always hyperphosphorylated and truncated is consistent with the involvement of abnormal phosphorylation and truncation of tau in the pathogenesis of neurofibrillary degeneration. Recent work (Zilka et al, 2006) suggests that pathological truncation and phosphorylation of tau is one of the major events leading to neurodegeneration. Consequently the inhibition of tau protein truncation and hyperphosphorylation is probably one of the most promising therapeutic targets for treatment of AD and other tauopathies. The approaches to block hyperphosphorylation of tau include inhibition of one or more tau kinases, activation of one or more tau phosphatases and enhancement of O-GlcNAcylation of tau. Other modifications of tau, such as ubiquitination and glycation are late events in AD. Detailed pathophysiological relevance of tau protein modifications in pathogenesis of AD and other tauopathies deserve further investigation.

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