

Review Article

Dysregulation of Protein Phosphorylation/Dephosphorylation in Alzheimer's Disease: A Therapeutic Target

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Studies during the last two decades have provided new insights into the molecular mechanism of Alzheimer's disease (AD). One of the milestone findings in AD research was the demonstration that neurofibrillary degeneration characterized by tau pathology is central to the pathogenesis of AD and other tauopathies and that abnormal hyperphosphorylation of tau is pivotal to neurofibrillary degeneration. This article reviews the recent research advances in tau pathology and the underlying dysregulation of the protein phosphorylation/dephosphorylation system. An updated model of the mechanism of neurofibrillary degeneration is also presented, and a promising therapeutic target to treat AD by correcting dysregulation of protein phosphorylation/dephosphorylation is discussed.

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INTRODUCTION

Although Alzheimer's disease (AD) and its main brain histopathology, that is, senile plaques and neurofibrillary tangles (NFTs), were described a century ago, significant research advances in the disease began only a few decades ago. The discoveries of the major protein components of senile plaques as amyloid β -peptide [1, 2] and of NFTs as abnormally hyperphosphorylated tau [3, 4] in the 1980s initiated a new era of AD research. Since then, much research has focused on the molecular mechanisms of initiation and formation of the senile plaques and NFTs and their roles in the pathogenesis of AD. Evidence accumulated in the last two decades indicates that malprocessing of both tau and β -amyloid precursor protein, which produces β -peptide, is pivotal, if not central, to the molecular mechanism of AD. The severity of dementia symptoms in AD strongly correlates to the number of NFTs, but not of senile plaques, in AD brains [5–9], suggesting that tau pathology might be associated with the disease mechanism more directly. Abnormal hyperphosphorylation of tau and its deposits in the brain is also seen in several other neurodegenerative diseases that are collectively named tauopathies (for review, see [10, 11]). The discovery of tau mutations that cause hereditary frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) [12–14] further indicates that tau abnormality alone is sufficient to produce dementia. Therefore, for developing

rational therapeutic treatment of AD, it is essential to understand the molecular mechanism by which tau abnormalities lead to neurofibrillary degeneration.

Because tau aggregated in the brain of AD and all other tauopathies is always abnormally hyperphosphorylated, numerous studies have focused on the roles of the abnormal hyperphosphorylation and the mechanism leading to tau hyperphosphorylation. Recent studies demonstrate that it is the abnormal hyperphosphorylation that makes tau lose its normal function to stimulate microtubule assembly, gain toxic activity, and aggregate into NFTs [15–23]. In addition to tau, several other brain proteins such as neurofilaments, microtubule-associated protein (MAP) 1 B, β -tubulin, and β -catenin are also found to be hyperphosphorylated [24–27], suggesting that the protein phosphorylation/dephosphorylation system might be dysregulated in AD brain. This article attempts to review the recent advances in this respect. Because abnormally hyperphosphorylated tau is pivotal to AD and has been extensively studied, this review focuses on tau hyperphosphorylation. Prevention and reversal of abnormal hyperphosphorylation of tau as a potential promising therapeutic strategy is also discussed.

TAU PROTEIN

Tau was first discovered by Weingarten et al [28] as a microtubule-associated protein that stimulates microtubule

assembly. There was not much research interest in tau protein until a decade later, when it was found to make up the paired helical filaments (PHFs) that form NFTs in AD brain [3, 4, 29]. Human tau gene was found on the long arm of chromosome 17 (position 17q21) and was found to contain 16 exons [30]. This single tau gene encodes six tau isoforms in adult human brain as a result of alternative splicing of its mRNA [31]. The six isoforms of tau differ from each other by the presence or absence of one or two inserts (29 or 58 amino acids) in the *N*-terminal part and by the presence of either three or four repeats in the *C*-terminal half. The *N*-terminal inserts are highly acidic. The repeats in the *C*-terminal half of tau are the domains that bind to microtubules [32–34]. The region upstream of the microtubule-binding domains contains many proline residues and, hence, is called the proline-rich region.

The best-known biological functions of tau are to stimulate microtubule assembly and to stabilize microtubule structure. Tau binds to microtubules via its microtubule-binding domains located at the *C*-terminal half of the molecule [32–34]. The *N*-terminal part projects from the microtubule surface, where it may interact with other cytoskeletal elements and the plasma membrane [35, 36]. Each of the six tau isoforms possibly has its particular physiological roles and differential biological activities, because they are differentially expressed during development and have different activities to stimulate microtubule assembly [37, 38]. Only the shortest isoform of tau is expressed in fetal brain, whereas all six isoforms are seen in adult brain [39, 40]. In addition to stimulating microtubule assembly, several studies have suggested that tau may have other physiological functions. It appears to interfere with binding of kinesin and kinesin-like motors to microtubules, leading to a preferential inhibition of plus-end-directed axonal transport [41]. Overexpression of tau inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum [42]. This may explain the symptoms of amyotrophic lateral sclerosis with neurofilament accumulation in motor neurons of several transgenic models of tau overexpression [43–46]. Tau has been found to interact with mitochondria [47], plasma membrane [36], and nucleic acids [48, 49], suggesting that it may act as a mediator between microtubules and these organelles. Tau also appears to interact with src-family nonreceptor tyrosine kinases such as *fyn* [50, 51] and phospholipase *C-γ* [52, 53] via its proline-rich region. These data suggest that tau may also play a role in the signal transduction pathways involving src-family tyrosine kinases and phospholipase *C-γ*. However, the physiological significance of these interactions remains to be elucidated.

As early as 1977, tau was found to be a phosphoprotein [54]. In 1984, it was demonstrated that phosphorylation of tau negatively regulates its activity in promoting microtubule assembly [55]. Because tau is abnormally hyperphosphorylated in AD and other tauopathies, tau phosphorylation has been studied extensively. Normal brain tau contains 2 or 3 moles of phosphates per mole of tau [56–58]. Studies on human brain biopsy tissue indicated that several serine and threonine residues of tau are normally phosphorylated

at substoichiometrical levels [59, 60]. A normal level of phosphorylation appears to be required for tau's optimal function, whereas the hyperphosphorylated tau loses its biological activity [15, 16, 61–69].

ABNORMAL HYPERPHOSPHORYLATION OF TAU IN AD BRAIN

The discovery that tau aggregated in AD brain is abnormally hyperphosphorylated has stimulated many studies on the extent and sites of tau hyperphosphorylation and their role in the pathogenesis of AD. The phosphorylation level of tau isolated from autopsied AD brains is 3- to 4-fold higher than that from normal human brains [56–58]. In addition, the hyperphosphorylated tau is accumulated in both brains [70, 71] and cerebral spinal fluid [72–80] of individuals with AD. All six isoforms of tau are aggregated into PHFs in the abnormally hyperphosphorylated forms in AD brains [3, 4, 31, 81]. To date, at least 37 serine and threonine residues have been found to be phosphorylated in PHF-tau (for review, see [82]). These residues include Thr39, Ser46, Thr69, Thr123, Ser137, Thr153, Thr175, Thr181, Ser198, Ser199, Ser202, Thr205, Ser208, Ser210, Thr212, Ser214, Thr217, Thr231, Ser235, Ser237, Ser238, Ser241, Ser262, Ser285, Ser305, Ser324, Ser352, Ser356, Ser396, Ser400, Thr403, Ser404, Ser409, Ser412, Ser413, Ser416, and Ser422. Many of these residues are also phosphorylated in normal human brains without NFTs at smaller extents, but they are rapidly dephosphorylated during postmortem delay and tissue processing [59, 60]. However, the phosphate groups at these sites are not readily dephosphorylated during the postmortem period and tissue processing of AD brain, probably because of the deficient protein phosphatase activities [83–89]. Some of the phosphorylation sites seen in PHF-tau are not phosphorylated at all in normal brains. These sites include Thr212/Ser214, Thr231/Ser235 [90], and Ser422 [91, 92].

Because all of the previously identified phosphorylation sites of normal tau and PHF-tau are at either serine or threonine residues, it was thought that tau was phosphorylated only at serine and threonine residues. However, recent studies suggest that tau in developing brain and in AD brain is also phosphorylated at tyrosine residues. The src-family nonreceptor tyrosine kinase *fyn* can bind to and phosphorylate tau *in vitro* and in transfected cells [50, 51, 93]. The phosphorylation site of tau was mapped as Tyr18. Tyrosine phosphorylated tau at this position is also seen immunohistochemically in the brain of transgenic mice that express mutated human τ_{P301L} [51]. Williamson et al [94] demonstrated that in primary human and rat brain cortical cultures tau is phosphorylated at Tyr 29 upon treatment with $A\beta$. The tyrosine phosphorylation of tau appears rapid and transient. Interestingly, antibodies specific to tyrosine phosphorylated tau labeled purified PHF-tau, but not normal tau, suggesting that PHF-tau is phosphorylated at the tyrosine residues [93, 94]. In addition, Tyr394 was also found to be phosphorylated in PHF-tau and in tau from fetal brains, and the phosphorylation at this site is catalyzed by another nonreceptor

tyrosine kinase *c-Abl* [95]. It is not clear if the phosphorylation at any of the above tyrosine residues is stoichiometrically significant. Therefore, whether the tyrosine phosphorylation of tau has any pathophysiological relevance remains to be elucidated.

Numerous studies have demonstrated the important role of abnormal hyperphosphorylation of tau in its aggregation into NFTs and in Alzheimer's neurofibrillary degeneration. In cultured cells, hyperphosphorylation of tau after treatment with phosphatase inhibitors impairs its activity to bind to microtubules and induces filamentous aggregation of tau [21]. Pseudohyperphosphorylated tau that simulates abnormally hyperphosphorylated tau by mutation of serine or threonine residues into glutamate at selected AD-related sites exerts a cytotoxic effect, whereas wild-type tau is neutral [22]. In contrast, neurons from tau-knockout mice are resistant to A β -induced neurotoxicity [96]. Overexpression of human tau in combination with phosphorylation by *Drosophila* GSK-3 β homologue Shaggy, but not tau overexpression alone, exacerbates tau-induced neurodegeneration and results in the formation of NFT-like filamentous tau aggregates [23]. This study shows a causal relationship between tau hyperphosphorylation and neurofibrillary degeneration *in vivo*. A study in Disabled-1 (an adapter protein) knockout mice further demonstrates that tau hyperphosphorylation causes early death of the animals [97]. Most importantly, tau in inclusions of all tauopathies in human and animal models is always hyperphosphorylated (for reviews, see [11, 98]). Abnormal hyperphosphorylation of tau appears to precede its aggregation into NFTs in AD brain [57, 99–101]. Taken together, these studies suggest that the abnormal hyperphosphorylation of tau is crucial to neurofibrillary degeneration in AD and other tauopathies.

The largest isoform of human brain tau (441 amino acids) contains 80 serine and threonine residues and five tyrosine residues [31]. Phosphorylation at nearly half of these residues has been reported in PHF-tau (see [82] for review). Many studies have demonstrated that phosphorylation of tau at different sites has different impacts on its biological function and on its pathogenic role. For instance, a quantitative *in vitro* study demonstrated that phosphorylation of tau at Ser262, Thr231, and Ser235 inhibits its binding to microtubules by ~35%, ~25%, and 10%, respectively [102]. In cultured cells, phosphorylation of tau at Ser214 and Ser262 decreases its binding to microtubules and appears to inhibit its assembly to filaments [103]. *In vitro* kinetic studies of the binding between hyperphosphorylated tau and normal tau suggest that phosphorylation of tau at Ser199/Ser202/Thr205, Thr212, Thr231/Ser235, Ser262/Ser356, and Ser422 are among the critical phosphorylation sites that convert tau to a toxic molecule to sequester normal MAPs from microtubules [19]. Further phosphorylation at Thr231, Ser396, and Ser422 promotes self-assembly of tau into filaments. Similarly, tau mutated at Ser396 and Ser404 (changing Ser into Glu) to mimic phosphoserine is more fibrillogenic than wild-type tau [104], and a tau construct in which Ser422 is mutated to Glu shows a significantly increased propensity to aggregate [105]. Consistent

with these observations is that mutation of Ser422 to Ala prevents A β -induced tau aggregation [106]. These results suggest that phosphorylation of Ser422 may play a key role in tau filament formation *in vivo*.

An important question is, by what mechanism is the tau abnormality involved in the pathological cascades that lead to neurodegeneration in AD and other tauopathies. Does a hyperphosphorylation-induced defect in its activity to stimulate microtubule-assembly contribute to cell dysfunction? Is it the formation of insoluble tau aggregates that is pathogenic? Although tau loses its activity to stimulate microtubules, lack of overt phenotype of tau knockout transgenic mice [107] suggests that it is very unlikely that tau abnormality contributes to neurodegeneration via loss of normal function due to its hyperphosphorylation. By a series of studies, we have found that both the abnormally hyperphosphorylated tau isolated from AD brain and *in vitro* hyperphosphorylated tau gain a toxic activity to sequester normal tau and other MAPs, such as MAP1 and MAP2, and cause microtubule disassembly [16, 18, 66, 108]. Upon dephosphorylation, they lose this toxic activity. Polymerization of the hyperphosphorylated tau into PHFs also abolishes this toxic activity (Alonso A et al, unpublished observation). Hence, we speculate that the abnormal hyperphosphorylation of tau causes neurodegeneration by gain of toxic activity rather than by loss of normal activity that can be compensated for by other MAPs and that formation of PHFs/NFTs from the hyperphosphorylated tau in neurons is a defense mechanism by which neurons aim to reduce the toxic activity of the abnormally hyperphosphorylated tau. This speculation is supported by recent *in vivo* studies. Conditional overexpression of GSK-3 β in the transgenic mouse brains induces tau hyperphosphorylation and neurodegeneration, but no tau aggregation [109]. In contrast, there are NFTs but no memory loss in several lines of tau transgenic mice (for review, see [110]). This phenomenon is probably common to other diseases characterized by abnormal protein aggregates such as Huntington disease and cardiomyopathy, in which the abnormal, nonfibrillar protein oligomers, rather than the aggregates themselves, appear to be pathogenic [111, 112].

IMBALANCE OF PHOSPHORYLATION/DEPHOSPHORYLATION IN AD BRAIN

To understand the mechanism leading to abnormal hyperphosphorylation of tau in AD, protein kinases and phosphatases that regulate tau phosphorylation level must be identified first. In the last two decades, numerous studies aimed to the identification of tau kinases and phosphatases have been carried out. It was found that *in vitro*, dozens of phosphoserine/phosphothreonine protein kinases and most of the major protein phosphatases could act on tau protein at various phosphorylation sites (for reviews in detail, see [82, 113, 114]). Tau appears to be a universal substrate for protein kinases and phosphatases *in vitro*. This may not be surprising, because nearly 20% of the amino acid residues of tau molecule are serines and threonines, and nearly 50% of these residues are phosphorylated to certain degrees in AD

brain (see [82] for review). However, it is unlikely that all these enzymes that act on tau in vitro catalyze tau phosphorylation/dephosphorylation in vivo. Immunohistochemical studies also have shown a colocalization of more than a dozen protein kinases and several protein phosphatases with NFTs of AD brain. As we now know that NFTs are very “sticky” structures that can be stained immunohistochemically by antibodies to numerous antigens, immunohistochemical colocalization with NFTs can only support other data that indicate a role of the specific protein or enzyme in the formation of NFTs, but itself cannot indicate such a role.

Further studies in cultured cells, in situ, and especially in vivo suggest that a few protein kinases and phosphatases may be involved in regulation of tau phosphorylation in the brain. The kinases that most likely play a role in phosphorylation of tau in the brain include glycogen synthase kinase-3 β (GSK-3 β), cyclin-dependent kinase 5 (cdk5), cAMP-dependent protein kinase (PKA), stress-activated protein kinases, and calcium/calmodulin-dependent kinase II (CaMK-II). Johnson and Stoothoff [115] have critically discussed this issue. The sites of tau phosphorylation by these kinases, except stress-activity protein kinases, have been summarized in our recent review [82]. Among protein phosphatases, PP2A has been shown to be the major tau phosphatase in the brain [69, 116–120]. In a recent study, we compared the catalytic kinetics of tau dephosphorylation by various major brain protein phosphatases and determined the relative contributions of these phosphatases to the regulation of tau phosphorylation quantitatively. We found that PP2A accounts for ~70% of the total tau phosphatase activity, whereas PP1, PP2B, and PP5 each accounts for only ~10% of the total tau phosphatase activity [88]. Because PP2B activity is upregulated rather than downregulated in AD brain, it is unlikely that it regulates tau phosphorylation in vivo [121].

Accumulated evidence indicates that tau phosphorylation is regulated by several protein kinases and that more than one kinase might be involved in abnormal hyperphosphorylation of tau in AD brain. Interestingly, GSK-3 β phosphorylates tau at both prime sites (ie, tau needs to be primed by phosphorylation with other kinases at other sites) and unprimed sites [122–126]. In a cotransfection study, Cho and Johnson [125] found that a GSK-3 β mutant (GSK-3 β -R96A) that only phosphorylates unprimed sites has no negative impact on tau’s ability to bind to microtubules, in contrast to wild-type GSK-3 β , which significantly impairs tau’s ability to bind to microtubules. Further studies demonstrate that primed phosphorylation of tau at Thr231 by GSK-3 β plays a critical role in decreasing tau’s ability to both bind to and stabilize microtubules [126]. In rat brains, activation of PKA not only induces primed phosphorylation of tau by GSK-3 β , but also impairs the spatial memory of rats [124, 127]. GSK-3 β appears to be regulated by both phosphoinositol-3 kinase and protein kinase C pathways [128–131].

An obvious approach to understanding how tau becomes abnormally hyperphosphorylated in AD is to study whether tau kinase(s) or tau phosphatase(s) are dysregulated in AD brain. Several studies have focused on whether the activities and expression of these enzymes are altered in AD brain.

Among protein kinases, cdk5 was reported to be upregulated in AD brain by one laboratory [132], but this result was challenged by others [133–136]. On the other hand, both the activity and the expression of PP2A as well as the activities of PP1 and PP5 are decreased in the selected areas of AD brain [83–89]. Consistent with this finding, several other neuronal proteins such as neurofilaments, MAP1B, β -tubulin, and β -catenin are also hyperphosphorylated in AD brain [24–27]. Hence, it appears that downregulation of the phosphatases, especially of PP2A, might underlie the abnormal hyperphosphorylation of tau and other proteins in AD brain. Studies of metabolically active rat brain slices and transgenic mice suggest that the downregulation of PP2A may produce hyperphosphorylation of tau, not only by the deficient dephosphorylation of tau, but also through the activation of several PP2A-regulated protein kinases, including PKA [137], CaMK-II [138], MAP kinases, and stress-activated protein kinases [139–141]. Nevertheless, inhibition of PP2A activity in animal brain could only induce hyperphosphorylation of tau at some of the hyperphosphorylation sites seen in PHF-tau, but does not result in NFTs. Attempts to produce massive tangles of PHFs in animal models merely via alteration of tau phosphatase and/or kinase activities have not yet been successful. These observations suggest that the downregulation of tau phosphatases in AD brain may be only partially responsible for the abnormal hyperphosphorylation of tau.

The causes leading to decreased PP2A activity in AD brain are not well understood. Downregulation of PP2A expression [85] and upregulation of PP2A endogenous inhibitor proteins I_1^{PP2A} and I_2^{PP2A} [142] in AD brain may both contribute to the downregulation of PP2A activity. Because the activities of PP1 [83, 88] and PP5 [88, 89], which contribute to regulation of tau phosphorylation to a much smaller extent than PP2A [88], are also decreased in AD brain, there might be a common factor that downregulates the activities of the major brain protein phosphatases in AD brain.

In addition to tau kinases and phosphatases, alterations of tau itself, the substrate of these enzymes, may also play an important role in its abnormal hyperphosphorylation and conversion into PHFs. Tau is also modified post-translationally by β -*N*-acetylglucosamine (GlcNAc) via a glycosidic bond at the hydroxyl groups of serine and/or threonine residues, and this modification is called *O*-GlcNAcylation [143–145]. Because *O*-GlcNAc could modify the same serine or threonine residues of tau as phosphate does and a reciprocal relationship between *O*-GlcNAcylation and phosphorylation has been seen in many proteins (for review, see [146]), *O*-GlcNAcylation could affect phosphorylation of tau. Recent studies in various systems found that tau phosphorylation is indeed regulated by *O*-GlcNAcylation inversely [144, 145, 147]. Most interestingly, fasting of mice induces downregulation of tau *O*-GlcNAcylation, which relies on glucose metabolism to supply UDP-GlcNAc as a donor for protein *O*-GlcNAcylation, and in turn leads to hyperphosphorylation of tau [145]. These findings led to the novel hypothesis that impaired glucose uptake/metabolism in AD brain, which was well established decades ago, contributes

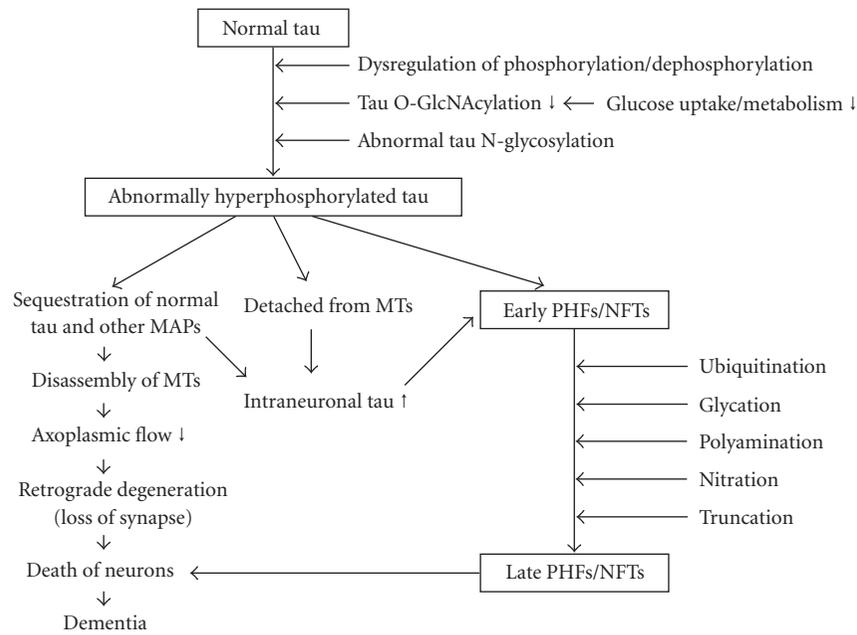


FIGURE 1: Proposed mechanism of neurofibrillary degeneration. MAPs, microtubule-associated proteins; MTs, microtubules; PHFs, paired helical filaments; NFTs, neurofibrillary tangles.

to the disease pathogenesis via downregulation of tau *O*-GlcNAcylation and, consequently, upregulation of tau phosphorylation that leads to neurofibrillary degeneration [148].

Classical *N*-linked glycosylation is a modification of protein at asparagine residues by oligosaccharides, which normally modifies only membrane proteins and secreted proteins. Tau in AD brain, but not in normal human brain, was found to be modified by *N*-glycosylation [68, 149, 150], and this aberrant tau modification appears to precede and facilitate abnormal hyperphosphorylation of tau [150–152]. This modification has been reviewed in detail in a recent article [82].

MECHANISM OF NEUROFIBRILLARY DEGENERATION

There is no doubt that the abnormality of tau plays a central role in neurofibrillary degeneration in AD and other tauopathies. A critical review of the literature accumulated in the last two decades sheds light onto the probable mechanism of neurofibrillary degeneration of AD (Figure 1).

Tau is the major microtubule-associated protein of mature neurons where it stimulates microtubule assembly and stabilizes microtubule structure. Tau is normally modified by both phosphorylation and *O*-GlcNAcylation. The phosphorylation level of tau is regulated by tau kinases and tau phosphatases, as well as by the alteration of tau itself. In AD and probably also in other tauopathies, metabolic and genetic abnormalities lead to dysregulation of signal transduction pathways, which in turn causes an imbalance of the

phosphorylation/dephosphorylation system, that is, downregulation of PP2A in the brain. This imbalance results in increased phosphorylation (ie, hyperphosphorylation) of tau. The impaired brain glucose uptake/metabolism that precedes AD also facilitates hyperphosphorylation of tau via downregulation of tau *O*-GlcNAcylation [148]. Aberrant *N*-glycosylation of tau in AD brain also makes tau a more favorable substrate for major tau kinases and less favorable for tau phosphatases [151, 152], thereby facilitating tau hyperphosphorylation.

The abnormally hyperphosphorylated tau resulting from any of the above causes not only loses its biological activity to stimulate microtubule assembly, but also becomes a toxic molecule, sequesters normal tau, MAP1, and MAP2, and causes disassembly of microtubules. The breakdown of the microtubule network in the affected neurons compromises axonal transport and leads to retrograde degeneration, which in turn results in neuronal death and dementia. On the other hand, the abnormally hyperphosphorylated tau detached from microtubules is not only easier to polymerize into PHFs as a result of hyperphosphorylation, but it also causes increased intraneuronal soluble tau concentration due to sequestration of normal tau from microtubules, which further facilitates tau aggregation into PHFs. The polymerized abnormal tau is further modified by ubiquitination, glycation, polyamination, nitration, and truncation (for review, see [82]), and forms mature PHFs/NFTs. Unlike the unpolymerized hyperphosphorylated tau that is toxic, PHFs/NFTs appears to be inert (Alonso A et al unpublished observation), but these lesions grow in size with disease progression

and eventually might physically choke the affected neuron to death.

THERAPEUTIC TARGET TO TREAT AD BY CORRECTING DYSREGULATION OF PROTEIN PHOSPHORYLATION/DEPHOSPHORYLATION

Because neurofibrillary degeneration plays a central role in the pathogenesis of AD, one of the most attractive therapeutic targets of AD is to inhibit neurofibrillary degeneration. As outlined in Figure 1, the most promising approaches to achieve this goal are to inhibit the abnormal hyperphosphorylation of tau and to inhibit its sequestration of normal MAPs. The former approach is more effective since it should both rescue the disruption of microtubule and axoplasmic flow and prevent further deposition of NFTs. Several academic groups and pharmaceutical companies have been investigating this approach by restoring PP2A activity or inhibiting tau kinase activity in the brain. Memantine, a low-to-moderate-affinity antagonist of NMDA receptor, which improves mental function and the quality of daily life of individuals with moderate to severe AD [153, 154], reverses the okadaic-acid-induced inhibition of PP2A activity and prevents tau hyperphosphorylation in hippocampal slice cultures from adult rats [155]. The restoration of PP2A activity to normal level by memantine also leads to restoration of the expression of MAP2 in the neuropil and a reversal of hyperphosphorylation and accumulation of neurofilaments. Wang's group has demonstrated that treatment of brain slices and rats with melatonin can restore PP2A activity that is inhibited by okadaic acid or calyculin A and reverse hyperphosphorylation of tau and neurofilament proteins as well as cytotoxicities [156–158]. Melatonin also prevents tau hyperphosphorylation and aggregation induced by overactivation of GSK-3 or PKA [131, 159]. These are examples showing that inhibition of dysregulation of protein phosphorylation/dephosphorylation is a promising target to treat AD. Further investigation of new compounds that can inhibit abnormal hyperphosphorylation of tau will likely provide new treatments for AD.

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