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Review

Tau pathology in Alzheimer disease and other tauopathies

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Abstract

Just as neuronal activity is essential to normal brain function, microtubule-associated protein tau appears to be critical to normal neuronal activity in the mammalian brain, especially in the evolutionary most advanced species, the homo sapiens. While the loss of functional tau can be compensated by the other two neuronal microtubule-associated proteins, MAP1A/MAP1B and MAP2, it is the dysfunctional, i.e., the toxic tau, which forces an affected neuron in a long and losing battle resulting in a slow but progressive retrograde neurodegeneration. It is this pathology which is characteristic of Alzheimer disease (AD) and other tauopathies. To date, the most established and the most compelling cause of dysfunctional tau in AD and other tauopathies is the abnormal hyperphosphorylation of tau. The abnormal hyperphosphorylation not only results in the loss of tau function of promoting assembly and stabilizing microtubules but also in a gain of a toxic function whereby the pathological tau sequesters normal tau, MAP1A/MAP1B and MAP2, and causes inhibition and disruption of microtubules. This toxic gain of function of the pathological tau appears to be solely due to its abnormal hyperphosphorylation because dephosphorylation converts it functionally into a normal-like state. The affected neurons battle the toxic tau both by continually synthesizing new normal tau and as well as by packaging the abnormally hyperphosphorylated tau into inert polymers, i.e., neurofibrillary tangles of paired helical filaments, twisted ribbons and straight filaments. Slowly but progressively, the affected neurons undergo a retrograde degeneration. The hyperphosphorylation of tau results both from an imbalance between the activities of tau kinases and tau phosphatases and as well as changes in tau's conformation which affect its interaction with these enzymes. A decrease in the activity of protein phosphatase-2A (PP-2A) in AD brain and certain missense mutations seen in frontotemporal dementia promotes the abnormal hyperphosphorylation of tau. Inhibition of this tau abnormality is one of the most promising therapeutic approaches to AD and other tauopathies.

Keywords: Neurofibrillary degeneration; Microtubule-associated protein tau; Microtubule; Microtubule-associated protein 2; Neurofilament; Memantine; Abnormal hyperphosphorylation of tau; Protein phosphatase-2A

1. Introduction

Microtubule-associated protein (MAP) tau, MAP1 (A/B) and MAP2 are the major microtubule-associated proteins of a normal mature neuron. These three MAPs apparently perform similar functions, i.e., the promotion of assembly and stability of microtubules. This excessive redundancy in biology, i.e., having three different proteins to maintain the microtubule network in a neuron, is probably due to the essential requirement of microtubules for axoplasmic flow, which, in turn, is critical to neuronal activity. Thus, a neuron has a capacity to compensate the loss of function of one MAP with the other two MAPs. Both tau and MAP2 knockout transgenic mice show apparent normal development into adult life [1,2], whereas tau and MAP2 and as well as MAP2 and MAP1B double knockout transgenic mice show defects in axonal elongation and neuronal migration [2,3].

The biological activity of tau, primarily a neuronal protein, in promoting assembly and stability of microtubules is regulated by its degree of phosphorylation. Normal tau

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contains 2–3 mol phosphate/mol of the protein [4], the level of phosphorylation for its optimal activity. Hyperphosphorylation of tau depresses its microtubule assembly activity and its binding to microtubules [5,6].

Human brain tau is a family of six proteins derived from a single gene by alternative mRNA splicing [7,8]. These proteins differ in whether they contain three (τ3L, τ3S or τ3) or four (τ4L, τ4S or τ4) tubulin binding domains (repeats, R) of 31 or 32 amino acids each near the C-terminal and two (τ3L, τ4L), one (τ3S, τ4S), or no (τ3, τ4) inserts of 29 amino acids each in the N-terminal portion of the molecule; the two amino-terminal inserts, 1 and 2, are coded by exon 2 and exon 3, respectively.

In Alzheimer disease (AD) and related disorders called tauopathies, tau is abnormally hyperphosphorylated and is accumulated as intraneuronal tangles of paired helical filaments (PHF), twisted ribbons and or straight filaments [9–13]. This hallmark brain lesion of these diseases directly correlates with dementia in these patients [14–16]. The etiology and the pathogenesis of neurofibrillary degeneration and therapeutic strategies to inhibit this lesion have been the subject of several recent reviews (see Refs. [17–19]). In this article, the pathobiology of tau and the molecular mechanism by which the abnormal hyperphosphorylation of this protein might lead to AD and other tauopathies and the role of various protein kinases and phosphatases and modifications of tau are updated.

2. Tau pathology

2.1. Abnormal hyperphosphorylation of tau

Tau in AD brain is abnormally hyperphosphorylated and in this state is the major protein subunit of PHF/SF which forms neurofibrillary tangles, a hallmark lesion of this disease [9–13]. Tau pathology, which is seen only as accumulation of abnormally hyperphosphorylated protein, is also seen in several other human neurodegenerative disorders (see Table 1). In everyone of these disorders, called tauopathies, the accumulation of the abnormally hyperphosphorylated tau is associated with neurofibrillary degeneration and dementia. The discovery of mutations in tau gene and their cosegregation with the disease in the inherited frontotemporal dementia with Parkinsonism linked to chromosome-17 (FTDP-17) has established that abnormalities in tau protein as a primary event can lead to neurodegeneration and dementia [20–22].

Table 1
Tauopathies characterized by abnormal hyperphosphorylation of tau

• Alzheimer disease, including tangle-only form of the disease
• Down syndrome, adult cases
• Guam parkinsonism dementia complex
• Dementia pugilistica
• Pick disease
• Dementia with argyrophilic grains
• Fronto-temporal dementia
• Cortico-basal degeneration
• Pallido-ponto-nigral degeneration
• Progressive supranuclear palsy
• Gerstmann–Sträussler–Scheinker disease with tangles

Taus from brain biopsies obtained from tissue adjoining the epilepsy focus from young adults and from fetal brains are phosphorylated at some of the same sites as those known to occur in PHF-tau [23,24]. However, only a few of the sites seen in PHF-tau are phosphorylated in the fetal or adult brains and the level of phosphorylation at the sites phosphorylated is less than 5% of that in AD tau [25].

The abnormal hyperphosphorylation of tau appears to precede its accumulation in the affected neurons in AD. The abnormally hyperphosphorylated tau was discovered not only in neurofibrillary tangles [10] but also in cytosol from AD brains [11]. Quantitative immunocytochemical studies with mAb Tau-1 have revealed deposits of only abnormally phosphorylated tau, but not normal tau, in neurons without tangles (stage "0" tangles) both in Alzheimer and in normal aged hippocampi [26,27]. Tau in tangles, mostly ghost tangles, is known to be ubiquitinated [28–30], whereas the abnormally hyperphosphorylated tau isolated from AD brain cytosol was found to have no ubiquitin reactivity. All these studies suggest that the abnormal hyperphosphorylation of tau precedes its accumulation into neurofibrillary tangles [4]. Employing monoclonal antibodies to mitotic phosphoepitopes, Vincent et al. [31] also showed that phosphorylation of tau precedes the presence of PHF in AD brain.

One of the possibilities is that the abnormal hyperphosphorylation of tau might be due to a conformational change(s) in tau in the diseased brain, which might make it a better substrate for phosphorylation and or a worse substrate for dephosphorylation. Davies and his colleagues have developed a series of monoclonal antibodies to conformational alterations of tau and employing these antibodies, have shown that tau is conformationally altered in AD [32–34] and in transgenic mice overexpressing human tau [35]. While in inherited cases of FTDP-17, where the disease is caused by certain missense mutations in tau and these mutations make tau a more favorable substrate for hyperphosphorylation by brain protein kinases [36], such a scenario is less likely in AD because tau is not the only neuronal protein which is hyperphosphorylated in AD as a result of the protein phosphorylation/dephosphorylation imbalance. Biochemically, tubulin and neurofilaments [37,38] and immunocytochemically neurofilaments and MAP1B [39–41] have been found to be hyperphosphorylated in AD brain. Furthermore, both the cytosolic- and PHF-abnormally hyperphosphorylated taus are readily dephosphorylated by phosphatases in vitro [10,12,42–45].

The neurofibrillary degeneration of the Alzheimer type is seen only sparsely in aged animals and in experimentally induced conditions. None of the mutations in β-amyloid precursor protein (β-APP), presenilin-1 or presenilin-2,

which have been found to cause familial AD, have, to date, shown to produce significant tau pathology in transgenic mice overexpressing these human mutant proteins [46,47]. On the other hand, overexpression of FTDP-17 mutant taus in transgenic mice has been found to produce neurofibrillary tangles of SF/PHF of abnormally hyperphosphorylated tau (e.g., Refs. [48–50]. A recent study has shown that, on hyperphosphorylation, murine tau self-assembles into tangles of filaments (PHF/SF) as readily as the corresponding human brain tau (Chohan et al., in preparation), suggesting that the protein phosphorylation/dephosphorylation system is probably more stable and resistant to changes in the lower order than the higher order species, such as humans. Consistent with these suggestions, overexpression of p25, the activator of cdk5 in transgenic mice which promotes the hyperphosphorylation of tau, has been found to result in self-assembly of filaments, though sparsely [51,52].

2.2. Truncation of tau

Tau in AD and other tauopathies appears to be mostly intact [9–13,25]. However, immunohistochemically, tau in AD neurofibrillary tangles has been shown to be truncated both at Glu 391 and Ser-421 [53,54]. These truncated taus have been shown to be associated with apoptosis in cultured cells [55,56]. However, what percentage of tau is truncated at these sites at what stage of neurofibrillary pathology in AD brain has not been reported, to date. Furthermore, unlike the monomeric truncated tau employed in the cell biological studies, this protein polymerized in neurofibrillary tangles/PHF might not have any biological activity. Since Alzheimer neurofibrillary degeneration takes place over a period of several months to years, it should not be surprising to have certain truncated taus in AD brain, especially resulting from neurofibrillary tangles which are exposed to hydrolases, both in the affected neurons and as well as in the case of the ghost tangles in the extracellular space [57]. Both the amino- and C-terminal regions flanking the microtubule binding domains of tau are inhibitory to its self-assembly into filaments [58]. Thus, neutralization of these inhibitory domains by abnormal hyperphosphorylation, a major mechanism probably involved in AD and other tauopathies, or partially by truncation might result in the formation of neurofibrillary tangles (see Ref. [58]). Consistent with this hypothesis, a transgenic rat model overexpressing truncated human tau has been shown to produce a significant number of neurofibrillary tangles and tau in these lesions is abnormally hyperphosphorylated [59].

2.3. Conformational changes and self-assembly of tau into filaments

Normal tau is immunohistochemically negative under most standard conditions of tissue fixation, whereas the opposite is true for tau aggregates. The latter is the only state in which tau pathology has been found, to date. In AD and other tauopathies, tau aggregates are seen as PHF, twisted ribbons or straight filaments of various diameters ranging from ~2.1 nm (protofilaments) to ~15 nm [60]. Both in human diseases, AD and other tauopathies, and as well as in experimentally induced animal conditions, the tau aggregates are invariably made up of abnormally hyperphosphorylated protein (for review, see Refs. [17,18,61]). Thus, ever since the discovery of tau as the major protein subunit of PHF/SF [9,10], there has been a considerable interest in understanding the aggregation of tau into filaments.

All of the six tau isoforms are present in a hyperphosphorylated state in PHF from AD brain [9–13,25]. In AD brain, abnormally hyperphosphorylated tau is present both as a cytosolic protein [4,11] and as polymerized into PHF [9–13]. Unlike normal tau, which contains two to three phosphate groups, the cytosolic hyperphosphorylated tau from AD brain (AD P-tau) contains 5 to 9 mol of phosphate per mole of the protein [4].

In vitro assembly of tau into SF and PHF-like structures has been achieved under different conditions, such as urea treatment for 60 h, incubations with unsaturated free fatty acids, tRNA, heparin or polyglutamic acid, employing a tau fragment, tau concentrations up to 12 mg/ml, and incubation times up to several days [62–72]. However, none of these conditions used for tau assembly is consistent with the presence in PHF of all six tau isoforms abnormally hyperphosphorylated as entire or nearly entire protein molecules. Although heparan sulfate and the hyperphosphorylated tau coexist in neurons in AD brain [67], the disassembly of PHF/neurofibrillary tangles by dephosphorylation [43] and the self-assembly of recombinant tau by hyperphosphorylation alone suggest that sulfated glycosaminoglyans might not play a critical role in neurofibrillary degeneration.

Abnormally hyperphosphorylated tau (0.4 mg/ml) isolated from AD brain polymerizes into tangles of PHF/SF in vitro at pH 6.9 under reducing conditions at 35 °C during 90 min [58] and these self-assembly conditions, which are consistent to the findings in AD and other tauopathies, do not require any co-factor. This self-assembly of tau requires hyperphosphorylation because dephosphorylation inhibits it. Unlike dephosphorylation, deglycosylation of AD tau does not inhibit its ability to self-assemble into filaments. Furthermore, on in vitro hyperphosphorylation, each of the six recombinant human brain tau isoforms self-assembles into PHF/SF. All these findings taken together suggest that the abnormal hyperphosphorylation is probably sufficient to cause the assembly of tau into filaments and might be the molecular mechanism involved in the formation of tau lesions in AD and other tauopathies.

Tau is an unusual protein that has long stretches of charged (positively and negatively) regions that are not conducive for intermolecular hydrophobic association [73]. Of the four microtubule binding repeats in tau, the predicted amino acids having β-structure are concentrated in R2 and R3 [72] and can self-assemble into filaments in vitro; R2

and R3 have also been shown to co-assemble with heparin into PHF [74]. It is likely that the way the charged regions are located, the rest of the molecule has an inhibitory effect upon the self-polymerization of tau. Of all of the tau isoforms, this inhibitory effect seems to be the least in τ4L. The N-terminal inserts are highly acidic, and the presence of these inserts markedly neutralizes the basic charge of tau. For instance, the theoretical isoelectric points of τ4, τ4S, and τ4L, respectively, are 9.46, 9.86 and 8.24. The presence of the extra repeat, the R2, and the two N-terminal inserts probably promotes the intermolecular hydrophobic interaction in τ4L sufficiently to result in its self-assembly into PHF, and hyperphosphorylation further enhances this process. The abnormal hyperphosphorylation that occurs in AD and other tauopathies neutralizes the basic inhibitory charge of tau. Most of the sites at which tau is hyperphosphorylated flank the microtubule binding domains. Neutralization of basic charge by hyperphosphorylation in these flanking regions probably neutralizes their inhibitory effect and allows tau to self-assemble into filaments. However, the nature of the neutralization by the two N-terminal inserts and that by the abnormal hyperphosphorylation are most likely different, as evidenced by the formation of filaments with different morphologies.

Tau self-assembles probably by intermolecular hydrophobic interaction and through its microtubule binding repeat R3 (in the case of 3R taus), and R2 and R3 (in the case of 4R taus), but only when the rest of the molecule (i.e., amino-terminal and carboxyl-terminal regions flanking the repeats, which are inhibitory) are neutralized. In AD and other tauopathies, these inhibitory regions are neutralized by abnormal hyperphosphorylation.

The FTDP-17 mutations appear to alter conformation of the protein such that it becomes a more favorable substrate

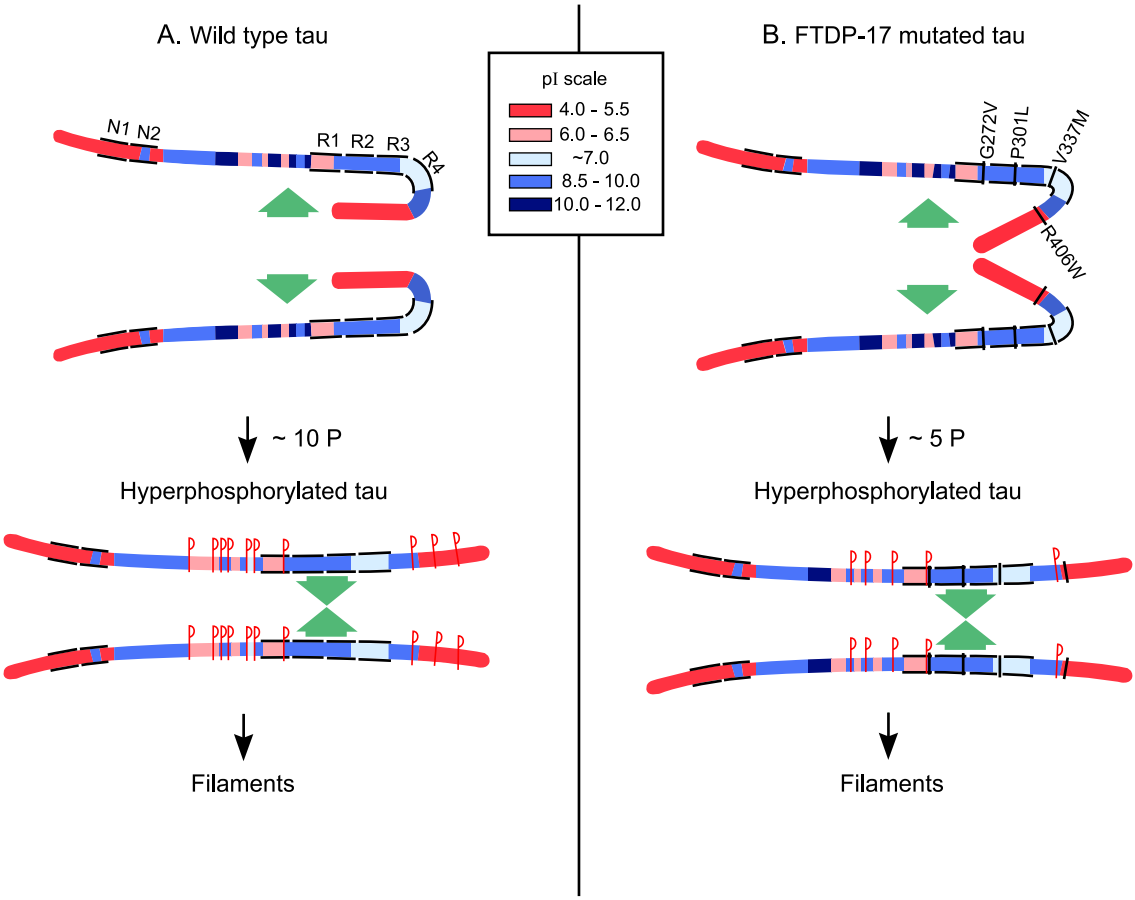

Fig. 1. A hypothetical scheme of the phosphorylation-induced self-assembly of wild-type and FTDP-17 mutated taus. Tau self-assembles mainly through the microtubule binding domain/repeat R3 in 3R taus and through R3 and R2 in 4R taus (R2 and R3 have β-structure). Regions of tau molecule both N-terminal and C-terminal to the repeats are inhibitory. Hyperphosphorylation of tau neutralizes these basic inhibitory domains, enabling tau–tau interaction (phosphorylation sites indicated by red Ps). In the case of the C-terminal region beyond Pro 397, i.e., 398–404, is a highly acidic segment that masks the repeats. Phosphorylation (red Ps) of tau at Ser396 and/or 404 opens this segment, allowing tau–tau interaction through the repeats.
FTDP-17 mutations make tau a more favorable substrate for phosphorylation than the wild-type τ4L, which in turn is more readily phosphorylated than τ3L. The mutated taus achieve the conformation required to self-assemble at a lower level of incorporated phosphate. Although the FTDP-17 mutant taus have conformations that are more prone to polymerize, in the absence of hyperphosphorylation, the highly basic segments and the C-terminal interfere with polymerization. The conformation of the mutant taus (mutation positions indicated in black) is more favorable for the action of kinases than τ4L. Upon hyperphosphorylation (phosphorylation positions indicated in red Ps), both wild-type and mutated taus adopt the conformation needed to polymerize into filaments. Reproduced with permission from Ref. [36].

to brain protein kinases [36]. The mutated taus are more rapidly hyperphosphorylated and can self-assemble at a lower level of hyperphosphorylation than the wild-type tau (see Fig. 1).

3. Mechanisms of neurofibrillary degeneration

In AD brains the levels of tau, but not the mRNA for this protein, are four- to eightfold increased as compared to age-matched control brains and this increase is in the form of the abnormally hyperphosphorylated tau [75]. The abnormally hyperphosphorylated tau is found in AD brain in two subcellular pools, i.e., (i) as polymerized into neurofibrillary tangles of PHF mixed with straight filaments (SF); and (ii) as non-fibrillized form in the cytosol [4,11,26]. The tau polymerized into neurofibrillary tangles is apparently inert and only on enzymatic dephosphorylation in vitro when released from PHF/tangles it behaves like normal tau in promoting microtubule assembly [42,43]. In contrast, the cytosolic abnormally hyperphosphorylated tau (AD P-tau), which can be as much as ~40% of the total abnormal tau in AD brain [4], does not interact with tubulin/microtubules but instead sequesters normal tau, MAP1A/MAP1B and MAP2, causing inhibition and disassembly of microtubules in vitro [6,77,78]. The association between AD P-tau and normal tau is not saturable and in vitro results in the formation of tangles of ~2.1 mm filaments [77]. The association between AD P-tau and MAP1A/MAP1B or MAP2 is weaker than that between the AD P-tau and normal

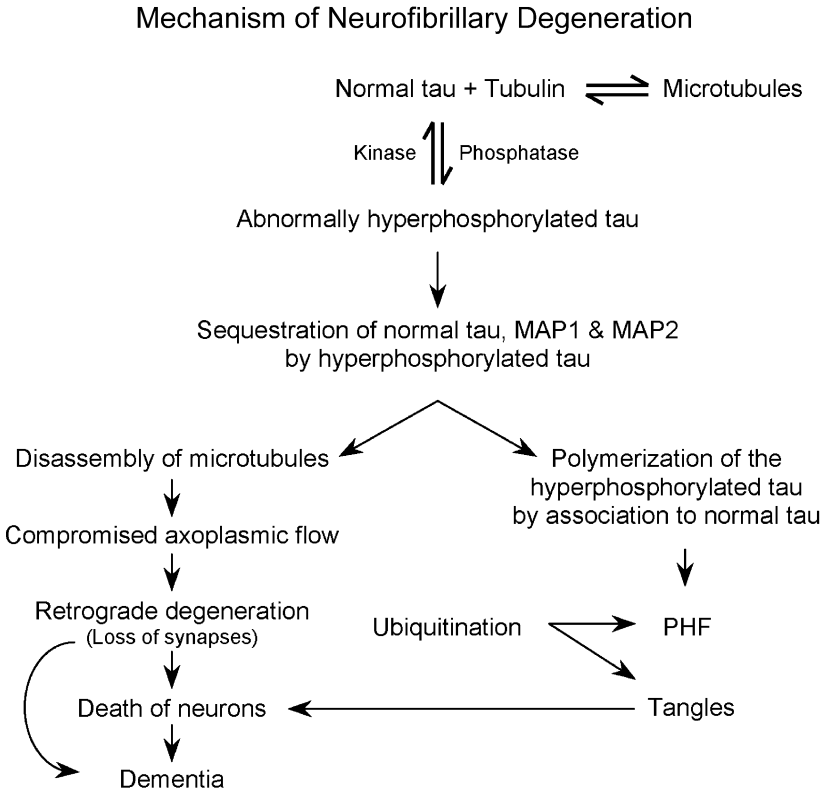

Fig. 2. Mechanism of neurofibrillary degeneration. Tau is a major microtubule-associated protein of a normal mature neuron where it stimulates assembly and establishes microtubules. Normal tau is both *O*-GlcNAcylated and phosphorylated. In normal brain, tau contains 2–3 mol phosphate/mol of the protein for its optimal activity. Hyperphosphorylation of tau depresses this normal biological activity. In AD and other tauopathies, tau becomes abnormally hyperphosphorylated. More than one mechanism appears to be involved in the abnormal hyperphosphorylation of tau. Unlike normal tau, this protein in AD is *N*-glycosylated, and this glycosylation makes tau a more favorable substrate for phosphorylation by PKA, GSK-3β, and cdk5, and less favorable for dephosphorylation by PP-2A. The level of protein *O*-GlcNAcylation, which reciprocally regulates phosphorylation, is reduced in AD brain and probably is another cause of abnormal hyperphosphorylation of the protein. The activity of PP-2A is reduced in AD brain and leads to abnormal hyperphosphorylation of tau not only by a decrease in dephosphorylation, but also by an increase in the activities of CaMKII, PKA and MAP kinases, which are negatively regulated by the phosphatase. In inherited FTDP-17, tau mutations G272V, P301L, V337M and R406W as well as mutations that lead to increase 4R/3R ratio make this protein a more favorable substrate for phosphorylation than the corresponding wild-type tau.
The abnormally hyperphosphorylated tau resulting from any one of the above causes behaves as an inhibitory/toxic protein; it not only is unable to stimulate microtubule assembly and bind to microtubules, but also sequesters normal tau, MAP1A/MAP1B and MAP2, inhibition of assembly and disruption of microtubules. The breakdown of the microtubule network in the affected neurons compromises axonal transport, leading to retrograde degeneration which, in turn, results in dementia. The association between the AD P-tau and normal tau in the presence of glycosylation results in the formation of neurofibrillary tangles. The tangles are ubiquitinated for degradation by the non-lysosomal ubiquitin pathway, but apparently this degradation, if any, is minimal. Unlike the non-polymerized abnormally hyperphosphorylated tau, the neurofibrillary tangles are inert but, with disease progression, these lesions grow in size and eventually might physically choke the affected cells to death.

tau and does not result in the formation of filaments [78]. This toxic property of the AD P-tau appears to be solely due to its abnormal hyperphosphorylation because dephosphorylation by alkaline phosphatase, protein phosphatase (PP)-2A, PP-2B and to a lesser degree by PP-1 converts the abnormal tau into a normal-like protein in promoting the microtubule assembly in vitro [6,42–44,77,78]. The loss of functional tau, which is critical for the maintenance of the cellular microtubule network (Li et al., in preparation), leads to neurodegeneration.

Several missense mutations in tau cosegregate with the disease in FTDP-17 [20–22]. Four of these missense mutations, G272V, P301L, V337M and R406W, which have been studied to date, make tau a more favorable substrate than the wild-type human tau for abnormal hyperphosphorylation by brain protein kinases in vitro [36]. These mutated taus become hyperphosphorylated at a faster rate and self-aggregate into filaments more readily, i.e., at a phosphorylation stoichiometry of 4–6 as compared with 10 or more in the case of the wild-type protein. These faster kinetics of the hyperphosphorylation of the mutated tau might explain a relatively early onset, severity and autosomal dominance of the disease in the inherited FTDP-17 cases.

The six human tau isoforms, τ4RL (4R, 2N), τ4S (4R, 1N), τ4 (4R, no N), τ3RL (3R, 2N), τ3RS (3R, 1N), and τ3 (3R, no N), also called fetal tau, are differentially sequestered by AD P-tau, in vitro [79]. The association of AD P-tau to normal human brain recombinant taus is τ4RL>τ4RS>τ4R and τ3RL>τ3RS>τ3, and that of τ4RL>τ3RL. AD P-tau also inhibits the assembly and disrupts microtubules pre-assembled with each tau isoform with an efficiency which corresponds directly to the degree of interaction with these isoforms. In vitro hyperphosphorylation of recombinant tau converts it into an AD P-tau-like state in sequestering normal tau and inhibiting microtubule assembly. The preferential sequestration of 4R taus and taus with amino-terminal inserts explains both (i) why fetal brain (fetal tau is with 3R and no N) is protected from Alzheimer neurofibrillary pathology and (ii) why intronic mutations seen in certain inherited cases of FTDP-17, which result in alternate splicing of tau mRNA, and consequently an increase in 4R/3R ratio, lead to neurofibrillary degeneration and the disease. In vitro, at a phosphorylation stoichiometry of ~4 and above, the hyperphosphorylated tau sequesters normal tau, whereas it requires a stoichiometry of 10 or more to self-aggregate into filaments [36]. Furthermore, on aggregation into filaments, tau loses its ability to sequester normal tau, indicating that the formation of filaments might be initiated as a self-defense response by the affected neurons (see Fig. 2).

The abnormal hyperphosphorylation of tau makes it resistant to proteolysis by the calcium activated neutral protease [43,44] and most likely it is because of this reason the levels of tau are several-fold increased in AD [75]. Some increase in tau level in AD brain can also result from the activation of p70 S6 kinase which up-regulates the translation of tau [80]. It is likely that to neutralize the AD P-tau's ability to sequester normal MAPs and cause disassembly of microtubules, the affected neurons promote the self-assembly of the abnormal tau into tangles of PHF. The fact that the tangle-bearing neurons seem to survive for many years [81] is consistent with such a self-defense role of the formation of tangles. The AD P-tau readily self-assembles into tangles of PHF/SF in vitro under physiological conditions of protein concentration, pH, ionic strength and reducing conditions [58]. Furthermore, dephosphorylation inhibits the self-assembly of AD P-tau into PHF/SF, and the in vitro abnormal hyperphosphorylation of each of the six recombinant human brain tau isoforms promotes their assembly into tangles of PHF/SF. Thus, all these studies taken together demonstrate the pivotal involvement of abnormal hyperphosphorylation in neurofibrillary degeneration.

4. Protein kinases and protein phosphatases involved in the abnormal hyperphosphorylation of tau

The state of phosphorylation of a phosphoprotein is a function of the balance between the activities of the protein kinases and the protein phosphatases that regulate its phosphorylation. Tau, which is phosphorylated at over 30 serine/threonine residues in AD (see Refs. [76,82]), is a substrate for several protein kinases [83,84]. Among these kinases, glycogen synthase kinase-3 (GSK-3), cyclin dependent protein kinase-5 (cdk5), protein kinase A (PKA), calcium and calmodulin-dependent protein kinase-II (CaMKII), mitogen activated protein (MAP) kinase ERK 1/2, and stress-activated protein kinases have been most implicated in the abnormal hyperphosphorylation of tau (see Ref. [85]). A large number of the abnormally hyperphosphorylated sites in tau are proline-directed, i.e., serine/threonine followed by proline which are canonical sites of proline-directed protein kinases (PDPKs). All the three major PDPKs, GSK-3β, cdk5 and ERK 1/2, have been shown to phosphorylate tau at a large number of the same sites seen in AD.

GSK-3β and cdk5 phosphorylate tau at a large number of sites, most of which are common to the two enzymes [45,86,87]. The expressions of GSK-3β and cdk5 are high in the brain [88–90] and both enzymes have been shown to be associated with all stages of neurofibrillary pathology in AD [91,92]. Overexpression of GSK-3β in cultured cells and in transgenic mice results in hyperphosphorylation of tau at several of the same sites seen in AD and inhibition of this enzyme by lithium chloride attenuates phosphorylation in these models [93–100].

Cdk5 requires for its activity interaction with p39 or p35 or, better, their proteolytic products p29 or p25, respectively, which are generated in postmitotic neurons by digestion with calpains (see Refs. [101,102]). Overexpression of p25

in transgenic mice, which results in an increase in the activity of this enzyme, also produces hyperphosphorylation of tau [51,52].

The MAP kinase family, which includes ERK1, ERK2, p70S6 kinase and the stress-activated kinases JNK and p38 kinase, have been shown to phosphorylate tau at several of the same sites as the abnormally hyperphosphorylated tau and so has been the association of these enzymes with the progression of neurofibrillary degeneration in AD [80,85,103–108].

Unlike the PDPKs, the non-PDPKs have been shown to phosphorylate tau at only a few of the sites. CaM Kinase II phosphorylates tau at Ser-262/356 and at Ser-416 [109–112]. Both PKA and MARK kinase have also been shown to phosphorylate tau at Ser-262 [113–116]. PKA phosphorylates tau at Ser-214, Ser-217, Ser-396/404 and at Ser-416 [45,117]. However, phosphorylation of tau by these non-PDPKs markedly increases the phosphorylation of tau by PDPKs, GSK-3β and cdk5 [45,118–122]. The priming of tau by PKA appears to be sufficient to enhance the abnormal hyperphosphorylation of tau by the basal level of GSK-3β activity in normal adult rat brain and lead to an impairment of spatial memory in these animals (Wang et al., in preparation). Although, to date, the activities of none of these protein kinases have been reproducibly shown to be up-regulated in AD brain, transient stimulation of these enzymes, especially the priming kinases such as PKA or CaMKII, might be sufficient to result in the abnormal hyperphosphorylation of tau.

The activities of PP-2A and PP-1 are compromised by ~20–30% in AD brain [123,124], and the phosphorylation of tau that suppresses its microtubule binding and assembly activities in adult mammalian brain is regulated by PP-2A and not by PP-2B [112,125]. PP-2A also regulates the activities of several tau kinases in brain. Inhibition of PP-2A activity by okadaic acid in cultured cells and in metabolically active rat brain slices results in abnormal hyperphosphorylation of tau at several of the same sites as in AD, not only directly by a decrease in dephosphorylation but also indirectly by promoting the activities of CaM Kinase II [112], PKA [126,127], MAP kinase kinase (MEK1/2), extracellular regulated kinase (ERK 1/2) and P70S6 kinase [80,85]. Thus, barring the fact that tau is not the only neuronal substrate of these protein kinases and phosphatases, it should be possible to inhibit the abnormal hyperphosphorylation of tau by inhibiting the activity of one or more tau kinases and or restoring or up-regulating the activity of PP-2A.

Although the brain has several tau phosphatase activities (Refs. [128,129], Rahman et al., in preparation), PP-2A and PP-1 make more than 90% of the serine/threonine protein phosphatase activity in mammalian cells [130]. The intracellular activities of these enzymes are regulated by endogenous inhibitors. PP-1 activity is regulated mainly by a 18.7-kDa heat stable protein called inhibitor-1 (I-1) [131,132]. In addition, a structurally related protein, DARPP-32 (dopamine and cAMP-regulated phosphoprotein of apparent molecular weight 32,000) is expressed predominantly in the brain [133]. I-1 and DARPP-32 are activated on phosphorylation by protein kinase A and inactivated at basal calcium level by PP-2A. Thus, inhibition of PP-2A activity would keep I-1, DARPP-32 in active form and thereby result in a decrease in PP-1 activity. In AD brain a reduction in PP-2A activity might have decreased the PP-1 activity by allowing the up-regulation of the I-1/DARPP-32 activity. PP-2A is inhibited in the mammalian tissue by two heat-stable proteins: (i) the I_1^{PP2A}, a 30-kDa cytosolic protein [134] that inhibits PP-2A with a Ki of 30 nM, and (ii) the I_2^{PP2A}, a 39-kDa nuclear protein that inhibits PP-2A with a Ki of 23 nM [135]. Both I_1^{PP2A} and I_2^{PP2A} have been cloned from human kidney [135,136] and brain (Tsujio et al., in preparation). I_1^{PP2A} has been found to be the same protein as the putative histocompatibility leukocyte antigen class II-associated protein (PHAP-1). This protein, which has also been described as mapmodulin, pp32 and LANP [137], is 249 amino acids long and has apparent molecular weight of 30 kDa on SDS-PAGE. I_2^{PP2A}, which is the same as TAF-1β or PHAPII, is a nuclear protein that is a homologue of the human SETα protein [138]. In AD brain there is a shift from nuclear to cytoplasmic localization of I_2^{PP2A} (Tanimukai et al., in preparation). Both I_1^{PP2A} and I_2^{PP2A} interact with the catalytic subunit of PP-2A (Chen et al., in preparation). The level of I_1^{PP2A} is ~20% increased in AD brains as compared with age-matched control brains, which probably is a cause of the decrease in PP-2A activity in AD brain.

5. Substrate regulation of the phosphorylation of tau

In addition to the activities of the tau kinases and phosphatases, the phosphorylation of tau is also regulated by its conformational state. Free tau is more readily hyperphosphorylated than the microtubule-bound tau. The rate and extent of tau phosphorylation by PKA, CaM Kinase II, C-kinase, casein kinase (CK)-I, cdk5 and GSK-3 are dependent on its initial phosphorylation state. For instance, when recombinant human brain tau is prephosphorylated by one of several non-PDPKs, i.e., PKA, CaM Kinase II or C-kinase, then its subsequent phosphorylations catalyzed by the PDPKs cdk5 or GSK-3 are stimulated several fold [118–120,139]. In addition, the rate and extent to which various tau isoforms are phosphorylated also depend on whether tau contains three repeats or four repeats and zero, one or two N-terminal inserts [140,141].

In addition to abnormal hyperphosphorylation, tau is also abnormally glycosylated and the latter appears to precede the former in AD brain [142,143]. In vitro studies indicate that the abnormal glycosylation promotes tau phosphorylation with PKA, GSK-3β and ckd5, and inhibits dephosphorylation of tau with PP-2A and PP5

[143,144]. In addition, like some other neuronal phosphoproteins, tau is also *O*-GlcNAcylated [145]. In contrast to classical *N*- or *O*-glycosylation, *O*-GlcNAcylation, which involves the addition of a single sugar at serine/threonine residues of a protein, dynamically posttranslationally modifies cytoplasmic and nuclear proteins in a manner analogous to protein phosphorylation (see Ref. [146]). *O*-GlcNAcylation and phosphorylation reciprocally regulate each other. In AD, probably due to impaired glucose uptake/metabolism, the *O*-GlcNAcylation of tau is significantly reduced and decreased glucose metabolism in cultured cells and in mice, which decreases the *O*-GlcNAcylation of tau, produces abnormal hyperphosphorylation of this protein [147].

In inherited frontotemporal dementia linked to chromosome-17 (FTDP-17), certain mutations in the tau gene cosegregate with the disease [20–22]. The most studied of these mutations are the missense mutations G272V, P301L, V337M and R406W. Tau with these mutations is a more favorable substrate for hyperphosphorylation than the wild-type tau; the mutated taus are hyperphosphorylated much faster and polymerize into filaments at lower stoichiometry than the identically treated wild-type tau [36]. Thus, all the studies described in this section, taken together, suggest that, in addition to the levels of the activities of tau kinases and phosphatases, the phosphorylation of tau is regulated at the substrate (tau) level.

6. Phosphorylation sites involved in converting normal functional tau into a toxic molecule

Tau is abnormally hyperphosphorylated at over 30 sites in AD. However, all of these sites might not be involved in converting normal tau into a toxic molecule. Identification of these critical sites has been most difficult. Phosphorylation of tau at Ser-262, Thr-231 and Ser-235 inhibits its binding to microtubules by ~35, ~25 and 10%, respectively [121]. Hyperphosphorylation of tau at the level of 4–6 mol phosphates/mol of the protein induces the toxic property where it sequesters normal tau [36]. Additional phosphorylation to a level of ~10 phosphates per mole of the protein is required to induce its self-assembly into filaments. Taus with FTDP-17 mutations, G272V, P301L, V337M, R406W, are much faster phosphorylated than the wild-type tau and self-assemble at lower levels of phosphorylation than the wild-type protein. Time kinetics of phosphorylation of these mutated and wild-type taus at various abnormally phosphorylated sites and the ability of these proteins to bind normal tau suggest Ser-199/202/205, Thr-212, Thr-231/Ser-235, Ser-262/356 and Ser-404 to be among the critical sites that convert tau to a toxic-like protein. Further phosphorylation at Thr-231, Ser-396 and Ser-422 promotes self-assembly of tau into filaments [36]. These sites are known to be substrates of PKA, CaMKII, GSK-3β and cdk5, among other protein kinases.

7. Inhibition of neurofibrillary degeneration and outcome measures

The most promising therapeutic approaches to inhibit neurofibrillary degeneration and consequently AD are (1) to inhibit sequestration of normal MAPs by the AD P-tau and (2) to inhibit the abnormal hyperphosphorylation of tau. The latter can be carried out either by restoring the PP-2A activity in the affected areas of the brain to normal levels or by inhibiting the activity of one or more tau kinase activities that are critically involved in converting normal tau into an abnormal state whereby it sequesters normal MAPs. Memantine, a low to moderate affinity NMDA receptor antagonist, which improves mental function and the quality of daily living of patients with moderate to severe AD [148,149], restores the okadaic acid-induced inhibition of PP-2A activity, the abnormal hyperphosphorylation of tau at Ser-262 and the associated neurodegeneration in hippocampal slice cultures from adult rats [127]. Furthermore, the restoration of the PP-2A activity to normal levels by memantine also results in the restoration of the expression of MAP2 in the neuropil and a reversal of the hyperphosphorylation and the accumulation of neurofilament H and M subunits.

In conclusion, tau pathology appears to be both pivotal and a primary cause of neurodegeneration in AD and other tauopathies. In all these diseases, tau pathology is seen in the form of the abnormally hyperphosphorylated protein. The abnormal hyperphosphorylation of tau leads to neurodegeneration by sequestration of normal MAPs and as well as promotes its self-assembly into bundles of PHF/SF. Inhibition of the abnormal hyperphosphorylation of tau and sequestration of normal MAPs by the hyperphosphorylated tau are among the most promising therapeutic targets for AD and other tauopathies. Approaches to inhibit the abnormal hyperphosphorylation of tau include the inhibition of the one or more tau kinase activities, activation of one or more tau phosphatases and the enhancement of *O*-GlcNAcylation of tau.

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