CENTRAL ROLE OF TAU PROTEIN IN ALZHEIMER'S DISEASE AND RELATED TAUOPATHIES

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INTRODUCTION

Alzheimer's disease, the major cause of dementia in middle- to old-age individuals, is characterized histopathologically by two brain lesions, namely the neurofibrillary degeneration of abnormally hyperphosphorylated tau and the amyloidosis. Neurofibrillary degeneration is seen as intraneuronal neurofibrillary tangles of paired helical filaments (PHF) mixed with straight filaments (SF), neuropil threads, and dystrophic neurites surrounding the amyloid core in neuritic (senile) plaques, the second hallmark lesion. The major protein subunit of PHF/SF is the microtubule associated protein (MAP) tau in an abnormally hyperphosphorylated state.[1,2] In AD, the number of neurofibrillary tangles directly correlates with the degree of dementia. The number of plaques does not correlate with dementia and some of the normal aged individuals have as much amyloid plaque load as in typical cases of AD. Thus, amyloid by itself might not be sufficient to produce the clinical expression of the disease but it might still play a synergistic role in individuals with neurofibrillary degeneration in producing the disease.

The etiology and pathogenesis of neurofibrillary degeneration and therapeutic strategies have been the subject of several recent reviews.[3,4] In this article, the central role of tau protein in the etiopathogenesis of AD and related tauopathies is described.

Normal and Pathological Taus:

Tau protein is the major neuronal MAP. It is encoded by a single gene but alternate splicing of its pre-mRNA in the brain results in several molecular isoforms.[5] In the human brain, there are six molecular isoforms, generated by exclusion (3R2N, 3R1N, 3R0N) or inclusion (4R2N, 4R1N, 4R0N) of exon 10 which codes for 31 or 32 amino acids near the carboxy-terminus, and 2 (3R2N, 4R2N), 1 (3R1N, 4R1N), or no (3R0N, 4R0N) inserts of 29 amino acids each near the amino-terminus by inclusion of exons 2, 3, or exon 2 or none, respectively. The major established function of tau is its interaction with the major microtubule protein subunit tubulin that results in the promotion of assembly of tubulin into microtubules and the maintenance of these structures. Microtubules are required for the axoplasmic transport which is too critical for neuronal function to depend solely on a single protein. The backup neuronal MAPs are the two high molecular weight proteins (HMW), MAP1 and MAP2 which, like tau, contain the microtubule binding domains/repeats and essentially perform the same function as tau in promoting assembly and maintaining the structure of microtubules. While tau in normal mature neurons is mostly localized in the axon, the HMW MAPs have largely somatodendritic distribution.

Tau is a phosphoprotein and its biological activity is regulated by its degree of phosphorylation. Hyperphosphorylation depresses the ability of tau to bind to tubulin and promote its assembly into microtubules. In Alzheimer disease (AD), tau is abnormally hyperphosphorylated.[6]

Since the discovery of tau as the major protein subunit of PHF in 1986, a number of post-translational modifications, i.e. abnormal hyperphosphorylation, ubiquitination, glycation, N-glycosylation, O-GlcNAcylation, polyamination, nitration and truncation, have been implicated in its pathology.[1,7] To date, the most established cause of dysfunction of tau in AD and related tauopathies is its abnormal hyperphosphorylation. Neurofibrillary degeneration of abnormally hyperphosphorylated tau is also seen in several other human neurodegenerative disorders, which include frontotemporal dementia, Pick disease, corticobasal degeneration, dementia pugilistica and progressive supranuclear palsy. In every one 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.[8]

The abnormal hyperphosphorylation of tau appears to precede its accumulation in the affected neurons in AD. In vitro hyperphosphorylation promotes tau's assembly into bundles of PHF and SF.[9,10] Induction of hyperphosphorylation of tau in metabolically active rat brain slices by inhibition of PP-2A activity with okadaic acid, and in normal adult rats by activation of protein kinase-A, leads to accumulation of tau.[11,12] The abnormally hyperphosphorylated tau was discovered not only in neurofibrillary tangles but also in cytosol from AD brains.[1,6] Quantitative immunohistochemical studies with monoclonal antibody (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.[13] Tau in tangles, mostly ghost tangles, is known to be ubiquitinated, whereas the abnormally hyperphosphorylated tau isolated from AD brain cytosol was found to have no ubiquitin reactivity.[14] All these studies suggest that the abnormal hyperphosphorylation of tau precedes its accumulation into neurofibrillary tangles.[15]

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. In inherited cases of FTDP-17, where the disease is caused by certain missense mutations in tau, these mutations make tau a more favorable substrate for hyperphosphorylation by brain protein kinases.[9] Such a scenario is less likely in AD because, in this disease, tau is not the only neuronal protein which is hyperphosphorylated as a result of the protein phosphorylation/ dephosphorylation imbalance. Biochemically, tubulin and neurofilaments and immunohistochemically neurofilaments and MAP1B 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. [2, 16, 17]

The neurofibrillary degeneration of the Alzheimer type is seen only sparsely in aged animals and in experimentally induced conditions. None of the mutations in amyloid precursor protein (APP), presenilin-1 or presenilin-2, which have been found to cause familial AD, have, to date, shown to produce AD-like extensive tau pathology in transgenic mice overexpressing these human mutant proteins. On the other hand, overexpression of FTDP-17 mutant taus and as well its co-expression with APP/PS1 mutations in transgenic mice have been found to produce neurofibrillary tangles of SF/PHF of abnormally hyperphosphorylated tau.[18,19] 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, 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.[20] 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.[21, 22]

Tau hyperphosphorylation

1. Inhibits its ability to bind to microtubules
2. Inhibits its ability to promote microtubule assembly
3. Sequesters normal tau and other MAPs, thereby preventing their association with microtubules
4. Disrupts preformed microtubules
5. Promotes its self assembly into bundles of paired helical filaments, i.e., neurofibrillary tangles

Tau in AD and other tauopathies appears to be mostly intact.[1,2,6] However, immunohistochemically, tau in AD neurofibrillary tangles has been shown to be truncated both at Glu 391 and Ser-421.[23,24] These truncated taus have been shown to be associated with apoptosis in cultured cells. 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. Both N- and C-terminal regions flanking the microtubule binding domains of tau are inhibitory to its self assembly into filaments.[10] Thus, neutralization of these inhibitory domains by abnormal hyperphosphorylation, a major mechanism probably involved in AD and related tauopathies, or partially by truncation might result in the formation of neurofibrillary tangles.[10] Consistent with this

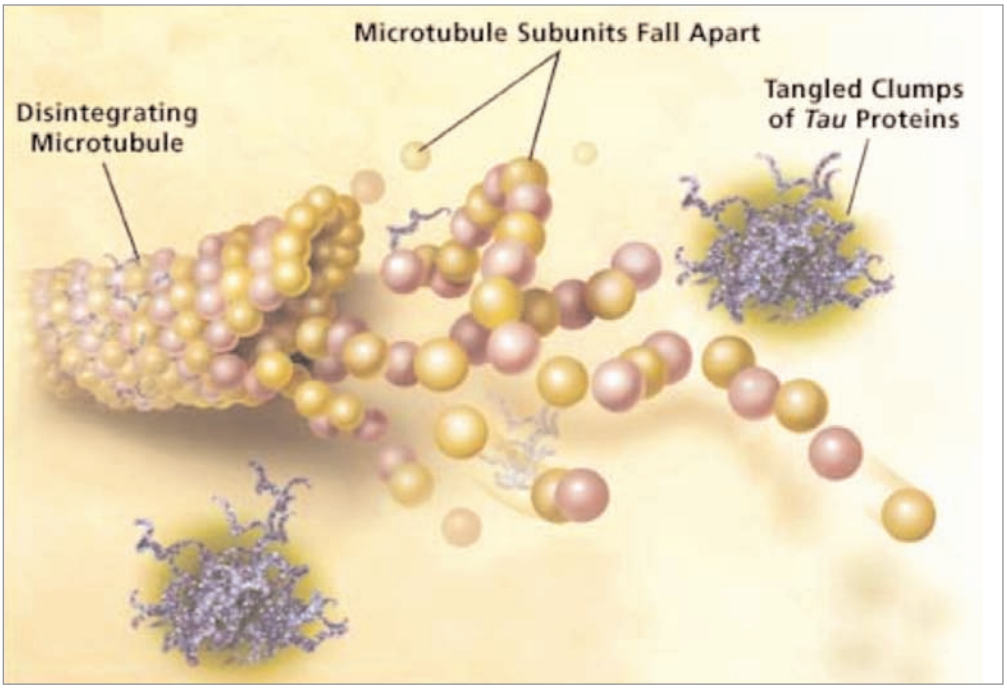

Figure 1. Neurofibrillary Degeneration. *Intracellular transport of proteins, lipids, synaptic vesicles filled with neurotransmitters, and other organelles are essential for the growth, survival, and functioning of a neuron. Microtubules serve as the railroad tracks along which the axonal transport takes place. Tau protein acts as the railroad ties, stabilizing the railroad tracks/microtubules. This activity of tau is regulated by its degree of phosphorylation; hyperphosphorylation depresses this activity. Abnormal hyperphosphorylation of tau in Alzheimer disease and related tauopathies not only results in the loss of the railroad tie function of this protein but also causes the disruption of the microtubule network, resulting in a seriously compromised axonal transport. Consequently the neurites start retracting and the affected neuron undergoes a retrograde degeneration. This neurofibrillary degeneration is a slow but chronic progressive process. A neuron with a neurofibrillary tangle lingers on up to several years in the diseased brain before the cell dies, leaving behind what is called a "ghost tangle" or a "tombstone" floating in the extracellular space in the brain parenchyma.*

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.[25]

Abnormally hyperphosphorylated tau isolated from AD brain readily polymerizes into tangles of PHF/SF in vitro and these self assembly conditions, which are consistent to the findings in AD and other tauopathies, do not require any co-factor.[10] 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.[26] Furthermore, on in vitro hyperphosphorylation, each of the six recombinant human brain tau isoforms self assemble into PHF/SF. All these findings taken together suggest that the abnormal hyperphosphorylation is probably required 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.

The FTDP-17 mutations appear to alter conformation of the protein such that it becomes a more favorable substrate to brain protein kinases.[9] The mutated taus are more rapidly hyperphosphorylated and can self assemble at a lower level of hyperphosphorylation than the wild type tau.

Molecular basis of neurofibrillary degeneration:

In AD brains the levels of tau, but not the mRNA for this protein, are four- to eight-fold increased as compared to age-matched control brains and this increase is in the form of the abnormally hyperphosphorylated tau.[27] 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.[6,13,15] 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.[16] 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 does not interact with

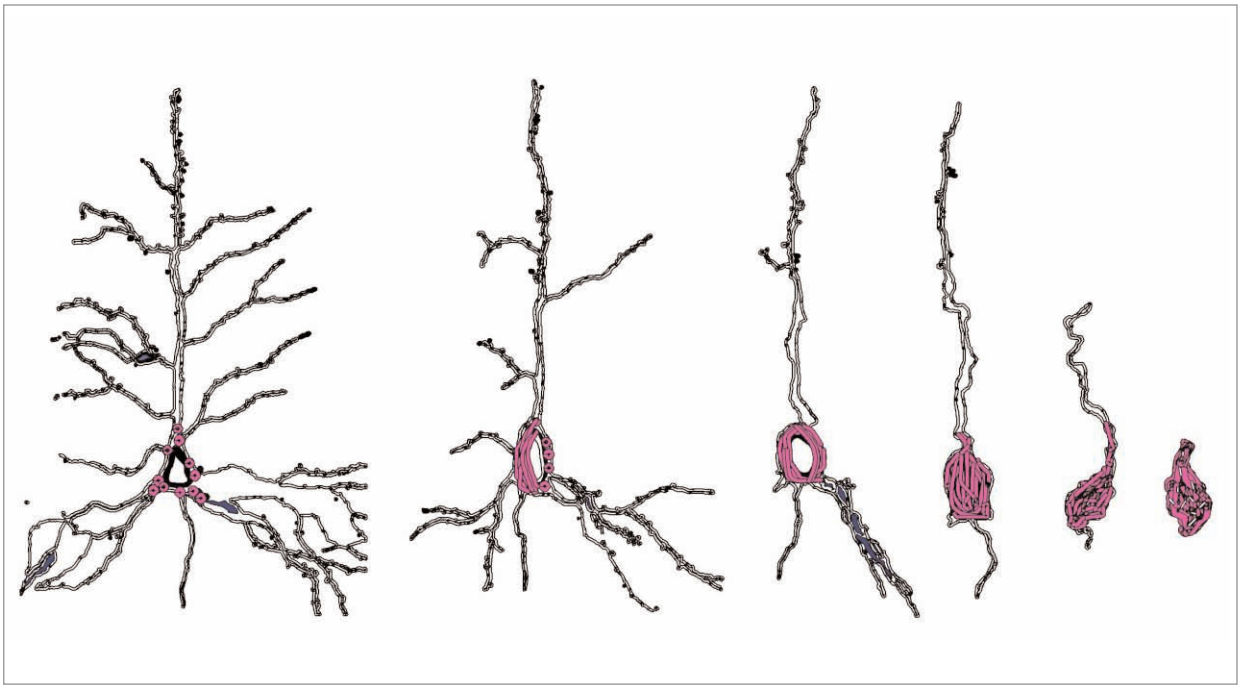

Figure 2. Molecular mechanism of neurofibrillary degeneration. *Normal tau interacts with tubulin and stimulates its assembly and stabilizes microtubules. In AD brain, because of an imbalance in tau kinase and phosphatase activities and a change in its conformation induced by other post-translational changes or mutations as in inherited cases of FTDP-17, tau becomes abnormally hyperphosphorylated. 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, MAP 1A / MAP1B and MAP2, and leads to 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. Illustration adapted from www.nia.gov*

tubulin/microtubules but instead sequesters normal tau, MAP1A/ MAP1B and MAP2, causing inhibition and disassembly of microtubules in vitro.[15,28-31] The association between ADP-tau and normal tau is not saturable and in vitro results in the formation of tangles of ~ 2.1 mm filaments.[29] The association between AD P-tau and MAP1A/ MAP1B or MAP2 is weaker than that between the AD P-tau and normal tau and does not result in the formation of filaments.[28] This inhibitory activity 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 which can promote the microtubule assembly in vitro.[16,28-30,32] The sequestration of functional tau by the abnormally hyperphosphorylated tau causes disruption of the microtubule network and thereby leads to neurodegeneration (Figs. 1 and 2).

Several missense mutations in tau cosegregate with the disease in FTDP-17.[8] 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.9 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 are differentially sequestered by AD P-tau, in vitro.[10] 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-terminal inserts) is protected from Alzheimer neurofibrillary pathology and (ii) why intronic mutations

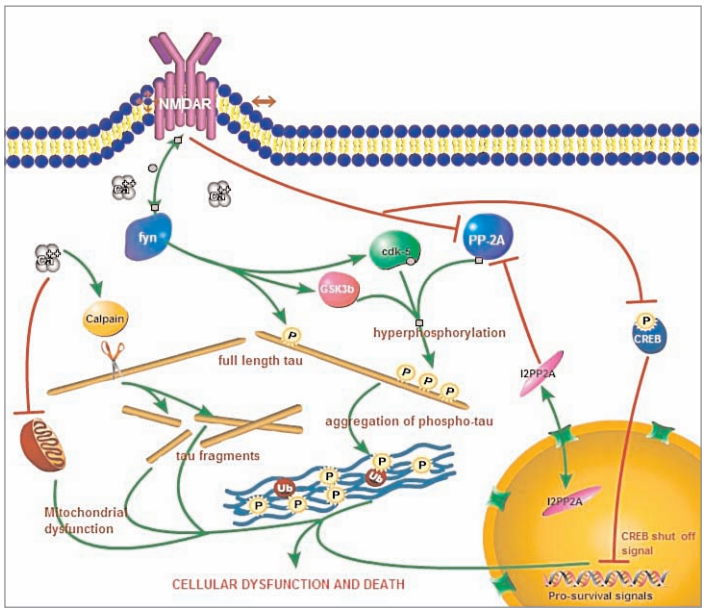

Figure 3. A proposed mechanism of tau-induced neurotoxicity in AD. *Chronic neuronal insult in AD leads to the activation of extra-synaptic NMDA receptors. Intracellular levels of a src family non-receptor tyrosine kinase, fyn, are increased in AD. Acute neuronal insults, like ischemia, bring about the formation of a ternary activator complex by which Fyn interacts with the NMDAR thereby activating the receptor channel. Fyn further activates extra-synaptic NMDA receptor by tyrosine phosphorylation of the NR2B subunit causing continued Ca+2 influx into the cytoplasm. Fyn also activates GSK-3? and cdk-5, which phosphorylate tau at several key serine/threonine residues. Fyn phosphorylates the N-terminal tyrosine 18 residue on tau. Hyperphosphorylation of tau leads to a change in its secondary structure that brings about its dissociation from the microtubules, and an "unmasking" of toxic domains probably near its N-terminal. Ca+2 activates calpain, which cleaves non-hyperphosphorylated tau into fragments, some of which are toxic to the cell. High intracellular Ca+2 leads to mitochondrial dysfunction. Chronic over-activation of extra-synaptic NMDAR sends a CREB shut-off signal whereby levels of phospho-CREB decline. This leads to decreased production of pro-survival signals like BDNF.*
Levels of I2PP2A, a PP-2A inhibitor, are increased in AD and the inhibitor is selectively translocated from the neuronal nucleus to the cytoplasm. PP-2A is also inhibited by activation of the NMDA receptor. This further contributes towards hyperphosphorylation of tau. All these events lead to cellular dysfunction and neuronal death over a period of time.
Adapted from ***Chohan and Iqbal (2006). J Alzheimers Dis. 10(1):81-7***

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.[9] On aggregation into filaments tau loses its ability to sequester normal tau. Furthermore, AD P-tau, but not PHF, inhibits regeneration of microtubule network in detergent-extracted PC12 cells, indicating that the formation of filaments might be initiated as a self defense response by the affected neurons.[31]

The abnormal hyperphosphorylation of tau makes it resistant to proteolysis by the calcium activated neutral protease and most likely it is because of this reason the level of tau is several-fold increased in AD.[16,27,32] Some increase in tau level in AD brain can also result from the activation of p70 S6 kinase which upregulates the translation of tau.[33,34] 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 many years is consistent with such a self-defense role of the formation of tangles.[35] 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.[10] 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 (Fig. 3).

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