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·Review·

Tau-induced neurodegeneration: mechanisms and targets

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The accumulation of hyperphosphorylated tau is a common feature of several dementias. Tau is one of the brain microtubule-associated proteins. Here we discuss tau's functions in microtubule assembly and stabilization and with regard to its interactions with other proteins. We describe and analyze important post-translational modifications: hyperphosphorylation, ubiquitination, glycation, glycosylation, nitration, polyamination, proteolysis, acetylation, and methylation. We discuss how these post-translational modifications can alter tau's biological function. We analyze the role of mitochondrial health in neurodegeneration. We propose that microtubules could be a therapeutic target and review different approaches. Finally, we consider whether tau accumulation or its conformational change is related to tau-induced neurodegeneration, and propose a mechanism of neurodegeneration.

Keywords: tau; phosphorylation; neurodegeneration; tauopathies; mitochondria; microtubules; tubulin; kinases; phosphatases; Alzheimer's disease

Introduction

Alzheimer's disease (AD), first described by Alois Alzheimer more than 100 years ago[1], is a progressive neurodegenerative disorder, characterized by an insidious onset with irreversible cognitive declines that lead to profound mental deterioration causing dementia[2]. Two major forms of lesion characterize AD: amyloid as diffuse neurotic plaques primarily composed of the Aβ peptide[3], which are mainly insoluble deposits of protein and cellular material, and neurofibrillary tangles composed of filamentous hyperphosphorylated tau protein that builds up inside the neuron[4, 5].

Amyloid precursor protein (APP) is a highly conserved transmembrane protein[6] believed to play a role in synapse formation, synaptic plasticity, and neuronal survival[7-9]. In AD, APP is cleaved by β- and γ-secretases leading to the overproduction of an abnormal proteolytic byproduct called amyloid beta (Aβ)[10]. Upon cleavage fragments of Aβ of various sizes are released from the membrane and aggregate in the brain to form the characteristic plaques seen in AD. Plaques are composed primarily of the 40 and 42 amino-acid peptide fragments Aβ40 and Aβ42, the latter being the predominant species. In addition, Aβ42 is more prone to aggregation and deposition and therefore the cause of neurotoxicity, as well as synaptic loss[11].

The second lesion in AD is formed by aggregates of the microtubule-associated protein tau, which forms intracytoplasmic neuronal inclusions or neurofibrillary tangles when hyperphosphorylated[12]. Tau is associated with neurons of the central nervous system[13] and its main biological function is promoting the *in vitro* assembly and stabilization of microtubules in the cytoskeleton[14, 15]. Tau is a phosphoprotein that is encoded by a single gene, *MAPT*, located on chromosome 17q21[16]; alternative splicing of the gene produces six major isoforms expressed in the adult human brain[17]. Isoforms derive their names from the number of microtubule-binding repeat sequences and which N-terminal exons are included[18].

Currently four million individuals in the United States

alone are affected and it has been estimated to cost the world $604 billion in 2010[2, 19]. Currently, there is no cure for AD and available drugs have been unable to intervene in the disease process enough to prevent or cure it[2].

Tauopathies

Tau pathology is seen in several other human neurodegenerative disorders called tauopathies. Although morphological differences exist in the tau lesions associated with each of these disorders, the disease mechanism is the same. Accumulation of abnormally hyperphosphorylated microtubule-associated protein tau results in the formation of intracellular filamentous tangles. The family of diseases includes AD, frontotemporal dementia with parkinsonism-17, corticobasal degeneration, Pick's disease, progressive supranuclear palsy, dementia pugilistica, amyotrophic lateral sclerosis, and tangle-only dementia[18, 20]. Recently, McKee and coworkers[21] reported an association between abnormal tau and chronic traumatic encephalopathy (CTE). In addition, UCLA researchers have used a brain-imaging tool to identify abnormal tau proteins associated with repetitive mild traumatic brain injury in retired National Football League players[22]. Binding patterns in the players' scans were found to be consistent with the tau deposit patterns observed in CTE. This may enable detection of those who are suffering from the syndrome while they are still alive, opening up possibilities for symptomatic treatment and prevention[22]. Finally, it has been established that abnormalities in tau protein as a primary event can lead to neurodegeneration and dementia[12].

Post-translational Modification of Tau

Tau Phosphorylation

Tau contains 80 Ser or Thr phosphorylation sites and five Tyr sites; ~71 of them can be phosphorylated under physiological or pathological conditions[23, 24]. Most of the phosphorylation sites surround the microtubule-binding domains in the proline-rich region, located between regions 181–235 and 396–422 which is outside the microtubule domain in the C-terminal region. When tau is phosphorylated, it is unable to polymerize tubulin into microtubules, which alters microtubule assembly and inhibits assembly promoted by normal tau (reviewed in[25]). Most of these sites are phosphorylated during fetal development and even in the normal adult brain, but with lower stoichiometry or with different isoform expression[26, 27]. Tau is hyperphosphorylated in the fetal brain because it contains higher than normal levels of tubulin needed for microtubule assembly suggesting that, at certain levels, phosphorylation is a normal mechanism used by neurons to maintain their activity and microtubule network[28]. Hyperphosphorylation of tau protein causes oligomerization and aggregation, eventually leading to paired helical filaments (PHFs) which are characteristic of pre-tangles in the neurons of AD patients[29]. In contrast, dephosphorylation of tau increases binding to tubulin, promotes microtubule growth, and normalizes the microtubule cytoskeleton[30, 31]. Phosphorylation of tau is mainly regulated through kinases and phosphatases

Kinases Tau can be phosphorylated by 20 or more protein kinases, including four groups: proline-dependent protein kinases (PDPKs), non-PDPKs, tyrosine protein kinases, and protein kinases that phosphorylate tau on serine or threonine residues followed or not by a proline[23]. About 50% of these phosphorylation sites are canonical (Ser/Pro, Thr/Pro) for PDPKs and the remaining sites are phosphorylated by non-PDPKs[25]. The non-PDPK group includes tau-tubulin kinases 1 and 2, casein kinases 1 and 2, DYRK1A (dual-specificity tyrosine-phosphorylated and regulated kinase 1A), phosphorylase kinase, Rho kinase, PKA (protein kinase A), PKB (protein kinase B)/Akt, PKC (protein kinase C) and PKN (protein kinase novel)[23]. Tyrosine kinases are Src kinases, c-Abl and c-Met. Protein kinases that phosphorylate tau on serine or threonine residues followed or not by a proline recognize the motif SXXXS or SXXXD/E and RXRXXS/T and include GSK (glycogen synthase kinase) 3α*,* GSK-3β and AGC kinases [such as MSK1 (mitogen- and stress activated protein kinase)][23].

From the groups of kinases shown to phosphorylate tau, GSK-3 has received significant attention because it can modify several sites of the tau protein into neurofibrillary tangles and is able to modulate the generation of Aβ[25]. GSK-3 has two isoforms, GSK-3α and GSK-3β, which share 85% sequence identity[32]. *In vitro*, GSK-3 phosphorylates tau on ~40 Ser/Thr residues, and

CK1 is the only other kinase comparable to GSK-3 in that it phosphorylates tau residues in similar numbers[31]. GSK-3 phosphorylation reduces the capability of tau to promote microtubule assembly *in vitro* and in cells[33, 34]. GSK-3 together with the activity of other kinases such as CK1, Cdk5, and MARK, has the ability to significantly affect tau phosphorylation and modulate its neuronal function[35-38].

Cyclin-dependent kinase 5 (Cdk5) is a member of the cyclin-dependent kinase family and, due to the expression of its regulator p35, its activity is highest in neurons. Cdk5/p35 plays a crucial role in brain development and function; Cdk5 complex p25 (a truncated form of p35) phosphorylates tau at epitopes similar to those phosphorylated during mitosis, suggesting that Cdk5/p25 is the cause of mitotic-like tau phosphorylation in the AD brain[24, 39, 40].

MARK (microtubule-associated protein-microtubule affinity regulating kinase) phosphorylates tau at specific sites (serines in KXGS motifs) in the microtubule-binding repeats and together with its homolog (Par-1) identified in diverse species such as yeast (KIN1 and KIN2) and fruit-flies, is involved in cell-cycle control, cellular polarization, neuronal migration, differentiation, and cell signaling[41]. They phosphorylate tau in its microtubule-binding domains, cause tau to lose its affinity to bind microtubules, and the detached tau becomes aggregated[42]. Overexpression of the fly homolog of MARK (dMARK) causes an increase in tau phosphorylation at Ser262/356 which increases tau toxicity[43, 44].

Tau phosphatases Phosphatases counterbalance the action of kinases and they are directly or indirectly associated with microtubules[45]. They control phosphorylation states and PP-1, PP-2A, and PP-2B have been shown to dephosphorylate tau *in vitro*[46]. Overexpression of PP5 in PC12 cells dephosphorylates tau both *in vitro* and *in vivo*[47]. PP5 dephosphorylates tau at sites similar to PP-2A[47]. PP-2A activity is regulated by endogenous protein inhibitors. PP-2A is considered a major tau phosphatase because it binds to tau and directly or indirectly regulates its phosphorylation by regulating GSK-3 activity[48-53]. In the human brain, PP-2A accounts for 70% of the tau phosphatase activity and in the AD brain this enzyme activity is reduced[47]. Phosphatase and tensin homolog (PTEN) is a protein that acts as a phosphatase and it has been demonstrated that the loss of PTEN in mouse cerebellar neurons causes neurodegeneration by hyperphosphorylation of tau and neurofilaments and activation of Cdk5 and pERK1/2, suggesting that deregulation of the PTEN/pAkt pathway mediates neurodegeneration[54].

Tyrosine phosphorylation Tau can be phosphorylated at tyrosine residues by kinases such as Fyn, Syk, and c-Abl. Out of the five tyrosines 18, 29, 197, 310, and 394 (according to tau441), 18, 197, and 394 are phosphorylated in the AD brain whereas 394 is the only residue that has been described to date to be phosphorylated under physiological conditions. PHF samples from AD brains have been analyzed and phosphorylation at tyrosines 18 and 394 has been reported[55]. Abl is known to phosphorylate tyrosine 394 (Y394) and, recently, the Tyr kinase Arg, which plays a role in the oxidative stress response and neuronal development, was also shown to phosphorylate Y394 in a manner independent of Abl activity[55]. In a transgenic mouse model of AD, tyrosine 18-phosphorylated tau occurs in neurofibrillary tangles and the expression of Fyn is increased in these cases[56, 57]. From these data it appears that Fyn, c-Abl, and Arg are critical kinases in the neurodegenerative process.

Other Post-translational Modifications

The mechanism leading normal tau to become hyperphosphorylated remains unknown and post-translational modifications besides phosphorylation could regulate tau function and aggregation[58], such as ubiquitination[59], glycation[60], glycosylation[61], nitration[62], polyamination[63], proteolysis[64], acetylation[58], and methylation[65].

Reversible lysine acetylation has emerged as a potential regulatory modification implicated in AD and other neurodegenerative disorders, and a recent study demonstrated that tau acetylation at a specific lysine residue (K280) impairs the tau-mediated stabilization of microtubules and enhances tau aggregation[58]. Using antibodies specific for acetylated tau, it has been shown that tau acetylation is elevated in patients at early and moderate Braak stages of tauopathy[66]. Meanwhile, they demonstrated that deacetylase SIRT1 deficiency leads to hyperacetylation of tau and accumulation of p-tau, while in contrast, promoting tau deacetylation eliminates p-tau. However, another study showed that the expression of histone deacetylase 6 (HDAC6) positively correlates

with tau burden, while a decrease in HDAC6 activity or expression promotes tau clearance[67], and its levels have also been shown to be elevated in the brains of patients with AD and transgenic mice[68, 69]. Thus, a better understanding of the relationship of these acetylation residues with phosphorylation could be crucial to identify potential targets for therapy in AD and other tauopathies. Further work is required in cell and animal models to elucidate the functional role of tau acetylation in the pathogenesis of neurofibrillary tangles.

Using mass spectrometry, K256, K311, and K353 within the tau microtubule-binding repeat region have been identified as ubiquitylation sites that are at least partially occupied in PHFs[70]. Moreover, in other biochemical pathways, certain acetylated Lys residues can be alternatively methylated, suggesting that the web of tau post-translational modifications is potentially complex, with Lys-directed modifications playing key regulatory roles with respect to rates of tau turnover and aggregation[58]. With the mass spectrometry approach expanding the search criteria to include both acetyl- and methyl-lysine modifications, it was found that seven Lys residues (K44, K163, K174, K180, K254, K267, and K290) in PHF-tau immunopurified from AD brain are monomethylated, and reveal tau methylation as a new tau post-translational modification accompanying PHF deposition *in vivo*[65].

The tau phosphorylation state is also modulated by competing modifications of hydroxy amino-acids such as O-linked beta-N-acetylglucosaminylation (O-GlcNAcylation), which is a key regulatory post-translational modification that is reversible and often reciprocal with phosphorylation of serine and threonine at the same or nearby residues. Gong and coworkers demonstrated that inhibition of O-GlcNAcylation leads to hyperphosphorylation of tau in cultured cells and in rat brain slices[47, 71]. They postulated that tau pathology and neurodegeneration can be caused by impaired brain glucose metabolism *via* the down-regulation of tau O-GlcNAcylation in AD. Furthermore, O-GlcNAcylation may also inhibit tau oligomerization directly[72]. Therefore, decreased O-GlcNAcylation may promote tau-mediated neurodegeneration by promoting tau oligomerization directly and also indirectly by inducing its abnormal hyperphosphorylation[73].

Small ubiquitin-related modifiers (SUMOs) belong to an important class of ubiquitin-like proteins. SUMOylation is a post-translational modification process that regulates the functional properties of many proteins, among which several are implicated in neurodegenerative disease. Many proteins that have functions relevant to AD pathology have been identified as SUMO substrates, including those involved in synaptic physiology, mitochondrial dynamics, and inflammatory signaling[74].

Glycation of tau can occur *via* the non-enzymatic formation of advanced glycation end-products[75]. Tau can be glycated *in vitro*, resulting in a loss of microtubule binding. PHF-tau is also modified by glycolipids. Mass spectrometry and NMR analysis of acid-resistant PHF tau revealed the presence of several glycolipids consisting of glucose pentamers, hexamers, and tridecamers variably associated with esterified fatty acids (C_{14}-C_{20})[13]. Formation of PHFs *in vitro* can be accomplished in a phosphorylation-independent manner *via* interaction of sulfated glycosaminoglycans (such as heparin and heparan sulfate) with microtubule-binding domains of adjacent tau three- and four-repeat monomers[76]. The interplay between phosphorylation as a targeting event, tau glycation, oxidation, and heparan-mediated PHF formation remains to be elucidated.

Mitochondrial Involvement with Tau

The number of neurodegenerative diseases (such as AD) that have been linked to changes in mitochondrial dynamics is increasing[10, 77-83]. Recent reviews have indicated that a general pathway, the mitochondrial dysfunction, may be a common link between them[10, 79, 84, 85]. When mitochondria do not function properly, numerous physiological processes, including oxidative phosphorylation (OXPHOS) and Ca^{2+} regulation, are affected[79]. Microtubule stabilization in neurons depends on the presence of normal tau, which is also important in organelle transport from the cell body to the synapses. Studies have shown that both Aβ and various forms of tau are linked to mitochondria and deficiencies in OXPHOS and/or apoptotic activity[84]. The deficiencies reported may be the cause or the effect of mitochondrial fragmentation, which may play a role in neurodegeneration. The cellular localization and respiratory function of mitochondria depend on the balance of fission and fusion[86]. If the mitochondria are not properly distributed in

the cell, in particular throughout the axons, the optimal ATP generation and Ca^{2+} regulation cannot occur.

A balance of mitochondrial dynamics is necessary for proper content and lipid exchange[87] and correct cellular distribution of mitochondria, which is especially significant in neurons that have synapses far from the nucleus[88]. Loss of this balance, mitochondrial dysfunction, has been linked to an increase in the production of reactive oxygen species, which can result in post-translational modifications of proteins throughout the cell. As described above, these post-translational modifications may modulate the formation of tau-containing neurofibrillary tangles and therefore may be important in the regulation of AD[63]. The proteins that are known to be involved in both the fusion and fission of mitochondria are nuclear-encoded and targeted to the organelle after expression. Fusion proteins include the GTPase proteins Mfn1, Mfn2, and optic atrophy 1 (OPA1), which are all located in the mitochondrial outer membrane (MOM) and/or the mitochondrial inner membrane (MIM). Mitochondrial fission is known to involve at least two mammalian proteins, Drp1, a cytosolic protein that is also found localized with mitochondria[89], and hFis1, which is anchored in the MIM by one carboxy-terminal TM domain[90]. The MOM-bound E3 ubiquitin ligase, MARCH5 (also known as MITOL), interacts with proteins for both fusion and fission, including Mfn1, Mfn2, Drp1, and hFis1[91-93].

These proteins involved in mitochondrial dynamics have been shown to play roles in many neurodegenerative diseases. For example, a decrease in ATP formation has been reported in Huntington's disease, where the overexpression of Mfn2 prevents fragmentation resulting in increased ATP levels (reviewed in[79]). Furthermore, mutant forms of other mitochondrial fusion proteins, Mfn2 and OPA1, have been shown to lead to neurodegenerative diseases[94-98]. When brains from AD patients and an AD mouse model, 3×Tg.AD, were studied, hyperphosphorylated tau was found to co-immunoprecipitate with the fission protein Drp1, whereas the control samples did not[83]. In this study, an increase in GTPase activity was also found, which could lead to an increase in mitochondrial fragmentation; this indicates a more definitive link between mitochondrial dynamics and AD. Another group has shown a link between mutant tau and Drp1 in neurodegeneration[99]. In hippocampal neurons from rTg415 transgenic mice that express Tau P301L, the length of the mitochondrion is significantly increased. This is also reported in the brains of *Drosophila* that express TauR406W. The overexpression of Drp1 or inhibition of MARF, an Mfn homolog, returns mitochondrial length to normal, suppresses superoxide production, and suppresses cell-cycle activation, all of which are linked to the rescue of neurodegeneration in TauR406W flies. The functions of the mitochondrial membrane-bound E3 ubiquitin ligase MARCH5 and Drp1 appear to be codependent as Drp1 co-immunoprecipitates with MARCH5 and the formation of stable mitochondrial fission complexes requires the presence of both proteins[93]. Preliminary work in our lab has shown that MARCH5 and tau interact when both are overexpressed in Chinese hamster ovary cells. This could be indicative of tau's involvement with mitochondrial dynamics.

Until recently, the majority of evidence that linked mitochondria to AD was the accumulation of Aβ in mitochondria (reviewed in[100]). The phosphorylation state of tau has been implicated in the degeneration of neurons[101] and linked to mitochondrial dysfunction[102]. Tau has also been shown to be ubiquitinated and to be involved in the cellular distribution of mitochondria in mouse neurons[103, 104]. In 2009, a transgenic mouse strain that mimics both Aβ plaque formation and tau tangles was analyzed and it was found that both the plaques and the tangles affected the OXPHOS of the mitochondria[105]. In a more recent work, it has been shown that reduction of the expression of soluble tau is important for the proper distribution of mitochondria in the neurons of rTg4510 mice[104]. The hypothesis linking mitochondria with AD that is most relevant to this study is the mitochondrial cascade hypothesis that relates the dynamic properties of these organelles with the hyperphosphorylation of tau and the aggregation of Aβ (reviewed in[100]). Due to the critical dependence of neurons on the proper distribution of mitochondria in axons, disruption of mitochondrial dynamics could result in degeneration due to decreased levels of ATP generated, increased accumulation of reactive oxygen species, and/or deficient regulation of Ca^{2+} levels. As a model of mitochondrial dysfunction, when ATP production is decreased chemically, the phosphorylation state of tau decreases except at positions in the microtubule-binding domain[102]. Furthermore, a study in mouse AD models indicated that soluble tau disrupts the mitochondrial

localization in the axon and this is reversed by decreasing the levels of soluble tau in the cells[104]. Both of these examples suggest a connection between mitochondrial dysfunction and tau.

Another pathological feature of AD is a loss of axonal transport. Overexpression of tau increases the stability of microtubules and causes a decrease in the number of spines on neurons in culture[106]. Under these conditions, it was also observed in neuronal culture that mitochondria are no longer transported along dendrites, the number of mitochondria is reduced, and they begin to decay. Furthermore, the absence of motor proteins in *Drosophila* results in loss of mitochondria from axons and synapses with the majority of these organelles found in the cell bodies[107, 108]. Tau overexpression in Neuro2a cells inhibits the trafficking of organelles, including mitochondria, peroxisomes, and vesicles, by stabilizing the microtubules[109]. An increase in H_2O_2 occurs when tau is present in these cells and leads to neurite degradation twice as quickly than in untransfected neurites. The trafficking of APP is also decreased in tau-expressing cells.

More recently, groups have attempted to deduce which phosphorylation sites are relevant to the loss of axonal trafficking and neurodegeneration. The Hisanaga group at Tokyo Metropolitan University began to study the AT8 phosphorylation sites that include Ser199, Ser202, and Thr205[110]. By transfecting COS-7, PC-12, and Sf9 cells as well as cultured neurons, they found a decrease in mitochondrial movement linked to an increase in the inter-microtubule distance and an increase in microtubule resistance. Neurodegeneration identified by the appearance of vacuoles in the lamina of *Drosophila* eyes was characterized by the overexpression of tau[111]. When tau is overexpressed and the expression of either Milton or Miro is decreased by RNAi, vacuole formation increases. Further analysis indicated that the tau proteins are phosphorylated at Ser262 by PAR-1 and that decreasing PAR-1 or mutating tau to S262A results in a decrease of vacuole formation. This study indicates that the restriction of mitochondria to the cell body may trigger a change in the phosphorylation state of tau and lead to increased neurodegeneration.

Both of the pathways presented here could be linked to neurodegeneration and the mitochondrial deficiencies in AD and other neurodegenerative disorders. The cause and effect are not yet understood. Does the mitochondrial dysfunction or loss of axonal transport precede the formation of neurofibrillary tangles and plaques or are these the cause? Much more research is needed to answer these questions.

Therapeutic Approaches Targeting Tau

Initially, compounds that inhibit acetylcholinesterase (AChE; a serine protease that breaks down ACh causing ACh deficiency) such as Tacrine, Donepezil, Rivastigmine, and Galantamine have been tested and eventually marketed for patient use to stop AChE from catalyzing ACh hydrolysis[112]. However, over time, significant patient improvement has not been seen[112]. Recently, approaches to find treatment have focused on compounds that either inhibit tau aggregation or stabilize microtubules[112].

With several failures of therapies based on the Aβ hypothesis, a different approach was taken to target stabilizing microtubules[113]. Naturally, the obvious target for treatment has been tau hyperphosphorylation and the kinases involved, such as GSK-3β. Significant research results have demonstrated the potential to stop cellular breakdown by the use of microtubule stabilizers such as Paclitaxel/Taxol in oncology[114, 115]. The idea of stabilizing microtubules fed interest into research on peptides that mimic tau to assist in this stabilization. For example, a study created a series of peptides with the NAP sequence similar to that of tau's binding sequence[116, 117]. The NAP sequence stimulated microtubule assembly upon binding with tubulin. Experimental findings from this study showed that the NAP-containing peptides increased microtubule density, yet decreased β-tubulin, thus suggesting that these peptides may play a role in regulating the composition of microtubule-stabilizing isoforms[116, 117]. Administration of NAP induces cognitive improvement in an AD mouse model, protects microtubules in a Parkinson model, and has neuroprotective effect in an amyotrophic lateral sclerosis model[118, 119].

Heavy emphasis has been placed on understanding what other potential natural treatment types could be investigated[120]. It has been shown that turmeric extract has anti-amyloidogenic properties[121]. There is also an emphasis on testing the ability of compounds to cross the blood-brain barrier (BBB). A sugar-modified form of curcumin has been shown to inhibit tau aggregation at

nanomolar concentrations[122]. Further work is being done with this compound to allow it to cross the BBB for more efficient drug delivery.

Natural antioxidants have also been explored. Green tea epigallocatechin-3-gallate (EGCG) moderates tau pathology in AD transgenic mice[123]. To demonstrate the effect of EGCG on tau, Tg mouse brain homogenates treated with EGCG or with PBS as control were compared and the levels of soluble phosphorylated tau were decreased in samples treated with ECGC. Hence, natural

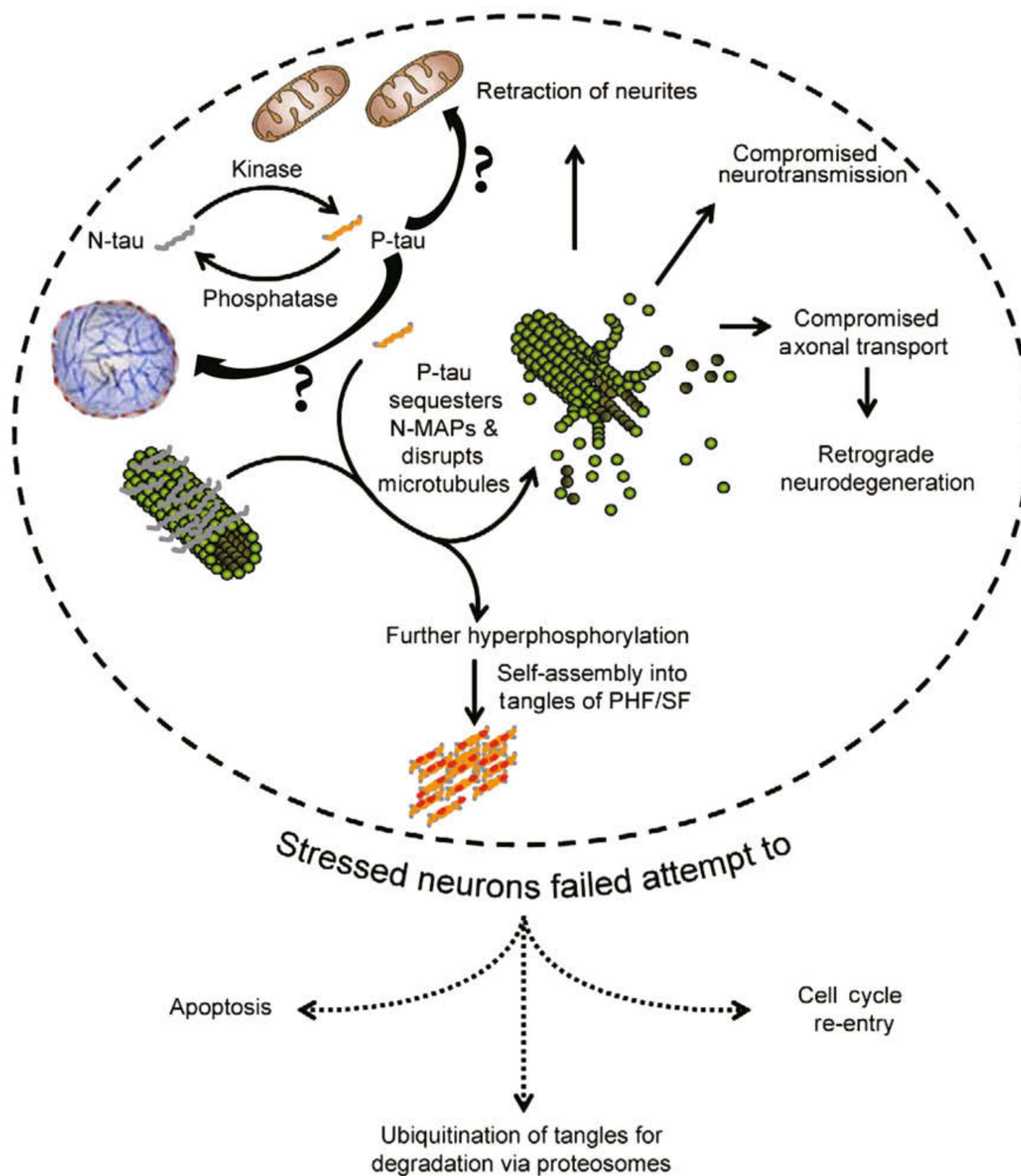

Fig. 1. Mechanism of tau-induced neurodegeneration. Tau is a phosphoprotein; in a disease situation, hyperphosphorylated tau disrupts microtubules, impairing axonal transport. P-tau impairs mitochondria and translocates into the nucleus. The consequences are under investigation.

antioxidant-containing compounds might be effective for treating tauopathies[123].

There are also synthetic compounds currently in phase II clinical trials such as Minocycline, Methylene Blue, and Tideglusib[113]. They have been successful in preventing tau aggregation, yet the effectiveness in therapy remains to be tested[121].

Conclusions

We have shown that hyperphosphorylated tau inhibits normal tau-promoted tubulin assembly into microtubules and disrupts preformed microtubules[30], probably by binding to normal tau[124] and other microtubule-associated proteins[125]. We have shown that phosphorylation of tau is sufficient to promote tau self-assembly into filaments[29] and that phosphorylation at Ser 199 and 262 and Thr 212 and 231 is sufficient to convert tau into an D-like protein in cells[101] and *in vivo* in transgenic *Drosophila*[126]. On the basis of our results and the evidence reviewed here, we propose that the hyperphosphorylation of tau leads to neurodegeneration through microtubule disruption and the consequent decreases in neurotransmission and axoplasmic transport (Fig. 1). Besides the microtubule disruption, we have evidence that abnormal tau translocates into the nucleus[101] and also impacts mitochondrial health in neurons. The consequences of these facts deserve further investigation. Neurons with neurofibrillary changes of hyperphosphorylated tau are under stress and respond in a number of ways including: (1) attempt to undergo apoptosis (a failed suicide attempt), (2) cell-cycle reentry, a failed attempt of a post-mitotic cell to divide, and (3) ubiquitination to get rid of the misfolded tau, i.e., hyperphosphorylated tau as neurofibrillary tangles; the proteasome activity is not sufficient to successfully operate this pathway. The only apparently successful rescue is the slow, progressive retrograde degeneration.

Abnormal phosphorylation is a root cause of tauopathies, so stabilizing microtubules, or even before that, controlling the abnormal phosphorylation, is a promising method and hypothesis that can provide the key to unlock the mysteries of tauopathy treatment. By further analyzing and experimenting with potential treatments such as NAP peptides, natural or synthetic compounds, and microtubule-stabilizing compounds, researchers may even understand how to target neurodegenerative disorders overall.

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