

The toxicity of tau in Alzheimer disease: turnover, targets and potential therapeutics

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Abstract

It has been almost 25 years since the initial discovery that tau was the primary component of the neurofibrillary tangles (NFTs) in Alzheimer disease (AD) brain. Although AD is defined by both β -amyloid ($A\beta$) pathology ($A\beta$ plaques) and tau pathology (NFTs), whether or not tau played a critical role in disease pathogenesis was a subject of discussion for many years. However, given the increasing evidence that pathological forms of tau can compromise neuronal function and that tau is likely an important mediator of $A\beta$ toxicity, there is a growing awareness that tau is a central player in AD pathogenesis. In this review we begin with a brief history of tau, then provide an overview of pathological forms of tau, followed by a discussion of the differential degradation of tau by either the proteasome or autophagy and possible mechanisms by which pathological forms of tau may exert their toxicity. We conclude by discussing possible avenues for therapeutic intervention based on these emerging themes of tau's role in AD.

Keywords: tau • Alzheimer disease • phosphorylation • truncation • oligomers • tau-based therapies • autophagy

Introduction

It has been more than 35 years since tau was first discovered as a protein that co-purified with microtubules through cycles of assembly and disassembly and was named for its ability to induce 'tubule' formation [1]. Two years later it was noted that tau was a phosphoprotein [2], and in 1984 it was demonstrated that tau in a more dephosphorylated state promotes microtubule assembly with greater efficiency, thus assigning a regulatory function to its phosphorylation [3]. Although these were intriguing findings at the time, interest in tau remained only moderate until it was demonstrated that tau made up the paired helical filaments which form the neurofibrillary tangles (NFTs) found in Alzheimer disease (AD) brain, and the tau that formed these filaments was abnormally phosphorylated [4–8]. These findings catapulted tau into the

limelight. In 1988, the first sequence of tau (from mouse) was published [9], and in the following year the sequences of both bovine and human tau were elucidated [10, 11]. These findings set the stage for an explosion of studies on tau, particularly on tau phosphorylation and function [12]. However, tau studies began to wane when it became clear that the causes of the familial form of AD were autosomal dominant mutations in genes that affect the production of β -amyloid ($A\beta$). $A\beta$ is a peptide generated from amyloid precursor protein (APP) that forms the core of the $A\beta$ plaques, which are the other predominant pathological lesion in AD brain [13–16]. These and other findings demonstrating the toxicity and pathogenic properties of $A\beta$ led to the notion that tau pathology was not central to the disease [17].

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In 1998, it was first discovered that mutations in the tau gene caused rare autosomal dominant neurodegenerative diseases (collectively known as frontotemporal dementia with parkinsonism linked to chromosome 17 [FTDP-17]) in which there was pronounced tau pathology but no A β pathology [18, 19]. Following these findings, a growing list of sporadic neurodegenerative tauopathies marked by abnormal tau-containing lesions in the brain and without significant A β pathology, were characterized [20]. These findings strongly suggested that alterations in tau could result in neurodegeneration and hinted at the importance of tau in the pathogenic processes in AD. This idea was further supported by the finding that neurons in which tau had been knocked out were relatively resistant to A β -induced cell death compared to neurons from wild-type mice expressing normal tau levels [21].

It is now very clear that tau plays a critical role in the pathogenesis of AD. However, it is also becoming evident that NFTs are not the 'toxic' tau species but rather non-aggregated forms are likely the culprits. For example, in an inducible tauopathy model, attenuating expression of FTDP-17 mutant tau ameliorated behavioural deficits and neuronal loss although NFT numbers either remained unchanged or increased [22]. More recent studies have shown that knocking out tau significantly attenuates A β -induced behavioural deficits but does not affect A β levels in an APP mouse model of AD [23], and that A β -induced deficits in axonal transport do not occur in neurons from tau^{-/-} mice [24]. These and other findings have contributed to a re-evaluation of the role of tau in neurodegeneration, including the consideration of a dual or synergistic relationship between tau and A β in AD pathogenesis [25, 26].

In this review, we will first present a brief overview of pathological modifications of tau and what may be the toxic species, followed by an overview of how tau is cleared from the cell and deficits that may occur in AD brain. We will then discuss possible targets of pathological tau that may contribute to disease progression and finally end with a presentation of potential 'tau-centric' therapeutic strategies for the treatment of AD.

Pathological modifications of tau

A defining feature of AD brain is tau pathology. Many different post-translational modifications of tau in AD brain have been described including phosphorylation, nitration, glycosylation, glycation, ubiquitylation and truncation [27, 28]. Given that abnormal phosphorylation is the most prominent modification of tau in AD brain, and truncation of tau also seems likely to play a key role in the evolution of tau inclusions, we will discuss these two modifications a bit further prior to exploring the role of tau in AD pathogenesis.

Tau phosphorylation

Tau is a phosphoprotein and phosphorylation plays a prominent role in regulating its physiological function. It is also clear that

aberrant tau phosphorylation occurs in AD brain and is associated with pathogenesis. Previous reviews have provided full discussions of the kinases and phosphatases that regulate tau phosphorylation, as well as possible involvement of tau hyperphosphorylation in the pathogenesis of AD [29–33]. Therefore, in this review we will highlight several recent studies on the phosphorylation of specific sites on tau, and how they affect tau function and their involvement in AD pathogenesis. It also should be noted that tau phosphorylation was detected in brain biopsies from non-AD patients at many sites that are not normally phosphorylated in autopsied non-AD material, although the extent of phosphorylation was less than observed in autopsied AD brain samples. In this study it was suggested that the hypophosphorylation of autopsy-derived adult human tau was due to rapid dephosphorylation post-mortem. Overall these data indicate that most tau phospho-epitopes detected in AD brain are not unique to the disease state [34], although the extent to which they are phosphorylated is greater than what occurs in the normal state.

In AD, phosphorylation of tau at specific epitopes appears to be a progressive event with phosphorylation at Threonine 231 (T231) occurring early, at 'pretangle' stages [35, 36]. Phosphorylation at this epitope appears to contribute to conformational changes in tau that are detected by the antibody TG-3 and subsequently by Alz-50 [36, 37]. It has also been shown that phosphorylation of T231 significantly reduces the ability of tau to bind microtubules [38]. Glycogen synthase kinase 3 β (GSK3 β) is often considered to be a 'tau kinase' as it efficiently phosphorylates T231 and other AD relevant tau epitopes *in vitro* and in cells and is likely deregulated in AD brain [38, 39]. Therefore, GSK3 β could contribute to the observed increase in pT231 in the disease state. Further, the peptidyl prolyl isomerase, Pin1, specifically binds to pT231 which results in a conformational change that can restore tau's ability to bind to microtubules or facilitate dephosphorylation of this site by protein phosphatase 2A, which also increases tau-microtubule interactions [40]. Aged Pin1^{-/-} mice exhibit increased phosphorylation of tau at T231 concomitant with increased tau aggregation and filament formation along with neurodegeneration [41], while soluble levels of Pin1 have been noted to decrease in AD brain [40]. Although Pin1 may act on other substrates in AD brain [40], these data suggest that increased phosphorylation of tau at T231 may play a role in the pathogenesis of AD.

Another phosphorylation site on tau that plays a pivotal role in regulating tau function is serine 262 (S262). S262 is located within the KXGS motif of the first microtubule binding repeat and is phosphorylated predominantly by the microtubule-associated protein microtubule affinity regulating kinase [42]. Phosphorylation of this site significantly decreases tau binding to microtubules [43], an effect that can be mimicked by pseudophosphorylation (mutation of serine or threonine to glutamate or aspartate to mimic phosphorylation) [44]. Increased phosphorylation at S262 was noted in pretangle neurons in AD brain suggesting that it was an early event in the pathogenic process. In flies, expression of tau with alanine mutations at both S262 and S356 (which is also part of a KXGS motif in the fourth microtubule binding domain) resulted in significantly less toxicity than expression of wild-type

tau [45]. In another fly study co-expression of A β and tau resulted in neurodegeneration, while expression of A β with tau containing a serine to alanine mutation at S262 (S262A) did not, strongly suggesting that phosphorylation of S262 is required for A β -induced, tau-dependent toxicity [46]. Intriguingly, S262A tau was phosphorylated at S202 to a lesser extent than wild-type tau in this model [46]. Likewise S262A/S356A tau was also phosphorylated to a lesser extent than wild-type tau at S202 as well as the PHF-1 epitope (S396/S404) in the fly. However, when GSK3 β was co-expressed with tau, both wild-type and S262A/S356A tau were efficiently phosphorylated at these same epitopes, although a significant reduction in the rough eye phenotype (indicative of neurodegeneration) was observed in flies expressing S262A/S356A tau compared to flies expressing wild-type tau [45]. These data suggest that the increased tau phosphorylation that results from increased GSK3 β activity is not the mechanism involved in the neurodegenerative process, at least in this model system [45].

When considering the role of tau phosphorylation in the pathogenesis of AD it is becoming apparent that a specific complement of phosphorylated residues enhance neurotoxicity and that phosphorylation of any one single site is likely not sufficient to convert tau to a toxic species. In addition, as alluded to above, the phosphorylation of one epitope on tau can influence the phosphorylation of other epitopes [45–48]. Expressing tau pseudophosphorylated at S262 and T231 resulted in increased toxicity in PC12 cells compared to wild-type tau or tau that was pseudophosphorylated at just S262 [49]. In flies, pseudophosphorylation of 14 key Ser/Thr-Pro sites in tau significantly enhanced toxicity [50], while mutating these same sites to alanine significantly blocked tau-induced toxicity [51]. Strikingly, this study revealed that no particular individual phosphorylation site was responsible for enhancing tau toxicity, and that recovery was only obtained when all 14 sites were mutated; when individual Ser/Thr-Pro sites were mutated to alanine, there was no recovery from tau-induced toxicity. It remains to be established whether phosphorylation of specific sites impact neurons in a sublethal, pathological manner, or alternatively compromise certain cellular functions that may contribute to decreased cell survival over time, rather than causing acute neuronal death.

Tau truncation

During the evolution of tau pathology in AD brain, tau appears to undergo sequential cleavage events [52]. Caspases, which are apparently elevated in AD brain [53–55], are likely involved in the proteolytic processing of tau. Several years ago it was shown that tau is cleaved by caspases at aspartic acid 421 (D421) in AD brain and appeared to be generated early in the pathogenic process [56, 57]. Tau truncated at D421 is more fibrillogenic than full length tau [56, 57] and early studies suggested that tau truncated at D421 significantly increased cell death [58, 59]. Recently it was demonstrated, using multiphoton imaging, that there are transient increases in caspase activity in neurons in a mouse tauopathy model, and this is followed by the formation of NFTs without acute

cell death. Additionally, expression of tau truncated at D421 in wild-type mouse brain also resulted in the formation of similar tau aggregates [60]. These data clearly indicate that caspase activation and tau cleavage precede the formation of tau fibrils and that caspase activation does not necessarily lead to acute cell death. Indeed, it is well documented that caspases play a number of roles in physiological processes [61–64]. Nonetheless, in AD inappropriate, albeit transient, increases in caspase activity and tau cleavage may compromise neuronal function. For example, inducible expression of tau truncated at D421 in a cortical neuronal cell line results in significant effects on mitochondrial function without profound cell death [65]. However, when the cells are subjected to stressors, the presence of tau truncated at D421 results in a significant decrease in cell survival compared to cells expressing wild-type tau [66]. Flies expressing tau truncated at D421 also displayed greater neuronal toxicity compared to what was observed in flies expressing full-length wild-type tau [67].

In addition to being cleaved by caspases at D421, tau may also be cleaved at D13 and D402 in AD brain by caspase 6 [68–70]. In one study the level of tau cleaved at D402 in NFTs and neuropil threads was associated with lower global cognitive scores in cases with no cognitive impairment, indicating that it may be an early event in the development of AD [68]. Finally, at later stages in the evolution of tau pathology tau undergoes further processing including truncation at glutamic acid (E) 391 which results in the formation of the MN423 epitope, along with further processing of the amino terminal [71]. These latter cleavage events appear after tau has formed the NFTs and seem to be part of the maturation process of the tangles [52, 71].

NFTs are not the primary toxic species

A final issue that needs to be addressed is: What is the toxic form of tau in AD? Although initially it was thought that the NFTs were the toxic species, it is becoming apparent that the mature fibrillar tangles are unlikely to be the primary mediators of toxicity, although at later stages of the disease they compromise cell viability. For example, cytotoxicity of an aggregation prone mutant tau occurred prior to the appearance of thioflavin S⁺ tau aggregates in an inducible cell model [72] and expression of tau in flies results in progressive neurodegeneration without NFT formation [73]. It has also been shown that in a repressible tau transgenic mouse model, turning off tau expression resulted in the attenuation of tau-induced memory impairment and neuronal loss, but NFTs continued to accumulate [22]. Furthermore, inhibition of tau hyperphosphorylation in the JNPL3 mouse model which expresses tau with an FTDP-17 mutation attenuated the severe motor deficits and reduced the amount of soluble tau aggregates without affecting NFT counts [74]. Another indication that NFTs are not the mediators of toxicity is that depletion of endogenous wild-type tau prevented behavioural abnormalities in an APP transgenic mouse model that does not develop NFTs [23]. Therefore, NFTs are likely not the major cause of neuronal toxicity and cognitive deficits, and in fact may play a protective role in the

initial stages of the disease. Indeed, it has been suggested that the formation of disease relevant protein aggregates may in general be protective by sequestering dispersed small aggregates, oligomers or misfolded proteins to minimize their toxicity and eventually facilitate their clearance by proteasomal activity or autophagy [75, 76]. Although this concept is supported by data from other neurodegenerative disease cell models [77, 78] it remains to be validated with respect to tau and AD.

Tau turnover and possible deficits in Alzheimer disease

AD results in deficits in numerous physiological processes, including degradative mechanisms. Proteasome activity in AD brain is lower than that in control brain, and this lowered activity is independent of the expression of proteasome subunits [79]. In addition, autophagic processes in AD brain are suggested to be compromised either as a result of decreased levels of macroautophagy regulatory proteins such as Beclin-1 [80], or impaired clearance of autophagic vacuoles by lysosomes [81]. Evidence of general degradative dysfunction in AD, along with co-labelling of tau with degradative markers (*i.e.* ubiquitin) [82], has led to a more detailed study of tau turnover in general and how it may be compromised in AD brain [83–85]. Tau, like other aggregative proteins involved in neurodegenerative diseases, has been described as a substrate for both proteasomal [86, 87] and autophagic [83] degradation and therefore its turnover may be impaired in the disease state. It should be noted that the majority of studies examining tau turnover have been done with exogenous, rather than endogenously expressed tau, a caveat that needs to be considered when evaluating the data.

Tau's susceptibility to proteasomal degradation has been reported to be influenced by phosphorylation, specifically at S199/S202/S205 with phosphorylation being required for tau's recognition by the E3 ligase C-terminus of Hsp70 interacting protein (CHIP) and subsequent proteasomal degradation [86]. However, another report [87] asserted the opposite: recognition of tau by CHIP occurred independent of its phosphorylation. Yet another report [88] showed that the ability of tau to be ubiquitinated by CHIP was dependent on the presence of exon 10 in the microtubule binding domain. Despite these differences, all three studies provided evidence that tau was ubiquitinated by CHIP. However, questions arise upon close examination of the findings that were presented. In separate studies, CHIP was found to catalyse the formation of forked ubiquitin chains *in vitro* on multiple substrates that consist of a mixture of lysine (K)11, K48 and K63 ubiquitin linkages and are insensitive to proteasome degradation [89]. This suggests that in the absence of other unidentified cofactors, CHIP behaves indiscriminately in *in vitro* reaction conditions. Second, degradation of the putative CHIP substrate nNOS is unaffected in cells derived from a CHIP-null mouse [90], which suggests that CHIP is dispensable in the degradation of its substrates,

and/or plays a role related to, but distinct from, acting directly as an E3 ligase towards its substrates. In fact, a recent study provided evidence that tau and tau truncated at D421 were not ubiquitinated by CHIP *in situ*, despite evidence of CHIP-mediated ubiquitylation in an *in vitro* reaction [91]. Overall, it is apparent that further studies are required to demonstrate definitively that tau is a bona fide substrate of CHIP *in situ*.

Although the proteasome primarily degrades ubiquitinated substrates, there is a growing awareness that it also can degrade proteins in an ubiquitin-independent manner and there is good evidence that tau's degradation by the proteasome can occur in the absence of direct ubiquitylation. Using SH-SY5Y neuroblastoma cells and *in vitro* techniques, David and colleagues [92] reported that clearance of full-length tau (without amino-terminal inserts) was sensitive to the proteasome inhibitor lactacystin, and that tau removal occurred independent of ubiquitylation. Similar results were reported with full-length tau in a cortical cell model [91]. A more recent study [93] also determined that phosphorylated tau is degraded by the proteasome independent of ubiquitylation through coexpression of dominant negative ubiquitin mutants with tau. The presence of these dominant-negative ubiquitin mutants was found not to affect the degradation of tau. Interestingly, the authors also found that phosphorylated tau co-immunoprecipitated with the chaperone Hsp70, but not with CHIP [93].

In addition to degradation by the proteasome, there is evidence that tau is processed by the lysosome, and more specifically, by autophagy. Treatment of a stable cell line expressing human tau with either the lysomotrophic drug chloroquine, the macroautophagy inhibitor 3-methyladenine (3-MA), or cathepsin inhibitors resulted in a delay to the degradation of tau and promoted formation of high molecular weight species [83]. In a separate study, D421-cleaved tau was produced in SK-N-SH neuroblastoma cells by treatment with prostaglandin J2, and the D421-tau cleavage product was degraded in a cathepsin-dependent manner [94].

Using a highly fibrillar internal fragment of tau, another study elucidated a pathway by which either inhibition of macroautophagy using 3-MA, or neutralization of the lysosome using NH₄Cl, slows the clearance of most forms of soluble tau, particularly pS262 tau [95]. However, this treatment resulted in the formation of a smaller cleavage product that engaged the machinery for chaperone-mediated autophagy, yet was not translocated into the lysosome. Despite this finding of involvement of macroautophagy and chaperone-mediated autophagy in the clearance of a small cleavage product of tau [95], further work is needed to determine the relative involvement of the autophagy pathway in the turnover of tau.

How to reconcile the equally compelling work describing both proteasomal and autophagic degradation of tau? The existence of an aggregative protein involved in neurodegeneration that is susceptible to either proteasomal or autophagic degradation depending on post-translational or aggregative state, is not unique. Huntingtin [96, 97], α -synuclein [98] and ataxin-3 [99, 100] have all been described as substrates of the proteasome as well as autophagy. Also, recent work examining mechanisms behind the selective autophagic clearance of aggregation-prone proteins

involved in neurodegeneration describes K63-linked ubiquitylation (rather than the more prevalent K48-linked ubiquitylation) of both tau and superoxide dismutase 1 (SOD1) leading to their autophagic clearance [101]. Interestingly, a mechanism for K63-linked ubiquitylation has been proposed for tau involving the E3 ligase TRAF6 [102]; the authors describe binding of K63-ubiquitylated tau to the shuttling factor p62, which has been linked to both the proteasome and autophagy [103]. Therefore it remains likely that if this mechanism for selective autophagy also occurs in AD brain, tau may be degraded by either the proteasome or autophagy, perhaps depending on conformational and aggregative state. In fact, work from our own lab has demonstrated that tau cleaved at D421 is more susceptible to autophagy-mediated clearance than full-length tau, which is prone to non-ubiquitin dependent degradation by the proteasome [91]. In agreement with our findings it was recently demonstrated that deletion of cathepsin D (a lysosomal protease and thus involved in autophagy) in flies resulted in the specific accumulation of tau truncated at D421 [67]. D421-tau has been reported to be more highly fibrillogenic than full-length tau [104], which may be a determining factor in the predominantly autophagic degradation of this form of tau, as aggregate-prone proteins are highly susceptible to autophagy [105]. It is also interesting to note that tau phosphorylated or pseudophosphorylated at S262 (which significantly changes the conformation of the protein [44]) appears to be degraded through the autophagy pathway and not the proteasomal pathway [95].

Tau toxicity in Alzheimer disease

Although for many years 'A β -centric' hypotheses dominated AD research [17], the importance of tau in the pathogenesis of AD is now much more appreciated [26], and seems to increase as new findings emerge. An early strong indicator of the importance of tau in A β -induced neurotoxicity came from a study in which neurons from tau^{-/-} mice were found to be very resistant to A β -induced neurite degeneration compared to neurons from wild-type mice or mice expressing human tau [21]. Complementing this study, reduction of tau levels in a human mutant APP mouse model by crossing them with tau^{-/-} mice resulted in marked improvements in performance in spatial learning, and reduced premature mortality, hyperactivity and excitotoxicity [23]. It was also recently demonstrated that reduction or elimination of tau was sufficient to prevent exogenous A β -induced axonal transport deficits in mouse primary neurons [24]. Finally, expression of a dominant negative form of tau in mice expressing human mutant APP resulted in the sequestration of Fyn kinase away from synapses, which prevented phosphorylation of the N-methyl D aspartate (NMDA) receptor and coincided with an attenuation of memory deficits. Similar results were obtained when tau was knocked out in these mice [106]. Overall these data strongly indicate that tau is an important mediator of A β -induced excitotoxicity, although its exact mechanism of action is unclear.

Tau was originally discovered as a microtubule-associated protein and the majority of studies involving tau have focused on its

ability to modulate microtubules; a function that is regulated by its phosphorylation state. Abnormal phosphorylation of tau, a prominent feature of AD brain, decreases its microtubule binding ability, which may destabilize microtubules and result in cellular damage [8, 107]. This 'loss of function' model could explain several aspects of tau toxicity; however, recent research points to possible 'gain of function' mechanisms of tau toxicity that may not be directly dependent on its microtubule binding ability [32]. For example, tau at approximately physiological concentrations did not inhibit axonal transport; however, tau filaments at the same concentration were found to be inhibitory and this inhibition was not due to direct microtubule binding [108]. Inhibition of anterograde axonal transport is now thought to be associated with the ability of tau to bind kinesin [109, 110]. Whether tau inhibits the interaction of cargoes with kinesin [109] or disrupts the attachment of kinesin to microtubules [111, 112] is still under investigation.

Tau-induced impairment of axonal transport has been implicated in the mislocalization of proteins and organelles to the soma. Pathological forms of tau have repeatedly been shown to cause abnormal somatic localization of mitochondria [112–115], which are the main source of energy production and calcium buffering in cells. Thus, sequestration of mitochondria away from axons, which contain areas of high energy demand and calcium influx, such as nodes of Ranvier and synapses, has a profound impact on cellular homeostasis [116]. For this reason, tau-induced mitochondrial mislocalization may be a key component in neurodegenerative processes [108, 112]. A recent paper provided some insight into a possible 'gain of function' mechanism by which pathologically modified tau may disrupt axonal transport of mitochondria and other kinesin cargoes. These studies were carried out using an FTDP-17 tau mouse model, and the hyperphosphorylated tau was found to selectively interact with c-Jun N-terminal kinase interacting protein 1 (JIP1), which regulates the association of cargo with the kinesin motor complex. This binding of JIP1 by hyperphosphorylated FTDP-17 tau caused JIP1 to mislocalize to neuronal cell bodies which was likely a contributing factor to the axonal transport deficits observed in these mice [116, 117].

Beyond impairment of mitochondrial transport, tau may disrupt several other aspects of mitochondria under pathological conditions. There is significant evidence indicating that mitochondria are compromised early in AD brain tissue [118–120], which has been replicated in experimental models exhibiting A β and tau pathologies [25, 121–126]. Abnormalities exist in mitochondrial fission/fusion and respiration protein levels in AD brain [120, 127], which may explain mitochondrial morphological alterations observed in several areas of AD patient brains [118] and apparent deficits in energy metabolism observed *via* PET scan [128]. Interestingly, stable expression of D421 truncated tau in cortical cell lines induced fragmentation of mitochondria [65], possibly replicating the imbalance of mitochondrial fission and fusion in AD brain. Reductions in several components of the tricarboxylic acid (TCA) cycle and the oxidative phosphorylation (OXPHOS) system have also been reported in AD transgenic mouse models [124, 125]. In fact, a recent study provided evidence that tau can synergize with A β at the level of mitochondria resulting in enhanced

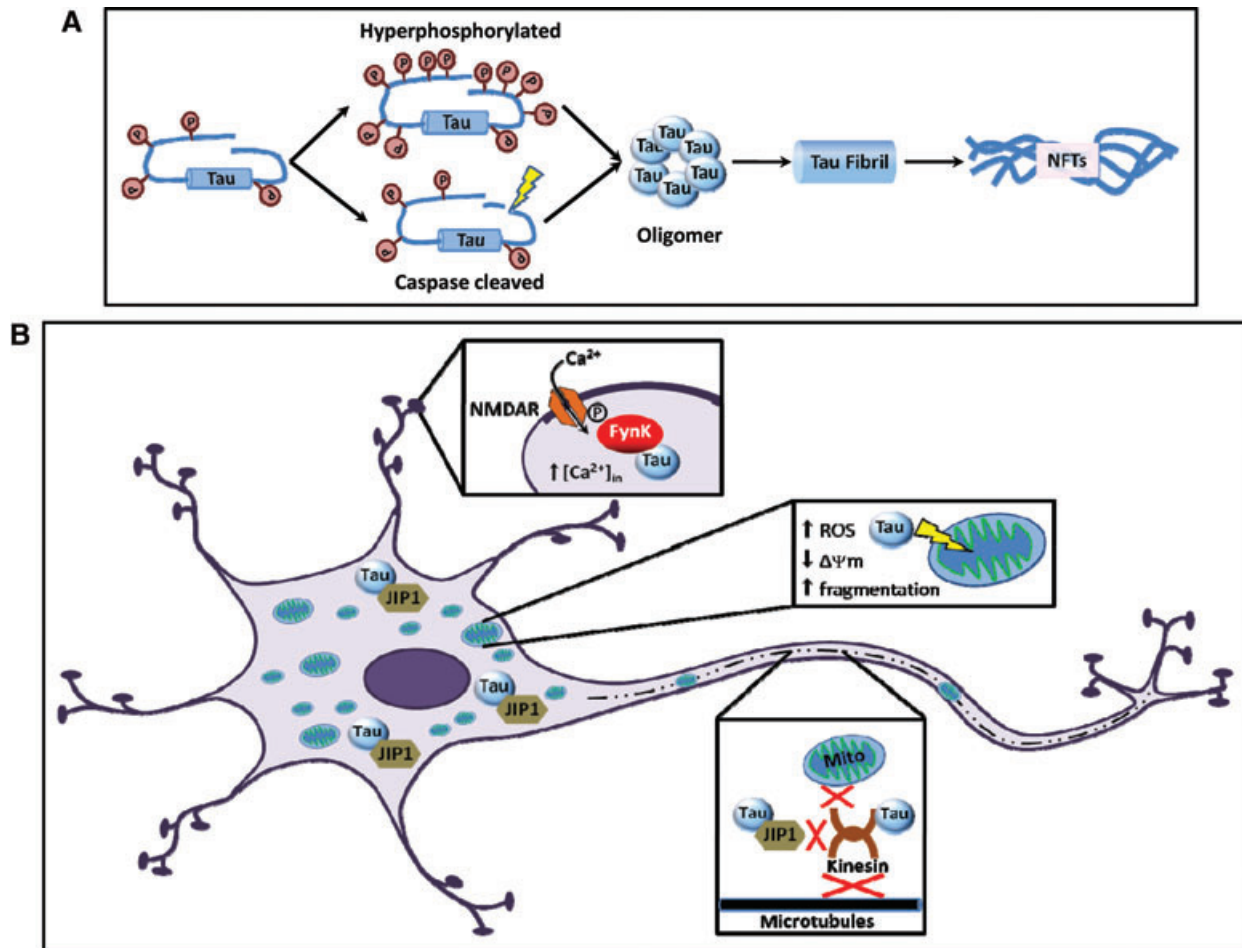


Fig. 1 Cellular targets of toxic tau. (A) Tau undergoes phosphorylation in physiological conditions. However, in pathological conditions, tau becomes hyperphosphorylated and/or cleaved, which facilitates aggregation and increases the toxicity of tau. In the continuum of the aggregation process, pathologically modified tau monomers first form oligomers, which further aggregate into fibrils and finally into NFTs. Recent studies suggest that monomeric or oligomeric species of tau are the more toxic than aggregated forms [22]. (B) Tau may manifest its toxicity by enabling or facilitating A β -induced excitotoxicity, mitochondrial damage and/or by disrupting axonal transport. Pathological tau may participate in the localization of Fyn kinase to the post-synaptic compartment, where it phosphorylates NMDAR subunits, causing increased inward Ca²⁺ conductance and leading to excitotoxicity [106]. Tau also contributes to mitochondrial dysfunction (*i.e.* decreased $\Delta\psi_m$, increased ROS, fragmentation) [65] and disrupts anterograde axonal transport of mitochondria and possibly other synaptic elements by binding to JIP1, which regulates the binding of cargos to the kinesin motor complex [116]. Transport deficits may cause energy depletion at synapses, thereby impairing synaptic transmission.

mitochondrial damage. This study compared mitochondrial measures between tau aggregate forming P301Ltau mice, A β plaque forming APP^{SWE}PS2^{N141I} mice, and triple transgenic (3xTg) mice which combine these mutations and pathologies. Proteomic analysis of 3xTg mice showed vast deregulation of several OXPHOS proteins related to complex I and IV, whereas tau transgenic mice only showed deregulation of complex I and A β plaque forming mice only showed deregulation of complex IV. Compared to either of the single pathology mice, 3xTg mice showed more severe or earlier onset of mitochondrial deficits, loss of mitochondrial membrane potential, and increased reactive oxygen species (ROS) production [25, 126]. This suggests that tau may exert its

toxicity through mitochondria, and may permit or facilitate A β -induced mitochondrial damage. Figure 1 summarizes some of the possible targets of pathological forms of tau.

Potential 'tau-centric' therapeutic strategies

There is a growing interest in developing pharmacological agents directed at pathological forms of tau. These treatment strategies

are largely in the early stages of development; nonetheless several studies have already yielded exciting and encouraging results. In this section, we highlight several of the emerging treatment strategies that are being explored. However, it should be noted that with many of the different approaches discussed herein the pharmacokinetic properties, bioavailability and possible side effects of these 'tau-directed' therapies are extremely important issues that remain to be fully addressed.

Tau aggregation inhibitors

It is well established that the NFT load can be used as a marker to clinically classify the stages of disease progression [129]. Also, the amount and progression of NFTs correlates positively with the extent of cognitive impairment [130]. Based on these and other findings it was originally suggested that NFTs were significant contributors to the pathogenic process in AD; however, more recently this has been called into question [22, 131]. Multiple potentially pathological forms of tau exist in the brains of AD patients, and there is significant debate over which forms are the toxic species. Nonetheless, there has been an effort to develop tau aggregate inhibitors as a therapeutic strategy for the treatment of AD with the presupposition that aggregated tau in some form (oligomeric, soluble or insoluble) is toxic. As mentioned previously, there are data to support the concept that soluble, aggregated tau species are toxic. For example, in a squid axoplasm model, monomeric tau at physiological concentrations had no effect on axonal transport, while tau filaments at the same concentrations selectively inhibited anterograde transport processes suggesting that pathological tau aggregation may be a contributing factor to neurodegeneration [108].

A number of labs have been investigating potential tau aggregation inhibitors, and in a recent review small-molecule inhibitors of tau aggregation were thoroughly described [132]. Therefore in this review we will just highlight a few key studies. Methylene blue is a phenothiazine that, along with other phenothiazine derivatives, has been used to treat a number of different medical conditions [133]. One important effect of methylene blue is the inhibition of aggregation of proteins that take on β -sheet conformation *in vitro*, including tau [134, 135]. Use of methylene blue *in vivo* has yielded mixed results. In a mouse tauopathy model, 1 mM methylene blue was administered to the right hippocampus using a mini-osmotic pump. This resulted in a reduction in total tau levels that were paralleled by a decrease in phosphorylated (S202/T205) tau along with improved behaviour but without any effect on pathology [136]. When the mice were administered a therapeutically relevant dose of 10 mg/kg of methylene blue in the drinking water for 12 weeks there was variable improvement in behaviour and decreases in soluble tau levels, with no change in tau tangle pathology. Interestingly, when the levels of methylene blue in the cerebellum were measured, they were found to positively correlate with Morris water maze performance and inversely correlate with soluble tau levels. These results are in agreement with previous findings showing that NFTs are likely not associated with func-

tional deficits but that reducing soluble tau levels is beneficial [136]. These results also suggest that the putative therapeutic effect of methylene blue may not be associated with its ability to inhibit aggregate formation. In Zebrafish, methylene blue inhibited the aggregation of a mutant form of huntingtin, but had no effect on tau pathology when an FTDP-17 mutant tau was expressed [137], which is similar to what was observed in the mouse model. However, in contrast to the mouse model, methylene blue treatment of the Zebrafish did not prevent or reverse the functional deficits that resulted from the expression of the FTDP-17 mutant tau or the mutant huntingtin [137].

N3 is a benzothiazole derivative that has been shown to protect against tau aggregation and tau-mediated neurodegeneration concurrent with neuroprotection in an *in vivo* model [138]. Based on *in vitro* assays, it was proposed that N3 was a direct tau fibrillization inhibitor and that this mechanism contributed to its neuroprotective effects. However, further studies demonstrated that N3 disrupts the binding of tau filaments to Thioflavin S, but does not directly antagonize the formation of tau fibrils. These studies again strongly suggest that the neuroprotective effects of the compound are not due to a reduction in tau aggregation [139]. Taken together, the results of these and other studies suggest that the reduction of tau fibril formation *in vitro* by certain small molecules may not be related to their therapeutic effects *in vivo*.

Microtubule stabilizing agents

Given that, in AD brain, tau is hyperphosphorylated and less functional as a microtubule stabilizer, therapies that compensate for this loss of tau function and stabilize microtubules are being considered. A reduction in microtubule integrity has been proposed to be an important factor in synaptic dysfunction [140]. In brain slice preparations a derivative of the microtubule stabilizing agent taxol protected against synaptic loss in response to lysosomal stress [140]. These findings are of interest, but unfortunately these taxol-based derivatives often show low blood-brain barrier permeability [141]. However, intranasal administration of an octapeptide (NAP), which stabilizes microtubules, in an AD mouse model reduced both tau and A β pathology indicating that it has therapeutic potential [142]. In addition, brain penetrant microtubule-stabilizing compounds have been identified which may allow for an *in vivo* analysis of the efficacy of this type of approach in the treatment of AD in appropriate animal models [141].

Tau immunotherapy

Although anti-A β immunotherapies yielded very promising results in AD animal models, results from clinical trials have been mixed. For example, A β levels and plaque loads can be reduced by A β immunotherapies, but improvements in cognition and disease progression have not been substantial [143]. Clinical trials using A β vaccines (such as Bapineuzumab; a humanized monoclonal antibody to A β) are still ongoing at this

time; however, the results thus far suggest that other targets may need to be considered.

Over the past several years, there has been a growing interest in immunotherapies targeted at reducing tau levels as a strategy for treating AD [144]. One of the possible immunotherapeutic approaches being considered is the selective reduction of pathological forms of tau. Asuni and colleagues demonstrated that immunization of mice expressing P301L-tau (JNPL3 mice) with a small phospho-tau peptide (amino acids 379–408 with S396/S404 phosphorylated) resulted in the production of antibodies that entered the brain, which reduced the extent and slowed the progression of the behavioural phenotype [145]. This group recently extended these studies by creating a new mouse model with accelerated development of tau pathology and immunizing with the same phospho-tau peptide. Using this model they demonstrated that immunization with this tau peptide resulted in a reduction in soluble and insoluble tau phosphorylated at S396/S404 and a significant attenuation of cognitive impairment [146]. A study from another group also demonstrated that immunization of other tauopathy mouse models with phospho-tau peptides also reduced tau pathology without significant side effects [147]. However, an earlier study found that immunization of mice with full length recombinant tau resulted in neurological deficits with neuropathology [148]. Given this finding and the fact that an active immunization protocol with A β resulted in an adverse response in a number of the human cases [149], it would seem that a passive immunization protocol may be a better therapeutic approach. In addition, the optimal AD relevant form of tau to be targeted for immunotherapy needs to be considered. Although the studies cited above focused on phospho-tau, there is a growing awareness that oligomeric tau forms may be the more toxic species, and therefore would make an appropriate target for tau immunotherapy [144]. In a recent study, relatively pure, homogenous populations of neurotoxic tau oligomers were prepared [150], thus providing a potential immunogen for the generation of antibodies to prefilament forms of tau.

Finally, there is a growing awareness that pathological forms of tau, as well as other disease relevant proteins such as α -synuclein (Parkinson's disease) and mutant huntingtin (Huntington's disease) may be capable of being spread from cell to cell and thus facilitate disease progression [151–154]. In addition, extracellular tau may also induce toxicity. Therefore, it has been proposed that extracellular tau may be a potential target for immunotherapy in the future [155].

Autophagy activators

Reducing the burden of aggregated proteins through up-regulation of autophagy has been proposed as a potential therapeutic approach for various neurodegenerative diseases [156]. Numerous studies have focused on the use of rapamycin, a well characterized clinically approved drug that up-regulates autophagy through inhibition of the central regulatory protein mammalian target of rapamycin (mTOR). In cell models, rapamycin mediates the reduc-

tion of aggregates composed of both polyglutamine and polyalanine expansions [157, 158]. In *Drosophila* models expressing wild-type or mutant forms of tau, rapamycin reduced toxicity and it was suggested that this was due to a reduction in insoluble tau [158]. Rapamycin also reduced both A β and tau pathology in the 3xTg AD mouse model, though it is unclear in this study if reductions of tau pathology occur as a direct result of rapamycin treatment, or as a downstream effect of A β reduction [159].

Though the use of rapamycin as an autophagic-inducing agent has shown promise in effecting the clearance of insoluble and pathologically modified tau in cell and animal models, questions about potential pharmacological side effects remain. mTOR is a central signalling molecule that regulates the cell's responses to nutritional and environmental stress. Many cellular processes besides autophagy are regulated by mTOR, including protein transcription and translation. The activity of mTOR is modulated by a variety of inputs such as varied nutrient conditions in the cell's microenvironment, cell stress and presence or absence of growth factors [160]. As such, in addition to up-regulating autophagy, inhibition of mTOR by rapamycin can also inhibit protein synthesis. It has been suggested that inhibition of protein translation may in fact be a reason for rapamycin's observed clearance of mutant huntingtin aggregates; in this study, the authors note Atg5-independent clearance of mutant huntingtin alongside global suppression of protein translation [161]. In addition to this finding, it has also been suggested that in isolated neurons, rapamycin is ineffective in promoting the conversion of LC3-I to LC3-II, a key indicator of autophagy up-regulation [162].

The myriad signalling responsibilities of mTOR, and the above-mentioned concerns over rapamycin's efficacy towards autophagy up-regulation in neurons have sparked searches for new specific autophagy inducing factors. A small molecule screen carried out to determine new effectors of autophagy identified multiple mTOR-independent candidates, some of which were effective in promoting both the clearance of huntingtin aggregates as well as decreasing levels of mutant α -synuclein in mammalian cell and *Drosophila* models of protein aggregation [163]. In addition, the disaccharide trehalose (found in multiple non-mammalian organisms) has been shown to reduce both mutant huntingtin aggregate burden and accumulation of mutant α -synuclein in an mTOR-independent manner [164]. Trehalose also promoted the clearance of phosphorylated and aggregated tau in a mouse model that was both parkin-null and expressed tau with multiple FTDP-17 mutations [165]. Concurrent with decreases in tau pathology, the study described improvements in behavioural deficits, as well as decreases in both astrogliosis and dopaminergic neuron death [165]. The findings of the above studies are quite promising, though further study of additional pharmacological effects of mTOR-independent autophagy is needed.

Mitochondria targeted therapies

Given that one of the primary targets of pathological forms of tau may be the mitochondria, therapies that increase mitochondrial

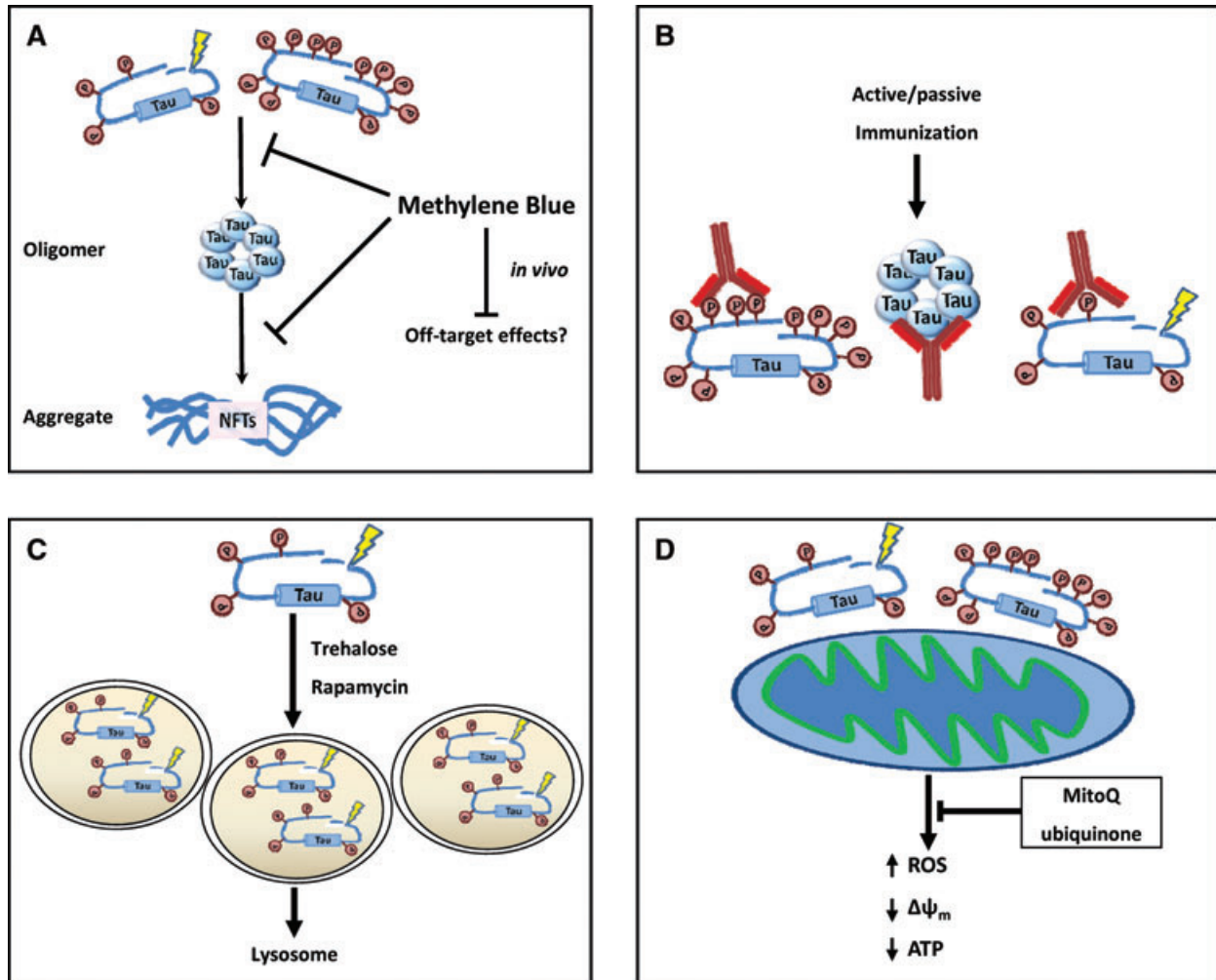


Fig. 2 Treatment strategies to reduce the impact of tau pathology. (A) *In vitro*, the formation of tau oligomers and aggregates is disrupted by the use of phenothiazine derivatives such as methylene blue. This results in a reduction of phosphorylated and total tau levels, though *in vivo* the effect of methylene blue treatment on the formation of aggregates is debated (see text). (B) Active immunization of tau in *in vivo* models has been shown to improve behavioural measures and reduce levels of phosphorylated and total tau. (C) Activators of autophagy have been shown to increase the degradation of pathological forms of tau, and improve pathological measures in mouse models of AD. (D) The detrimental effects of expressing pathological forms of tau on mitochondrial function may be slowed or reversed with antioxidants such as mitoQ, ubiquinone and other antioxidants.

function in concert with therapies that target tau may be a reasonable strategy. Indeed, mitochondria have been increasingly recognized as a beneficial therapeutic target in neurodegenerative diseases, including AD [166]. Many proposed therapies target mitochondrial ROS production and cellular oxidative stress which result from malfunctioning mitochondria and compromise cell viability.

Due to the well-documented oxidative damage associated with neurodegenerative diseases, antioxidant molecules, such as vitamins C and E, glutathione, Coenzyme Q₁₀ (ubiquinone), and a mitochondrial targeted derivative of ubiquinone, MitoQ, have been popular therapeutic candidates. Vitamins C and E show neuroprotective effects in AD mouse models including decreased A β plaque load

and enhanced cognitive function [167, 168]. However, conflicting results exist as to the efficacy of these antioxidants in AD patients, which in part may be due to differences in study design and the characteristics of the subjects [169]. Ubiquinone is an important component of the electron transport chain that can act as a potent lipid oxidant scavenger. Therapeutic ubiquinone has shown positive results in animal models of AD and Parkinson's disease [170, 171] and appears to show some efficacy in clinical trials with Parkinson's disease [172, 173] and Huntington's disease [174, 175] patients. MitoQ is a lipophilic cation analogue of ubiquinone, which accumulates specifically in mitochondria in a mitochondrial membrane potential dependent manner and can be recycled by the electron

transport chain [176]. Currently, MitoQ clinical trials for Parkinson's disease have yielded encouraging results suggesting that MitoQ may prove to be a useful therapeutic molecule [177]. However, using these molecules as therapies has several drawbacks such as poor translocation across the blood–brain barrier and the non-universal contribution of these oxidants to cellular oxidative damage. Therefore, treating with a single antioxidant may be beneficial but will not abate all oxidative stress [166].

Summary and conclusions

Studies over the past decade have brought about a maturation of our understanding of the role of tau in AD pathogenesis. These discoveries have breathed new life into a number of encouraging avenues for future tau research including: (1) investigation of soluble, non-aggregated forms of tau as a primary disease agent, (2) exploring the role of tau as an enhancer, or even gatekeeper, of A β -induced degeneration, (3) elucidating the mechanisms/pathways regulating the degradation of tau as determined by its post-translational state and (4) examining the mechanisms by which pathological forms of tau may negatively impact mitochondrial biology. Going forward, studies focused in these areas as well as further mechanistic dissection of the contribution of different tau isoforms in the disease process, will provide us with foundational

information for the development of therapies for the treatment of AD. Figure 2 provides a summary of possible tau-directed therapeutic strategies.

Although alterations in tau are likely a significant component of AD pathogenesis, it is unlikely that a monotherapeutic approach will be highly efficacious in slowing or halting disease progression given the complex nature of the disease. Therefore, combining therapies is likely a strategy that needs to be pursued. Conceptually, combinatorial approaches for treatment of AD are already being used (*e.g.* treating with both cholinesterase inhibitors and NMDA antagonists) and more are being considered [178–180]. Overall, it is clear that understanding the role of tau in AD pathogenesis is fundamental to the development of successful therapeutic strategies for the treatment of AD.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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