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Evidence That Non-Fibrillar Tau Causes Pathology Linked To Neurodegeneration And Behavioral Impairments

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Abstract

The discovery that mutations within the tau gene lead to frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) provided direct evidence that tau alterations can lead to neurodegenerative disease. While the presence of tau fibrils and tangles is a common feature of all tauopathies, including Alzheimer's disease (AD), data are emerging from biochemical, cell-based and transgenic mouse studies which suggest that a pre-fibrillar form of pathological tau may play a key role in eliciting central nervous system (CNS) neurodegeneration and behavioral impairments. Herein we review recent findings that implicate diffusible tau pathology in the onset of neurodegeneration, and discuss the implications of these findings as they relate to tau tangles and possible therapeutic strategies for the treatment of AD and related tauopathies.

Keywords

Fibrils; Neurodegeneration; Oligomers; Tangles; Tau; Transgenic

Introduction

A large number of neurodegenerative dementias and movement disorders, including Alzheimer's disease (AD), Pick's disease, corticobasal degeneration, progressive supranuclear palsy and certain frontotemporal dementias, are characterized by the intraneuronal accumulation of aggregates comprised of the microtubule-associated protein, tau (reviewed by [12,40]). Collectively, these disorders comprise the neurodegenerative tauopathies, with AD being by far the most prevalent example. In each of these diseases, tau monomers assemble into higher order structures that ultimately form defined filaments which take on characteristic morphologies that can differ between specific tauopathies, but share the common features of all amyloids in deposits known as neuronal and glial tangles or dystrophic processes [10].

In AD brain, tau fibrils are generally found as paired-helical filaments (PHFs) which aggregate to form neurofibrillary tangles (NFTs) that, along with Aβ peptide-containing senile plaques, are hallmark pathological lesions of AD [11]. The observation that the degree of cognitive impairment in AD correlates better with the number of NFTs than senile plaques [7,8,57] has led to the suggestion that it is these tau aggregates that lead to neuronal dysfunction. However, while there are mutations in inherited forms of AD that clearly affect Aβ levels and thereby directly implicate it in this disease [25], there have been no defined mutations in tau that lead

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to familial AD. Thus, the discovery that a number of tau mutations cause frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) was pivotal in establishing that tau abnormalities could directly cause neurodegenerative disease [26,27], and strengthened the hypothesis that tau plays a critical role in AD pathogenesis.

While tau alterations result in FTDP-17 and, by extension, likely contribute to the pathology of all tauopathies, the mechanism(s) by which tau causes neuronal dysfunction and death is still a matter of debate. In general, the existing literature can be interpreted to support either a tau toxic "gain-of-function" or a "loss-of-function" that compromises a normal tau activity, likely through a loss of its ability to stabilize microtubules. In this brief review, we examine these mechanistic hypotheses with an emphasis on what has been learned from *in vitro* and *in vivo* studies of tau, including those tau isoforms linked to FTDP-17. We also consider whether it is fibrillar tau, or perhaps instead a soluble pre-fibrillar tau species, that causes neuropathology leading to behavioral impairments. Contemplation of the possible mechanisms of tau toxicity, particularly the possible role of soluble pre-fibrillar structures, is timely given a variety of recent studies which suggest that diffusible Aβ oligomers may play a critical role in AD [23]. As is the case with Aβ, the proposition that non-fibrillar tau may lead to neurodegenerative changes begs the question of whether pathological tau deposited into aggregates such as NFTs might, in fact, ultimately be benign or perhaps even protective due to their ability to sequester harmful diffusible species of tau.

In Vitro Studies of Tau Function

The major function ascribed to tau is that of microtubule binding, where it has been demonstrated to stabilize microtubules and hasten their assembly [20,55]. There are six isoforms of tau in the adult brain that are predominantly localized to axons, and these arise through the alternative splicing of exons 2, 3 and 10 [6,28]. Within the tau sequence are four carboxyl-terminal tandem repeat sequences that encode the microtubule-binding domains, and one of these resides in alternatively-spliced exon 10 such that tau isoforms with both three (3-R) and four (4-R) microtubule-binding repeats exist in the adult CNS. There is evidence that 4-R tau binds with greater avidity to microtubules than does 3-R tau [49], and the ratio of these 3-R and 4-R tau isoforms, which is ~1:1 in the adult human brain, may therefore affect microtubule function.

Tau has a large number of potential phosphorylation sites, and ~30 of these appear to be phosphorylated in adult brain [17]. There is a profound hyperphosphorylation of tau in neurodegenerative tauopathies [9,10], and this has been shown to decrease tau interaction with microtubules [14,16,20]. Accordingly, one of the possible consequences of tau hyperphosphorylation is a destabilization of microtubules that results in impaired axonal function, and a number of studies have provided evidence of this. For example, isolated hyperphosphorylated tau from AD brain was shown to be a poor promoter of microtubule polymerization, but normal polymerization could be restored upon treatment with phosphatase [3,16]. Subsequent studies revealed that hyperphosphorylated tau could sequester normal tau that was bound to assembled microtubules, resulting in microtubule depolymerization [1]. Furthermore, increasing the degree of tau phosphorylation in cell-based models, either through inhibition of protein phosphatase activity [45] or by co-transfection with glycogen synthase kinase (GSK)-3β [56], resulted in an alteration of microtubule function. Besides affecting microtubules, an increase of highly phosphorylated tau could result in enhanced tau aggregation and this has been demonstrated with hyperphosphorylated tau [1], although phosphorylation at only some of the known sites seems to result in increased fibrillization [48]. It should be noted that recombinant non-phosphorylated tau is fibrillization competent, albeit requiring the presence of negatively charged co-factors [40]. While increased tau phosphorylation may lead to enhanced aggregation, this would likely be secondary to effects

on microtubule dynamics as it has been demonstrated that pseudophosphorylated forms of tau can induce cellular toxicity without signs of fibril formation [22]. These data thus provide evidence that pre-fibrillar, hyperphosphorylated tau may be cytotoxic.

The effects of FTDP-17 mutations on tau function have been extensively studied. These mutations can be grouped into two general categories; those that are intronic and affect the splicing of exon 10 (and its corresponding microtubule-binding domain), and those that alter the protein sequence. The former group comprises approximately 50% of the tau mutations and the primary effect seems to be an increase in the 4-R/3-R tau ratio [26,27,40]. Since 4-R tau binds to microtubules more effectively than 3-R tau [49], one possible result of the exon 10 splicing mutations is an increased microtubule stabilization that might alter normal polymerization/depolymerization dynamics. FTDP-17 cases with exon 10 splicing mutations show only 4-R tau within brain inclusions [52], suggesting that perhaps another effect of an elevated 4-R/3-R ratio is increased fibrillization of 4-R tau. Nearly all FTDP-17 mutations that affect protein sequence are found within or near one of the microtubule-binding domains, and most cause an increased propensity of tau to aggregate [13,24,47] and a decreased ability to promote microtubule assembly [19,32,33]. The impaired capacity of mutated tau isoforms to enhance microtubule formation may be further exacerbated by elevated levels of phosphorylation, as it appears that many FTDP-17 tau mutations cause the protein to be a better kinase substrate than normal tau [2].

Tau Transgenic Mouse Models

A number of research groups have generated transgenic mouse models in which various tau isoforms are over-expressed. While it is beyond the scope of this short treatise to review all of these models (see [41] for greater detail), it is worthwhile to consider some of the findings from these transgenic mice in the context of mechanism(s) of tau-induced pathological changes. It is interesting that over-expression of normal human tau has generally resulted in relatively mild phenotypes in transgenic mice. For example, expression of tau40 (the longest 4-R tau isoform) in two different transgenic lines did not lead to the formation of PHFs or tangles [30,53], although there was a somatodendritic relocalization of the expressed tau and, in one model [53], an observation of axonal dilations with motor impairment. Incorporation into mice of a tau transgene that encodes the entire human tau gene, such that all six human isoforms were synthesized, also led to an age-dependent re-localization of human tau from axons to somatodendritic compartments, but again resulted in an absence of frank tau inclusions or other neuropathology [21]. Interestingly, when these mice (designated "*8c*") were crossed with tau knock-out mice to generate progeny that only expressed human tau, filamentous tau inclusions and neuronal death were observed as these *"htau"* animals aged [5]. These data suggest that the absence of mouse tau, which is exclusively of the 4-R form, somehow resulted in increased tau neuropathology relative to the original *8c* mice. Since *htau* mice were shown to have a higher 3-R/4-R tau ratio than is normally found in humans, and since the *8c* mice had a more normal 3-R/4-R proportion due to the contribution of endogenous mouse 4-R tau, the authors suggested that perhaps the imbalance in tau isoforms led to the *htau* phenotype. That excess 3-R tau was pivotal to the phenotype of these mice was bolstered by the finding that *htau* mice accumulated only 3-R tau within inclusions [5]. This explanation would imply that a delicate balance of 3-R-to-4-R human tau must be maintained in healthy cells, and would be consistent with the onset of FTDP-17 when this ratio is affected by mutations that alter exon 10 splicing such that there is an increase of 4-R tau expression relative to 3-R tau [26,27,40]. A change in 4-R/3-R tau balance could affect microtubule function due to the stronger binding of 4-R than 3-R tau to microtubules [49]. In this regard, it is noteworthy that crossing tau40 (4-R) mice that developed axonal swellings and motor deficits [53] with transgenic mice expressing GSK-3β resulted in a normalization of phenotype due to increased tau phosphorylation [54]. While this finding would appear to be counterintuitive given the evidence that tau

phosphorylation reduces microtubule binding and enhances fibrillization, the authors hypothesized that the axonal dilation in the tau40 mice resulted from excessive microtubule binding by tau40 and that its phosphorylation allowed for a more normal level of tau/microtubule interaction.

Unlike what has been observed with 4-R tau, expression of the shortest human tau 3-R isoform in transgenic mice led to an age-dependent formation of insoluble, hyperphosphorylated tau inclusions [36], although it took 18–24 months for these mice to develop NFT-like deposits that were thioflavine-positive [37]. Notably, by 12 months of age the transgenic mice showed a reduction in both microtubule numbers and axonal transport within peripheral nerve as well as an impairment in motor function [36,59], and microtubule density and motor behavior could be improved by treating the mice with the microtubule-stabilizing drug, paclitaxel [59]. Interestingly, the ameliorative effects of paxlitaxel occurred despite the fact that paclitaxel did not reduce the burden of axonal tau aggregates or spheroids [57]. These data provide important *in vivo* evidence that tau abnormalities affect microtubule dynamics, and that these alterations can manifest as behavioral changes.

A variety of transgenic mouse lines have been created in which mutated tau is over-expressed. These include several lines that express the FTDP-17 P301S or P301L mutations found within exon 10 [41]. In general, these tauopathy animal models show an age-dependent deposition of hyperphosphorylated tau filaments and associated signs of neurodegeneration in brain areas wherein the mutant protein is expressed. Moreover, a recent report [58] revealed that synapse loss can be detected ~3 months before filamentous tau accumulations appear in a tau P301S transgenic strain, and that microglial activation as well as astrogliosis also preceded the development of filamentous tau inclusions. However, there is evidence of pathological tau hyperphosphorylation and insolubility concomitant with the onset of synaptic loss, microgliosis and astrogliosis. These observations suggest that a pre-fibrillar form of pathological tau causes early synaptic changes, gliosis and neuroinflammation, all of which are consistent with the concept that a pre-fibrillar species of pathological tau, and not necessarily fibrillar species of tau, is responsible for early pathological changes and neurodegeneration in these transgenic mouse models of tauopathy. Further support for this idea is provided by the study of SantaCruz et al. [50], who examined mice expressing human P301L tau under a repressible promoter. These animals showed profound tau inclusions and neuron loss by 5.5 months of age. Surprisingly, repression of the human tau transgene after initial brain deposition of tau fibrils resulted in improvements in cognitive testing and decreased neuron loss relative to non-repressed mice, even though tau tangles continued to accumulate. These data would imply that tau tangles themselves do not lead to synaptic dysfunction and neurotoxicity, but that non-fibrillar, albeit pathological, human tau is responsible for these events. Finally, a recent study utilizing the previously discussed *htau* transgenic mice [4] showed that neuron death in aged animals occurred in cells that did not have direct evidence of NFT formation. This again suggests that tau fibrils are not in and of themselves required for pathological tau to have neurotoxic effects. Thus, results from at least three tau transgenic models implicate non-fibrillar tau as being at least partially culpable as a proximal cause of neuropathological changes and neurodegeneration, thereby bringing into question whether mature tau fibrils further contribute to the disease process.

Finally, as we consider what transgenic mice can teach us about mechanisms of tau-mediated neurotoxicity, it should be noted that tau knockout mice do not have an obvious phenotype other than altered microtubule organization in small-caliber axons [18,31]. On the other hand, primary hippocampal cultures from these animals show a delay in axonal extension and development [18] and Hirokawa's group showed that tau knockout mice develop cognitive and motor abnormalities with advancing age [35]. Nonetheless, the absence of significant neuronal abnormalities in tau knockout mice has led some to suggest that the role of tau in microtubule

stabilization and dynamics might not be critical, and that a tau gain-of-function toxicity is more likely. However, developmental changes cannot be excluded in these constitutive knockout mice, whereby another one of the many microtubule binding proteins in brain could compensate for the loss of tau. In fact, it has been reported that tau knockout mice have an elevated level of the microtubule-binding protein, MAP1a [31]. It would be of interest to determine the effects of repressing mouse tau after animals have reached adulthood, such that compensatory changes are minimized. It should also be noted that the exclusively 4-R mouse tau is unlikely to fully model its human counterpart, particularly with respect to the possible importance of maintaining the critical 4-R/3-R tau ratio of ~1 in the adult human brain.

Mechanisms of Tau Toxicity: Diffusible or Fibrillar Pathological Tau?

Historically, dogma has been that the aggregated filaments which accumulate in neurodegenerative CNS and other amyloid diseases are responsible for pathology. However, this notion is being critically re-examined in light of a variety of recent data which suggest that soluble oligomeric structures, including those formed by Aβ peptide, may be the most biologically relevant form of amyloid peptides and proteins (reviewed in [23]). In fact, among the multiple activities recently attributed to Aβ oligomers is an ability to increase the activity of GSK-3β [34] and to initiate tau-dependent microtubule disassembly [38]. The increasing evidence that oligomeric Aβ, and not fibrillar Aβ peptides, might be more meaningful in AD has led to speculation that Aβ fibrils as well as the senile plaque into which they accumulate are not harmful and, instead, may be beneficial by sequestering toxic oligomers into Aβ fibrils. Simply stated, this premise is based on the notion that diffusible Aβ oligomers are structural precursors of fibrils, and that their assembly into insoluble fibrillar deposits removes the oligomers from “circulation” so that they can no longer exert their noxious biological effects.

The possibility that diffusible tau, either as monomer/dimer or as oligomers, could contribute to disease pathology has not been extensively studied. However, as noted above, data from *in vitro* studies and several tau transgenic mouse models [4,50,58] are consistent with this possibility, and recent manuscripts that have examined tau fibril assembly have identified oligomeric tau intermediates that might be analogous to their Aβ counterparts [39,43]. In particular, Maeda et al. [43] report the presence of what they term “granular” tau oligomers that have been proposed to be comprised of ~40 monomers, and this group shows that these oligomers can ultimately assemble into filaments. Our laboratory has identified a similar pre-fibrillar tau oligomer that can be visualized by atomic force and transmission electron microscopy, and tau protofibrils were identified which were comprised of an aligned series of these oligomeric structures (Xu et al., submitted). Notably, there is evidence that oligomeric tau is elevated in the frontal cortex of brain samples from individuals displaying varying degrees of Braak-staged NFT pathology, and these species were detected in brains where NFTs had not yet formed [44]. The authors thus speculated that increases in these tau oligomers may represent a very early marker of AD.

As of this writing, there have been no published studies that have directly tested the biological activities of tau oligomers. Therefore, the mechanisms by which these species might contribute to disease processes are generally unknown. Since tau oligomers presumably form within the cellular cytoplasm, it is likely that any biological effect that they exert will be quite different from those ascribed to extracellular Aβ oligomers. However, one can speculate that oligomeric tau might confer either a gain-of-function toxicity, such as has been proposed for Aβ oligomers, or a loss-of-function that results from a decrease of tau microtubule binding upon formation of oligomers. When contemplating diffusible tau forms, it is also important to recall that there is evidence of highly phosphorylated tau species which impair microtubule function in the absence of overt fibril formation [22,45,56]. Assuming that tau phosphorylation affects microtubules through a reduction of tau monomer binding, oligomer formation may not be

important to this process and may simply represent structural intermediates on the pathway to fibril formation.

Conclusions

The discovery that mutations within the tau gene can result in FTDP-17 was a transforming event that provided direct evidence that tau alterations can lead to neurodegenerative disease in the absence of Aβ deposits or any other neurodegenerative disease lesions. While a common feature of tauopathies is the formation of tau fibrils and tangles, data are emerging from biochemical, cell-based and transgenic mouse studies that a pre-fibrillar form of pathological tau may play a central role in eliciting neurodegeneration. In fact, the involvement of a soluble tau species in disease is concordant with the observation that some FTDP-17 patients with the P301L mutation can have profound neurodegeneration with relatively few tau tangles [15], and that neuron loss in AD far exceeds the number of NFTs [29]. However, it is worth noting that while tangles are far easier to quantify than neurotic tau lesions, >90% of the burden of tau pathology is located in dystrophic tau neurites which rarely have been correlated with clinical manifestations of AD or other tauopathies [46].

While there is support for the hypothesis that diffusible tau can contribute to neuropathology, the evidence of well-defined tau oligomers playing an important role in disease is largely speculative. There is a paucity of studies that have directly tested the biological effects of oligomeric tau and such studies are not straightforward since tau oligomers would presumably exert their effect from an intracellular compartment. Nonetheless, data generated over the last several years have provided evidence of some form(s) of non-fibrillar tau being a toxic agent, and this calls into question whether the hallmark fibrillar tau deposits seen in all tauopathies are meaningful to disease onset or progression. As has been suggested for Aβ, it is possible that the deposition of tau into insoluble aggregates may serve as a mechanism to reduce the levels of toxic soluble species. Of course, it is also conceivable that both a diffusible tau species and tau aggregates contribute to CNS neurodegeneration in AD and related tauopathies. Gaining a better understanding of the relative toxicities of diffusible and insoluble tau forms is important, as this has profound implications when considering therapeutic interventions for treating tauopathies. For example, one current drug strategy that has been discussed is the development of inhibitors of tau fibrillization [42,51]. While this tactic is sensible if tau fibrils lead to neurotoxicity, it may be less attractive if pre-fibrillar pathological tau is biologically active since inhibiting tau fibrillization could increase the level of this toxic diffusible species of non- or pre-fibrillar tau. We therefore anxiously await future experiments that will further elucidate the nature and activities of the non-fibrillar toxic tau species that have been hinted at in recent studies.

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