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Review

Tau, tangles, and Alzheimer's disease

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Abstract

Neurofibrillary tangles (NFT) are comprised of the microtubule-associated protein tau, in the form of filamentous aggregates. In addition to the well-known changes in phosphorylation state, tau undergoes multiple truncations and shifts in conformation as it transforms from an unfolded monomer to the structured polymer characteristic of NFT. Truncations at both the amino- and carboxy-termini directly influence the conformation into which the molecule folds, and hence the ability of tau to polymerize into fibrils. Certain of these truncations may be due to cleavage by caspases as part of the apoptotic cascade. In this review, we discuss evidence that strongly suggests that these truncations occur in an orderly pattern and directly influence the ability of tau to polymerize into filaments.
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Keywords: Neurofibrillary tangle; Tau; Alzheimer's disease

1. Introduction

In the mid 1980s, a number of laboratories discovered that the main protein composing neurofibrillary tangles (NFTs) was the microtubule-associated protein, tau [1–3]. NFTs are aggregates of filamentous tau polymers that comprise a portion of the fibrillar pathologies in Alzheimer's disease (AD)—the other elements being the neuropil threads and the dystrophic neurites that invade a subset of amyloid plaques (neuritic plaques) [4]. These structures occur in the regions of the brain responsible for the various cognitive domains that are compromised during the course of AD, the density of tau inclusions correlating well with regional and global aspects of cognitive decline [5–7]. Hence, if one wishes to follow cognitive decline during the progression of AD, the tau inclusions provide fairly reliable pathological signposts along the way. However, it is the transition from seeming normality into the disease state that interests us most. How does a protein normally associated with microtubules leave this functional docking site and transit into a polymeric state? What are the seminal changes in tau structure that drive aggregation? Once in a polymer, does tau continue to change state? And finally, do molecular changes and conformational changes in polymeric tau render it more toxic to crucial cellular mechanisms?

Much work remains to be accomplished to understand and answer these fundamental questions. Certainly, defining the role of phosphorylation in tau pathogenesis is of considerable importance and this is dealt with in many reviews [8–12] and in contributions to this volume as well. However, very early in the annals of tau in AD, it was noted that, in many NFTs, the molecule was truncated [13,14]. This is an alteration in tau that has been little discussed and to which we have recently devoted much of our effort, with the goal of understanding how tau polymers form and

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evolve during the course of AD. Additionally, tau is known to undergo substantial conformational changes prior to and after polymer formation. Herein, we will attempt to place our recent findings documenting folding and proteolytic truncation in the context of the contributions from numerous laboratories regarding the molecular and structural alterations of this protein during AD pathogenesis.

2. The role of tau filaments in neurodegenerative disease

Because the tau pathology occurs within the cell bodies and processes of neurons and/or glia, it has always been assumed to be harmful. The discovery of tau's involvement in certain genetic forms of frontotemporal dementia (FTD) (see Refs. [15,16] for reviews) strongly suggested that tau's aggregation into filaments results in a toxic gain of function much like that hypothesized for Aβ accumulation in AD. In vitro data obtained using mutated tau [17,18] and specific tau isoforms [19] in assembly paradigms support this proposition. These studies demonstrated that many of the exonic mutations result in tau molecules that form filaments more efficiently than their wild-type counterparts. On the other hand, the intronic mutations result in overproduction of tau with four microtubule-binding repeats (4R tau), and 4R tau forms filaments more readily than its 3R counterpart. Additionally, plasmids containing human tau cDNA constructs microinjected into lamprey neurons in situ produce tau filaments that accumulate and lead to neuronal degeneration [20,21]. Control experiments in which neurofilaments are overproduced in the same manner are apparently harmless to lamprey neurons [21]. Likely, therefore, it is tau filament formation that causes neurodegeneration; in AD this process may be induced by Aβ [22], while in familial FTD it is a mutation, rather than an extracellular insult, which enhances tau filament formation. Findings such as these compel an understanding of the changes that drive, accompany, and sustain the formation of tau polymers in AD.

3. Tau conformation and tangle formation

The functional consequences of tau alterations, of which the prime example is phosphorylation, remained obscure until the realization that, in order to form filaments, tau had to undergo a massive conformational change to begin the aggregation/polymerization process. The extent of this change was not appreciated until the discontinuous epitope of the Alz50 monoclonal antibody was discovered [23,24]. This antibody only binds efficiently to tau when its amino-terminus comes into contact with the microtubule-binding repeats (MTBR). In order to aggregate into filaments, the tau molecule must undergo a shift from an essentially unfolded random coil configuration [25] to this more compact "Alz50 state". We have subsequently shown that once in the polymer/tangle state, tau can shift into the "Tau-66 state," defined by reactivity to the Tau-66 antibody. This conformation is dependent upon the proline-rich region of the tau molecule contacting a region of the MTBR that partially overlaps the putative N-terminus binding site in the Alz50 state [26,27] (Fig. 1).

While numerous studies have suggested that phosphorylation acts as an inducer of the Alz50 state, our data indicate that C- and/or N-terminal truncation events either induce or accompany attainment of the Tau-66 state [27] (see below). Interestingly, according to the epitope mapping studies referred to above, the Alz50 state of tau is attained when the extreme amino-terminus contacts the third microtubule-binding domain (a.a. 312–322), while the Tau-66 state requires binding of the proline-rich region to a partially overlapping sequence of the MTBR (a.a. 305–314). If the binding sites are indeed coextensive, the two tau conformations cannot coexist within the same molecule [26]. Our findings appeared to underscore this when it was discovered that the Alz50 and Tau-66 antibodies never colocalized, labeling distinctly different populations of NFTs [27] (Fig. 2). In this study, the amino-terminus appeared to be absent from the tau molecule when it attained the Tau-66 state. Cleavage at the N-terminus could abolish tau's ability to assume the Alz50 conformation or destroy it in the NFT. This would allow tau to attain the Tau-66 state. Studies

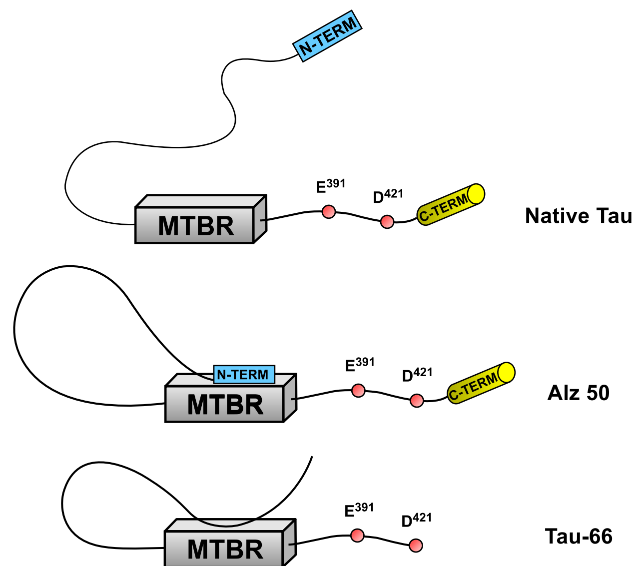

Fig. 1. Diagrammatic representation of the conformational states of tau in NFTs. Native tau is in an extended or random coil conformation. Tau can also assume the Alz50 state in which the amino portion of the molecule folds and contacts the MTBR. It is in this state that tau first forms aggregates in AD-vulnerable neurons. Amino and carboxy truncation accompany or drive tau into the Tau-66 state, characterized by the proline-rich region of the molecule contacting the same general region of the MTBR. The Tau-66 conformation succeeds the Alz50 state after tau is in the NFT. E^{391} and D^{421} are two carboxy truncation sites known to exist in AD NFTs.

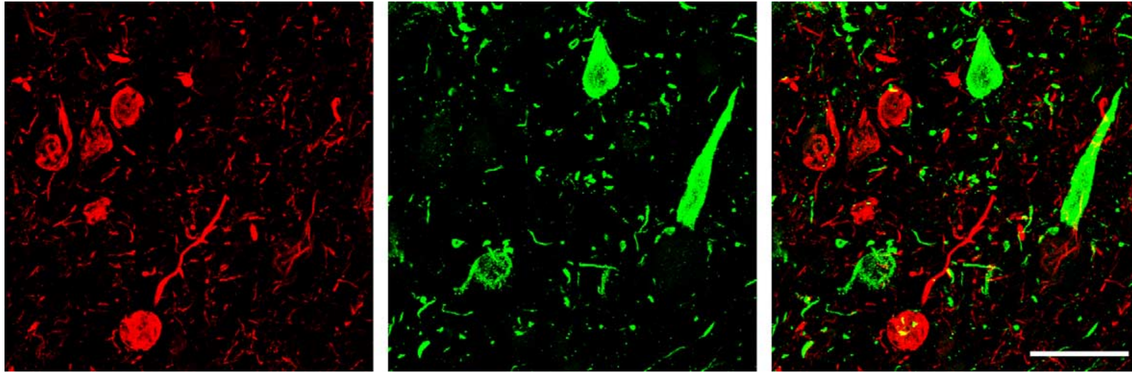

Fig. 2. Confocal microscopic images demonstrating that Alz50 and Tau-66 do not colocalize in AD brain. The lack of colocalization of Alz50 (red channel) and Tau-66 (green channel) indicate the mutually exclusive nature of the two tau conformations (merged image). Evidence supports an ordered change in conformation where the Alz50 state occurs first and gives way to the Tau-66 state. Bar = 50 μm.

using N-terminally modified tau in our in vitro polymerization paradigm support this hypothesis.

4. The role of the amino-terminus in tau filament formation

As discussed above, folding of the tau molecule, as well as its ability to form polymers, appears to be in some way driven or accompanied by proteolytic truncation at both ends of the molecule. Although truncated tau was discovered some time ago and shown to be resident in the PHF core [14,28], its significance and its place in the AD cascade has remained somewhat obscure. However, SDS-soluble PHFs purified from AD brain appear to contain full-length tau molecules [29,30], suggesting that truncation occurs *after* tau polymer formation. If this is the case, then removal of, for example, portions of the amino-terminus key to formation of the Alz50 epitope should negatively impact tau polymerization. When we tested this, we found that a small internal deletion in the amino-terminus (a.a. 5–18) greatly inhibited filament formation in vitro [31]. This finding again indicates that the Alz50 folding event and the interaction of this region of the molecule with the MTBR is an important prerequisite to tau aggregation. Additionally, N-terminal truncation in situ would likely occur after initial filament formation (see below).

5. The role of the carboxy-terminus in tau filament formation

In studying tau filament formation in vitro, we discovered that the carboxy tail of the tau molecule inhibits filament formation [32]. Tau constructs lacking the tail assembled much faster and to a greater extent than wild-type. However, until recently, the only tau truncation known to occur in AD (E^{391}) was that recognized by the MN423 monoclonal antibody [33] and this was known to occur fairly late in NFT evolution [27]. Recently, we discovered that caspase could cleave tau at D^{421} [34] (see below), releasing a discrete peptide (a.a. 422–441) that was capable of forming an amphipathic α-helix [35]. Tau truncated at D^{421} assembled more readily than the full-length molecule in our in vitro assay [34,35], and when a synthetic peptide comprising the caspase cleavage fragment was added back to the tau molecule, assembly was inhibited. Interestingly, the site on the molecule crucial for carboxy end inhibition (a.a. 321–375) [35] partially overlaps with the site that interacts with the amino-terminus [24] (see above) in forming the Alz50 epitope. These results suggest a role for carboxy truncation in filament formation and hence, NFT genesis in AD where the carboxy-terminus of the tau molecule behaves as an aggregation inhibitor that competes with assumption of the Alz50 conformation [35].

6. Caspases may cause carboxy and amino truncations of tau in situ

Caspases are cysteine proteases that cleave on the carboxy side of the terminal aspartic acid residue in the canonical consensus sequence, DXXD. These enzymes are participants in a proteolytic cascade leading to cell death via apoptosis; in neurons, the major “killer caspase” is thought to be caspase-3 [36]. Members of the caspase family of cysteine proteases play a critical role in Aβ-induced neuronal apoptosis [37,38] and are activated in apoptotic neurons in AD [39–45]. Reports of tau cleavage by caspases during apoptosis in neuronal cells in culture [38,46] led us to a series of productive investigations into the potential role of caspase cleavage in the progression of tau pathogenesis in AD. Of the three potential canonical caspase cleavage sites in tau (D^{25}, D^{341}, D^{421}), we found that only the last is readily cleaved in vitro. Significantly, this site is located in the C-terminal tail region of the molecule. As mentioned above, caspase cleavage at D^{421} appears to drive tau assembly in vitro [34].

The combination of in vitro cleavage and assembly results encouraged us to produce a monoclonal antibody,

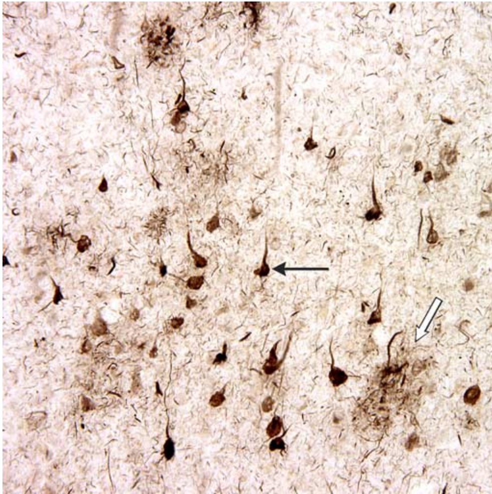

Fig. 3. Bright-field immunohistochemistry demonstrating that Tau-C3 reacts with all forms of the fibrillar pathologies in AD: NFTs (black arrow), neuritic plaques (white arrow), and neuropil threads (hair-like structures scattered throughout the neuropil).

Tau-C3, which only recognizes tau when truncated at D^{421} [34,47]. This antibody stains NFTs, neuropil threads and dystrophic neurites in AD brain, the three most common pathological manifestations of in situ tau aggregation (Fig. 3). Hence, not only does caspase cleavage release the assembly-inhibiting peptide from tau, but this previously unidentified tau truncation occurs in AD; this was recently confirmed by another group using a truncation-specific polyclonal antibody [48].

We have also observed the apparent disappearance of amino epitopes shortly after NFT formation in AD brain [27,49], and it would appear that amino-terminal truncation is nearly contemporaneous with carboxy truncation. That said, the sites of the amino truncations are still a matter of conjecture. Studies of the amino acid sequence suggested—in addition to the canonical caspase sites mentioned above—a semi-canonical caspase 6 site at D^{13} (VMED). In vitro digestion experiments followed by mass spectrometric analyses indicated that tau could indeed be cleaved at D^{13} by caspase 6 [49]. However, specific immunological probes have yet to be developed that recognize this site-specific cleavage during NFT evolution in situ, making carboxy cleavage at D^{421} the only established caspase cleavage event in AD.

7. Carboxy truncation and cellular toxicity

The presence of tau truncated at E^{391} in NFTs has been known for some time. Subsequent to this discovery, a number of studies have indicated that the E^{391} form of tau is toxic to cultured cells, suggesting that this is also the case in the NFTs of AD [50]. Similarly, reports of toxicity of tau truncated at D^{421} have also emerged [51]. It can be inferred from this group of findings that C-terminally truncated tau in monomeric or polymeric form may be hazardous to the neuron and perhaps even facilitate the neuronal dysfunction that precedes cell loss in AD.

This work prompted us to investigate tau truncation in a model of neurodegeneration [34]. We found that when cultured cortical rat neurons are treated with aggregated Aβ, apoptosis is initiated, and tau is cleaved at D^{421}, as the neurites begin to die back. Since previous work indicated that cultured neurons do not undergo neurodegeneration in the absence of tau expression [52], it is possible that tau cleavage is an essential part of a deleterious cascade leading to neuronal dysfunction and death.

8. The evolution of tau structure within NFTs

The aggregation of tau within the neuron to form NFTs appears to involve an ordered series of events. During this process tau changes conformation at least twice and becomes progressively truncated at both its amino- and carboxy-termini. The Alz50 conformation of tau appears first in pretangle neurons but persists in neurons containing NFTs as well [53]. Apparently, soon after tangle formation both carboxy and amino truncation events ensue. Many Alz50-positive NFTs are also reactive to Tau-C3, the antibody that binds to tau truncated at D^{421} [47]. Tau-C3 reactivity also colocalizes with a few Tau-66-positive structures, indicating persistence of the D^{421} truncation through the transition from the Alz50→Tau-66 conformational states. Once in the Tau-66 state, tau is cleaved even more extensively in the NFT, becoming positive for MN423, an antibody that reacts with tau truncated at E^{391}. A high incidence of colocalization exists between MN423 and Tau-66, suggesting that the deeper C-terminal truncation, like the Tau-66 state, is a somewhat later event (Fig. 4). The ordering of these events now makes it possible to discern the sequence of site-specific phosphorylation occurrences and other events during NFT formation and evolution.

These findings further suggest that truncation at the caspase site of D^{421} appears to occur after filaments form from full-length tau. Hence, the role of the C-terminus in the initiation of assembly is not known definitively. Perhaps cleavage rapidly increases the amount of filaments in an NFT by positively affecting nucleation or elongation. Alternatively, it could stabilize filaments after they form. Stabilization suggests an effect on the reverse rate constant for assembly; lowering this parameter would result in an increased rate and extent of filament formation, as was observed in vitro [34,35]. Stabilization of formed filaments may explain the difficulty we have experienced in solubilizing D^{421} tau from AD brains [34,47].

It should be noted that others [48] have reported the presence of C-terminally D^{421}-truncated tau in pretangle neurons, suggesting a role for caspase cleavage in the initiation of polymer formation. These findings are at

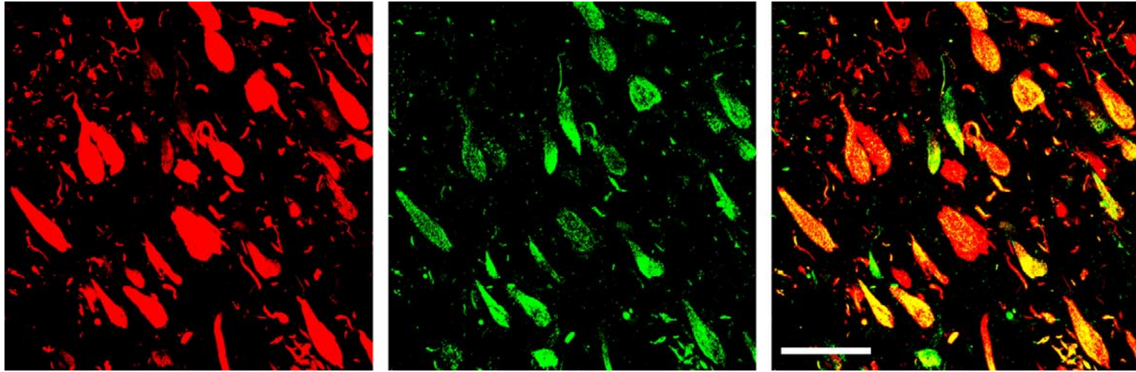

Fig. 4. Confocal microscopic images demonstrating that MN423 (red channel), an antibody that recognizes tau only when truncated at E391, colocalizes with some, but not all, NFTs that stain with Tau-66 (green channel). The merged image is at the far right; the yellow profiles indicate colocalization. Bar=50 μm.

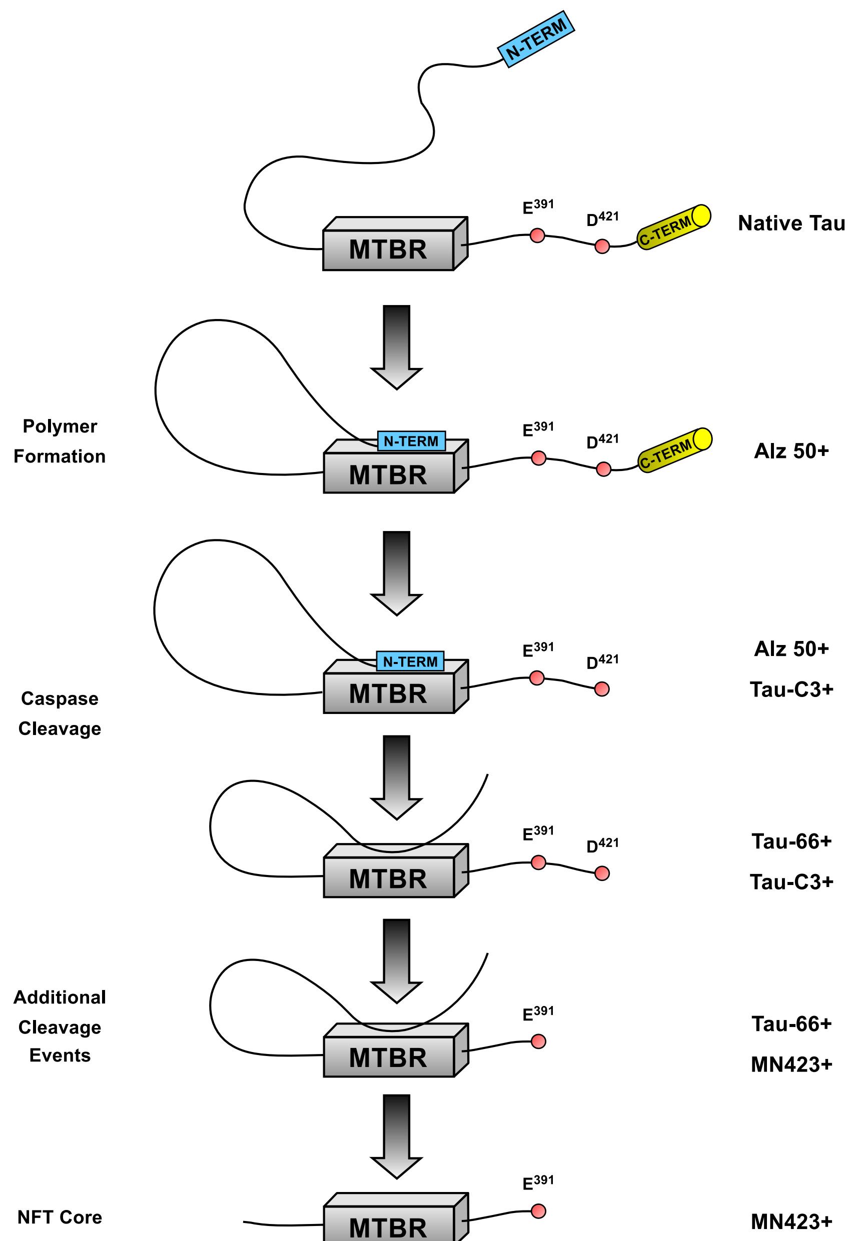

Fig. 5. Maturation of NFTs follow an ordered pattern of conformational alterations involving, and perhaps driven by, cleavage of the tau molecule. Two of the carboxy truncation sites are noted; however, although truncation of the amino-terminus is depicted, the exact cleavage sites have yet to be identified.

variance with our quantitative data [47], which clearly indicate the existence of Alz50-positive pretangles and tangles prior to colocalization with Tau-C3, our monoclonal antibody specific to D^{421}-truncated tau. There is no simple way to reconcile these disparate findings. Although it is conceivable that the polyclonal antibody is more sensitive to the presence of D^{421}-truncated tau, it is also possible that it is recognizing non-truncated tau at higher antibody titers. Specificity of polyclonal antibodies for a truncation site is commonly accomplished by absorption with full-length tau to remove cross-reacting immunoglobulin species, leaving behind those that are specific for the truncated protein. By contrast, Tau-C3 is a monoclonal antibody that, by definition, is a single homogeneous immunoglobulin species recognizing a single epitope. This antibody demonstrates no cross-reactivity with intact tau on ELISAs or Western blots at any concentration tested [47]. In support of our findings, PHFs purified from AD brains contain an intact carboxy-terminus, as they are reactive against Tau 46.1, an antibody that recognizes an epitope encompassed by a.a. 420–441. Likely, therefore, C-terminal truncation occurs after tau is initially in the filamentous state. In summary, the formation of the Alz50 epitope and the onset of polymer formation appear to be followed quickly by both carboxy and amino truncation; these proteolytic events seem to coincide with the loss of Alz50 reactivity and formation of the Tau-66 conformation. Once this has occurred, the carboxy end becomes further truncated to E^{391}. Similar events probably occur at the amino end.

9. Conclusion and unanswered questions

We have discovered that tau filament formation in vitro is governed in part by control elements within the tau molecule itself. The carboxy-terminus of tau is inhibitory to filament formation while a folding event involving the extreme amino-terminus facilitates polymer formation. The findings discussed above indicate that tau-filament/tangle formation involves seemingly ordered shape changes (i.e., polymerization=folding) associated with molecular alterations of the normally random coil tau monomer. In situ evidence obtained using specific monoclonal antibodies demonstrates the occurrence of tau folding leading to PHF/NFT formation as well as truncation of both the amino and carboxy ends of the tau molecule after initial polymer formation in AD. These events appear to comprise an evolutionary process that extends from tau deposition in pretangle neurons to neurons containing NFTs containing truncated tau (Fig. 5).

The significance of NFT evolution is not known. Since certain truncation events have been shown to produce tau that is more toxic than the full-length protein in cell models, it is tempting to suggest that NFTs with, for example, tau truncated at D^{421} or E^{391}, are more toxic to the cell. That is to say, tangles alone may not be toxic to the cell until they become modified by truncation. An intriguing possibility is that NFTs reflect the state of tau not only in tangle-bearing neurons but in surrounding vulnerable cells as well. In a sense, they supply a "window" into the molecular changes that occur to tau during the disease process. We are able to observe alterations in tau in tangles because they are perhaps more amenable to fixation than smaller aggregates. One might even imagine that NFTs are sequestering the toxic tau and prolonging the survival of the neuron. By extension, toxic tau in non-tangle bearing neurons may rapidly cause the dysfunction and demise of the affected cell. In this hypothesis, NFTs would merely serve as a harbinger of what is happening to tau in rapidly dying neurons. This may also supply an explanation as to why neurons die in cell and animal models of tauopathies in a toxic tau-dependent manner without producing NFT-like inclusions.

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