The Role of Caspase Cleavage of Tau in Alzheimer Disease Neuropathology

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Abstract

Alzheimer disease (AD) is characterized by the accumulation of amyloid plaques and neurofibrillary tangles within selective brain regions. In addition, cell death pathways become active leading to neurodegeneration. Caspase activation, a key step in the programmed cell death pathway known as apoptosis, occurs in AD and leads to the proteolytic cleavage of several neuronal proteins. Previously, it was hypothesized that the development of the classical hallmarks of AD, amyloid plaques and neurofibrillary tangles, occur independently and do not involve the activation of caspases. However, recent studies suggest that plaques, tangles, and caspase activation share a common pathway. β-Amyloid, the main component of amyloid plaques, activates caspases. Activated caspases can in turn cleave tau, the main component of neurofibrillary tangles. Caspase-cleaved tau (Δtau) may initiate or accelerate the development of tangle pathology. Tau, when cleaved by caspases at Asp421, "seeds" filamentous aggregates in vitro. Caspase-cleaved tau also adopts the MC1 conformation, one of the earliest pathologic events in tangle formation. Importantly, Δtau occurs early in the development of tangle pathology within AD brains and in a transgenic mouse model of AD. This review summarizes recent evidence suggesting that caspase cleavage of tau plays an important role in the development of neurofibrillary tangle pathology. In addition, a model is presented whereby caspase cleavage of tau provides a mechanistic link between the development of amyloid and tangle pathologies.

Key Words: Alzheimer disease, Amyloid, Caspase activation, Tau.

INTRODUCTION

Neuronal and synaptic loss occurs during the progression of Alzheimer disease (AD) (1–4) and correlates with cognitive decline. AD is also characterized by the gradual accumulation of extracellular β-amyloid (Aβ, amyloid) in the form of senile plaques and intracellular tau inclusions in the form of neuropil threads and neurofibrillary tangles (NFTs). Aβ is a 40- to 42-amino acid peptide derived from the amyloid precursor protein (APP). NFTs consist of hyperphosphorylated and aggregated forms of the protein tau. Tau is a microtubule-binding protein that stabilizes the neuronal cytoskeleton and participates in vesicular transport and axonal polarity (5–8). For many years, there has been an ongoing debate as to whether plaques or tangles are the primary driving force underlying AD pathogenesis, the so-called “Baptists versus Tauists” controversy. The Baptists support the amyloid cascade hypothesis as the mechanism driving AD pathogenesis, while the Tauists propose that phosphorylation and aggregation of tau lead to neuronal and synaptic dysfunction. The identification of tau mutations that lead to tangle pathology in frontotemporal dementias implicates a role for tau in neurogenerative diseases. However, tau mutations do not lead to amyloid accumulation and are not associated with familial forms of AD. In contrast, mutations in the APP or presenilin genes are associated with early-onset AD and lead to the formation of both amyloid plaques and neurofibrillary tangles. The presenilin genes are thought to encode the enzyme(s) that cleave APP to generate Aβ. Taken together, this and other evidence suggests that, while tau is necessary for tangle formation in AD, its role in the pathology is likely downstream of amyloid.

While neither the Baptists’ nor Tauists’ hypothesis provides a complete explanation for AD etiology, growing evidence suggests that both Aβ and tau pathologies may be interconnected and together lead to synaptic and neuronal loss. In this review, we discuss recent data supporting the hypothesis that Aβ induces caspase cleavage of tau, which in turn accelerates NFT formation.

AMYLOID AND AD

Extracellular Aβ accumulation is a primary hallmark of AD. Aβ is generated from the sequential proteolysis of APP by two enzymes, β-1 and γ-secretase (reviewed in [9]). In addition to forming senile plaques, Aβ can cause degenerative changes, activate cellular stress responses, and disrupt signal transduction pathways. Several studies have shown that extracellular Aβ activates caspases in vitro (10–15). However, recent evidence suggests that accumulation of intracellular Aβ may precede the deposition of Aβ in senile plaques (16–19) and contribute to neuronal stress and degeneration (20–22).
While Aβ is generated at the cell membrane (extracellular), it can also be produced within the secretory pathway or internalized from the extracellular milieu (intracellular) (9, 19, 23). Intracellular Aβ accumulates in multivesicular bodies (particularly within post-synaptic compartments) of transgenic mice and AD brains (24). Increased intracellular Aβ is correlated with both abnormal cellular morphology and reduced synaptic density (24). Although the mechanism(s) by which intracellular Aβ induces these pathologic changes remains unclear, Aβ-mediated caspase activation may lead to tangle pathology and neuronal compromise (15, 25).

NEUROFIBRILLARY TANGLES AND AD

NFTs are another major pathologic hallmark of AD. NFTs are composed of hyperphosphorylated tau and initially accumulate within the entorhinal cortex and CA1 subfield of the hippocampus (26–28). Hyperphosphorylated tau is also found in several other neurodegenerative disorders (29). The binding of tau to microtubules is regulated by phosphorylation at numerous sites (for a review, see [8]). Tau phosphorylation has been demonstrated to cause decreased binding and destabilization of microtubules, and hyperphosphorylated tau from AD brains fails to bind microtubules (26, 30–37). Interestingly, phosphorylation may initially function to dissociate tau and tau aggregates from microtubules and thereby restore microtubule function, serving a neuroprotective role (38). However, the majority of studies suggest that hyperphosphorylation of tau is a major component of the pathologic progression that leads to tangle formation (39).

Antibodies have been developed against pathologic tau that have helped characterize both conformational and hyperphosphorylated tau alterations that progress to NFTs. These include the antibody MC1, which recognizes a conformational change due to aberrant folding in tau and appears to be one of the earliest tau pathologic events (40–42). The antibody AT8 recognizes tau phosphorylated at Ser202 and Thr205, which is one of the first residues to be hyperphosphorylated, whereas PHF-1 recognizes phosphorylation at Ser396 and Ser404 and reacts with more mature hyperphosphorylated forms of tau found primarily within late-stage tangles (43–45).

ACTIVATION OF CASPASES IN AD

Caspases are a family of serine-aspartyl proteases that are activated as part of the programmed cell death pathway known as apoptosis. During AD and other age-related degenerative diseases, neurons induce a series of proteases, including caspases, and a number of key proteins are cleaved by caspases including APP presenilin (PS1, PS2), tau, actin, fodrin, huntingtin, and atrophin-1 (46–50). This and other evidence has led to the suggestion that the extensive neuronal loss observed in AD may result from the activation of apoptotic-related pathways or possibly even apoptosis itself (51).

Caspases can be activated within a cell without undergoing classical apoptosis (52), and there is evidence for prolonged caspase activation without neuronal death. For example, transgenic familial amyotrophic lateral sclerosis mice expressing mutant superoxide dismutase-1 have prolonged activation of initiator caspases (caspase-1), but motor neuron death occurs only after a few months coincident with executioner caspase activation (caspase-3) (53). This delay may be due to the upregulation of inhibitory apoptosis proteins (54), which inhibit downstream executioner caspases (53). Thus, upregulation of inhibitory apoptosis proteins within neurons may serve as a compensatory mechanism. A similar situation may occur in AD, where chronic caspase activation may lead to cleavage of tau and other essential cellular proteins and contribute to neuronal pathology prior to cell death.

THE CONVERGENCE OF PATHOLOGIES

Recent data support a model suggesting a convergence of AD pathologies that includes caspase activation (Fig. 1). Tau is required for Aβ toxicity (38), and Aβ exposure can lead to the formation of paired helical filaments (PHFs) in vitro (55). In addition, tau pathology in transgenic mouse models is exacerbated by Aβ (56–59). Within a triple-transgenic model of AD (3xTg-AD), Aβ not only preceded but accelerated the development of tau pathology (58, 59). Currently, the mechanism(s) by which Aβ drives tangle formation are unresolved. However, APP or Aβ may activate kinases implicated in tau phosphorylation, including glycogen synthase kinase-3β (GSK-3β), mitogen-activated protein kinase (MAPK), and cyclin-dependent kinase 5 (Cdk5) (60–63).

In addition to regulation of tau hyperphosphorylation, Aβ may disrupt the proteosomal degradation of tau (64). Aβ also activates caspases (10, 13, 14) that can cleave tau (15, 25, 65–68). Caspase-cleaved tau has been shown to accelerate tau aggregation in vitro, suggesting it may initiate and/or catalyze tangle formation (15, 25). Furthermore, caspase-cleaved tau is pro-apoptotic (66–69). Other in vitro evidence suggests that caspase-cleaved tau can precede hyperphosphorylation of tau and may therefore be a catalyst for NFT formation (25). In the AD brain, caspase-cleaved tau colocalizes with both intracellular Aβ and activated caspase-3 (25). Caspase-cleaved
TAU CLEAVED AT ASP\textsuperscript{421} IS DETECTED IN THE AD BRAIN

Caspase activation is detected in the AD brain, and active caspases are found within tangle-bearing neurons (70–72). Furthermore, tau is cleaved by caspase-3, specifically after Asp\textsuperscript{421} (15, 25, 65, 66, 69). Besides caspase-3, other caspases are capable of cleaving tau at Asp\textsuperscript{421} (15). In addition to cleavage at Asp\textsuperscript{421}, tau can also be cleaved at other sites (Fig. 2). Since the most detailed studies have been performed on tau cleaved at Asp\textsuperscript{421}, this discussion will focus on the properties and importance of this tau cleavage product.

Caspase-cleaved tau can be detected in vivo (15, 25). Using a rabbit polyclonal antibody directed against the caspase-cleaved carboxyl-terminus of tau after cleavage at Asp\textsuperscript{421}, analysis of temporal cortex brain lysates revealed that caspase-cleaved tau was found in MCI and AD but not in control (Fig. 3). Specifically, \( \Delta \text{tau} \) was detected in the soluble RAB fraction of MCI and AD but not control, suggesting that \( \Delta \text{tau} \) production coincides with the early stages of AD cognitive decline. In detergent-soluble RIPA fractions, \( \Delta \text{tau} \) was only detected in higher pathology MC1 and AD cases and suggests that \( \Delta \text{tau} \) becomes more insoluble as AD progresses. Since further truncated species of tau have been detected in AD brain (73), it is interesting to speculate whether this results from further tau cleavage after caspase.

\( \Delta \text{tau} \) can also be detected by immunohistochemistry (15, 25). There are more \( \Delta \text{tau} \)-immunoreactive cells within CA1 of the hippocampus in AD compared with control subjects (Fig. 4A, B). Furthermore, a positive correlation between the number of \( \Delta \text{tau} \)-immunoreactive cells within CA1 and increasing Braak stage score was observed (e.g. numbers of PHF-1 tangles). In addition, the number of \( \Delta \text{tau} \) cells within CA1 was inversely correlated \( (r = -0.72, p < 0.05) \) with cognitive function (Mini-Mental State Examination score) in AD (Fig. 4C). Thus, both biochemical and immunohistochemical data suggest that \( \Delta \text{tau} \) production may be a mechanism contributing to neuronal dysfunction and cognitive decline.

\( \Delta \text{tau} \) IS COLOCALIZED WITH ACTIVE CASPASE-3 IN THE AD BRAIN

Caspase cleavage of tau implies the activation of caspases. This was confirmed in AD brain (25). \( \Delta \text{tau} \) colocalized with active caspase-3 within pretangle bearing neurons. However, \( \Delta \text{tau} \) in mature/extracellular tangle pathology was devoid of active caspase-3 labeling. A recent in vitro study found that active caspase-3 rapidly degrades itself (74). This suggests that detection of active caspase-3 in AD brain may be greatly diminished by postmortem delay. Interestingly, active caspase-3 has been observed primarily within lysosomal-like compartments that are thought to be granulovacuolar degeneration. It is interesting to speculate whether the acidic pH present in lysosomal-like compartments could inactivate active caspase-3 and thereby prevent auto-degradation. Therefore, one would predict that the only active caspase-3 that should be detectable in postmortem tissue would be within such subcellular compartments. It is likely that cells with active caspase-3 within granulovacuolar degenerations would therefore also have cytoplasmic active caspase-3 if postmortem delay were not a factor.

FIGURE 2. \( \Delta \text{tau} \) is cleaved by caspases at multiple sites in the AD brain. The longest tau isoform, Tau40, is depicted. The known caspase cleavage sites (arrows) are shown. Both caspase-3 and -6 have been reported to cleave tau in the AD brain. While caspase-3 appears to cleave tau after Asp\textsuperscript{25} or Asp\textsuperscript{421}, caspase-6 can cleave tau after Asp\textsuperscript{3}, Asp\textsuperscript{102}, or Asp\textsuperscript{211}.

FIGURE 3. \( \Delta \text{tau} \) becomes increasingly insoluble with AD pathologic progression. Temporal cortex samples from control, mild cognitive impairment (MCI), and AD were fractionated into high-salt RAB and detergent-soluble RIPA fractions. \( \Delta \text{tau} \) was detected in the soluble RAB fraction of MCI and AD but not control cases, suggesting that \( \Delta \text{tau} \) production coincides with the early stages of AD cognitive decline. In detergent-soluble RIPA fractions, \( \Delta \text{tau} \) was only detected in higher pathology MC1 and AD cases, which suggests that following caspase cleavage, \( \Delta \text{tau} \) becomes more insoluble and coincides with AD progression. The multiple bands correspond to the multiple tau isoforms that exist. All tau isoforms contain the Asp\textsuperscript{421} caspase cleavage site. *This patient was considered “transitional,” between MCI and AD. (Reproduced courtesy of the Journal of Clinical Investigation.)
CASPASE CLEAVAGE OF TAU INDUCES FILAMENT FORMATION

NFTs are formed from the assembly of tau into PHFs. Caspase-3-cleaved tau increases light scattering over time, indicative of filament formation and aggregates more rapidly than full-length tau (15, 25, 75). In addition, Δtau accelerated filament formation of full-length tau. The incubation of full-length tau in the presence of Δtau leads to a dramatic increase in the rate of light scattering (Fig. 5). These results not only show that caspase cleavage of tau leads to increased filament formation but that Δtau may also act as “seeds” similar to filaments isolated from AD brain, to enhance filament formation of full-length tau. These data further support a nucleation-dependent mechanism of PHF assembly (76).
to filament formation. Interestingly, a 3:1 ratio of full-length tau dramatically accelerated light scattering, suggesting that Δtau may nucleate the assembly of full-length tau filaments. (Reproduced courtesy of the Journal of Clinical Investigation.)

RELATIONSHIP BETWEEN CASPASE CLEAVAGE OF TAU AND TAU PATHOLOGIC ALTERATIONS

Tau undergoes a conformational change very early in the generation of AD tau pathology, possibly even prior to hyperphosphorylation (42). This conformational change can be detected by the antibody MC1 (42). The proposed MC1 conformation involves the intramolecular interaction between the N- and C-termini of tau (folded conformation) and precedes PHF formation in AD (40, 42, 77). The antibody MC1 preferentially immunoprecipitates Δtau versus full-length tau (25). Therefore, Δtau may more readily adopt the MC1 conformation and further support the notion that caspase cleavage of tau is an early event in tangle formation.

Phosphorylation of specific residues is another important stage in the pathogenesis of tau. Glycogen synthase kinase-3β (GSK-3β) is one of a number of kinases implicated in the hyperphosphorylation of tau (78–80). Incubation of Δtau in the presence of GSK-3β leads to hyperphosphorylation (PHF-1 immunoreactivity) (25). Thus, caspase-cleaved tau can be hyperphosphorylated and may contribute to the formation of PHF.

ΔTAU IS DETECTED WITHIN A TRIPLE-TRANSGENIC MODEL OF AD (3XTG-AD) THAT PROGRESSIVELY DEVELOPS BOTH Aβ DEPOSITION AND TANGLE PATHOLOGY

The 3xTg-AD mouse provides an ideal model to study the convergence of Aβ, tau, and caspase pathologies (58). At 6 months, mice exhibit both intracellular and extracellular Aβ deposits but lack both neurofibrillary pathology and Δtau immunoreactivity. By 12 months, initial stages of neurofibrillary pathology are observed and Δtau immunoreactivity colocalizes with intraneuronal Aβ within hippocampal CA1 neurons. At 18 months, when both plaque and NFT pathologies are well established, Δtau and intraneuronal Aβ colocalize within CA1 and the neocortex. Δtau also colocalizes with MC1 within CA1 pyramidal neurons of 12- and 18-month-old 3xTg-AD mice. Interestingly, the subcellular distribution of Δtau evolves with increasing age. At 12 months, the great majority of Δtau-immunoreactive cells display punctate cleavage-product within the soma and proximal dendrites of neurons. However, by 18 months, many Δtau-bearing neurons exhibit aggregated filamentous structures that appear strikingly similar to AD tangle pathology (25).

Aβ induces caspases and Δtau in vitro (10, 13, 14). In addition, Δtau colocalizes with intraneuronal Aβ42 accumulation within both neurons and dystrophic neurites of the AD brain and within 3xTg-AD neurons. Intraneuronal Aβ accumulation is proposed to precede plaque deposition and is an early event in AD pathogenesis (21, 24, 81, 82). Therefore, the colocalization of Aβ with Δtau further supports the hypothesis that Δtau is an early event in the development of tangle pathology.

OTHER APOPTOTIC INSULTS LEAD TO CASPASE CLEAVED TAU

Δtau is generated in a dose-dependent manner upon staurosporine treatment and is not generated when caspases are inhibited (Fig. 6) (66). Treatment of neurons with the lipid peroxidation product 4-hydroxynonenal also induces caspase activation and leads to an almost 10-fold increase in Δtau immunoreactivity (Fig. 6B, left panel) versus vehicle (Fig. 6B, right panel). Quantification of Δtau-immunoreactive cells following 4-HNE treatment is statistically significant when compared with controls (Fig. 6C). Therefore, apoptotic insults aside from Aβ, including oxidative stress, can lead to caspase cleavage of tau (83). Interestingly, oxidative stress has also been implicated in AD pathogenesis.

There are many neurodegenerative diseases that contain tau pathology in the absence of amyloid plaques (i.e. tauopathies). Since multiple apoptotic stimuli can lead to caspase-cleaved tau, it is important to determine if caspase activation and tau cleavage are a general mechanism associated with tauopathies. A comparative approach of various tauopathies will provide information on whether Aβ is essential for caspase cleavage of tau. In addition to AD, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration, dementia with Lewy bodies, and the AD variant of dementia with Lewy bodies are associated with the accumulation of tau or α-synuclein. Preliminary studies indicate that certain tauopathies demonstrate Δtau accumulation (Elizabeth Head, personal communication). Therefore, caspase cleavage of tau may represent a common pathway associated with abnormal accumulation of intracellular tau.
Confocal microscopy demonstrated that treatment of neurons with 10-fold increase in m with 10

**TAU CAN BE CLEAVED AT MULTIPLE SITES IN AD BRAIN**

In addition to tau cleavage at Asp\(^{421}\), other caspase cleavage products of tau have been identified in the AD brain. Caspase-6 cleaved tau (at Asp\(^{392}\) based on the longest tau isoform) has been identified in intracellular tangles, extracellular tangles, pretangles, neuropil threads, and neuritic plaques (67). Colocalization with active caspase-6 within pretangles suggests that this cleavage event may also occur early in AD pathogenesis. In addition to C-terminal caspase cleavage of tau, tau can be cleaved at the N-terminus by caspase-6 (68). Loss of N-terminal specific epitopes suggestive of N-terminal cleavage coincided with the appearance of Asp\(^{421}\) cleaved tau. Interestingly, caspase-6 is also able to cleave at Asp\(^{431}\), but not as effectively as caspase-3 (15). Therefore, Asp\(^{421}\) may be the result of caspase-6 as well as caspase-3 cleavage. Taken together, these data demonstrate that tau is targeted at numerous sites by caspases and that this may be one of the earliest pathologic changes occurring in tau pathology.

In conclusion, in addition to creating a degenerative state in neurons, caspase activation is a part of a complex mechanism that can enter several pathologic pathways, including tangle formation. Several conclusions can be drawn from these collective data. First, caspases can cleave tau at the N- and C terminus. Second, caspase cleavage at Asp\(^{421}\) can be triggered by Aβ, a pathologic hallmark of AD. However, tau cleavage may also occur in other tauopathies in which Aβ does not accumulate, suggesting several potential insults that may be present in neurodegenerative diseases can initiate caspase activation and tau cleavage. Third, caspase-cleaved tau adopts the MC1 conformation, one of the earliest markers in incipient AD tau pathology, and can enter into the hyperphosphorylation cascade. Fourth, there is a striking parallel between the course of pathology in the triple transgenic mouse and AD brain. Thus, a caspase-dependent mechanism may converge with that of tangle formation and become part of the pathologic cascade causing dysfunction.

For many years, there has been an ongoing debate as to whether amyloid or tangles are the primary pathology driving AD progression. The idea that caspase activation accelerates tangle formation and that β-amyloid is one of the factors driving caspase activation suggests a more unified mechanism where caspase activation links β-amyloid and NFT in AD. Aβ
FIGURE 7. Proposed model by which caspase cleavage of tau leads to neurofibrillary tangle formation. Stimuli that activate caspases lead to caspase cleavage of the microtubule-associated protein tau after Asp421 (1). Caspase-cleaved tau rapidly adopts the MC1 conformational epitope (2), which leads to increased filament formation and tau aggregation involving both caspase-cleaved tau and full-length tau (3). Tau aggregation may block kinesin-mediated transport. The cellular response for tau aggregation, may involve the targeting of tau by kinases, dissociating tau from the microtubule (4). The resulting hyperphosphorylated tau leads to PHF assembly (5).

may also amplify the consequences of other insults that activate caspases, such as oxidative stress, oxygen/glucose deprivation, and excitotoxicity (85). Overall, the serial cascade model, as depicted in Figure 1, implies that early intervention at one or more steps may slow the progression of AD.

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