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Propagation of tau pathology in Alzheimer’s disease: identification of novel therapeutic targets

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Abstract
Accumulation and aggregation of the microtubule-associated protein tau are a pathological hallmark of neurodegenerative disorders such as Alzheimer’s disease (AD). In AD, tau becomes abnormally phosphorylated and forms inclusions throughout the brain, starting in the entorhinal cortex and progressively affecting additional brain regions as the disease progresses. Formation of these inclusions is thought to lead to synapse loss and cell death. Tau is also found in the cerebrospinal fluid (CSF), and elevated levels are a biomarker for AD. Until recently, it was thought that the presence of tau in the CSF was due to the passive release of aggregated tau from dead or dying tangle-bearing neurons. However, accumulating evidence from different AD model systems suggests that tau is actively secreted and transferred between synaptically connected neurons. Transgenic mouse lines with localized expression of aggregating human tau in the entorhinal cortex have demonstrated that, as these animals age, tau becomes mislocalized from axons to cell bodies and dendrites and that human tau-positive aggregates form first in the entorhinal cortex and later in downstream projection targets. Numerous in vitro and in vivo studies have provided insight into the mechanisms by which tau may be released and internalized by neurons and have started to provide insight into how tau pathology may spread in AD. In this review, we discuss the evidence for regulated tau release and its specific uptake by neurons. Furthermore, we identify possible therapeutic targets for preventing the propagation of tau pathology, as inhibition of tau transfer may restrict development of tau tangles in a small subset of neurons affected in early stages of AD and therefore prevent widespread neuron loss and cognitive dysfunction associated with later stages of the disease.

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In AD, NFTs accumulate in a hierarchical pattern affecting selectively vulnerable brain regions that are essential for learning and memory [4]. Progression of NFT pathology throughout the brain correlates well with decline in cognitive function [5]. The entorhinal cortex (EC) is the first cortical region affected by NFTs, followed by the hippocampus, and ultimately the neocortex [6]. Progression of NFTs appears to propagate across limbic and association cortices, creating a predictable pattern that permits the neuropathological diagnosis of different AD stages [6,7]. The loss of NFT-affected neurons results in disconnection and deafferentation of critical neural circuits [8] and thus is likely to contribute to the defects in memory and higher-order cognitive functions in AD.

The spread of protein aggregates during disease progression is a common theme in many neurodegenerative disorders, including α-synuclein in Parkinson’s disease [9] and superoxide dismutase-1 in amyotrophic lateral sclerosis.
Models of tau propagation

Substantial progress has recently been made in understanding the pathogenic mechanisms of tau spreading between neurons and across brain regions. Three independent research groups modeled the process of tau spreading by using transgenic mouse lines with localized expression of aggregating human tau. To generate these mouse lines, neurepsin promoter-driven tTa mice that express this transcriptional activator mainly in the medial EC [14] were crossed with a responder mouse line that expresses human tau carrying the P301L mutation [15]. The resulting mice express transgenic mutant tau almost exclusively in the medial EC [16-18]. As these animals age, the distribution of tau in the brain alters. Tau becomes mislocalized from axons to cell bodies and dendrites in the EC, and human tau-positive NFT-like aggregates form first in the EC and later in downstream projection targets of EC neurons, suggesting trans-synaptic propagation of tau [16-18].

Until recently, the spread of tau pathology in AD was attributed to the passive release of aggregated tau from dead or dying tangle-bearing neurons. However, a number of recent studies demonstrated active secretion and interneuronal transfer of tau in different AD model systems [17-25], suggesting that neuronal tau release is a regulated process that could facilitate the trans-neuronal spread of tau misfolding. Therefore, understanding how inter-neuronal transfer of tau occurs may provide potential therapeutic targets to interrupt the proliferation of tau pathology early in disease. In this review, we discuss the cellular mechanisms of tau propagation and potential therapeutic approaches.

Mechanisms of neuronal tau release

How do neurons secrete tau? This question has been the subject of several recent studies but remains controversial. Specifically, whether tau is released in a free soluble form or packaged into small membrane vesicles such as exosomes is unclear. Since exosomal membrane is derived from the plasma membrane [35] and tau is associated with the plasma membrane [36,37], it is possible that tau packaged in exosomes is actively secreted in this manner. Consistent with this hypothesis, tau was detected in exosomes isolated from CSF of both healthy, age-matched controls and AD patients [22]. Earlier studies of tau secretion performed in human tau overexpressing non-neuronal cell lines – COS-7 and human embryonic kidney (HEK) – showed that tau release in microvesicles, in the absence of cell death, is an actively regulated process [38]. Furthermore, neuroblastoma M1C cells that inducibly express wild-type human tau secreted both free soluble and exosomal tau [22]. Human tau overexpression in giant neurons of the lamprey caused the release of tau contained within various types of vesicles [20] and subsequent dendritic degeneration. These results suggest that non-microtubule-associated intracellular tau may be packaged and secreted within neuron-derived vesicles. However, whether exosomal tau release is a regulated neuronal process in vivo or results from overexpression and missorting of tau in these cell models remains unclear.

However, a number of recent studies report that tau may be released from cells by a mechanism that does not involve membrane vesicles. Analysis of culture medium...
from HEK cells inducibly expressing human tau, rather than transiently overexpressing tau, revealed soluble extracellular tau but no detectable tau in the exosome fraction [33]. Exosomes isolated from SH-SY5Y neuroblastoma cells, infected with a lentivirus to express human GFP-tagged tau, also did not contain tau [39]. Furthermore, increased neuronal activity in cortical neuron cultures stimulates the release of non-exosomal endogenous tau [23]. The absence of tau in neuronal exosomes is supported by proteomic analysis of exosomes derived from cortical neurons [40] and from human neural stem cells [41]. These studies showed that neuron-derived exosomes contain cytoskeletal proteins, including β-tubulin, but do not possess detectable amounts of tau. Taken together, these studies suggest that free soluble, rather than membrane vesicle-associated, tau is released in the brain. However, under which conditions neurons release tau and whether multiple secretion mechanisms are involved need further investigation.

The discrepancy between the reported exosomal versus soluble secreted tau may result from the use of different model systems: studies showing exosomal or vesicular secretion of tau all involve overexpression of the protein, whereas physiological tau expression levels seem to favor the release of soluble tau. Depending on the cell type, high levels of intracellular tau may lead to toxicity; indeed, exosomal localization of tau was observed in degenerating cells [20]. Furthermore, in a cell culture model, in which tau is inducibly expressed, tau release was detectable in the absence of cytotoxicity and was not associated with vesicles [21,33]. In cortical neurons and neuroblastoma cells, the level of tau secretion appears to be low, suggesting a constitutive release of endogenous tau. The amount of tau secreted is greatly increased after stimulation of neuronal activity and seems to depend on changes in intracellular calcium [23,34]. Such activity-regulated tau release opens a new view on the progression of tau pathology in AD. There is evidence that disease-relevant tau mutations may have an impact on tau release as well: in vitro expression of tau harboring the P301S mutation in human neuroblastoma cells decreases tau release [34], whereas mice expressing human P301S tau show higher tau levels in ISF compared with wild-type mice [31]. Therefore, further research using animal models of the disease is needed to reveal whether tau release is dynamic and regulated by neuronal activity in vivo and whether it may be altered in neurodegeneration. Importantly, understanding the mechanism of tau secretion in the brain could open the way to new plausible drug targets for the treatment of AD, as reduction of tau secretion may prevent the spread of tangle pathology.

**Characterization of extracellular tau**

Since tau has been demonstrated to propagate between neurons, determining which species of tau are involved in its intercellular transfer is of great interest. The phosphorylation state and length of extracellular tau have been reported in numerous studies, but obtaining a global picture is difficult since different epitopes were analyzed in various model systems. Analysis of tau in human CSF found that levels of tau phosphorylated on T181 are increased in patients with AD compared with healthy controls [42]. Phosphorylation of this epitope was also observed in extracellular tau in vitro, released from HEK cells [33] and neuroblastoma M1C cells [22] both inducibly expressing wild-type human tau, and in endogenous tau from SH-SY5Y neuroblastoma cells [43] and mouse cortical neurons [34]. However, tau secreted from cultured cortical neurons was reported to be dephosphorylated at the Tau1 (S199/202) and PHF1 (S396/404) epitopes [23]; tau released from transfected HeLa cells was also shown to be dephosphorylated at the AT8 (S202/T205) and PHF1 epitopes [21]. The phosphorylation status of tau as it is released may be difficult to ascertain, however, as extracellular phosphatases may dephosphorylate tau once it is outside the cell [44]. Examination of tau released from human neuroblastoma SH-SY5Y cells revealed that when activity of tissue-nonspecific alkaline phosphatase was blocked, levels of extracellular tau phosphorylated at the AT8 and PHF1 epitopes were significantly increased [44]. These results suggest that tau phosphorylation may be altered extracellularly, and this is of particular interest as changes in extracellular tau phosphorylation may underlie tau propagation in AD. Truncation of extracellular tau is the subject of conflicting reports. HeLa cells overexpressing GFP-tagged human tau have been demonstrated to release tau that is cleaved at D421, a caspase-3 cleavage site [21]. However, additional studies report intact, full-length extracellular tau in vitro [23,34] and in vivo [31].

Although it is known that neurons readily take up tau aggregates [45,46] (as will be discussed in the next section of this review), the extracellular species of tau that propagates between neurons in vivo is not known. Various forms of tau, including monomers, oligomers, or fibrils, could be involved in the spread of tau aggregates. Furthermore, soluble tau monomers or oligomers may be released from cells before tangle formation. A recent study suggests that various types of tau may be transferred between cells, since inoculation of tau-enriched extracts of human brain from individuals with various tauopathies, including AD, frontotemporal dementia, and corticobasal ganglionic degeneration, propagated to neurons in a mouse expressing wild-type human tau and even to mice that express only endogenous mouse tau [47]. To date, reports analyzing tau release in cell culture systems [34] or in a mouse model of tauopathy [31] suggest that extracellular tau released from healthy cells is largely monomeric. However, extracellular tau has not...
been characterized in mouse models in which intracerebral inoculation aggregated tau is used to initiate propagation of tangles [46-48]. In these models, released tau may have abnormal phosphorylation or be oligomeric; however, further studies are required to characterize the species of tau that propagates under these conditions.

**Neurons internalize extracellular tau**

Knowledge about neuronal uptake of tau, which may facilitate tau spreading in AD and other tauopathies, is still very limited. As discussed above, tau can be released by neurons into extracellular space and therefore may be available for uptake by neighboring cells. Several recent studies have explored the nature of the tau that is taken up by neighboring neurons and aimed to uncover the mechanism by which this process occurs.

Propagation of aggregated tau has been recently explored *in vivo* by brain inoculation of tau aggregates and monitoring their uptake and propagation in mouse cortex. Injection of tau filaments from transgenic mice expressing human mutant P301S tau into hippocampi of young mice expressing wild-type human tau caused filamentous tau inclusions of the wild-type tau and the spreading of pathology from the site of injection to neighboring brain regions [24]. In a follow-up study, brain extracts from sporadic human tauopathy cases were injected intracerebrally into wild-type mice. Injected mice displayed highly phosphorylated tau inclusions in several brain regions, indicating that, in this model, the presence of mouse tau was sufficient for propagation of pathology [47]. Interestingly, aggregates were found in both astrocytes and neurons [47], suggesting that multiple cell types may be able to internalize tau and participate in propagation of tau pathology.

However, it is not clear which component of the inoculated brain material – the tau aggregates or a soluble tau species – is responsible for transmitting tau pathology. Several studies have directly tested the uptake of various forms of tau to determine whether cells preferentially internalize specific types of tau. For example, in cultured neurons treated with either low-molecular-weight aggregates, short fibrils, or long fibrils composed of recombinant full-length tau, only aggregates and short fibrils were internalized [49]. Cells also take up paired helical filaments (PHFs) of tau isolated from AD brain; both HEK and SH-SY5Y neuroblastoma cells internalized exogenously applied PHFs by endocytosis [39]. Furthermore, in C17.2 neural stem cells treated with a monomeric or oligomeric recombinant tau microtubule-binding domain construct (amino acids 243 to 375), a majority of cells took up oligomeric tau, although a small percentage internalized monomeric tau [45]. Interestingly, HEK cells overexpressing this tau construct formed aggregates which were released and internalized by other HEK cells [50], providing direct evidence that microtubule-binding domain fragments of tau aggregates can be transferred between cells *in vitro*.

Recent *in vivo* studies have explored whether certain forms of tau are preferentially propagated. To determine whether different types of tau aggregates could be taken up by neurons, synthetic preformed fibrils of recombinant human full-length tau or a truncated form of tau, containing only the microtubule-binding domain, were injected intracerebrally into young mice expressing human mutant P301S tau [46]. Both forms of fibrils were internalized by cells, likely by endocytosis [51], and over time human tau-positive inclusions were found throughout the brain, indicating that both full-length or truncated tau may be propagated *in vivo* [46]. However, another study, using tau isolated from AD brain, suggests that cells might discriminate in the types of tau internalized *in vivo*. When oligomeric or PHF tau was injected in the hippocampus of young, wild-type mice, only the tau oligomers, not brain-derived PHF, induced tau pathology 11 months post-injection [48]. Interestingly, both oligomeric and PHF tau were internalized by cells near the injection site, but only oligomeric tau was propagated to other brain regions, suggesting that PHF tau may not be transported and released by neurons [48]. This study supports a critical role of mouse tau in propagation of tau pathology in mouse models since, in tau knockout mice, no propagation of oligomeric tau was observed. Furthermore, the aggregates found in brain areas distal to the injection site were composed of mouse and not human tau, indicating that the tau propagation was not simply transfer of the injected material [48], in agreement with recently reported findings using a different mouse model [47].

The exact mechanism by which cells internalize tau remains unknown; it may be dependent upon the tau species (for example, monomers, small soluble oligomers, or aggregates). Further work is required to better understand whether the spread of tau inclusions is due to a prion-like mechanism, in which misfolded tau induces other tau molecules to become similarly misfolded [52]. In this manner, misfolded or aggregated tau might act as a template, altering the conformation of tau in each neuron that internalizes it. However, in the case of tau aggregates, it remains unclear whether misfolded tau can alter conformation of other tau molecules directly or whether these aggregates interact with other cellular factors, like kinases, to alter tau in a way that would induce changes in conformation [53]. Internalization of tau may be different for isoforms that vary in number of microtubule repeat domains and N-terminal inserts or in post-translational modifications such as phosphorylation and truncation state. The studies discussed above indicate that soluble oligomeric tau is taken up and released...
both in vivo [48] and in vitro [45] and that this species facilitates the transmission of tau misfolding between cells, suggesting that therapies such as small-molecule tau aggregation inhibitors (reviewed in [54]), which might reduce accumulation of extracellular tau oligomers, might also slow transmission of tau pathology.

**Therapeutic intervention and tau propagation**

Accumulating evidence therefore suggests that inhibiting the intercellular transfer of tau may slow or halt the progression of tau pathology in AD. Furthermore, as tau pathology is associated with synapse loss [55] and subsequent cell death [56], prevention of its spread may also impact disease-related cognitive decline. Therapeutic strategies for preventing or reducing the interneuronal transfer of tau may take several approaches: blocking tau release, reducing accumulation or oligomerization of extracellular tau, or preventing tau uptake (Figure 1). Targeting tau release may enable reduction of extracellular tau levels, thereby reducing its availability for uptake by neighboring neurons. Since neurons release tau in vivo [31], possibly in an activity-dependent manner [23], further research is needed to determine the precise mechanism, which would be crucial for identifying targets for therapeutic intervention. Interestingly, the presence of extracellular tau in the absence of neurodegeneration, as described above, suggests that perhaps tau may have a yet unknown function outside the cell (for example, by participating in cell-cell signaling). Indeed, exogenously applied recombinant tau has been shown to bind and activate muscarinic acetylcholine receptors in cultured hippocampal neurons with an affinity higher than that of acetylcholine [57, 58]. Additional work is needed to determine whether tau can stimulate these receptors in vivo, as these studies suggest that, although inhibition of tau release may halt intraneuronal transfer of tau pathology, care must be taken to ensure that normal functions of extracellular tau are preserved. Furthermore, although recent studies suggest that neurons may preferentially internalize and propagate oligomeric tau in vivo [47, 48],

![Figure 1 Potential therapeutic targets to slow or prevent the spread of tau pathology in the brain.](image-url)

The mechanisms by which toxic tau species are transferred between cells are not known; further investigation is required to understand how tau is released into extracellular space and internalized. It is also unclear which species of tau are involved at each stage; neurons may release monomeric or oligomeric tau, which may include toxic forms of soluble tau. Released tau may also be misfolded, and may have modifications such as abnormal phosphorylation, truncation or both. As these mechanisms are better understood, potential therapeutic targets that will prevent or slow the spread of pathological tau may be uncovered. For example, the release and uptake of tau may be inhibited pharmacologically, and immunotherapy might lower extracellular tau concentration. Furthermore, anti-aggregant drugs may prevent tau oligomerization, reducing levels of potentially toxic forms of tau that are available to be internalized.
mechanisms of uptake of extracellular tau also require additional research in order to identify which type (or types) of tau may be internalized and how this process may occur. The spread of pathogenic tau may also be slowed or prevented by treatments that reduce levels of extracellular tau or that alter tau such that neurons are no longer able to internalize it. For example, inhibition or reversion of extracellular tau oligomerization might interfere with tau pathology spreading [54]; however, care needs to be taken with this strategy, as disruption of tau aggregates may result in formation of other soluble species of tau, which may also be toxic [59]. Therefore, prevention of extracellular tau accumulation or removal of pathological tau species may prove to be a more cautious method. Interestingly, recent studies of passive immunization of a tauopathy mouse model with antibodies directed against tau significantly prevented the formation of NFTs [60] or ameliorated existing tau pathology in older animals [61]. Active immunization, in which mice were inoculated with tau peptides, also proved effective at preventing and improving tau pathology in mice overexpressing human P301L tau [62]. Although tau immunotherapy has been successful in animal models, clinical trials will provide important information about whether anti-tau immunotherapies may slow or reverse disease progression in AD [63]. These trials may demonstrate a reduction in tau inclusions in AD brain, as found in an animal model of disease [61]. Furthermore, inhibition of tau transfer may restrict development of tau tangles to brain regions affected in early stages of AD and therefore prevent the cognitive dysfunction associated with later stages of the disease.

Conclusions

Propagation of tau pathology in the brain is a hallmark of AD. However, only recently have the mechanisms underlying the interneuronal transmission of tau been explored. Further understanding of these processes will be important for identification of novel therapeutic targets aimed at halting the spread of tau aggregation. Although progress has been made in identifying the tau species involved in propagation, further research is needed both in vitro and in vivo in order to identify precisely which forms of tau are involved in transmission of pathology. Identifying these pathological tau species is particularly important therapeutically, since these transmissible forms of tau may represent a specific target for immunotherapy. Crucially, it will be important to target pathological tau to minimize interference with normal tau function.

Numerous questions with regard to how tau pathology might be propagated in disease remain. For example, further research may provide insight into whether non-neuronal cell types regulate intercellular tau transfer, since glia are able to internalize tau aggregates in a mouse model of tauopathy [47] and glial tangles are also found in frontotemporal dementia [64]. Furthermore, although tau pathology appears to propagate along neural networks [17,18] and its release is regulated by synaptic activity [23], whether propagation of pathology occurs at synapses remains to be demonstrated [26]. Therefore, further identification and characterization of mechanisms of tau release and uptake will be critical for therapeutic interventions that may prevent or slow neurodegeneration in AD.

Note: This article is part of a series on Tau-based therapeutic strategies, edited by Leonard Petrucelli. Other articles in this series can be found at http://albres.com/series/tau_therapeutics.

Abbreviations


Competing interests

The authors declare that they have no competing interests.

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