ACS Chemical Neuroscience

Review

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The Role of Tau Oligomers in the Onset of Alzheimer's Disease Neuropathology

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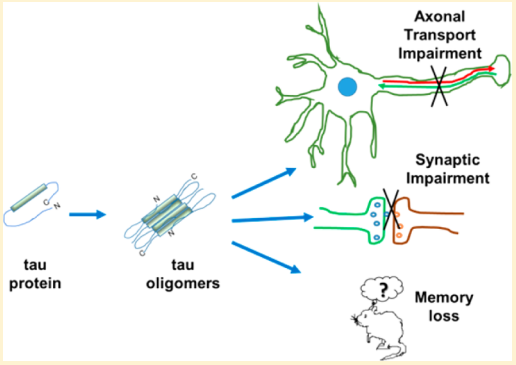
ABSTRACT: Most neurodegenerative diseases are characterized by the presence of protein aggregates. Alzheimer's disease (AD) is the most common cause of dementia in people over age 60. One of the histopathological hallmarks of AD is the presence of tau protein aggregates. Historically, it has been thought that paired helical filaments (PHFs) were the toxic form of tau that assembled to form neurofibrillary tangles (NFTs), but recently there has been evidence that tau oligomers, which form before PHFs and NFTs, could be the structures mediating neurodegeneration even before the fibrillary tau is deposited. Here, we discuss the recent advances in tau oligomer research, their implications on AD and other tauopathies, the mechanisms of tau turnover by the principal protein clearance systems (the proteasome and autophagy), and the potential use of tau oligomers as drug targets for the development of new therapeutic approaches.



KEYWORDS: *Tau oligomers, aggregation, neurodegeneration, Alzheimer's disease, protein spreading, synaptic impairment, new drug targets, proteasome, autophagy*

Dementias are complex human brain diseases, characterized by the progressive deterioration of cognitive function. AD is the most common cause of dementia in elderly people. Total new cases of dementia each year worldwide are predicted to be 7.7 million, or one every four seconds. In the US, AD impacts 5.5 million people, and it is projected that the total number of AD cases for 2050 will be 13.8 million,[1,2] with 7 million aged 85 years or older in the US alone. Although, to date, outstanding progress has been made to elucidate the cellular and molecular mechanisms involved in AD neuropathology, there is still no effective cure or treatment for this disease. Thus, more research in this field is needed, and we also probably need to rethink the hypotheses of the contribution of aggregated proteins to the neurodegeneration observed in this type of dementia, generating new research paradigms that allow for a better understanding of the disease and the design of improved therapeutic interventions.

Many mechanisms have been proposed to contribute to AD, such as mitochondrial dysfunction,[3] oxidative stress,[4] imbalance of metal ions,[5] disturbances of cholesterol and lipid metabolism,[6] damage of cellular membranes by amyloid toxins,[7] metabolic disturbances with increase in homocystein,[8,9] alterations in glucose metabolism,[10] neuroinflammation,[11] accumulation of amyloid peptides (Aβ) that form plaques, and deposits of microtubule-associated protein tau (MAPT) that forms PHFs and NFTs.[12] In this review, we will focus on tau aggregates from oligomeric (soluble) to fibrillary (insoluble aggregates), and we will discuss their possible role in AD pathology.

Tau protein was first known for its function as a microtubule stabilizer. In its native conformation, it binds microtubules and favors polymerization; once hyperphosphorylated, tau changes its conformation and detaches from the microtubules, destabilizing them. Tau is more abundant in axons, but under pathological conditions, it delocalizes and accumulates in the cell soma, where it forms aggregates. Insoluble aggregates of tau protein are one of the typical hallmarks of AD and other neurodegenerative diseases grouped as tauopaties (i.e., Niemann−Pick disease,[13] corticobasal degeneration,[14] progressive supranuclear palsy,[15] frontotemporal lobar dementia linked to chromosome 17 (FTLD-17),[16] and tangle-only dementia (TOD)[17]). Tau protein stabilizes the microtubules in the axons and may have alternative functions in axonal transport and synapsis. Under physiological conditions, tau is soluble with limited secondary structure, but under pathological conditions, tau dissociates from the microtubules and self-associates to form prefibrillar oligomeric (2 or more molecules in multimeric structure) and fibrillar aggregates known as PHFs and NFTs. For many years, it was thought that the major neurotoxic tau species were the NFTs, but much evidence from animal models

Received: July 6, 2014
Revised: September 26, 2014
Published: September 30, 2014

   dx.doi.org/10.1021/cn500148z | *ACS Chem. Neurosci.* 2014, 5, 1178−1191

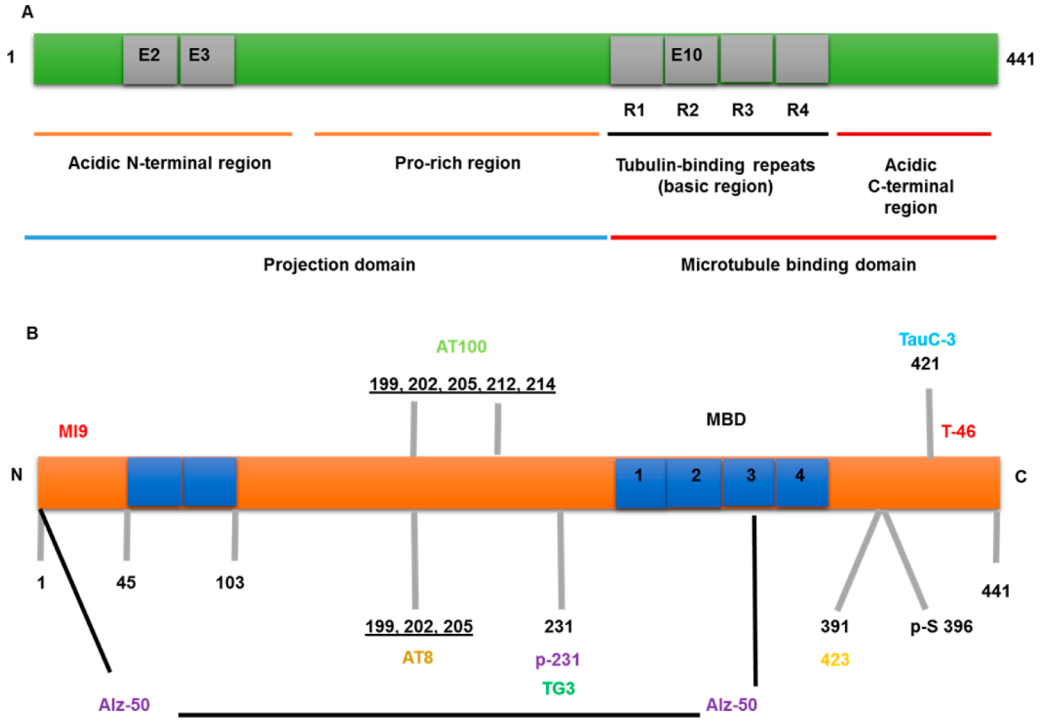


Figure 1. Tau protein. (A) Schematic representation of the longest human tau isoform of 441 amino acids that contains 4 repeat domains (R1−R4) in the microtubule binding region, exon 10 (E10), and 2 N-terminal inserts (E2,E3). The most important regions of tau protein are depicted: the projection domain, which comprises the N-terminal region, the proline-rich region, and the microtubule binding domain, which contains the tubulin binding repeats and the C-terminal region. (B) Schematic representation of tau protein showing the epitopes susceptible to phosphorylation or conformational changes that are recognized by the most common tau antibodies used in Alzheimer's research.

and human brains from AD or tauopathy cases has shown that the generation of NFTs can be dissociated from memory loss and neurodegeneration in the early stages of AD neuropathology.[18−20] In animal models, cell death and synaptic loss occur independently of the formation of NFTs, and soluble tau correlates better with memory loss and behavioral deficits than the levels of NFTs. Furthermore, isolated oligomers, but not monomers or NFTs, induced memory impairments and synaptic dysfunction, including mitochondrial damage, when given intracerebrally to wild-type mice.[21] Thus, attention is now being given to the study of the multimeric tau aggregates known as tau oligomers, which are intermediate aggregates, that form prior to filamentous tau in AD and may be the mediators of early neuronal damage, including axonal transport and synaptic impairment, and that may have the consequence of affecting memory and learning. Soluble aggregates (i.e., Aβ and α-synuclein) have been implicated as the primary toxic species in many degenerative diseases.[22] These new findings in AD research may change our point of view about filamentous tau aggregates because now we may consider them to be protective instead of the structures responsible for the toxicity. It is possible that the cell forms these structures (PHFs and NFTs) in order to put aside the toxic tau oligomers, packed filamentous structures, to avoid its toxic effects. Interestingly, tau in its oligomeric form is conformationally changed (and may be truncated) from its native structure. Once tau is structurally modified, it becomes more prone to self-aggregate (self-assembly) and it can also bind native tau and induce similar changes to it, causing a self-propagation of the tauopathology in a prion-like and autocatalytic way. Moreover, the presence of annular protofibrils (APFs) that form annular pore-like structures that evade fibrillary fate have recently been discovered, and they may function like pore-forming protein toxins, possibly causing an ion imbalance and permeability changes, leading to cellular damage and possibly contributing to tau oligomer spreading-modified proteins to the extracellular space and to unaffected brain regions.[20] Previously, it was discovered that other amyloidogenic proteins, such as Aβ and α-synuclein, form these pore-like structures. This was shown first in in vitro studies with recombinant proteins and later with isolated protein inclusions from patient brains.[23−25] In relation to intercellular tau propagation, tau oligomers may enter the cell in different ways: by stressing the cell membrane, through the formation of pore-like structures, or by endocytosis.[26] Both macropinocytosis and receptor-mediated endocytosis have been implicated as tau entry mechanisms. Additionally, tau may also be secreted either within exosomes through synaptic vesicle release or directly without an enclosed membrane,[27] possibly by a direct interaction with Fyn receptor (Src kinase). Fyn−tau interactions have also been colocalized with exosomes, suggesting a possible role for Fyn in tau transport.[28]

Another important aspect that needs to be considered when studying tau aggregation is the mechanism that the cell uses to clear the aggregates. It is well-known that the cell has two major protein clearance mechanisms: the proteasomal and autophagy−lysosomal pathways. Here, we discuss the possibility that tau may be degraded by both clearance mechanisms and that one or both turnover pathways may be impaired during AD and other tauopathies. Finally, we will talk about past and ongoing clinical trials directed at inhibiting tau aggregation and the new therapeutic strategies to target tau oligomers and their possible advantages, including immunotherapy approaches and the use of phenothiazine colorants to inhibit tau aggregation as well as new drug designs considering the use of small molecules.

dx.doi.org/10.1021/cn500148z | ACS Chem. Neurosci. 2014, 5, 1178−1191

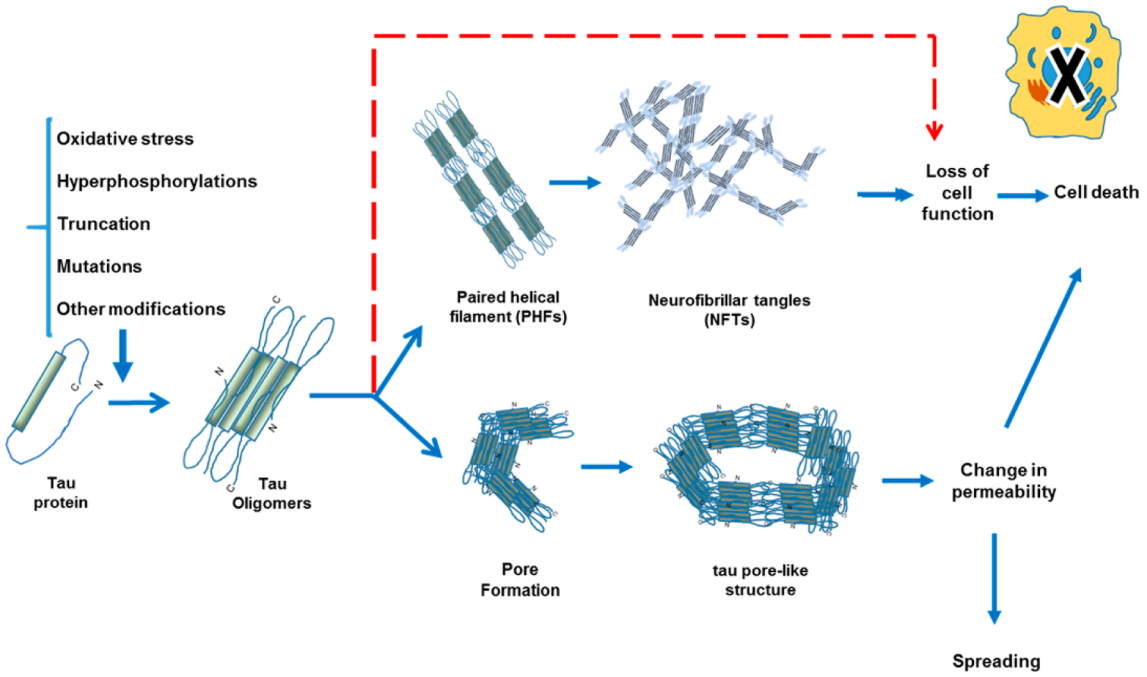


Figure 2. Tau oligomers could be the early mediators of neuronal damage before PHFs and NFTs are formed (red dashed line). Factors that may contribute to the generation of tau oligomers include oxidative stress, hyperphosphorylation, truncations, and mutations, among others; these factors could cause the detachment of tau from the microtubules and cause conformational changes that promote its aggregation. According to Lasagna-Reeves et al.,[20] the tau pore-like structure may be involved in Alzheimer's pathology as well as in other tauopathies. Their working model proposes that tau oligomers could either form PHFs and NFTs or align to form spherical pore-like structures consisting of annular protofibrils (APFs) that can disrupt membrane permeability, causing ion imbalance and possibly release tau oligomers, which contributes to the spread of tau pathology.

TAU PROTEIN

Tau protein is a MAP with an important role in maintaining the complex neuronal cell microarchitecture, particularly the axon and dendrites. These processes (cellular projections) are relevant for neural transmission; thus, changes in its arrangement could have functional and pathological consequences. Tau gene localizes in chromosome 17q21, contains 16 exons, and is 100 kb in size.[29] There are different tau isoforms, produced by alternative splicing. Isoforms lacking exon 10 are expressed at early developmental stages. Exons 2, 3, and 10 are alternatively spliced and are specific to the adult brain. In the adult human and mouse central nervous system (CNS), the alternative splicing of exons 2, 3, and 10 results in the generation of six isoforms of tau protein, consisting of 352−441 amino acids residues.[30] Apart from that, there is a fetal isoform of 316 amino acids with modified N-terminal sequence. Since exon 10 codes for one of the microtubule binding regions (repeats domain), alternative splicing of exon 10 produces tau isoforms with three (tau 3R without exon 10) or four repeats (tau 4R with exon 10) of the tubulin/microtubule binding region.

Thus, adult human isoforms contain from 3 to 4 microtubule binding sites and have different alternative splicing of exons 2 and 3 (E2 and E3): 3R taus (0N3R, 1N3R, 2N3R) and 4R taus (0N4R, 1N4R, 2N4R). 2N4R tau is the largest brain isoform, 441 amino acids, whereas the shorter isoform is the fetal, which lacks the two amino terminal inserts, has only 3 repeats (0N3R), and is 352 amino acids in length. The high molecular weight isoform that is expressed in the peripheral nervous system (PNS) is approximately 100 kDa.[31] Tau protein has little secondary structure; it is mostly random coil, with β structure in the second and third microtubule binding repeats.[32]

Brain tau isoforms have two large domains: the projection domain, which consists of two-thirds of the molecule including the amino-terminal region, and the microtubule binding domain, consisting of the remaining one-third of the molecule, which includes the carboxy-terminal domain (Figure 1). The projection domain comprises two regions: the amino-terminal region with a high proportion of acidic residues and the proline-rich region. The microtubule binding domain is divided into the basic tubulin binding region and the acidic carboxy-terminal region. The projection domain may play a role in the interaction with other molecules or cation binding due to its acidic residues. Additionally, this domain includes motifs like KKXK, which is involved in heparin binding, and PPXXP and PXXP in the proline-rich region that may play an important role in the interaction of tau with proteins containing SH3 domains and proteins associated with the plasma membrane.[33] Tau repeat domains bind to the microtubules and promote its assembly.[34] Tau is mainly a neuronal protein, although it is expressed in glial cells in degenerative diseases.[35] In neuronal cells, tau could be associated with the plasma membrane[36] as well as with the microtubules. Normally, tau is more abundant in the axons than in the soma, but during pathology, its distribution changes and becomes more abundant in the cell soma. The distribution of tau could be mediated by its level of phosphorylation; thus, in AD, it is hyperphosphorylated and therefore detached from the microtubules.[37] The longest tau isoform has 79 putative serine or threonine phosphorylation sites on the longest CNS tau (comprising 441 amino acids). Some of the epitopes that are recognized by antibodies used in tau research[38,39] are depicted in Figure 1. Among the kinases that can phosphorylate tau are GSK3, cdk5 p38 MAP kinase, JNK, protein kinase A, protein kinase C, and calmodulin kinase II (CamKII).[30]

dx.doi.org/10.1021/cn500148z | ACS Chem. Neurosci. 2014, 5, 1178−1191

Apart from phosphorylation, several other post-translational modifications have been described for tau protein (glycosylation, ubiquitination, deamination, oxidation, nitration, cross-linking or glycation, and truncation).

Tau protein is an amyloidogenic protein; thus, it is able to self-aggregate, and some of the prost-translational modifications of tau, like phosphorylation and truncation, potentiate its self-aggregation capability.

ALTERNATIVE FUNCTIONS OF TAU PROTEIN AND TAU OLIGOMERS

For years, the tau microtubule hypothesis was accepted as the general explanation of the role of tau protein on neurodegeneration in AD, in which hyperphosphorylated tau does not bind effectively to microtubules and is no longer able to function in stabilizing them and thus axonal transport cannot proceed efficiently, leading to synaptic dysfunction.[40−44] Nevertheless, the precise mechanism of tau-mediated neurotoxicity remains unclear. Recently, it has been proposed that tau protein may have additional functions other than just stabilizing microtubules. Currently, there is a debate as to the form of tau that is linked to neurotoxicity: tau oligomers or tau fibrillar aggregates. It has been shown that pretangle tau aggregates in brains in the early stages of Alzheimer's disease,[45] indicating that fibrillar tau might not be the toxic species of this protein. These pretangle tau aggregates are known as tau oligomers. The first in situ immunohystochemical demonstration of the presence of tau oligomers, described as amorphous aggregates in the neuronal cytoplasm, reported as "stage 0" tangles, was published by Bancher et al.[46] Tau oligomers were biochemically characterized in a conditional mouse model (rTg4510) that expresses the P301L mutant human tau (Figure 5). In this transgenic model, the best correlation between neuronal loss and behavior deficit was through the accumulation of oligomeric tau, not NFTs.[18]

Tau oligomers are any complex of two or more tau molecules (commonly, 3−10 monomers) in a multimeric structure. They consist of either hyperphosphorylated or nonphosphorylated tau proteins (Figure 2). They can be soluble (consisting of a small number of molecules) or insoluble (consisting of a larger number of molecules). Dimers have an apparent size of 94−130 kDa,[47,48] and trimers have an apparent size of 150−190 kDa,[49] whereas large insoluble oligomers that consist of an average of 40 molecules of tau have an average size of 1800 kDa.[50] Oligomers have been identified in AD, FTPD-17, and PSP[51] brains, and it is generally accepted that they are formed early in the disease process, possibly as a consequence of inadequate clearance of misfolded tau by the chaperone/ubiquitin-proteasome system or by the autophagy/lysosomal system; as the disease progress, the tau oligomers can form filaments and then tangles.[50] In recent years, many amyloidogenic proteins including tau have been implicated in neurotoxicity and neurodegeneration observed in amyloid diseases.[19,22,48,52,53] According to Lasagna-Reeves et al.,[21] the accumulation of oligomers results in learning impairment through the disruption of synaptic (by reducing the levels of synaptic vesicle-associated proteins synaptophysin and septin-11) and mitochondrial (by decreasing the levels of NADH-ubiquinone oxidoreductase, electron transport chain complex I) functions. Additionally, the oligomers can activate caspase-9, which is related to the mitochondrial apoptotic pathway.

Furthermore, it has been published that tau pathology may spread in the brain via a prion-like mechanism, possibly involving a transynaptic mechanism of spreading along anatomically connected neuronal networks.[54,55]

POSSIBLE ROLE OF TAU PHOSPHORYLATION IN TAU OLIGOMER FORMATION

According to Iqbal and colleagues,[56] normal brain tau contains two to three phosphates per mole of protein, and in this condition, it is a soluble protein, whereas in AD brain, tau is 3- to 4-fold hyperphosphorylated compared to that in normal brain. Tau self-aggregates and can also bind native tau instead of microtubules, leading to the formation of tau oligomers. According to this group,[56] tau oligomerization in AD and other taopathies is hyperphosphorylation-dependent.

Some of the evidence of the role of hyperphosphorylation in tau aggregation that they discussed includes (1) in vitro studies showing that the treatment of AD-phosphorylated tau (AD-ptau) with protein phosphatase 2A (PP2A) inhibits its oligomerization, whereas rephosphorylation of AD-tau (previously treated with PP2A) with kinases promotes its oligomerization, (2) missense mutations in FTD promote abnormal tau hyperphosphorylation, possibly leading to tau oligomerization and fibrillation,[57] and (3) dysregulation of tau alternative splicing that modifies the ratio of the 1:1, 3-repeat/4-repeat tau (in Down syndrome, Pick's disease, and progesive supranuclear palsy), leading to abnormal hyperphosphorylation.[58]

Iqbal and colleagues noted that in AD brains altered tau was present not only in NFTs but also in the cytosol, and this abnormally phosphorylated tau sequesters some of the normal native tau and can be sedimented by centrifugation at 200 000*g*, whereas the normal soluble nonhyperphosphorylated tau remains in the supernatant.[40,59] This suggests that AD-tau oligomers are hetero-oligomers of hyperphosphorylated tau and nonhyperphosphorylated tau. However, since oligomers could be composed of either phosphorylated or nonphosphorylated tau, it is not clear whether phosphorylation is a requisite to its formation. According to Wischik et al. and the group of Mandelkow, tau phosphorylation could be inhibitory for tau−tau interaction.[60,61] This groups mention that the hyperphosphorylation that causes the detachment of tau from microtubules does not prime tau for its aggregation, but rather inhibits it, implying that the hyperphosphorylation of tau in AD may not be responsible for the pathological aggregation of tau; on the contrary, they claim it could protect against it. Moreover, the group of Wischik proposes that the inherent binding affinity at the tau−tau site in the repeats domain is sufficient to promote tau aggregation. According to their data, the repeat domain tau fragment that they originally isolated from the core of the PHFs[62,63] has prion-like properties in vitro. Therefore, the repeat domain of tau is able to catalyze and propagate the conversion of normal native soluble tau into accumulations of aggregated and truncated oligomeric forms. More research is warranted to elucidate the role of tau phosphorylation and/or truncation in tau oligomerization.

TAU OLIGOMERS IN NEUROPATHOLOGY AND MECHANISMS OF TAU AGGREGATE CLEARANCE

Recently, conformational changes and multimeric aggregates of tau protein have been getting more attention as a possible first step in the pathology of AD, and the amyloid hypothesis is being questioned since Aβ deposition in amyloid plaques appears to be a late, nonspecific event. Clinical trials with drugs

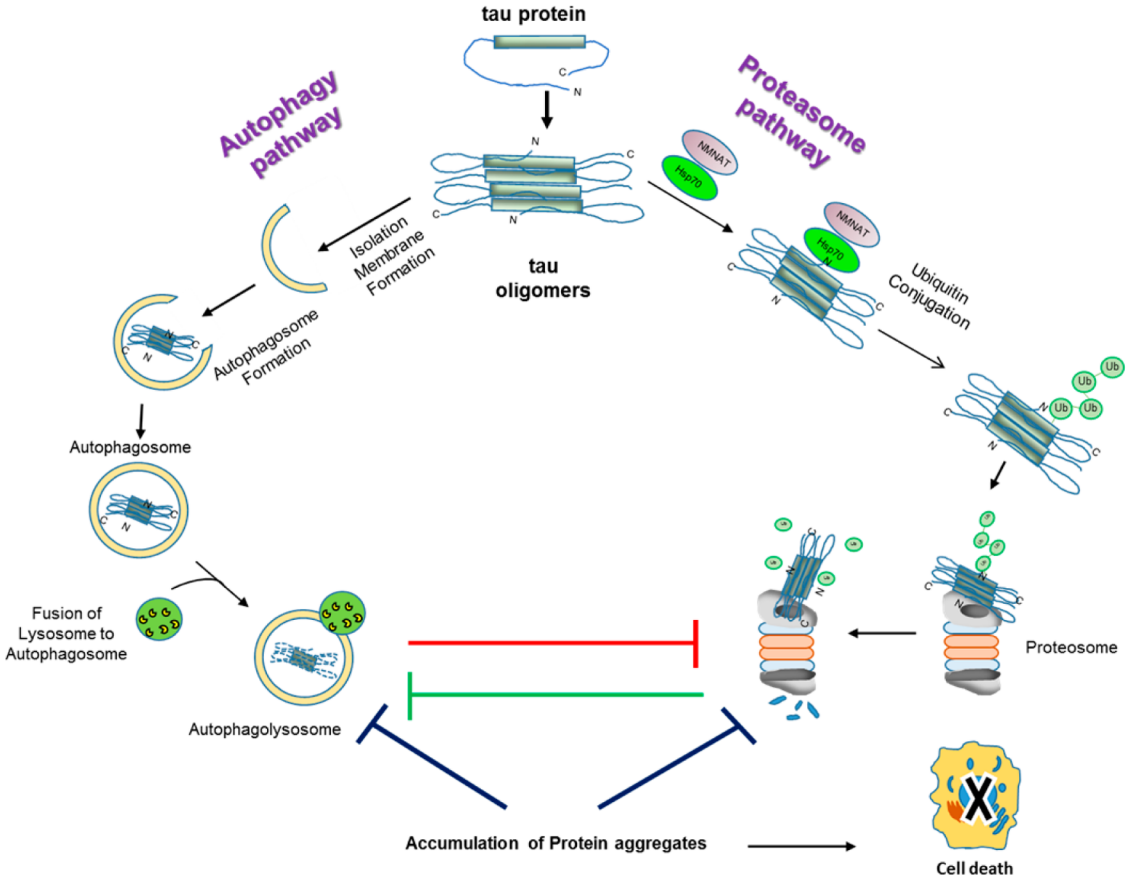


Figure 3. Tau oligomer turnover through the principal clearance mechanisms. It is possible that tau oligomer turnover occurs via both proteasomal and autophagy mechanisms, although evidence (see text for details) supports the possibility that larger tau aggregates (i.e., fibrillary tau) are preferentially degraded by autophagy, whereas soluble tau may be preferentially degraded by the proteasomal pathway. However, the preferred route of degradation for each modified form of tau remains to be determined. The autophagic pathway consists of the formation of the isolation membrane, which engulfs part of the cytoplasm, possibly including tau oligomers, giving rise to the double membraned autophagosome or autophagic vacuole (AV). This AV is then fused to a lysosome to form the autophagolysosome structure in which the lysosomal enzymes degrade the material sequestered in this vesicle. Then, the resulting material can be recycled. The 26S proteasomal pathway involves the participation of chaperones such as CHIP, Hsp70, or NMNAT as well as the tagging of tau oligomers with ubiquitin. Then, the tau oligomers are targeted to the proteasome, where the regulatory cap subunit, together with chaperones, unfolds the proteins and removes the ubiquitin tag prior to feeding the protein into the catalytic core where it is enzymatically degraded. Impairment of one or both clearance pathways causes further protein accumulation and toxicity that affects cell function and could lead to cell death.

that attempt to eliminate or delay the formation of Aβ deposits have failed to restore deficits in cognitive impairment.[64] The amyloid hypothesis[65] was based on the self-polymerization of Aβ over years that forms amyloid plaques which could then cause brain damage, but recent evidence shows that oligomers of Aβ and/or tau appear to be responsible for the brain damage, in particular synaptic impairment that occurs even before the accumulation of fibrillary Aβ or tau (PHFs and NFTs).[66−68,21,69] Studies of pathological species of tau have been focused on hyperphosphorylation and aggregation of large insoluble filaments; however, recently, it has been shown that tau oligomers could be the main toxic tau species in early stages and correlate with memory deficits.[70,21] Tau oligomers or multimers could be synaptotoxic in the early stages of neuropathology.[70]

According to Takashima[71] and others, the increased levels of granular tau oligomers in the frontal cortex could be an early sign of AD, suggesting that increased levels of granular oligomers may occur before NFTs formation and before any clinical manifestation of AD. Tau oligomers have also been identified in AD and FTPD-17 brains and accumulate probably as a consequence of inadequate clearance of misfolded tau.[50] The two major protein degradation pathways in the cell are the ubiquitin−proteasomal system and the autophagic−lysosomal system, and there is an important crosstalk between these two degradation systems. Inhibition of the proteasome induces autophagy, but autophagy impairment does not seem to elevate proteasomal function and, in fact, inhibits it, probably because of the accumulation of large aggregated substrates that may impair the proteasome.[72−75] The clearance of pathological forms of tau could be mediated by both degradation systems.[76] The proteasome is a large barrel-shaped multiprotein complex involved in soluble cytosolic protein degradation. The 26S proteasome degrades substrates tagged with polyubiquitin chains as the targeting sequence, and it consists of a regulatory cap (19S or 11S) on either end of its catalytic core (20S), which has the proteolytic activity. The regulatory cap, together with chaperones, unfolds the substrate protein and removes the ubiquitin tag in an ATP-dependent way, prior to feeding it into the catalytic core to be enzymatically degraded (Figure 3). The 20S proteasome consists of the catalytic core without its regulatory caps; thus, it is able to degrade natively unfolded

substrates directly in an ATP- and ubiquitin-independent way. Monomeric tau is natively unfolded; therefore, it could be a substrate for the 20S proteasome. Additionally, tau may be ubiquitinated[77] and targeted to the 26S proteasome,[78] and much experimental evidence support this possibility.[79−82] Molecular chaperones, such as the Hsp70/Hsp90 family and NMNAT, are potent inhibitors of tau aggregation in vitro. Hsp70 family proteins are upregulated in AD,[83] whereas NMNAT is downregulated in this disease.[84] These chaperones can bind to hyperphosphorylated tau oligomers, preventing its further aggregation and favoring its ubiquitination and clearance through the proteasomal system. The blockage of the proteasomal pathway with its inhibitor MG-132 led to a reduction of total tau levels, suggesting a compensatory upregulation of the other major degradation system (i.e., autophagy).[85] Autophagy (self-eating) is a lysosomal degradative intracellular process used to recycle obsolete cellular components and eliminate damaged organelles and protein aggregates.[72] Thus, autophagy maintains cell homeostasis, working like a housekeeping mechanism, participating in the continuous turnover of intracellular constituents.[86] There are three major forms of autophagy: macroautophagy, microautophagy, and chaperon-mediated autophagy. Macroautophagy (hereafter refered to as autophagy) is the most common form of autophagy, in which the cytosolic substrates that must be degraded are sequestered by an isolating membrane that seals to form the autophagosome (or autophagic vacuole, AV). Then, this AV fuses to a lysosome (to form the autophagolysosome) that provides the enzymes necessary for the degradation of the engulfed material. The membrane of the AV is then degraded to allow lysosome hydrolases access to the internalized substrates (Figure 3). In microautophagy, a similar process of sequestration of the material occurs, but, in this case, the lysososmal membrane itself deforms to engulf the cytosolic substrate. In the third type of autophagy, chaperone mediated, cytosolic proteins selectively bind a lysosomal membrane receptor that mediates their translocation into the lysosomal lumen and depends on the selectivity of recognition of a target signal in the amino acid sequence of the substrate by a cytosolic chaperone.

Autophagy can degrade protein aggregates; thus, tau oligomers and fibrillary tau could be substrates of this degradation system. Previous publications support the possibility that tau (native monomeric or pathologically aggregated) can be degraded by autophagy, but more studies are necessary to elucidate the mechanisms of tau turnover.[78] One of these studies was an in vitro cleavage of tau by cathepsin D (an aspartyl protease) from human liver.[87] In another study, the treatment of brain slices with chloroquine (CQ), which impairs lysosomal function by raising the pH, caused an increase in full-length tau levels.[88] Similarly, in an inducible cellular model that expresses full-length tau, CQ treatment slowed tau degradation, favoring its accumulation.[89] Likewise, in a *Drosophila* model of tauopathy that expresses mutant human tau, the levels of cathepsin D are elevated, suggesting the importance of these enzymes in degrading tau. Thus, if cathepsin D is genetically ablated in this experimental model, then there is enhanced neurotoxicity and a reduction of the lifespan.[90] In other studies, the overexpression of only the repeat domain of tau containing a FTDP-17 mutation in neuroblastoma cells causes tau proteolysis and aggregation. Furthermore, the use of the autophagy inhibitor 3MA led to an increase in soluble and insoluble tau[91] in this cellular model. On the other hand, it has been shown that activation of autophagy leads to enhanced tau clearance. In one of these studies, treatment with methylene blue in a hippocampal slice preparation induced autophagy and decreased phosphorylated and insoluble tau.[92] Similarly, in a cell line expressing the repeat domain of tau containing the mutation of FTDP-17, the treatment with the disaccharide trehalose (an mTOR-independent autophagy activator) significantly reduced aggregated tau levels as well as total soluble and insoluble tau.[85] Accordingly, in a mouse model expressing FTDP-17 mutant P301S, the promotion of autophagy with trehalose treatment beginning at weaning significantly reduced insoluble tau as well as phosphorylated tau at T212/S214, with improved neuronal survival in cortical layers I−III.[93]

Activation of autophagy with rapamycin in cultured cells and in a *Drosophila* model overexpressing human tau reduced tau levels.[94] Furthermore, mice with Atg7 (critical autophagy gene) knocked out in forebrain neurons develop age-dependent neurodegeneration with accumulation of intracellular inclusions that contain tau phosphorylated at AT8, AT100, and TG3 epitopes.[95] Finally, Mandelkow et al.[96] has shown the possible role of autophagy in soluble and insoluble tau degradation in inducible cell models.[91] According to them, unlike the proteasome, autophagy degrades tau regardless of its phosphorylation. Their experimental data in primary neurons suggests that endogenous tau is preferentially degraded by autophagy and not by the proteasome.[85] It is important to mention that tau can modulate the autophagy pathway; thus, tau pathology may lead to impairment of the autophagy/lysosomal system.[96]

Several reports support the idea that aging impairs autophagy; both the formation of AVs and their removal by lysosomal fusion decrease with age.[97−99] According to Nixon et al. and others,[100−102] neurons without competent autophagy accumulate ubiquitinated protein aggregates and degenerate. It has been shown that autophagy is defective in neurodegenerative diseases, including AD.[103] In AD brain, AVs accumulate in dystrophic neurites and correlate with the presence of filamentous tau.[103] Accordingly, activation of autophagy at early stages of a neurogenerative disease could play a protective role because it is crucial for cell adaptation to adverse conditions since it permits the elimination of stressed or damaged organelles or toxic aggregated proteins. Furthermore, the degradation of intracellular macromolecules provides the energy required when nutrients are scarce.

Recently, the group of Wischik has proposed the following hypothesis of tau aggregation in relation to autophagy dysfunction:[60] defective autophagy later in life could release partially digested mitochondrial degradation products that accumulate in the cytosol as lipofuscin. These deposits are the most likely substrate for initial seeding (nucleation) of tau aggregation; thus, a key factor triggering tau aggregation is binding to a nonspecific substrate. Then, nucleation of tau (PHF core fragment) could generate tau oligomeric aggregates that can capture normal or mutated tau.

According to this group, Tau oligomers are preferentially degraded by autophagy because they are resistant to cytosolic proteases. These aggregates could further accumulate due to congestion and dysfunction in lysosomal processing.

Hence, in neurodegenerative disease, one or both of these main protein degradation pathways are impaired, and this may contribute to further protein aggregate accumulation and neurotoxicity.[104]

Understanding the tight regulation of chaperones as well as the proteasomal and autophagy pathways to maintain protein homeostasis will allow therapeutic approaches to be better designed to focus on the early stages of the disease and on the appropriate targets.

PRION-LIKE SPREADING OF TAU OLIGOMERS

Tau oligomers, but not fibrillary tau, are capable of seeding and propagating AD pathology[105] (Figure 4). The propagation of tau pathology is similar to a self-propagating prionosis. Once a tau molecule is pathologically modified (hyperphosphorylated, conformationally changed, or truncated), it detaches from the microtubules and self-aggregates. It can bind other altered tau proteins or native tau and induce pathological changes to it. Tau oligomers may act as templates for the misfolding of native tau, seeding the spread of the toxic forms of the protein.[27] The spreading of the pathology occurs first intracellularly and later (as the recent findings suggest) extracellularly and intercellularly.[106−108] Under pathological conditions, most tau oligomers escape proteolytic degradation and could be transported through cytoplasmic flow to the nerve terminals, where they can cause synaptic impairment and can be released to neighboring neurons, initiating the same cascade of damage in them.

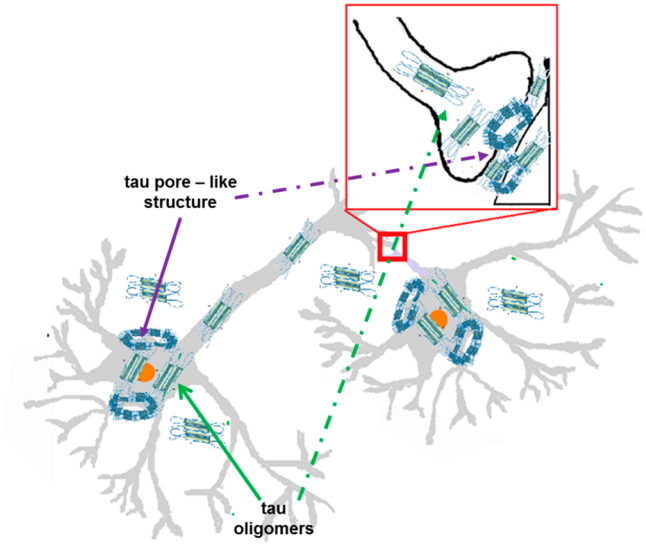


Figure 4. Model of tau oligomer spreading pathology. Tau oligomers act as a template for the misfolding of native tau, seeding the spread of altered forms of tau. Recent evidence suggests that tau oligomers could also embed themselves into the membrane and alter its permeability, mimicking the action of pore-forming protein toxins and causing ion imbalance and possibly releasing tau oligomers to the extracellular space, thereby disseminating tau pathology to unaffected regions.

Although tau is an intracellular protein, tau aggregates are observed in the extracellular space (Figure 4). Moreover, tau can be detected in the cerebrospinal fluid of AD patients. In AD, tau pathology begins discretely in specific brain regions (i.e., entorhinal cortex) but ultimately involves much larger areas of the brain (i.e., hippocampus), thereby suggesting a spreading mechanism (intercellular transfer of tau aggregates).[108] Many in vitro and in vivo studies support this possibility of intercellular spreading. In one of these studies, brain extract obtained from transgenic mice that overexpress mutant tau (P301S) were injected into the brains of ALZ17 mice that overexpress wild-type human tau (which does not normally form tau aggregates), and they found tau deposits not only in the site of injection but also in the neighboring areas.[107] Duff et al.[109] have demonstrated a trans-synaptic spread of tau pathology in vivo in a transgenic mouse that differentially expresses pathological human tau in the entorhinal cortex. This mouse recapitulates the tauopathy that defines the early stages of AD. Furthermore, Hyman and colleagues[110] generated another transgenic mouse model in which mutated tau (P301L) expression is restricted to layer II of the entorhinal cortex. In this model, tau pathology progresses from entorhinal cortex transgene-expressing neurons to neighboring neurons (not expressing the transgene), followed by propagation of neurons downstream in the synaptic circuit such as the dentate gyrus, hippocampus CA, and cingulate cortex. Finally, Lasagna-Reeves and colleagues[105] confirmed that the tau species responsible for the spreading is the oligomeric form. They injected a pure extract of tau oligomers from AD brain cortex into wild-type mice (nontransgenic) and observed the propagation of the pathology, as human brain-derived tau oligomers propagated the abnormal tau conformation to endogenous murine tau.

Recently, the presence of tau pore-like structures,[20] which consist of annular protofibrils (APFs), in the AD brain has been reported (Figures 2 and 4). This structure mimics the membrane-disrupting properties of the pore-forming protein toxins of bacteria.[111] These APFs can be formed by several amyloidogenic proteins including Aβ, α-synuclein, and tau; in particular, when these amyloidogenic proteins are mutated, they are more prone to form pores. Once tau oligomers are formed after conformational changes and misfolding, they can either continue to aggregate to form PHFs and NFTs or align to form spherical pore-like structures (APFs). Pores may cause ion imbalance and also cause changes in membrane permeability that may lead to cell damage and eventually cell death, possibly contributing to the spreading of tau pathology (Figures 2 and 4).

Tau oligomers may also enter the cell by endocytosis.[26] Both macropinocytosis and receptor-mediated endocytosis have been implicated as tau entry mechanisms. In addition, tau may be secreted within exosomes through synaptic vesicle release or directly without an enclosed membrane,[27] possibly by a direct interaction with Fyn receptor (Src kinase). Fyn−tau interactions have also been colocalized with exosomes, suggesting a possible role for Fyn in tau transport.[28]

TAU OLIGOMERS IMPAIR FAST AXONAL TRANSPORT

Since neuronal damage precedes the formation of fibrillary tau aggregates, it is suggested that prefibrillar tau oligomers may be neurotoxic. Binder and colleagues have demonstrated by using a squid axoplasm assay that aggregated oligomeric tau and not monomeric soluble tau inhibit anterograde fast axonal transport (FAT).[112,113] First, they showed that aggregated tau inhibits anterograde kinesin-based FAT by activating axonal protein phosphatase 1 (PP1) and GSK3 independent of microtubule binding. Next, they found that tau amino acids 2−18, containing a phosphatase-activating domain (PAD), are sufficient for activation of this pathway in the squid axoplasm assay. This PAD epitope was detected in AD brain, and it could be considered as an early marker of neuropathology, similar to that for the AT8 tau epitope (Figure 1). Thus, their model proposes that the conformational changes of tau cause the exposition of this epitope, activation of PP1 and GSK3, and

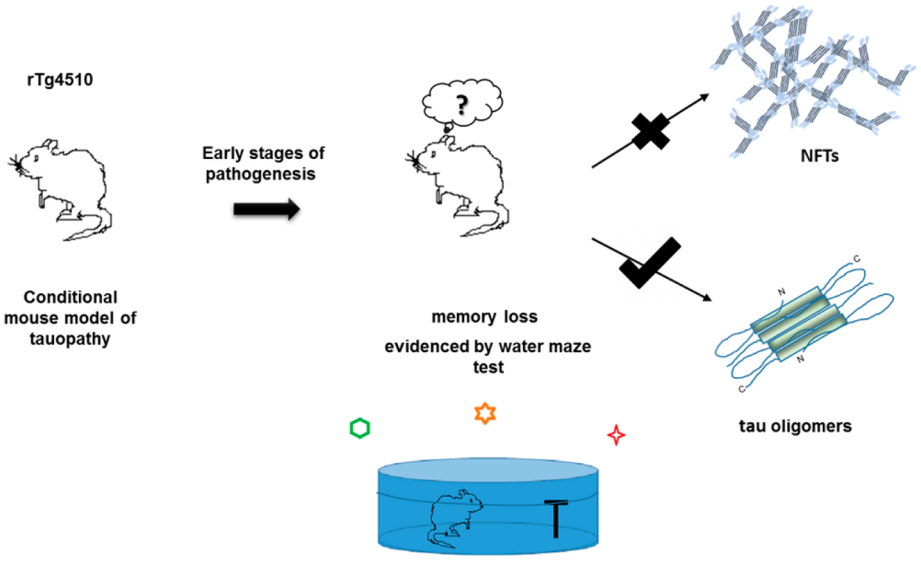


Figure 5. Tau oligomers correlate with memory loss. According to Berger and colleagues,[18] the presence of tau multimers (140−170 kDa) correlates with memory loss in the rTg4510 transgenic conditional mouse model of tauopathy. The functional deficits were associated with early stage tau aggregates (oligomers) and not with the late aggregates known as NFTs.

inhibition of FAT, pointing to a toxic gain of function of tau in the early stages of AD.[114] Originally, it was thought that the tau aggregates responsible for FAT impairment were fibrillary tau, but experiments using Hsp70, which preferentially binds tau oligomers over fillaments, prevented the anterograde FAT inhibition observed with a mixture of both forms of aggregated tau, supporting the hypothesis that tau oligomers are the toxic tau species in neurodegenerative diseases, at least in the early stages of the pathology.[113] This is in agreement with recent evidence suggesting that memory function and neuronal loss can be restored despite the continuing accumulation of NFTs[115] and the fact that NFTs persist in neurons for 20−30 years,[116] making them unlikely candidates for immediate toxicity.

TAU OLIGOMERS AFFECT LEARNING AND MEMORY

According to Lasagna-Reeves et al.,[105] tau oligomers are potent inhibitors of long-term potentiation (LTP) and disrupt memory in wild-type mice (C57BL/6) that were injected with a pure extract of human AD brain tau oligomers or treated with oligomers obtained from recombinant full-length human tau.[21] In particular, tau oligomers of recombinant human tau injected near the hippocampus were responsible for immediate memory impairment by acute disruption of anterograde memory storage. Additionally, these tau oligomers could be responsible for the neuronal death in the CA1 region of these mice and the mitochondrial damage detected in the brain areas where the tau oligomers were injected.

As mentioned before, Berger et al.[18] were the first to biochemically characterize oligomers, and they also reported memory deficits and neuronal loss in a conditional mouse model expressing human mutant tau P301L. The memory loss was associated with the accumulation of tau oligomers and not with the NFTs (Figure 5). They found that levels of 140 and 170 kDa tau (aggregates) in total protein extracts from the transgenic animal rTg4510, and a 64 kDa tau in the sarkosyl-insoluble fraction, display a significant negative correlation with memory index in mice 5.5 and 8 months of age. Thus, their data clearly imply that the formation of aggregated tau species is necessary for neuronal dysfunction and memory loss, at least in this transgenic animal (Figure 5).

PHFS AND NFTS AS NEUROPROTECTIVE STRUCTURES AGAINST TAU OLIGOMER NEUROTOXICITY

Although there is still a debate about the protective role of tau aggregation, much evidence supports the possibility of a protective role of tau aggregation into PHFs and NFTs.[27,30,69,117−121] This aggregation is a way to "set aside" cytosolic pathologically modified tau to avoid its toxic effects (like a detoxifying process). This new interpretation is changing our way of understanding AD pathology.

Even though the cognitive decline in AD is known to correlate with the degree of neurofibrillary pathology, the role of the preceding nonfibrillary abnormal tau (phosphorylated and or truncated) in neurodegeneration needs to be reconsidered.

It has been demonstrated that cytosolic, abnormally phosphorylated tau in AD brain sequesters normal tau and other microtubule-associated proteins (MAP1 and MAP2), resulting in the inhibition of microtubule assembly and disruption of microtubules.[120] Conversely, the polymerization of AD phosphorylated tau inhibits its binding to normal tau and prevents the disruption of microtubule assembly.

Taken together, the evidence reported by many groups[27] shows that the nonfibrillized tau (in the form of oligomers) is most likely the responsible entity for microtubule depolymerization and is a mediator of neurodegeneration. Moreover, pathological tau oligomerization, but not fibrillary tau, correlates with cognitive decline. Thus, the formation of fibrillar aggregates may be protective.[56,121]

PHARMACOLOGICAL APPROACHES: TAU OLIGOMERS AS DRUG TARGETS

Many phase II and III clinical trials with at least 20 different drugs meant to clear Aβ pathology have failed to demonstrate efficacy.[64,122] These unsuccessful trials suggest the need for new

dx.doi.org/10.1021/cn500148z | ACS Chem. Neurosci. 2014, 5, 1178−1191

strategies based on other targets in AD disease, such as targeting tau oligomers. The first phase III clinical trial based on tau is currently ongoing.[60] This trial is based on the use of a tau aggregation inhibitor (TAI), methylthioninium chloride (or methylene blue, MB), and its reduced form, LMTX. Pharmacological strategies for tau aggregation inhibitors take advantage of the structural differences that distinguish between the binding of tau−tau and the binding of tau−tubulin because it is critical that the tau aggregation inhibitor does not impair the normal tau−tubulin interaction.[60]

Prior to this new clinical trial, the use of dyes to inhibit tau aggregation had been evaluated. Among these dyes are the phenothiazine MB[123124] (a cationic dye) that, in addition to being an antiseptic, inhibits tau and Aβ aggregation in vitro[125] and in vivo in animal studies. Phase II clinical trials with MB in patients with mild to moderate AD had encouraging results: significantly improved cognitive functions compared to that in placebo controls and slowed AD progression for over a year. MB is able to block the tau−tau binding interaction through the repeats domain, but it does not affect tau−tubulin interactions that also occur through the repeats domain.[123] This is the reason that MB could also be useful for inhibiting tau oligomerization, not only tau fibrillization. A phase III clinical trial is ongoing.[108,126]

It is possible that other dyes, such as cynine dyes, and other molecules, such as anthraquinones, could also be useful as drugs to inhibit tau aggregation.[12,69,127]

The use of chemically derived small molecules has advantages over protein-based drugs in facilitating delivery to the target tissue (due to its small size) and having a low manufacturing cost. Such small molecules include dyes and other compounds such as porphyrins[128] (which cause the loss of β-structure in tau oligomers, thereby interfering with the formation of tau fillaments), polyphenols[129] (extracted from grape seed; they have shown a reduction in tau inclusion formation in an animal model), and inhibitors of the deubiquitinating enzyme USP14 that enhances the degradation of tau aggregates by the ubiquitin−proteasome system.[130]

Since aggregates are probably not degraded efficiently by the proteasome, it is possible that they may be degraded by the autophagy/lysosomal system. It has been reported that trehalose, which activates autophagy in an mTOR-independent manner, is able to reduce tau inclusions and improve neuronal survival in animal models.[93] However, it is important to mention that an excessive activation of autophagy in neurodegeneration may not be beneficial, since the pathway may be impaired and the clearance of the material engulfed in the autophagolysosome may not be completed due to the lack of the a proper lysosomal acidic environment.[131,132] According to Nixon, when lysosomal clearance is impaired, inducing autophagy exacerbates the pathology. Thus, the success of any autophagy intervention depends on first relieving the lysosomal block or the activation of autophagy at an early stage of the disease.[72]

Inhibitors of tau kinases, such as k252a[133] (a nonspecific thyrosine kinase inhibitor), inhibitors of GSK3[134,135] (NP-12 or lithium, a nonspecific inhibitor of GSK3), or the activation of tau phosphatases[136,137] (for example, by the use of the antidiabetes drug metformin or sodium selenite) are among other therapeutic approaches that are in the experimental phase. Some of them are already in clinical trials.

An alternative to the above strategies is tau immunotherapy. Antibodies directed against tau oligomers have been developed: TOC1,[48] T22,[19] and TOMA.[138] These antibodies do not recognize filaments, but they selectively label tau dimers and oligomers and have been useful to reverse tauopathy phenotypes such as locomotor and memory deficits in mouse models.[139] There are several reports of active or passive immunization against tau that show promising effects in models of tauopathy.[139−146]

CONCLUSIONS

Over the last 10 years, there has been a significant advance in tau protein research and elucidation of its alternative functions under both physiological and pathological conditions. The recent discovery of the role of toxic soluble tau oligomers is changing our understanding of the pathogenesis of tau. Oligomers have been proposed to be the main toxic form of the tau pathology observed in tauopathies. Fibrillary tau aggregates, such as NFTs, are now considered a late event and not directly responsible for early synaptic dysfunctions; rather, they could be protective structures. Thus, oligomers or multimers could be synaptotoxic and cause memory deficits in early stages of the disease. As discussed here, there is much evidence of the intercellular spreading of tau pathology, which is consistent with the fact that neurodegeneration begins in a restricted brain area from where it spreads to other previously unaffected brain regions.

More studies are needed to elucidate the initial mechanisms that trigger the formation of tau oligomers in order to design appropriate therapeutic interventions to block the progression of neurodegeneration and, possibly, to prevent it.

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M.C.C.-A. designed the review, directed its implementation, wrote the review, contributed to the literature search, drafted the role of tau oligomers in AD pathology and the relevance of tau clearance mechanisms, and contributed to the design and creation of the figures. L.G.-V. contributed to the literature search and to the design and creation of the figures. S.D. contributed to the writing of this review. M.A.M.-R. contributed to the writing and review of the manuscript. All authors read and approved the final version of the manuscript.

Funding

This work was supported by the grant 203584 from the National Research Council (CONACYT), Mexico City.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

AD, Alzheimer's disease; MAP, microtubule associated protein; PHFs, paired helical filaments; NFTs, neurofibrillary tangles; APFs, annular protofibrils; ROS, reactive oxygen species; AV, autophagic vacuole; NMNAT, nicotinamide mononucleotide adenylyltransferase

GLOSSARY

Tau oligomers: Two or more tau molecules in a multimeric structure. Dimers have a size of 94−130 kDa, and trimers have an apparent size of 150−190 kDa.

Aggregation: Assembly of two or more molecules. In many neurodegenerative diseases, self-aggregation of proteins occurs with pathological consequences.

Alzheimer's disease: The most common cause of dementia in the elderly. It is characterized by cognitive impairment; in late stages, it is characterized histopathologically by the presence of Aβ deposits in the form of neuritic plaques and tau aggregates in PHFs and NFTs.

Protein spreading: A prion-like protein with altered conformation and function that binds and affects native proteins and induces the same changes in the native protein; thus, the newly affected proteins can alter the remaining native proteins, and the propagation of a pathological protein from an affected cell to the extracellular space and to other healthy cells can then occur.

Synaptic impairment: Synaptic transmission that does not occur properly or is blocked. This could occur for many reasons, including an imbalance in the cytoskeletal architecture or by cell toxicity due to stress signals like ROS, hyperphosphorylation, and truncation of key proteins that stabilize microtubules, such as tau.

New drug targets: Discovery of new molecules or new conformational changes and aggregation stages of a known molecule that contribute to pathology and that can be the objective for drug intervention in order to stop or slow disease progression.

Proteasome: A multimeric barrel-shaped complex that clears soluble cytosolic proteins. The 26S proteasome consists of a regulatory cap (19S or 11S) on either end of its catalytic core (20S) and degrades polyubiquitin-tagged proteins. The 20S proteasome degrades nonubiquitin-tagged cytosolic proteins.

Autophagy: A self-eating and cell recycling mechanism in which part of the cytoplasm is engulfed in double membrane vesicles (autophagosomes) that fuse to a lysosome to degrade cellular components including proteins, lipids, and organelles.

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