Tau in Alzheimer's Disease: Pathological Alterations and an Attractive Therapeutic Target*

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Summary: Alzheimer's disease (AD) is an age-related neurodegenerative disease with two major hallmarks: extracellular amyloid plaques made of amyloid- β (A β) and intracellular neurofibrillary tangles (NFTs) of abnormally hyperphosphorylated tau. The number of NFTs correlates positively with the severity of dementia in AD patients. However, there is still no efficient therapy available for AD treatment and prevention so far. A deeper understanding of AD pathogenesis has identified novel strategies for the generation of specific therapies over the past few decades. Several studies have suggested that the prion-like seeding and spreading of tau pathology in the brain may be a key driver of AD. Tau protein is considered as a promising candidate target for the development of therapeutic interventions due to its considerable pathological role in a variety of neurodegenerative disorders. Abnormal tau hyperphosphorylation plays a detrimental pathological role, eventually leading to neurodegeneration. In the present review, we describe the recent research progresses in the pathological mechanisms of tau protein in AD and briefly discuss tau-based therapeutic strategies.

Key words: Alzheimer's disease; tau protein; hyperphosphorylation; propagation of tau pathology

Alzheimer's disease (AD) is a dementia associated with age marked by gradual memory loss and cognitive impairment. AD primarily afflicts the elderly and inexorably degenerates the brain tissue of patients. About 50 million people worldwide have been estimated to currently experience dementia and the figure can be more than 152 million in 2050. AD is the sixth major cause of death in the U.S. and the dementia prevalence in people over 65 years in China is 5.14%. Extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) are the two distinctive pathological features of AD. NFTs are intraneuronal fibrillary aggregates containing paired helical filaments (PHFs) of hyperphosphorylated tau^[1]. The NFTs number is positively associated with the dementia severity in AD patients^[2, 3]. Tau is abnormally hyperphosphorylated at many sites in AD brain^[4, 5]. Hyperphosphorylated tau loses its normal function, gains neurotoxicity, and

aggregates into NFTs^[6, 7]. Besides phosphorylation, tau truncation also promotes its aggregation^[7]. In AD brain, the tau pathology initiates in the coeruleus/subcoeruleus complex and in the trans-entorhinal area, progresses gradually toward the limbic system and, occasionally, to the limbic regions and isocortex^[8, 9]. The geographic spread of the tau pathology is seemingly correlated with the AD progression^[10, 11]. The application of tau aggregates from brains with AD can cause tau pathology in the injection sites and anatomically link brain regions. *In vitro* experiments have shown that incubation of recombinant tau with heparin can produce tau pathology, similar to the tau pathology propagation in the AD brain^[12–14]. Prion-like propagation of tau pathology collaborates to the AD evolution^[15]. Despite joint efforts to investigate the pathophysiological basis of AD, the exact mechanisms remain poorly understood. Worse yet, therapies that looked promising in preclinical research have failed in human clinical trials. Here, we review the progresses in the biology and pathophysiology of AD-related tau and briefly discuss tau-based drug therapies.

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1 TAU PROTEIN AND TAU PATHOGENESIS

1.1 Tau Protein

Tau is a neuronal protein associated with microtubules that supports the assembly of tubulins in microtubules and steadies them in the brain. The microtubule-associated protein tau (*MAPT*) gene encoding human tau is localized on chromosome 17q21.31 and it consists of 16 exons. Six tau isoforms varying from 352 to 441 residues, containing two (2N), one (1N), or zero (0N) N-terminal inserts and four (4R) or three (3R) C-terminal microtubule-binding repeats (MTBRs) are expressed in the adult human brain owing to alternative splicing of exons 2, 3, and 10^[16, 17] (fig. 1A). Approximately equimolar quantities of 4R- and 3R-tau are expressed in adult human brain^[16, 18]. Rodent tau has about 90% identity with human tau, and 4R-tau is expressed primarily in adult rodents and 3R-tau is expressed only in fetal and newborn rodents^[18, 19]. 4R-tau has greater binding affinity to microtubules than 3R-tau and can deslocate the heretofore bound 3R-tau^[20]. Tau is formed by three domains: a projection domain in the N-terminal portion, which protrudes away from the microtubule surface; a Proline-rich domain in the central region, which is responsible for the interaction with proteins containing Src homology 3 (SH3) domains; and an assembly domain in the C-terminal portion, which composes the MTBRs and the flanking regions, and supports microtubule assembly and tau aggregation^[21] (fig. 1B). Tau is expressed primarily in axons but it is

also observed in the somatodendritic compartment^[22, 23].

1.2 Tau Phosphorylation

Tau is a phosphoprotein whose phosphorylation regulates the tau binding to microtubules. Soluble hyperphosphorylated tau from AD patients holds 5 to 9 phosphate moles per mole of tau, whereas normal tau in healthful human brain contains a mean of 1.9 phosphate moles^[4].

Tau can be modified by phosphorylation at multiple threonine (Thr, T), serine (Ser, S) and tyrosine (Tyr, Y) residues. The longest human brain tau isoform, tau441 (Clone #, tau40), holds 85 possible phosphorylation sites comprising 35 T, 45 S and 5 Y residues, respectively. Multiple phosphorylation sites are found in PHF-tau in AD brains^[24–26] (fig. 2A). Tau phosphorylation at S199, S202/T205, T231, S262, S396 and S422 residues has been indicated to assess the AD progression^[27]. Tau phosphorylation controls many processes, including binding affinity to microtubules, subcellular distribution and axonal transport^[28–30]. Aberrant tau hyperphosphorylation induces the dismantle of microtubules and alters their function in the neuronal cytoskeleton organization. Meanwhile, phosphorylated tau accumulates in the neuron cytoplasm, leading to the emergence of tau oligomers and aggregates, such as tangles and fibrillar filaments^[31].

Tau hyperphosphorylation in AD brains may result from increased phosphorylation by kinases and/or decreased dephosphorylation by phosphatases. Tau is abnormally hyperphosphorylated by proline-

Fig. 1 Human *MAPT* gene and isoforms of tau proteins

A: Human *MAPT* gene is localized on chromosome 17q21.31 and consists of 16 exons. The 5'-UTR of h*MAPT* mRNA is encoded by exons 0 and 1, whereas part of the 3'-UTR is encoded by exon 14. Exons 4a, 6, and 8 are present only in big tau in peripheral tissues. Exons 2 and 3 encode two N-terminal insertions of 29 residues each (N1 and N2, in orange and yellow, respectively) and exons 9–12 encode microtubule-binding repeats (MTBRs) (R1, R3, and R4 in blue, R2 in green). Differential splicing of exons 2, 3, and 10 generates isoforms containing zero (0N), one (1N), or two (2N) N-terminal insertions and three (3R-tau) or four (4R-tau) MTBRs, resulting in six tau isoforms varying from 352 to 441 residues. **B:** Tau is formed by 3 domains: a projection domain in the N-terminal portion (a.a. 1–197), a Proline-rich domain (a.a. 198–244) and an assembly domain in the C-terminal portion (a.a. 245–441). *MAPT*, microtubule-associated protein tau; UTR, untranslated region; MTBR, microtubule-binding repeat

directed kinases (PDPKs) and non-PDPKs^[32]. S/T residues of tau preceded by a proline (Pro, P) residue are phosphorylated by PDPKs, including cyclin dependent kinase 5 (cdk5), C-Jun amino-terminal kinase (JNK), glycogen synthase kinase-3 β (GSK-3 β), extracellular signal-regulated kinases (ERKs), dual specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A) and cdc2-like kinase 1 (CLK1)^[33–36] (fig. 2B). Proline-independent tau S/T residues are phosphorylated by non-PDPKs, including cAMP dependent protein kinase A (PKA), casein kinase 1 δ/ϵ (CK1 δ/ϵ), calcium/calmodulin-activated protein kinase II (CaMK II), microtubule affinity-regulated kinases (MARKs), protein kinase R (PKR) and adenosine-monophosphate activated protein kinase (AMPK)^[36–40] (fig. 2B). Each of these kinases has different roles regarding tau pathology in AD. In AD brains, cdk5 phosphorylates tau40 at Ser residues 199, 202, 235, 396 and 404, and Thr residues 181, 205, 212, 217 and 231; GSK-3 β phosphorylates all sites phosphorylated by cdk5, except S235^[41]; ERK-2 phosphorylates tau at Ser residues 46, 202, 235, 396 and 404, and Thr

residues 175, 181, 205, 212, 217, 231^[27] and Dyrk1A phosphorylates tau *in vitro* at Ser residues 199, 202, 396, 400, 404 and 422, and Thr residues 181, 205, 212, 217 and 231^[42, 43] (fig. 2A). Tau phosphorylation by several of these protein kinases prepares the protein for additional phosphorylation by other kinases^[42, 44, 45]. In contrast, there are different protein phosphatases (PPs) types, including protein phosphatases 1 (PP1), 2A (PP2A), 2B (PP2B) and 5 (PP5), as well as tissue-nonspecific alkaline phosphatase (TNAP), calcyclin binding protein and Siah-1 interacting protein (CacyBP/SIP)^[46–52] (fig. 2B). PP2A is responsible for about 70% of all tau dephosphorylation activities in the human brain^[47] and its role regarding hyperphosphorylated tau is prejudiced in AD^[53, 54]. We recently reported that dysregulations of PP2A and GSK-3 β affect tau phosphorylation indirectly and directly by affecting activities of each other^[55–57]. PP2A can also regulate tau phosphorylation by affecting the functions of other tau protein kinases, including cdk5, PKA and CaMK II^[17].

1.3 Tau Truncation

Tau is abnormally truncated at several sites^[58, 59],

Fig. 2 Phosphorylation of human tau

A: Schematic and sequences of human tau441 showing phosphorylation sites. Tau in PHFs is phosphorylated (red underline) at Ser residues 46, 195, 198, 199, 202, 208, 210, 214, 235, 262, 396, 400, 404, 409, 412, 413 and 422, and Thr residues 175, 181, 205, 212, 217, 231 and 403. Cdk5 phosphorylates tau (blue underline) at Ser residues 199, 202, 235, 396 and 404, and Thr residues 181, 205, 212, 217 and 231. GSK-3 β phosphorylates all sites phosphorylated by cdk5, except S235 (orange underline). ERK-2 phosphorylates tau (green underline) at Ser residues 46, 202, 235, 396 and 404, and Thr residues 175, 181, 205, 212, 217 and 231. Dyrk1A phosphorylates tau (purple underline) at Ser residues 199, 202, 396, 400, 404 and 422, and Thr residues 181, 205, 212, 217 and 231. B: protein kinases and protein phosphatases that mediate tau phosphorylation

catalyzing by many proteases, including calpains, caspases, asparaginyl endopeptidases (AEPs) and cathepsins^[60–62]. In NFTs of AD brain, at least three specific tau cleavage sites (N368, E391 and D421) have been recognized and are associated with the Braak stages progression^[63–65]. Tau in NFTs is C-terminal truncated and the tau predominant forms in the cerebrospinal fluid (CSF) do not have half of the C-terminal portion containing MTBRs. In contrast, tau aggregates from AD brain with high molecular weight (HMW-tau) resistant to SDS and reducing agents do not have the N-terminal region^[66, 67]. Truncated tau is more likely to aggregate than full-length tau^[62, 68, 69]. We recently reported that several tau truncations modulate specific sites of phosphorylation, increase tau self-aggregation, and promote the binding to oligomeric tau from AD brain (AD O-tau) and aggregation seeded by AD O-tau^[70]. Among all the truncated forms, Tau151-391 causes the most serious pathological disorders and the most potent aggregation effects templated by AD O-tau in cultured cells *in vitro*^[70]. Tau truncation can also promote mitochondrial dysfunction and synaptic deficits^[71, 72]. Tau truncation has an essential function in the tau pathogenesis.

1.4 Other Post-translational Modifications (PTMs)

Tau can be also modified at many sites by other PTMs, including acetylation, O-GlcNAcylation, methylation, S-Guanylation, ubiquitination, SUMOylation, nitration, carbamylation and glycation.

1.4.1 Tau Acetylation More than 20 lysine (Lys, K) residues located in the the MTBRs and in the tau flanking region are acetylated by CREB-binding protein (CBP) or histone acetyltransferase p300, and tau deacetylation is catalyzed by histone deacetylase (HDAC) 6 or sirtuin 1 (SIRT1)^[73–75]. Tau acetylation blocks tau degradation, prevents the tau binding to microtubules and elevates tau aggregation^[76]. Tau acetylation level is increased in the tauopathy brains. Acetyltransferase p300 is up-regulated in AD brains and AD mice models^[76]. Intrinsic auto-acetylation of tau is associated to following proteolytic cleavage and the tau fragments production^[74, 77]. SIRT1 levels are decreased in AD brains^[78] and A β treatment reduces SIRT1 expression in cultured neurons^[79], perpetuating acetylated tau accumulation. Therefore, enhanced acetylation or reduced deacetylation contributes to the pathological elevation of acetylated tau in AD^[76].

1.4.2 Tau O-GlcNAcylation O-GlcNAcylation is a modification of nucleocytoplasmic proteins characterized by monosaccharide β -N-acetylglucosamine (GlcNAc) bound to the hydroxy of S or T residues. Normally, tau is modified by O-GlcNAcylation^[80, 81]. O-GlcNAcylation is performed by the O-GlcNAc transferase (OGT) and O-GlcNAc modification is detached by the O-GlcNAcase (OGA)^[82–84]. The relative activities of OGT and OGA

and the UDP-GlcNAc amount in the cell control the O-GlcNAcylation level^[85]. Tau site-specific phosphorylation and O-GlcNAcylation compete each other. The reduction in the O-GlcNAcylation level promotes abnormal tau hyperphosphorylation and NFTs formation^[86]. Calpain I overactivation resulting from Ca²⁺ overload proteolyzes the neuronal specific glucose transporter 3 (GLUT3), which can impair neuronal uptake of glucose, consequently leading to a decrease in tau O-GlcNAcylation in AD brain^[87]. Thus, an improvement in brain glucose uptake may be an attractive target for preventing and treating AD.

1.4.3 Tau Ubiquitination Ubiquitination is a very versatile PTM that regulates many aspects of protein functions, such as protein degradation as part of the ubiquitin-proteasome system (UPS) and proteasome independent functions^[88, 89]. Several Lys residues in the tau protein are ubiquitin modified by ubiquitin ligases (E3 ligases), including the C-terminal region of heat shock protein 70-interacting protein (CHIP) and tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6)^[90, 91]. Tau ubiquitination was found in neuritic plaques and NFTs in AD brains, in Lewy bodies and filaments related to Parkinson disease (PD) and Pick's disease (PiD)^[92, 93].

The hippocampus shows decreased proteasome activity in the initial stages of AD patients^[94]. The proteasome function is reduced in the presence of aggregated proteins, whose levels can be elevated by tau and A β aggregation^[95]. Soluble PHFs from AD brains are mainly ubiquitylated at residues K254/311/353 of MTBRs^[96]. Tau monoubiquitylation in PHFs, rather than poly-ubiquitylation, is not sufficient to promote the proteolysis of tau aggregates regulated by UPS^[97]. Therefore, increasing tau polyubiquitylation and degradation may be a promising therapeutic strategy for AD treatment^[98].

1.5 Tau Aggregation

The natively unfolded soluble tau monomers generally display a random coil structure without propensity to aggregate^[99]. Two motifs formed by six residues (₂₇₅VQIINK₂₈₀ and ₃₀₆VQIVYK₃₁₁) in tau MTBRs are fundamental for aggregation and formation of tau filaments. These motifs have the propensity to switch their conformation to β -sheet structures, which are responsible for aggregation of tau monomers to dimers and for pathological aggregation of soluble tau oligomers to insoluble PHFs, and eventually NFTs^[100]. PHFs in AD have all the six tau isoforms^[15].

Aggregation is necessary for neurodegeneration mediated by tau, although the complete mechanism underlying tau aggregation needs to be clarified. Tau aggregation can be promoted by aberrant PTMs and phosphorylation is considered the main trigger for this aggregation. The number of NTFs is positively associated to the dementia severity in AD patients

and tau aggregation was originally hypothesized to cause NFTs toxicity^[3]. Interestingly, researches on transgenic mice and AD patients indicate that most neurons may die owing to the formation of NFTs. Thanks to the occurrence of dysfunction, synapse loss and cognitive impairment that arise well before NFTs formation, soluble tau oligomers may be the leading neurodegeneration cause, although the precise oligomeric tau species driving neurodegenerative processes have not been identified^[37]. Phosphorylation or pseudo-phosphorylation of 4R2N (tau40), the largest of the tau isoforms, increases aggregation of tau, while phosphorylation of 4R0N (tau24) or 3R2N (tau39) decreases it^[101, 102].

Truncation influences tau aggregation. Tau is truncated at several sites by different proteases^[58, 103] and the acidic terminal interacts with MTBRs preventing tau aggregation^[104]. We recently constructed 11 truncations from both the C- and N-terminal portions of the tau protein according to the reported truncation sites, and then compared the pathological activities of all the truncated forms^[70]. We observed that deletion of the first 150 or 230 residues of the tau protein increased its self-aggregation, site-specific phosphorylation, and binding to and aggregation seeded by AD O-tau, while the first 50 residues deletion did not have those effects^[70]. Deletion of the last 50 residues regulated the tau site-specific phosphorylation and promoted tau self-aggregation, which could be seized and seeded to aggregation by AD O-tau, while the last 20 residues deletion did not show significant effects^[70]. Among all the truncated forms, Tau₁₅₁₋₃₉₁ was the most pathologically active. AD O-tau induced Tau151-391 aggregation in cultured cells and *in vitro*. The deletion of the first 150 and the last 50 residues protected tau from pathological features and facilitated the tau pathological activities^[70]. Thus, suppressing tau truncation can be a possible treatment method to inhibit tau pathology in AD and related tauopathies^[70].

Other PTMs can also affect tau aggregation. Similar as phosphorylation, acetylation at some Lys residues, like K18/163/280/281/369 increases the aggregation of tau^[74], while acetylation at many other lysines decreases it^[105]. O-GlcNAcylation at S400^[106], nitration at Y18/394^[107], methylation at multiple residues^[108], and S-Guanylation at certain cysteines also decrease tau aggregation^[109]. SUMOylation at K340^[110], carbamylation at K280 and/or K311^[111], and glycation at multiple lysine residues increase tau aggregation^[101].

In addition to PTMs, mutations also affect tau aggregation. Tau missense mutations, including G272V, P301L, V337M, and R406W, have a greater tendency to form aggregates, while the MTBR deletion mutation ΔK280 reduces the binding affinity to microtubules, strengthens the β-sheet structures and promotes

fibrillization^[112, 113]. Tau exon 10 alternative splicing and the 4R-tau/3R-tau appropriate ratio are important to prevent abnormal fibrillization. 4R-tau, which contains an additional MTBR, binds and promotes microtubule assembly more easily than 3R-tau^[114]. 4R-tau is more likely to trigger polymerization induced by heparin than the association of 4R- and 3R-tau *in vitro*^[115]. Besides, several negatively charged cofactors, such as dextran sulfate, heparin, RNA and arachidonic acid, can also promote tau aggregation *in vitro*^[116]. Some proteins, including 14-3-3ζ and FK506 binding protein 4 (FKBP4), possibly induce tau aggregation by stabilizing the structure prone to aggregation *in vitro*^[117, 118].

1.6 Prion-like Propagation

Tau pathology initiates in the coeruleus/subcoeruleus complex and in the transentorhinal area, then progresses gradually towards the limbic system and, eventually, can reach the isocortex^[8, 9]. The regional distribution of tau lesions is positively related to the cognitive impairment in AD. Tau pathology progresses according to predictable patterns in patients and has been proposed to involve brain networks^[9, 119, 120]. Some processes, such as templated seeding, cellular uptake, secretion and intercellular transfer by non-synaptic and synaptic paths, are expected to occur for the tau pathology proliferation in the prion-like manner^[116, 121]. It is not clear that tau accumulation can be absorbed by cells through accurate pathways. The accumulation of tau is ingested through macro-pinocytosis^[122, 123] and heparan sulfate (HS) proteoglycans are required^[122]. Tau seeds in endosomes in contact with the cytosol, then prompt the accumulation of non-accumulated tau. HS participates in tau hyperphosphorylation in AD brain, then precedes the tau accumulation^[124].

Prion-like mechanisms of tau pathology propagation were first proposed in 2009^[125]. Since then, several studies have suggested that assemblies of tau, when administered extracellularly, may “seed” the generation of aggregates, which then spread to other cells. The propagation of intracellular tau requires seeding, aggregate uptake and release. Even if the tau monomer is absorbed by the cells, from which it can be discharged, it is likely not capable to seed the aggregation. Expressed 4R-tau is not able of being seeded when it does not contain the 275–280 and 306–311 regions^[123]. Tau aggregation inhibitors (TAIs) can then be capable to decrease tau-induced seeding and spreading.

Once a normal protein comes in contact with a misfolded tau protein “seed”, it can be transformed into a pathogenic form. The mechanism of this transformation is not yet clear, but it is known that it requires template-mediated conformational changes and that its propagation is carried out by neuronal networks^[126, 127]. Tau proteins are hubs for their own

aggregation and then serve as “seeds” for further misfolding^[40]. Dujardin *et al* examined several aspects of tau obtained from postmortem AD brains in P301S mice and found that the seeding activity correlates with oligomeric/hyperphosphorylated tau levels, rather than the total tau amount^[128]. The plenty of oligomeric/hyperphosphorylated tau forms is also associated with disease evolution, and high phospho-tau (P-tau) levels are correlated with seeding activity. We recently reported that AD O-tau effectively induced tau aggregation *in vivo* and *in vitro* in a prion-like manner^[67, 70, 129, 130], which can be the amplification and spread basis of tau pathology throughout AD brain. AD O-tau can arrest normal tau and template it to form nerve fiber bundles in an unsaturated way^[31], indicating that the tau pathology can propagate in the human brain via prion-like characteristic of tau “seeds”^[131].

In vitro studies have important contributions to the understanding of the basic mechanism of intercellular tau pathological metastasis. For example, in cultured cells, tau “seeds” are able to promote a similar type of tau aggregation in recipient cells and clearly reproduce the morphological characteristics of tau aggregation. Pathological tau involves a variety of molecular pathways in the process of intercellular metastasis^[132].

2 TAU-BASED THERAPEUTIC STRATEGIES

There are several opportunities for therapeutic intervention at each stage of tau pathology development. However, meaningful significant advances in

understanding the AD pathology, a treatment that has been shown to be effective in humans has not yet been found. To date, treatments based on A β appear to be ineffective in improving the AD symptoms. On the other hand, potential therapeutic strategies against tau have appeared to be very promising for the AD treatment (fig. 3). Tau targeted therapies are currently in the early developmental stages, but have great potential for evolution.

2.1 Tau Expression Inhibition

Tau protein, as a central molecule in AD pathology, is toxic to cells directly and mediates A β toxicity^[133]. Thus, inhibition of tau expression may be a promising approach for the AD treatment. Decreased levels of endogenous tau have been indicated to exhibit protective effects against behavioral abnormalities and A β -induced cognitive impairments in AD model mice. Tau knockout (KO) has few side effects in model mice^[134], possibly because other proteins associated with microtubules can compensate for tau deficiency to a great extent. If tau monomer levels in cells decrease, the balance governing the formation of aggregates determines that tau assembly will depolymerize, decrease oligomeric tau and aggregate as PHFs^[135]. Antisense oligonucleotides (ASOs) or small interfering RNA (siRNA) can reduce tau expression. siRNAs decrease tau pathology and associated functional impairments in both cell and animal models^[71]. To date, siRNAs have not been examined in clinical tests for AD or other tauopathies, although they have been employed for other diseases, such as cancer^[136, 137].

Fig. 3 Therapeutic approaches targeting tau

Tau expression and its pathogenesis and the possible mechanism of disease-modifying agents. MT, microtubule; MAPT, microtubule-associated protein tau; NFTs, neurofibrillary tangles; p-tau, hyperphosphorylated tau

Several microRNAs, including miR-106b, miR-125b, miR-132/122, and miR-219, regulate tau expression and phosphorylation^[138–141]. ASOs have been a common experimental method for about 25 years ago, but have lost preference due to side effects. Recently, ASOs have been found to alleviate the progress of spinal muscular atrophy, which can lead to a resurgence of ASOs treatment for various diseases, including tauopathies^[142, 143]. However, additional studies are needed to evaluate whether tau expression inhibition would be advantageous for the AD treatment.

2.2 Tau Phosphorylation Inhibition

Hyperphosphorylated tau proteins are prone to form aggregates and determine tau pathology propagation. Tau hyperphosphorylation inhibition is a therapeutic approach, and tau protein kinases and protein phosphatases exert key roles in evolution of AD. Tau is phosphorylated by kinases, such as cdk5, GSK-3 β , ERK and Dyrk1A. Lithium chloride (LiCl), a specific inhibitor against GSK-3 β and K252a (a non-specific inhibitor against cdk5, ERK1 and GSK-3 β), decreases insoluble and hyperphosphorylated tau levels in mice models^[40]. Some small molecule inhibitors that target GSK-3 β , such as SRN-003-556, CHIR-98014 and SB 216763 are currently at the preclinical stage. Tau protein kinases activities are directly or indirectly associated to tau pathology in several ways, making them an indubitable potential target.

The main phosphatase of tau is PP2A, which can be regarded as the most decisive phosphatase because it is involved in more than 70% of the total phosphatase events upon tau^[47]. PP2A mediates tau dephosphorylation and the levels of PP2A and its activators have been found to be down-regulated in AD brains in comparison with the age-matched controls^[53, 54]. We recently reported the cross-talk between PP2A and GSK-3. PP2A increased GSK-3 β activity by GSK-3 β dephosphorylation at S9 residue. Up-regulated GSK-3 β , in turn, increased PP2A activity by PP2A methylation through leucine carboxyl methyltransferase 1 (LCMT-1) and/or protein phosphatase methylesterase-1 (PME-1)^[55]. Therefore, GSK-3 β and PP2A mutually regulate and affect tau phosphorylation. Several drugs that target PP2A activity are presently being developed or assessed in clinical tests^[24]. Tau hyperphosphorylation is a predictive event in the AD process and can be regarded as a leading strategic therapy against tau protein.

2.3 Tau Aggregation Inhibition

Several PTMs decrease the microtubule binding ability of tau and enhance its detachment from the microtubules. This effect conducts to a gradual rise in the intracellular tau levels, which increases the probability of interaction with tau protein, and eventually leads to the tau aggregation. Tau oligomers are the most toxic species that induce neurotoxicity

and neurodegeneration in AD. Tau aggregation inhibitors (TAIs) therapy aiming the prevention of the tau pathology prion-like propagation is a considerable strategy that targets tau.

To date, most TAIs are derivatives from methylene blue, including methylthioninium chloride (MTC), Rember TM, and LMT. These TAIs were reported to disrupt tangles and tau filaments, and hinder cognitive deficits in tau transgenic mice^[144]. MTC is a drug authorized by the FDA for the methemoglobinemia treatment and has been reintroduced to treat AD and other related tauopathies^[40]. Although extensive studies have demonstrated that MTC and other methylene derivatives can reverse the PHF proteolytic stability through blocking tau-tau binding without interfering with the tau-tubulin interaction *in vitro*, their effects are not the same *in vitro/in vivo* or in clinical trials^[145]. So far, the benefits of these drugs have been limited^[145]. As an example, LMTX is a second-generation TAI that has not been able to ameliorate functional and cognitive skills in mild to moderate AD patients in phase III clinical trials^[24]. In contrast, NPT088 is currently used as a TAI in phase I trials^[146]. It is a fusion protein that recognizes and remodels a number of misfolded proteins, including A β and tau. NPT088 can reduce A β plaque and phosphorylated tau pathology and enhance cognitive performance in transgenic mice. Another strategy to inhibit tau aggregation is to interfere with the steric zippers formed by two hexapeptide motifs^[147]. This approach may be fundamental for the design of new drugs against tau.

2.4 Intercellular Transfer of Tau Inhibition

Tau aggregates, as well as pathologically misfolded proteins, can migrate from cell to cell and release into the extracellular compartment in a prion-like manner, causing the tau pathology to spread to different regions of brain. This is a therapeutic direction to curb the evolution of tauopathies and other neurodegenerative disorders^[148]. Numerous studies have shown that targeting tau protein by blocking its intercellular transference can delay the tau pathology in patients with AD^[148]. Tau pathology is harmful to neurons, so preventing the pathological propagation of tau aggregates can reasonably reduce the disease impact^[40, 147]. There are three different pathways to block or reduce tau interneuronal transfer, including blocking the tau release, inhibiting tau uptake, and decreasing tau oligomerization and the extracellular tau levels. The tau release block can be targeted on two different sides. After the tau release has been blocked, extracellular levels and availability of tau will decrease, thereby preventing tau uptake by neighboring neuronal cells^[149].

2.5 Microtubule Stabilization

Impaired normal tau function leads to abnormal axonal transport and microtubule assembly.

Pathological tau proteins detach from microtubules, leading to microtubules rupture in AD patients. Therefore, microtubule stabilization is considered to compensate for tau-induced neurotoxicity. Targeting microtubule stabilization is a potential therapeutic strategy in which tau is not the primary focus. The rationale for this therapeutic approach is that microtubule stabilizers have beneficial neuroprotective effects in patients with AD^[150]. Taxol-derived epothilone is a small molecule microtubule-stabilizing molecule that can traverse the blood brain barrier (BBB) easily. Thus, it can recover spatial memory defects by reducing hippocampal neuron loss and tau pathology, and reduce the axon number with abnormal morphology and increase the microtubule number in transgenic mice^[151–153]. Abeotaxane and Davunetide are recently found microtubule stabilizers that have provided advances towards microtubule stabilization, but whose effectiveness in animal models and human trials is unbalanced^[135, 151]. Some other microtubule stabilizers, such as TPI287 and NAP, are in phase I / II clinical trials^[135]. Thus, there are large gaps to be filled for the development of this strategic approach. This therapeutic strategy targeting microtubule-stabilizing drugs still needs more effective clinical trials results and requires further evaluation.

2.6 Tau Immunotherapy

To date, the most promising tau-targeted methodology may be tau immunotherapy. Passive or active immunization through antibodies against P-tau peptides or phosphorylated tau reduces tau pathology and behavioral abnormalities and effectively improve cognitive performance^[154]. A promising tau-directed monoclonal antibody-based tau immunotherapy using an antibody (BIIB092) against the tau protein N-terminal region lately demonstrated great toleration in a phase Ib clinical trial for progressive supranuclear palsy (PSP)^[154]. But, the phase II trial was interrupted after analysis of futility and a potential reason for the failure was the relatively advanced stage of the disease [<https://www.alzforum.org/news/research-news/abbvie-tau-antibody-flops-progressive-supranuclear-palsy>]. Many other tau antibodies, including those obtained from serum, display great selectivity for pathological tau and are promising in preclinical studies^[135]. Among them, it was highlighted those targeting tau central portions, such as UCB0107, which have been well tolerated and acceptably safe in recent PSP trials. Some antibodies against p-tau (RO6926496, RO7105705), tau fragments (BMS-986168, C2N-8E12), tau conformations (anti-tau oligomer-specific antibody), or total tau (ABBV-8E12) are presently being assessed in clinical trials^[24, 25]. Clearly, it is impossible to remove the existing neurodegeneration in AD, but inhibiting the tau propagation to non-affected brain regions can delay or stop progression of

tau pathology. Our recently studies have shown that tau passive immunization with antibody 43D (targeting tau 6-18) blocked the seeding and spreading of Alzheimer hyperphosphorylated tau-induced pathology. This led to a decrease in A β pathology and enhanced cognition in 3xTg-AD mice, indicating a promising therapeutic approach for AD and related tauopathies^[155–157]. The tau immunotherapy mechanisms remain unclear and this knowledge would promote the faster development of clinical trials and increase the probability to deliver the most successful immunotherapy for AD patients.

3 CONCLUSIONS AND PERSPECTIVES

Tau is a central molecule in the AD pathogenesis. Tau hyperphosphorylation, truncation, aggregation, and prion-like propagation provide novel paradigms for our present understanding of the AD pathogenesis. Recent studies have shown that tau oligomers, instead of fibrillar aggregates, are cytotoxic through disrupting synaptic function, causing neuronal death and the tau pathology spread in preclinical models of tau-mediated neurodegeneration *in vitro* and *in vivo*^[158–160]. However, to properly understand AD and to create and improve novel disease-modifying treatments and diagnostic tools, knowledge of the detailed underlying mechanisms and the relevance of these alterations to clinical AD cases will be needed. Thus, the detection and elimination of toxic tau oligomers or seed-competent monomers, prior to the NTF and PHF formation in the AD early stages, can be crucial in the prevention of tau pathologies. Essays to improve tau immunotherapies are of similar relevance. Anti-tau vaccines and other passive/active immunotherapies presently submitted to clinical tests have great potential for future therapeutic applications.

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Conflict of Interest Statement

The author declares there is no conflict of interest.

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