Chapter

Tau in Health and Neurodegenerative Diseases

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Abstract

Tau, one of the major microtubule-associated proteins, modulates the dynamic properties of microtubules in the mammalian nervous system. Tau is abundantly expressed in the brain, particularly in the hippocampus. Insoluble and filamentous inclusions of tau in neurons or glia are discovered in neurodegenerative diseases termed 'tauopathies', including Alzheimer's disease (AD), argyrophilic grain disease (AGD), corticobasal degeneration (CBD), frontotemporal dementia (FTD), Pick's disease (PiD) and progressive supranuclear palsy (PSP). Accumulation of intracellular neurofibrillary tangles (NFTs), which are composed of hyperphosphorylated tau, is directly correlated with the degree of Alzheimer's dementia. This chapter reviews the role of tau protein in physiological conditions and the pathological changes of tau related to neurodegenerative diseases. The applications of tau as a therapeutic target are also discussed.

Keywords: tau, neurodegenerative diseases, pathology, Alzheimer's disease, therapy

1. Introduction

The human hippocampus is critically involved in spatial navigation, the forming, storing and retrieval of episodic memory and the processing of many other types of memory [1]. In the normal human brains, the hippopotamus volume remains relatively stable before the age of 60, and then gradually atrophies. In Alzheimer's disease (AD) patients, the pattern of brain atrophy follows a stereotypical pathway that initiates from the entorhinal cortex and the hippocampus, and then spreads to the medial parietal, lateral temporal and frontal regions, eventually to the neocortex. As the connections between the hippocampus and its neighboring cortical structures are selectively vulnerable to neurodegeneration in AD, hippocampal volume loss is considered an important indicator of AD neuropathology [2]. In addition, since neurogenesis in adult brain only occurs in the dentate gyrus of the hippocampus and the olfactory bulb, hippocampus atrophy in AD also alters the production of newborn neurons [3].

Tau, initially isolated as a microtubule-associated protein from the porcine brain in 1975, is predominantly expressed in the hippocampus [4, 5]. In the previous year, neurofibrillary tangles (NFTs) and a paired helical filament (PHF) protein had been identified from the brains of patients with Alzheimer's disease (AD) [6], but it was not until 1986 that tau was discovered to be a major component of PHF [7]. Subsequently, tau was implicated in the pathogenesis of over 25 human neurological disorders (termed "tauopathies"), including Alzheimer's disease (AD), argyrophilic grain disease (AGD), corticobasal degeneration (CBD), frontotemporal dementia

(FTD), globular glial tauopathy (GGT), primary age-related tauopathy (PART), Pick's disease (PiD) and progressive supranuclear palsy (PSP) [8–10].

As the most common neurodegenerative disease, AD is characterized by early impairments in learning and memory, followed by progressive loss of complex attention, executive function, language, orientation and self-care ability, changes in mood, loss of motivation, and impairments in thinking, behavior and/or social comportment [11]. The two major neuropathological hallmarks of AD are the extracellular deposition of β-amyloid (Aβ) plaques and the intracellular neurofibrillary tangles (NFTs) consisting of aggregated hyperphosphorylated tau [12]. NFTs pathology in AD is initiated in the locus coeruleus and transentorhinal cortex, from where it spreads to the limbic system (e.g., entorhinal cortex and hippocampus) and further to the neocortex, leading to six Braak stages [13]. The progression of cognitive decline in AD correlates with the accumulation of NFTs and loss of hippocampal volume, but not deposition of Aβ plaques [14, 15]. Since most therapies targeting Aβ failed in late-stage clinical trials for AD in the past decades, increasing research revealing the roles of tau in disease has inspired tau-targeting approaches in the treatment of AD and related tauopathies [16, 17].

This chapter reviews the expression and functions of tau in physiological conditions, the pathological changes of tau in diseases, such as genetic variants, post-translational modifications (PTMs) and prion-like seeding and propagation. Recent advances in the development of tau-based therapies for AD and other neurodegenerative diseases are also discussed.

2. Tau gene

Human tau protein is encoded by the microtubule-associated protein tau (MAPT) gene, which locates on chromosome 17q21.31 and consists of 16 exons. In the central nervous system (CNS), the alternative splicing of exons 2, 3, and 10 gives rise to six tau isoforms with zero (0 N), one (1 N) or two (2 N) N-terminal inserts and three (3R-tau) or four (4R-tau) microtubule-binding repeats (**Figure 1**) [18]. The longest isoform of human brain tau consists of 441 amino acids (2N4R, tau441) with an apparent molecular weight (MW) of 46 kDa. Exons 4a and 6 are predominantly expressed in the peripheral nervous system (PNS), producing proteins of apparent MW of 110 kDa, named big tau [19].

2.1 Alternative splicing of tau pre-mRNA

The expression of tau isoforms is developmentally and pathologically regulated. 3R-tau isoforms are expressed throughout life, including in the fetal brain, whereas 4R-tau isoforms are specifically expressed in adults, resulting in approximately equal levels of 3R-tau and 4R-tau in the adult human brain [18]. Rodent tau shares about 90% homology with human tau. Unlike humans, rodents express 3R-tau only in fetus and infant, and mainly 4R-tau in adulthood [20].

Tau pre-mRNA contains multiple cis-elements that allow the interaction of trans-acting factors like the serine and arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs), including an SC35-like enhancer, a polypurine enhancer (PPE) and an A/C-rich enhancer (ACE) at the 5′ terminus, and an exonic splicing silencer (ESS) and an exonic splicing enhancer (ESE) at the 3′ end of tau exon 10, and an intronic splicing silencer (ISS) and an intronic splicing modulator (ISM) at 5′ end of intron 10 [21]. Binding of trans-acting factors to these cis-elements either suppresses (SRSF3, SRSF4, SRSF7, SRSF11, U2AF, PTB

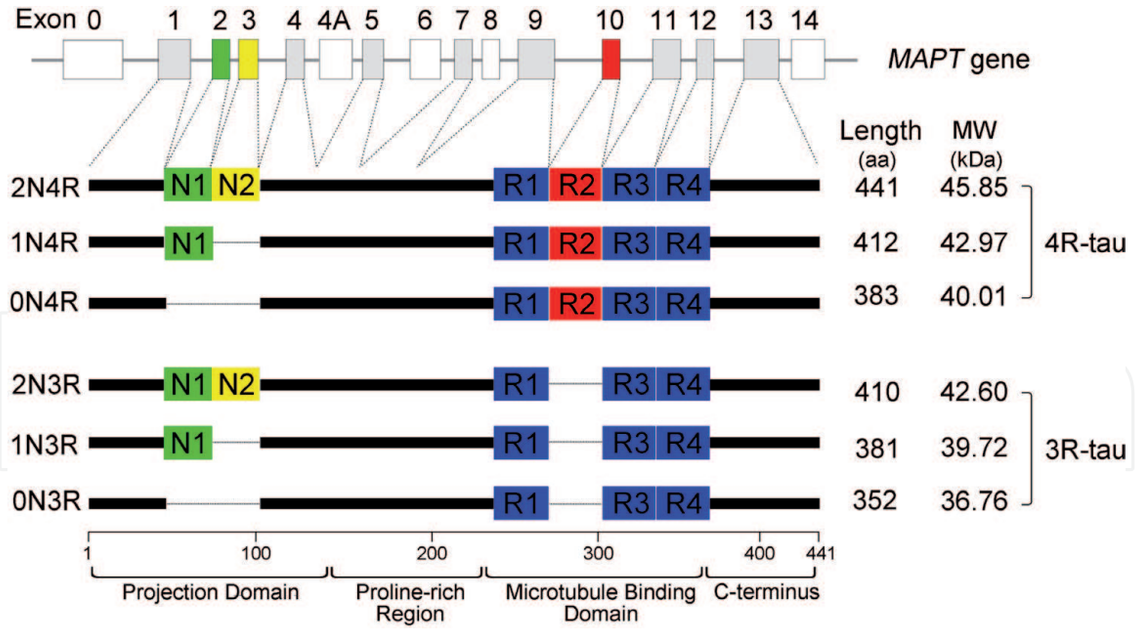

Figure 1.
Gene structure and expression of human MAPT gene. MAPT gene is localized to chromosome 17 and consists of 16 exons. Exons 4a, 6, and 8 are expressed in PNS. Alternative splicing of tau exon 2 (green, encodes N1) or exon 3 (yellow, encodes N2) produces isoforms with zero (0 N), one (1 N) or two (2 N) N-terminal inserts. Exons 9, 11 and 12 encode the microtubule-binding repeats R1, R3, and R4 (blue). Alternative splicing of exon 10 (red, encodes R2) generates isoforms containing four (4R-tau) or three (3R-tau) microtubule-binding repeats.

and hnRNP G) or promotes (hTRA2-beta1, CELF3, CELF4, SRSF1, SRSF2, SRSR6, SRSF9, RNA helicase p68, RNA binding motif protein 4 and Tar DNA-binding protein 43) the inclusion of exon 10 [22].

Mutations in the MAPT gene or dysregulation of the splicing factors that alter the expression of tau exon 10 are involved in the pathogenesis of neurodegenerative diseases [21]. PiD is a prototypical 3R-tauopathy with spherical tau inclusions (Pick bodies), whereas PSP, CBD, AGD and GGT are primary 4R-tauopathies. AD, FTD and chronic traumatic encephalopathy (CTE), progressive neurodegeneration associated with repetitive mild traumatic injury, comprise both 3R and 4R-tau pathologies [23]. Studies on the amount of the six tau transcripts in AD brain have been contradictory [24, 25]. However, 4R- and 3R-tau proteins appear equally in the early-stage and some late-stage AD cases [26, 27]. In some cases of late-stage AD, 3R-tau protein is predominantly expressed in the subiculum, entorhinal cortex and area CA1 of the hippocampus [27], implying that dysregulation of tau exon 10 splicings could be related to AD progression rather than initiation.

2.2 Tau mutations

So far, 112 mutations have been identified in human the MAPT gene, of which 13 were located in an intron (https://www.alzforum.org/mutations). Not all the mutations are pathogenic, and at least 27 benign mutations are not responsible for significant clinical symptoms. The largest number of MAPT mutations (55) is found in FTD. Second, 15 mutations are identified in AD, most of which are benign mutations causing no significant neuropathology. The rest mutations are correlated to PSP (10 mutations), Parkinson's disease (PD) (8), PiD (7), CBD (4), AGD (1) and other tauopathies (**Table 1**). As genetic risk factors for neurodegenerative diseases, pathogenic tau mutations alter the protein sequence or/and the balance between 4R- and 3R-tau by changing alternative splicing [28].

Clinical Phenotype	Mutations
Argyrophilic grain disease	S305I
Alzheimer's Disease	A90V, A152T, G213R, V224G, Q230R, K280del, V287I, A297V, S318L, R406W, L410F*, S427F*, P512H* Duplication 17q21.31, IVS10 + 16 C > T
Corticobasal Degeneration	A152T, C291R, P301T, N410H
Frontotemporal Dementia	R5H, G55R, V75A, A152T, A239T, D252V, I260V, L266V, G272V, G273R, N279K, K280del, L284L, N296D, N296N, N296H, P301L, P301P, P301S, P301T, S305N, S305S, L315L, L315R, S320F, P332S, G335A, G335S, G335V, Q336R, V337M, E342V, S356T, V363I, P364S, G366R, K369I, E372G, G389_I392del, G389R, G389R, P397S, R406W, T427M IVS9–10 G > T, IVS9–15 T > C, IVS10 + 3 G > A, IVS10 + 4 A > C, IVS10 + 11 T > C, IVS10 + 12 C > T, IVS10 + 13 A > G, IVS10 + 14 C > T, IVS10 + 15 A > C, IVS10 + 19 C > G, IVS10 + 16 C > T
Pick's disease	G272V, K280del, S320F, Q336R, Q336H, K369I, G389R
Progressive Supranuclear Palsy	R5L, A152T, L284R, S285R, N296N, N296del, G303V, S305S, D285N* IVS10 + 16 C > T
Parkinson's Disease	R5C, A41T, A152T, N296del, I360V, S427F*, T427M, R448*

**The position of this variant is about the longest isoform of peripheral tau, which is 776 amino acids in length.*

Table 1.
MAPT mutations found in main neurodegenerative diseases.

Two major extended haplotypes cover the MAPT gene: H1 and H2 [29]. The frequency of the haplotypes differs between population groups; the H2 haplotype is found primarily in Caucasian and southwest Asians, but barely reported in the Chinese Han population [29, 30]. H1 haplotype was shown overrepresented in Caucasian patients with PSP [31]. Nevertheless, the relationship between MAPT haplotype and AD is still contradictory [32].

3. Tau protein

3.1 Protein structure of tau

Tau is a highly water-soluble and basic protein with little secondary structure. As an intrinsically disordered protein, tau consists of a large number of serine/threonine and arginine/lysine/histidine residues, which makes the protein easy to be hyperphosphorylated. Full-length human tau (tau441) is composed of four domains: (i) an acidic amino-terminal projection domain that projects away from the surface of the microtubule, (ii) a proline-rich region required for the interaction with SH3-domain-containing proteins like tau kinases, (iii) a microtubule-binding domain involved in mediating tubulin assembly and tau aggregation, and (iv) a C-terminus (**Figure 1**) [33].

3.2 Localization of tau

Human tau is highly expressed in the frontal and temporal cortices and is decreased to approximately a quarter in the cerebellum. In rodent brains, the highest levels of tau are detected in the hippocampus and entorhinal cortex. The cerebellum and olfactory bulb showed the lowest total tau level, being about 2/3 of that in

the frontal cortex [5]. The expression of 3R-tau is comparable throughout different regions in the adult rat brains, such as the hippocampus, entorhinal cortex, frontal cortex, occipital-temporal cortex, parietal–temporal cortex, striatum, thalamus, olfactory bulb and cerebellum. However, the distribution of 4R-tau showed significant regional differences, with the highest levels in the entorhinal and frontal cortices and the lowest in the cerebellum. The uneven expression of tau protein in brain regions may contribute to distinct vulnerability/resistance to tau pathology [5].

In the human brain, tau is primarily expressed in neurons and also expressed at lower levels in oligodendrocytes and astrocytes [34]. In physiological conditions, tau was believed to be predominantly localized to axons, but limited in the soma and dendrites of neurons. Tau levels are comparable in gray matter and white matter in normal elderly brains, but higher in gray matter than whiter matter in AD brains [35]. Normally, tau monomer is difficult to be immunostained. Only aggregated tau or microtubule-binding tau can be detected by immunostaining. Although high levels in axons, a pre-synaptic abundance of tau is low. The mechanism for polarized neuronal distribution of tau could be (i) relocalization of tau from axon to soma may be blocked by the axon initial segment (AIS), (ii) Annexin A2 in the distal part of the axon interacts with tau and provides a sink for the redistribution of tau [16]. Under pathological conditions, endogenous tau translocates from axon to the soma and dendrite, and into the post-synapse. Early studies in AD and FTD have revealed that NFTs composed of aggregated hyperphosphorylated tau are localized in the soma and dendrites [36]. Translocation of tau depends on its interaction with microtubules [37] and are modulated through multiple mechanisms involving pathological post-translational modification (e.g., hyperphosphorylation) and/or non-physiological overexpression of tau protein [38], imbalanced expression of tau isoforms (e.g., 2 N tau) [39] and dysregulation of extracellular signals [16]. Furthermore, tau messenger RNA (mRNA) might be recruited to the dendrite and post-synapses, resulting in local somatodendritic translation of tau protein [40, 41]. Lastly, tau is also detected in the nucleus, where it is likely to protect DNA integrity from stress [42].

3.3 Function of tau

Microtubules are more stable in mature neurons than in non-neuronal cells [43], probably due to posttranslational modifications of the tubulin subunits as well as their interaction with specific neuronal microtubule-associated proteins (MAP) such as tau [44]. Tau binds to the interface between tubulin heterodimers through its microtubule-binding repeats [45] and stabilizes microtubules in the test tube and cultured cells [46, 47]. 4R-tau isoforms have a stronger affinity for microtubules than 3R-tau isoforms [48] and are more prone to promote microtubule assembly [49]. Besides, tau can nucleate and bundle microtubules in vitro [50] as well as in axons of mammalian neurons [51]. Recent studies have shown that tau not only acts as a microtubule stabilizer but also positively regulates the elongation of labile domains of microtubules at the plus ends [43]. Additionally, tau is involved in mediating intracellular transport along the axon [52], synaptic structure and function, and signaling pathways in neurons [53].

In AD and other tauopathies, pathological tau would detach from microtubules, leading to decreased microtubule stability, impaired axonal transport and synaptic function [54]. First, tau is more abundant on the labile domains of microtubules to protect them from severing proteins like katanin. Under a pathological condition, hyperphosphorylated tau disassociates with the microtubules and causes rapid and selective shrinkage of microtubule labile domains [55]. Disease-associated tau mutants, like K369I, G389R found in FTD, also showed decreased associations rate with microtubules, resulting in reduced ability in promoting microtubule assembly

[56]. Second, the ability of tau to regulate axonal transport alters under pathological conditions. Amino acids (aa) 2–18 of tau protein, termed the phosphatase-activating domain (PAD), activate a signaling cascade involving protein phosphatase 1 (PP1) and glycogen synthase kinase 3β (GSK-3β) that results in disruption of kinesin-1-mediated anterograde fast axonal transport. Y18 (tyrosine 18) phosphorylation of tau, which is stronger in monomers than in filaments, shows reduced inhibition of kinesin-1 and is significantly reduced in disease-associated tau species [57]. Moreover, intracellular tau aggregates have been shown to impair fast axonal transport by increasing the run length, run time and instantaneous velocity of membranous organelles [58]. Third, pathological tau accumulates on both pre- and post-synapses in the AD brain. Presynaptic accumulation of tau induces the depletion of the synaptic vesicle pool, followed by impaired synaptic transmission and plasticity [59]. On the other hand, toxic Aβ oligomers trigger N-methyl-D-aspartate receptor (NMDAR)-mediated excitotoxicity depending on the presence of endogenous tau [16].

Besides, glial tau pathology is also a common feature of many tauopathies and contributes to pathogenesis [34]. Oligodendrocytic tau pathology disrupts the maintenance of myelin sheath [60]. Although found at trace levels in astrocytes [61], tau has been shown to accumulate in astrocytic end feet directly apposed to vascular endothelial cells, and therefore impair the blood–brain barrier (BBB) integrity [62] and cause neuron degeneration without neuronal tau inclusions [63]. Filamentous recombinant tau also activates astrocytes via integrin signaling [64]. The expression and function of tau in microglia remain unclear [34]. However, microglia may regulate the uptake and exosomal secretion of tau, therefore involved in the spreading of tau pathology across the brain [65].

4. Post-translational modifications (PTMs) of tau

Tau is post-translationally modified by multiple mechanisms, including phosphorylation, ubiquitination, acetylation, methylation, glycosylation, glycation, nitration, lipoperoxidation, sumoylation and truncation [22]. Recently, up to 95 PTMs have been identified in human brains by high-resolution quantitative proteomics (**Table 2**) [70]. In neurodegenerative diseases, tau undergoes a series of pathological changes, such as PTMs alterations and the prion-like seeding and propagation, many of which not just accompany the diseases and indicate the pathology progression and individual heterogeneity, but are also the driving force of diseases.

4.1 Phosphorylation

Tau is a phosphoprotein. Theoretically, 80 serine/threonine and 5 tyrosine residues of the longest CNS tau isoform-tau441 can potentially be phosphorylated. In the brains of normal elderly, tau contains 2–3 mol phosphate/mole of the protein [71]. Phosphorylation sites like pT231, pT181, pS404 are found with high frequency (> 50%) [70]. However, tau phosphorylation increases 2–3 times in AD brains, i.e. hyperphosphorylation [71]. At least 55 phosphorylation sites are detected on pathological tau (**Table 2**) [70]. Hyperphosphorylation of soluble, oligomeric and seed-competent tau all exhibit substantial patient-to-patient heterogeneity, even though certain hot spots are present in AD brains, including pT181, pT217, pT231/S235, pS262, pS396 and pS400/T403/S404 [70, 72]. Among these sites, the phosphorylation levels of pT231/S235 and pS262 are positively correlated to the seeding capacity of tau species [72].

Rodent tau exhibits approximately 90% homology with human tau. Profiling of PTMs showed modifications on up to 63 sites on tau protein in the wild-type mouse brain, of which 27 were phosphorylation sites [73]. Phosphorylation of tau at specific

Modification	Sites
Phosphorylation	Y29, T30, T39, S46, S56, S68, T69, T71, T102, T111, S113, T153, S185, S184, T181, T175, S191, S198, S199, S202, T205, S210, T212, S214, T217, T220, T231, S235, S237, S238, S241, S258, S262, T263, S289, S293, S305, Y310, S316, S352, S356, T361, T386, Y394, S396, S400, T403, S404, S409, S412, S413, T414, S422, S433, S435
Ubiquitination	K163, K180, K190, K224, K228, K234, K240, K254, K257, K259, K267, K274, K280, K281, K290, K298, K311, K317, K321, K331, K340, K343, K353, K369, K375, K383, K385, K395
Acetylation	K24, K44, K240, K267, K274, K280, K281, K298, K311, K317, K331, K343, K347, K353, K375, K370, K369, K385, K395
Methylation	K44, K67, K87, K163, K174, K180, K254, K267, K290, R406, K438
SUMOylation	K340
Nitration	Y18, Y29, Y197, Y394
Truncation	M1-A2, M11-E12, V10-M11, D13-H14, D25-Q26, K44-E45, T102-A103, T123-Q124, R126-M127, A152-T153, R155-G156, I171-P172, A173-K174, G186-E187, Y197-S198, P223-K224, R230-T231, S237-S238, A239-K240, R242-L243, N255-V256, S258-K259, I260-G261, N279-K280, K281-L282, S305-V306, Q307-I308, I308-V309, Y310-K311, D314-L315, H330-K331, K340-S341, N368-K369, A390-E391, E391-I392, Y394-K395, D402-T403, D421-S422

Table 2.
Tau PMTs found in AD brains [50, 66–69].

sites was different in the brains of human and AD model mice. For example, 3 × TG-AD mice, the commonly-used AD model which contains three mutations associated with familial AD (APP Swedish, MAPT P301L, and PSEN1 M146V), are not significantly hyperphosphorylated at pS199, pS214, pS396/S400 and pS422 as in AD brains, implying a limitation of a mouse model in studying human tau pathology [74].

Abnormal hyperphosphorylation of tau is a pivotal step in neurofibrillary degeneration in AD and other tauopathies [71]. Taking the AD brain as an example, tau can be separated into three pools according to the phosphorylation state and solubility: (i) non-hyperphosphorylated normal tau (AD-tau), (ii) hyperphosphorylated tau (AD P-tau) and (iii) polyubiquitinated, hyperphosphorylated and aggregated tau in the insoluble PHFs (PHF-tau) [75]. Hyperphosphorylation of tau induces pathology through multiple mechanisms. First, hyperphosphorylation reduces tau affinity for microtubules. Natural tau forms a "paper clip" structure, with the N- and C-terminus fold over the microtubule-binding domain to prevent self-aggregation [76]. Hyperphosphorylation changes the net charge of tau protein and alters tau conformation to expose the microtubule-binding domain, thereby facilitating self-oligomerization and aggregation. Hyperphosphorylated and/or aggregated tau detach from the microtubules and lose their ability to stabilize microtubules [71]. Besides normal, AD P-tau captures microtubule-associated proteins other than tau, such as MAP1 and MAP2 [77], leading to further disruption of microtubules. Second, hyperphosphorylated tau redistributes from axons to the somatodendritic compartment and impairs synaptic function (see Section 3.3) [16, 54]. Third, phosphorylation may change the interaction of tau with other regulatory proteins [28]. Recently, 75 proteins specifically bound to phosphorylated tau in NFTs have been identified by quantitative proteomics coupled with affinity purification-mass spectrometry; most enriches in the protein ubiquitination pathway and phagosome maturation [78]. Whether hyperphosphorylation of tau alters its affinity to these proteins and directly leads to the damage of relevant pathways deserves more extensive investigation.

Tau phosphorylation is regulated by both proline-directed [GSK-3β, cyclin-dependent-like kinase-5 (CDK5), dual-specificity tyrosine phosphorylation regulated

kinase 1A (DYRK1A) and extracellular signal-related protein kinase (Erk)] and non-proline-directed protein kinases [calcium/calmodulin activated protein kinase II (CaMKII), protein kinase A (PKA), casein kinase 1 (CK1) and microtubule affinity-regulated kinase 110 (MARK p110)] in vivo, which are activated and/or overexpressed in AD brains [22]. On the contrary, tau is dephosphorylated by protein phosphatases, in particular protein phosphatases 2A (PP2A), which is responsible for over 70% of the total tau phosphatase activity in the human brain [79]. Dephosphorylation of AD P-tau with PP2A restores tau activity in promoting microtubule assembly in vitro and diminishes AD P-tau-induced propagation of tau pathology in mouse brain [80]. In disease conditions, phosphatase activity against tau is reduced to half, further increasing the imbalance between kinase and phosphatase activities, eventually resulting in excessive phosphorylation of tau [79]. It should be noted that tau is rapidly dephosphorylated during postmortem in a site-specific manner, suggesting timely dissection and proper cooling of the brain tissues [81].

4.2 Ubiquitination

Ubiquitination, a PTM that covalently conjugated ubiquitin (a highly conserved 76 amino acid protein) to the ε-amino group of target lysine residues in a protein, is usually involved in cellular protein degradation as well as non-degradative pathways including cell signaling, mitochondrial homoeostasis and DNA damage responses [82]. Ubiquitin positive pathological aggregates are present in AD, FTD, PD, CBD and other neurodegenerative diseases [83]. Quantitative analysis of ubiquitylome in AD brain reveals 28 ubiquitination sites in tau protein, which are the most abundant PTMs except phosphorylation (**Table 2**) [83]. Most of these ubiquitylation sites are located in the proline-rich region and the microtubule-binding domain.

Ubiquitination of tau is catalyzed by various ubiquitin ligases (E3 ligases), for instance, the C-terminus of the Hsc70-interacting protein (CHIP), TNF receptor-associated factor 6 (TRAF6) and axotrophin/MARCH7 [22]. High molecular weight (HMW) tau extracted from AD brain is shown to be polyubiquitinated, likely through K6-, K11- or K48-linkages [83], while PHF-tau is mostly monoubiquitylated, making it insufficient to trigger the ubiquitin-proteasome system (UPS)-mediated proteolysis [84]. Both polyubiquitylation and monoubiquitylation of tau contribute to the formation of insoluble protein inclusions [85, 86].

As a natively unfolded protein, tau is degraded by ATP/ubiquitin-independent 20S proteasome in physiological conditions [87]. Misfolded tau is typically ubiquitylated and is sent to the proteasome for degradation [88]. However, misfolded oligomers and aggregates cannot be fully degraded by the proteasome, but also directly damage the proteasome activity [89]. The autophagy-lysosome system provides a more potent pathway to degrade tau aggregates, which also relies on ubiquitination modification for recognition [88]. Therefore, activating ubiquitin degradation for toxic tau species is considered as one of the potential therapeutic strategies for the treatment of AD and related tauopathies.

4.3 Acetylation

Acetylation is a co- or post-translational modification that utilizes acetyl-CoA as the acetyl source to modify the N-termini or specific lysine residues in proteins [84]. Tau acetylation is catalyzed by the histone acetyltransferase p300 (EP300) or CREB-binding protein (CBP), and removed by sirtuin 1 (SIRT1) and histone deacetylase 6 (HDAC6) [22]. Tau is also able to catalyze self-acetylation by using cysteine residues C291 and C322 in the R2 and R3 repeats, respectively. 4R-tau displays the higher activity of autoacetylation than 3R-tau because the latter lacks

the R2 repeat [90]. 19 distinct acetylation sites have been mapped in tau protein isolated from AD brains, most of which are located within the microtubule-binding repeats and the flanking region (**Table 2**) [70].

The pathological effect of tau acetylation depends on specific modification sites. For example, high levels of tau acetylation are found at Lys163, Lys174, Lys180, Lys274, Lys280, Lys281 and Lys369 in AD brains, which may be related to the impairment of tau function [84]. Acetylation at these sites could prevent the polyubiquitylation and degradation of hyperphosphorylated tau, thus accelerating the accumulation of phosphorylated tau and promoting NFTs formation, accompanied by increased cognitive impairment. Acetylated tau also mislocalizes to the somatodendritic compartment and disrupts cytoskeleton dynamics, postsynaptic protein localization and receptor trafficking, consequently giving rise to synaptic plasticity deficits and memory loss [91]. Moreover, auto-acetylation of tau in C291 and C322 is coupled to its auto-proteolysis at K281-L282 and K340-S341 [90]. On the contrary, Lys259, Lys290, Lys321 and Lys353 within the KXGS motifs, which are found hypoacetylated in the AD brain, are normally acetylated to inhibit tau phosphorylation and aggregation [92].

4.4 Truncation

As an intrinsically disordered protein, tau is sensitive to proteolysis. In addition to acetylation-induced auto-proteolysis, tau can be cleaved by a variety of proteases both in vitro and in vivo including a disintegrin and metallopeptidase domain 10 (ADAM-10), asparagine endopeptidase (AEP), Calpain-1, Calpain-2, Caspase-3, Caspase-6, chymotrypsin, thrombin, cathepsins, human high-temperature requirement serine protease A1 (HtrA1) and puromycin-sensitive aminopeptidase (PSA). Besides, many fragments of tau in the brain have been found with undefined proteases (**Table 2**) [74, 93, 94]. In NFTs, at least three site-specific cleavages of tau (N368, E391 and D421) have been identified to be correlated with the progression of Braak stages [95]. Similar cleavage sites are also detected in several mouse models of tauopathies [28]. To date, more than 50 truncated forms of tau have been identified and over 30 are present in AD brains [94].

Truncation of tau plays a crucial role in its pathology. Truncation opens up the "paper clip" tertiary structure of tau protein, increases its site-specific phosphorylation, self-aggregation and affinity to oligomeric tau derived from AD brain (AD O-Tau), and thus promotes tau capture and seeded-aggregation by AD O-Tau [28, 96]. Tau truncation alone is sufficient to trigger hyperphosphorylation and aggregation [97]. Some fragments of tau can spread transcellularly, resulting in the propagation of tau pathology [94]. Additionally, Tau truncation can also induce toxic responses like apoptosis which is independent of its function on aggregates [98].

The characteristics of tau fragments depend on their amino acid composition. The C-terminal truncation increased tau fibrillization in vitro [66], while N-terminal truncations are found more associated with hyperphosphorylated high molecular weight tau oligomers (HMW-tau) isolated from AD brains [74, 93]. Tau fragments containing the aggregation-prone elements (**Table 3**) are prone to assemble the protease-resistant pathological core, which has various compositions in the different tauopathies [99]. Some specific fragments are secreted from the cytosol into the interstitial fluid and further released to the cerebrospinal fluid (CSF) or plasma, making them potentially biomarkers to indicate the progression of AD and other tauopathies [100]. Early study using immunoprecipitation showed that tau in CSF is predominantly the N-terminus fragments with an apparent molecular weight of approximately 20–40 kDa [101]. A 20–22 kDa NH_2-truncated form of tau (aa 26–230) identified in CSF is found to be neurotoxic due to its damages to mitochondrial oxidative phosphorylation [102].

Disease	Aggregation-prone elements
AD	3R Q273-E380, 4R-tau: G304-E380
CBD	K274-E380
CTE	3R-tau: K274-R379, 4R-tau: S305-R379
PiD	K254-F378

Table 3.
Aggregation-prone elements of tau in representative tauopathies [5, 188].

High-resolution mass spectrometry revealed at least 19 tau fragments in the CSF, of which tau aa 156–224 is the most abundant peptide [67, 68]. Nonetheless, the prion-like seeding activity of CSF tau fragments is quite limited [69].

4.5 Other PMTs

In addition to the above modifications, tau can also be modulated by methylation, SUMOylation, nitration, glycosylation and glycation. The contributions of these PMTs to tau pathology are mostly unelucidated.

Tau methylation mainly occurs on lysine residues in the proline-rich region and the microtubule-binding domain, and a few arginine residues [73]. Up to 11 methylation sites were found in the human brain [70]. Methylated tau is highly concentrated in NFTs particularly in late-stage AD brains [103]. Lysine methylation suppresses tau binding to the microtubule-binding domain, increases abnormal phosphorylation of tau and blocks the UPS-mediated tau degradation. However, the role of methylation on tau self-aggregation is still controversial [84].

K340 residue is the major site of tau that be modified by the small ubiquitin-like modifier protein (SUMO) [104]. SUMO-1 colocalizes with phosphorylated tau in the AD brain. Sumoylation reciprocally stimulates tau phosphorylation at T231 and S262, and competes against K340 ubiquitylation and consequently suppresses degradation [105].

Tau can be nitrated on four Tyr residues, Y18, Y29, Y197 and Y394, which are found in AD and non-AD tauopathies [106]. The effect of nitration on tau assembly depends on the specific nitration sites in vitro. Y18 nitration is reported to be associated with astrocyte activation [107].

In addition, the complexity of PTM is reflected more in the cross-talk between various modifications, not just in the types of modifications. First, a single amino acid residue could be modified by different PMTs. Taking lysine residues as an example, some of these residues in tau protein are competitively modified by ubiquitylation, methylation, acetylation, or SUMOlyation in the AD brain (**Table 2**). The competition between these PMTs will determine whether tau will undergo degradation or pathological transformation [84]. Secondly, PMTs at different sites cross-talk with each other. Many phosphorylation and ubiquitination sites of tau are located within the KXGS motifs in the microtubule-binding domain. Tau hyperphosphorylation is shown to facilitate ubiquitylation of NFT tau [83]. Therefore, further investigations focusing on the cross-talk between tau PMTs are required to reveal and intervene in the pathological changes of tau.

5. Propagation of tau aggregation

In AD brains, the progression of tau aggregation follows a stereotypical pattern of spread (the Braak stages): initiates from the locus coeruleus and transentorhinal cortex

(Braak stages I and II), gradually spread to the limbic system (Braak stages III and IV) and eventually to the neocortex (Braak stages V and VI) [108]. The stereotypical transmission of tau pathology is highly correlated with the cognitive impairment in AD [13]. Remarkably, tau pathology can be induced in rodent brains by injecting aggregates isolated from AD brains, and propagating to anatomically connected brain regions, in a similar spreading pattern to that observed in AD patients [80]. Besides, injection of tau aggregates extracted from other neurodegenerative diseases, such as AGD, CBD and PSP, also recapitulated the hallmark lesions of corresponding diseases [109]. A large amount of evidence indicates that the prion-like propagation of misfolded tau may explain the diverse characteristics of tauopathies [10].

5.1 Tau aggregation

The term "prion", originally defined by Prusiner, refers to a 'proteinaceous infectious particle' that causes degeneration of the CNS [110]. In addition to prion, other misfolded proteins, most notably Aβ, tau, α-synuclein and TAR DNA-binding protein 43 (TDP43), also act as proteopathic seeds to template the physiological alterations of the same protein, transmit between cells and spread to neuroanatomical connected regions, described as the 'prion-like property' [110].

The natively unfolded and highly soluble tau monomer contains a minimal content of ordered secondary structure, which shows little tendency to undergo aggregation [111]. An initial disruption likely changes tau conformation to obtain more β-sheet structures required for the interaction between monomers [94]. Two hexapeptide motifs of tau, ^{275}VQIINK^{280} and ^{306}VQIVYK^{311}, are crucial for the conformational switch and filament assembly [112]. As the intrinsic cause of fibrillation, the aggregation-prone sequence elements are found in multiple tauopathies (**Table 3**) [10]. Once the partially folded tau monomers are stimulated, they may sequentially aggregate to form dimmers, soluble oligomers, and eventually the insoluble PHFs and NTFs [113]. The microtubule-binding domains that contain β-sheet structures assemble into the rigid core of PHFs, while the N- and C-terminus of tau protein form a 'fuzzy coat' surrounding the core [99]. All six isoforms of CNS tau are detected in AD PHFs [114]. Oligomeric tau and PHF-tau isolated from AD brains can serve as prion-like seeds to induce the aggregation of normal tau both in vitro and in vivo, while monomeric heat-stable and straight filament (SF)-tau showed limited prion-like properties. However, AD O-tau is the most potent toxic species to induce pathological tau aggregation and propagation in vivo [80, 96, 115–117].

Tau fibrils isolated from the brains of patients with different tauopathies show disease-specific folding. In some cases, even in the same disease, the protofilaments of tau pack in distinct ways to form different polymorphs although the conformation of tau monomer is relatively preserved [10, 118]. Tau strains from different sources exhibit variant ability in inducing aggregation in vivo. For example, both the structures of PHF and SF in AD contain eight β-sheets in a C-shaped fold, but the intermolecular organization between the two kinds of protofilament is different [119]. PHF-tau, but not SF-tau, dramatically seeds tau aggregation in vitro and triggers the propagation of tau pathology in vivo [116]. Furthermore, the induced pathology in specific cell types is related to the origin of tau species. Injection of AD pathological tau to mouse brain induces pathology only in neurons, while injection of CBD or PSP pathological tau gives rise to pathology in neurons as well as astrocytes and oligodendrocytes [120].

Truncation and hyperphosphorylation are two major PMTs that contribute to tau aggregation. Truncation of tau may expose the microtubule-binding repeats that are responsible for aggregation. Tau truncation alone is sufficient to induce site-specific phosphorylation and self-aggregates [97]. Among various truncations of tau,

deletion of the first 150 aa and the last 50 aa (tau151–391) promotes its pathological characteristics most significantly. Compared with full-length tau, tau151–391 is more prone to phosphorylation, self-aggregation and seeded aggregation by AD O-tau [96]. Hyperphosphorylation alone is not sufficient to induce tau aggregation, but only when it occurs together with truncation [121]. Phosphorylation at several sites (e.g. pT231, pS235 and pS262) flanking the microtubule-binding domains inhibits tau-mediated microtubule assembly and facilitates tau aggregation into PHFs [122]. Interestingly, the phosphorylation levels of these sites are positively correlated to the seeding capacity of tau species isolated from AD patients. Notably, the effect of phosphorylation on tau seeding activity seems to be site-specific. For instance, phosphorylation levels at pS198/S199/S202 and pS400/T403/S404 show a negative correlation with tau seeding activity [72].

Mutation, dysregulation of tau alternative splicing and local contextual factors are also risks for tau aggregation [22]. Moreover, some chemical factors with strong negative charges (e.g. heparin, RNA, dextran sulphate and arachidonic acid) can induce tau aggregation in vitro [10]. Nevertheless, it should be pointed out that none of the fibrils synthesized in vitro resemble patient-derived fibrils so far, because the structures of chemical-induced tau filaments are quite different from those in diseases [118]. In addition to neurons, microglias also take up both soluble and insoluble tau [123] and are involved in promoting the spread of tau pathology [124].

5.2 Propagation of tau pathology

Once formed in the coeruleus/subcoeruleus region, the prion-like tau seeds are transmitted from a "donor cell" to a "recipient cell" to template more aggregation, and progressively spread the pathology along synaptically connected neurons to large-scale human brain networks. Propagation of tau pathology involves the following steps: 1) uptake of tau seeds, 2) seeded aggregation, 3) secretion of new seeds and 4) transcellular transmission of the toxic seeds [125].

The proteopathic seeds released from neurons can be engulfed by interconnected neurons at the somatodendritic compartment and the axon terminals, and are transported both anterogradely and retrogradely [126]. Endocytosis is the primary pathway for the internalization of proteopathic seeds, although the specific mechanisms vary [120]. It has been reported that pathological tau could be taken up by macropinocytosis, heparan sulfate proteoglycans (HSPGs)-dependent endocytosis, clathrin-mediated endocytosis, phagocytosis and tunneling nanotubes (TNT)-dependent direct intercellular transport, of which HSPGs-mediated endocytosis is the most predominant way [127]. The mechanisms of tau uptake are mainly similar for both vesicle-bound and free proteins [126]. Diverse tau strains display different uptake efficiency. Tau trimers are the minimal fragment that can be spontaneously internalized by primary neurons, while no clear upper limit is observed [128]. However, the soluble HMW-tau isolated from AD brain is the most efficiently internalized species [129].

After internalization, tau seeds are transported to the endo-lysosomal system, and some of them are degraded in the lysosomes [130]. Due to age-related dysfunction or other unknown mechanisms [131], a part of the seeds disrupt the endosomal vesicle and enter the cytoplasm, where they template amplification of new fibrils [132]. The danger receptor galectin-8 could protect against the release of seeds by monitoring endomembrane integrity and activating autophagy [133].

Tau contains no signal peptides. But it is secreted into the culture medium, interstitial fluid or CSF in a monomeric and/or truncated non-phosphorylated form lacking the C-terminal portion in physiological conditions [125], implying a potential physiologic role. Nonetheless, the transmission of tau monomer is unlikely to mediate transcellular propagation of tau pathology [134].

The proteopathic tau seeds are released from neurons through multiple pathways, including exocytosis [135], exosome [136], synaptic vesicles [137], presynapse membrane penetration [138] or even direct translocation across neurons [139]. Truncation and hyperphosphorylation of tau or increased synaptic activity facilitate its secretion [94]. The released proteopathic seeds are taken up by postsynaptic neurons and subsequently propagate tau pathology in the interconnected cells.

6. Tau-based therapeutics strategies

So far, the US Food and Drug Administration (FDA) has granted seven prescription drugs for the treatment of AD. Three of these drugs, Donepezil (Eisai Co., Ltd., Pfizer), Galantamine (Janssen, Ortho-McNeil Pharmaceutical, Sanochemia Pharmazeutika, Shire, Takeda Pharmaceutical Company) and Rivastigmine (Novartis Pharmaceuticals Corporation), are cholinesterase inhibitors that prevent the breakdown of acetylcholine in the brain. Rivastigmine has also been approved for the treatment of PD. Applications of Donepezil in the treatment of dementia with Lewy bodies, Down's syndrome and PD are under clinical trial. The forth drug Memantine (Forest Laboratories, Inc., H. Lundbeck, Merz Pharma) is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist. The fifth drug Suvorexant (Merck), an orexin receptor antagonist, is approved for treating sleep disorders in AD. The sixth drug Tacrine is a reversible acetylcholinesterase inhibitor, but has been discontinued because of its hepatotoxicity (Pfizer, Shionogi Pharma). In 2021, Aduhelm (Aducanumab), a human IgG1 monoclonal antibody against Aβ (Biogen, Neurimmune), was approved as the first immunotherapy for AD. However, most therapies exhibit limited benefits and do not prevent or slow down the progression of the disease [122].

Based on the extensive understanding of AD pathogenesis, a large number of therapies targeting tau have been developed recently. To data, among the 137 active therapeutic clinical trials for AD, FTD and PSP, 17 targeted tau (https://www.alzforum.org/therapeutics).

6.1 Immunotherapy

Although the mechanism is not fully elucidated, tau immunotherapy, either active or passive, shows protective effects on tau pathology and cognitive performance in AD model animals and is consequently becoming an essential strategy in the development of AD therapies [17]. Two vaccines for active immunotherapy of AD, AADvac-1 which is a synthetic peptide of tau (aa 294–305) and ACI-35 which is a phosphorylated tau peptide containing pS396/S404 are now in clinical trials (**Table 4**) [140]. Since active immunization may increase the risk of autoimmune reaction and other disadvantages [141], passive immunization currently accounts for the majority of the immunotherapy for tau (**Table 4**).

Passive immunotherapy is a short-term immunization administered by continuous injection of antibody that is designed specifically to pathological epitopes. Intravenously injection of tau antibody decreases both Aβ and tau pathologies in animal models, implying a therapeutic potential in the treatment of AD and related tauopathies [142]. There are 8 specific tau antibodies in clinical trials up to now. Their targets mainly focus on the N-terminus (ABBV-8E12 and Semorinemab), phosphorylation sites (Lu AF87908 and JNJ-63733657) or microtubule-binding region (Bepranemab and E2814) of tau protein (**Table 4**). Some antibodies (e.g. Bepranemab) also show potential in the treatment of other neurodegenerative diseases

Approach	Agent	Mechanism of action	Sponsor	Clinical trial	Status
Active	AADvac1	A synthetic peptide derived from tau	Axon Neuroscience SE	Phase 2	Completed
Active	ACI-35.030 JACI-35.054	Synthetic tau fragment phosphorylated at pS396/S404 anchored into a lipid bilayer	AC Immune SA	Phase 1 Phase 2	Recruiting
Passive	ABBV-8E12	A monoclonal antibody recognizes tau's N-terminus	AbbVie	Phase 2	Completed
Passive	BIIB076	A monoclonal anti-tau antibody	Biogen	Phase 1	Completed
Passive	Semorinemab	A monoclonal antibody recognizes tau's N-terminus	Genentech, Inc.	Phase 2	Completed
Passive	LY3303560	A humanized antibody against soluble tau aggregates	Eli Lilly and Company	Phase 2	Active, not recruiting
Passive	Lu AF87908	A monoclonal antibody recognizes phosphorylated tau	H. Lundbeck A/S	Phase 1	Recruiting
Passive	JNJ-63733657	A monoclonal antibody recognizes phosphorylated tau	Janssen Research & Development, LLC	Phase 2	Recruiting
Passive	Bepranemab	A monoclonal antibody recognizes tau235–250	UCB Biopharma SRL	Phase 2	Recruiting
Passive	E2814	A monoclonal antibody recognizes an HVPGG epitope of tau	Eisai Inc.	Phase 1 Phase 2	Recruiting
PTM	Salsalate	Inhibit tau acetylation	Adam Boxer	Phase 1	Active, not recruiting
PTM	LY3372689	A inhibitor of the O-GlcNAcase	Eli Lilly and Company	Phase 1	Completed
PTM	ASN51	A small-molecule inhibitor of O-GlycNAcase	Asceneuron Pty Ltd.	Phase 1	Recruiting
ASO	BIIB080	An antisense oligonucleotide (ASO) targeting tau expression	Ionis Pharmaceuticals, Inc.	Phase 1 Phase 2	Active, not recruiting

Data from: https://clinicaltrials.gov/, https://www.alzforum.org/therapeutics/

Table 4.
Tau-based therapies for AD.

like PSP (https://www.alzforum.org/therapeutics) [143]. Of course, tau-based passive immunization also faces some challenges, of which the key problem is how to deliver the antibodies more efficiently through the blood–brain-barrier (BBB) into specific brain region with tau pathology [144].

6.2 Therapies targeting tau PTMs

Acetylation at specific sites of tau is shown to inhibit the degradation of hyperphosphorylated tau, obstruct synaptic plasticity and promote cognitive impairment in AD mouse models [91]. Salsalate, a non-acetylated dimer of salicylic acid commonly used as a non-steroidal anti-inflammatory drug, can inhibit acetyltransferase p300-induced tau acetylation, thus enhancing tau turnover and reducing tau levels [145]. O-GlcNAcylation negatively regulates tau phosphorylation by competing for the phosphorylation sites [146]. Two small molecular inhibitors of O-GlycNAcase (LY3372689 and ASN51) are currently undergoing clinical trials.

Hyperphosphorylation is the crucial PTM that determines the propagation of tau pathology. Inhibition of tau hyperphosphorylation has long been considered as a potential therapeutic strategy. Tau phosphorylation can be modulated by the balance between protein kinases and phosphatases. Nasal insulin and the GSK-3 inhibitor Lithium that inhibit tau phosphorylation via activating PI3K signaling, and PP2A activator Metformin aimed at tau dephosphorylation are currently under development or evaluation in clinical trials [122].

6.3 Other therapies

Knockout of MAPT gene induces no obvious phenotype except for behavioral deficits in aged mice [147]. Reducing the levels of endogenous tau shows protection against cognitive impairments and behavioral abnormalities in AD mice [148]. BIIB080, the first antisense oligonucleotide (ASO) targeting the translation of tau mRNAs, has just started Phase 1 and Phase 2 clinical trials. Recently, a selective protein degradation approach achieved by the proteolysis targeting chimeras (PROTACs) is utilized to decrease tau protein in the brain [82, 149]. PROTACs form a ternary complex with the target protein and ubiquitin E3 ligase. E3 ubiquitin ligase stimulates polyubiquitination of the targets and facilitates its following recognition and degradation by the 26S proteasome [149]. PROTACs targeting tau remarkably decreased tau levels and improved synaptic and cognitive functions in wildtype and AD mice [150].

In the past few years, inhibitors of tau aggregation were considered as potential therapies for AD for their roles in preventing the prion-like seeding and propagation of tau pathology. Unfortunately, the clinical trials of corresponding small molecular drugs, such as methylene blue, Rember TM, and LMTX, did not show the expected effect and are thus discontinued. NPT088, a fusion protein consisting of human-IgG 1-Fc and an active fragment binds to and remodels misfolded aggregates of tau, also exhibited no effect on brain plaques, tau aggregates or AD symptoms [151].

What's more, the disruption of microtubules is one of the main consequences of tau-induced neurotoxicity. Therefore, stabilization of microtubules may also be a potential therapeutic approach associated with tau. However, the efficacy of microtubule stabilizers (e.g. TPI-287) in the treatment of AD still requires further evaluation.

7. Conclusions and perspectives

In summary, tau-mediated microtubule dynamics and assembly play essential roles in neuronal transport and the maintenance of synaptic structure and function. In neurodegenerative diseases, tau undergoes a series of pathological changes, including mutation, abnormal alternative splicing, abnormal PTMs and prion-like seeding and propagation. Certain benefits of therapeutic approaches targeting

pathological tau have emerged in the treatment of AD and related tauopathies. Recently, proteomics results indicate significant heterogeneity of tau pathology in different patients, raising the possibility that personalized approaches according to the biochemical characteristics of tau may achieve better therapeutic effects [10]. It is also worth noting that tau interacts with other risk factors of neurodegenerative diseases, such as Aβ [152], apolipoprotein E [153], α-synuclein [154], metal dysregulation [155], defective mitophagy [156], stress and inflammation [157] and so on. Therefore, further investigation of the comprehensive map of tau interactions will better reveal the pathogenesis of neurodegenerative diseases.

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Conflict of interest

The authors declare no conflict of interest.

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