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REVIEW

# Behind the curtain of tauopathy: a show of multiple players orchestrating tau toxicity

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**Abstract** tau, a microtubule-associated protein, directly binds with microtubules to dynamically regulate the organization of cellular cytoskeletons, and is especially abundant in neurons of the central nervous system. Under disease conditions such as Pick's disease, progressive supranuclear palsy, frontotemporal dementia, parkinsonism linked to chromosome 17 and Alzheimer's disease, tau proteins can self-assemble to paired helical filaments progressing to neurofibrillary tangles. In these diseases, collectively referred to as "tauopathies", alterations of diverse tau modifications including phosphorylation, metal ion binding, glycosylation, as well as structural changes of tau proteins have all been observed, indicating the complexity and variability of factors in the regulation of tau toxicity. Here, we review our current knowledge and hypotheses from relevant studies on tau toxicity, emphasizing the roles of phosphorylations, metal ions, folding and clearance control underlining tau etiology and their regulations. A summary of clinical efforts and associated findings of drug candidates under development is also presented. It is hoped that a more comprehensive understanding of tau regulation will provide us with a better blueprint of tau networking in neuronal cells and offer hints for the design of more efficient strategies to tackle tau-related diseases in the future.

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**Abbreviations**

| | |
|---|---|
| Aβ | β-Amyloid |
| AD | Alzheimer's disease |
| BBB | Blood–brain barrier |
| CHIP | Carboxyl terminus of Hsc70 interacting protein |
| CMA | Chaperone-mediated autophagy |
| CNS | Central nervous system |
| CQ | Clioquinol |
| FKBP51 and FKBP52 | FK506-binding protein 51 and 52 |
| FTDP-17 | Frontotemporal dementia and parkinsonism linked to chromosome 17 |
| LTP | Long-term potentiation |
| MAP | Microtubule-associated protein |
| MAPT | Microtubule-associated protein tau |
| MARK | Microtubule/MAP-affinity regulating kinase |
| MT | Microtubule |
| NFTs | Neurofibrillary tangles |
| NMNAT2 | Nicotinamide mononucleotide adenylyltransferase 2 |
| PHFs | Paired helical filaments |
| PP2A | Protein phosphatase-2A |
| PSP | Progressive supranuclear palsy |
| PTP1B | Protein tyrosine phosphatase 1B |
| TMAO | Trimethylamine N-oxide |
| 3-MA | 3-Methylamphetamine |

## Introduction

### tau protein

During a search for factors promoting microtubule (MT) assembly, tau was first purified from porcine brains by taking advantage of its thermal stability and was characterized as a member of the Microtubule-associated protein family (MAP) [1, 2]. tau proteins are broadly distributed in the neurons of the central nervous system, but are also expressed at low levels in astrocytes and oligodendrocytes [3]. Under physiological conditions, tau proteins predominantly localize in the distal portions of axons, whereas MAP6s, another member of MAPs, distribute in the proximal portions of axons and MAP2s mainly localize in the dendritic compartments [4]. This restriction of distribution indicates that a possible barrier may exist to confine the diffusion of these MAP members (please see review from Mandelkow [2] for a summary of this).

In humans, tau has six isoforms that are the products of alternative spicing from a single gene (MAPT or microtubule-associated protein tau) which is localized on chromosome 17q21, and contains 16 exons [5]. The differences among these six isoforms are due to alternative splicing of exons 2, 3 and 10 [6]. Both exons 2 and 3 encode 29-amino acid fragments, and alternative splicing can generate the 2N (contains exons 2 and 3), 1N (contains only exon 3), and 0N (contains neither) forms of tau. Exon 10 encodes a 31-amino acid fragment of the second microtubule-binding domain (R2) in the C terminal of the protein; its alternative splicing creates 4R (contains 4 microtubule-binding domains) or 3R forms (contains 3 microtubule-binding domains) of tau [7, 8]. Altogether, MAPT alternative splicing creates six isoforms of tau proteins: 0N3R, 1N3R, 2N3R, 0N4R, 1N4R and 2N4R. A relatively constant 1:1 ratio of 3R/4R isoforms in the adult brain is maintained; however, can be shifted under certain pathological conditions [9]. The exact physiological roles of the various tau forms are not well elucidated.

The primary known function of tau is its ability to bind to microtubules. It is known that the binding domains of tau proteins contain positively charged amino acid residues, allowing them to bind to the negatively charged microtubules and resulting in better microtubule stabilization of 4R isoforms compared to 3R isoforms [10, 11]. In addition to alternative splicing, tau is also subject to several kinds of post-translational modification including phosphorylation and glycosylation. Phosphorylation in particular is a highly complex event: tau contains 85 potential serine (Ser, S) threonine (Thr, T) and tyrosine (Tyr, Y) sites on the longest isoform, and it has been shown that phosphorylation can happen on approximately 30 of them [12] (for details, see review from Iqbal [13]). In addition, the phosphorylation of tau is highly dynamic during development. For example, the embryonic central nervous system (CNS) expresses more highly phosphorylated tau proteins than the adult CNS does [14], and the degree of phosphorylation of tau proteins (in all 6 isoforms) decreases during aging [15]. The biological significance of tau variations and phosphorylation during neurogenesis remains unknown, especially while considering that tau knock-out mice do not present obvious abnormalities in the brain development, likely due to compensatory effects from other MAPs [16]. Phosphorylation levels of tau can change dramatically under certain disease conditions. In post-developmental brain tissues, the phosphate to tau protein ratio is on average 2–3:1, whereas under certain pathological conditions it can rise to a 7–8:1 ratio, which is recognized as "hyperphosphorylation" [17, 18]. This process will be discussed in detail later.

tau is considered a prototypical "natively unfolded" protein or "intrinsically unstructured protein" based on observations in biophysical studies [19]. The dynamic structure of human tau (441 residues) has been recently described at single residue resolution using nuclear magnetic resonance (NMR) spectroscopy [20]. The data revealed that tau has a highly dynamic structure with a distinct domain in the aqueous phase and an internal network of transient long-range interactions that are important for pathogenic aggregation. Although limited information has been obtained regarding how phosphorylation might change tau structure, biochemical studies have revealed that phosphorylation can reduce the binding affinity between tau and microtubules, an event which is greatly affected by the protein conformation [21].

### Tauopathy diseases

Although MAPs have irreplaceable functions in neurogenesis, tau protein is not known for its normal biological functions, but rather its pathogenic potential in a series of human neurodegenerative diseases, including Alzheimer's disease (AD) [22], the most severe and common form of dementia worldwide. Tauopathy is a term used to classify a collection of neurodegenerative diseases with abnormal aggregations of tau proteins [22]. In AD, for example, in addition to the extracellular plaques composed of β-amyloid aggregates, tau was found to form intracellular neurofibrillary tangles (NFTs) accompanied by abnormal phosphorylation [23]. These pathogeneses always correlate with phenotypic symptoms such as progressive memory loss and cognitive impairment in AD patients [24]. The abnormally phosphorylated and aggregated tau also can be found in other types of neurodegenerative diseases, such as Down syndrome, Pick's disease, progressive supranuclear

palsy, corticobasal degeneration, frontotemporal dementia, amyotrophic lateral sclerosis/parkinsonism–dementia complex, and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [25]. Mutations in the tau gene itself (Fig. 1) are considered to be the pathogenic cause for some of the cases, such as FTDP-17 [26], an autosomal dominant neurodegenerative disorder with severe degeneration in the patients' frontal and temporal lobes and also parietal cortical atrophy [27–31]. Autopsies from FTDP-17 patients have revealed abundant NFTs similar to those found in AD, but without β-amyloid plaques. The kinds of tau mutations in FTDP-17 are diverse, including missense mutations, small deletions in the exons, and intronic mutations that are close to the splice-donor site of exon 10 [32–34]. Missense mutations such as P301L/S, V337M and G272V can impair the MT binding ability of tau and these mutants are prone to the formation of paired helical filaments (PHFs), progressing to NFTs. Notably, different mutations may induce distinct patterns of neuronal or glial pathology [35]. The tau intronic mutations can influence the alternative splicing of exon 10, and the resultant ratio change of 4R/3R tau isoforms leads to consequent protein aggregation [34, 35]. A significant observation is that FTDP-17 mutants, when compared to wild-type tau, can elicit more severe neuropathology in transgenic animal models independent of Aβ, indicating that tau alone can explain the etiology of tauopathies [35–37].

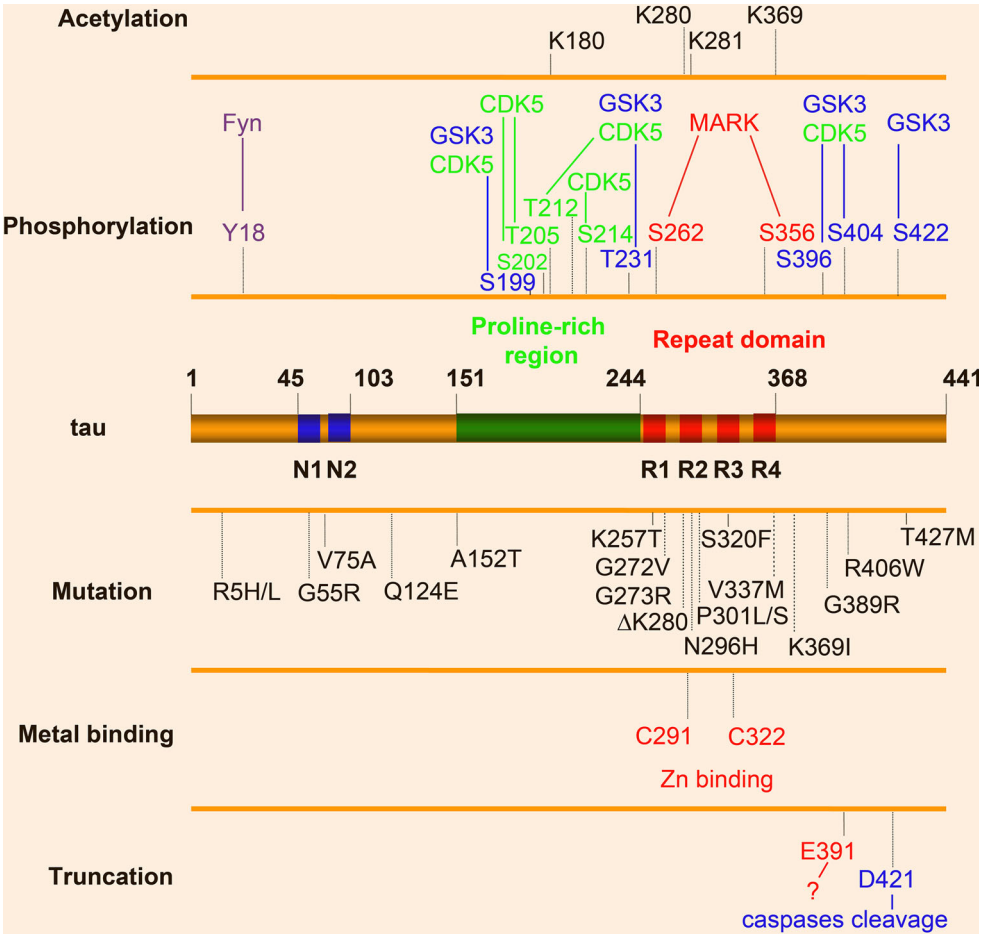


**Fig. 1** The multiple layers of modifications on tau proteins. The major domain composition of tau protein (the longest isoform, 441 amino acid), including the N-terminus, proline-rich region, microtubule-binding repeat (microtubule-binding domain), and the C-terminus, is illustrated here. Modifying events such as phosphorylation, acetylation, metal binding, truncations and disease-related mutations are listed *above* or *below* the schematic tau protein. These represent only a partial list of tau modifications. Some other modifications such as glycosylation, glycation, prolyl-isomerization, nitration and sumoylation, which could also affect the function and toxicity of tau, are not shown. Specifically, acting sites of kinases (MARK, GSK3 and CDK5) and the metal binding sites of tau are marked; several tau mutations such as K257T, G272V, ΔK280, P301L/S, K369I, V337M, G389R and R406W have been widely used in model organism studies and are listed; some known acetylation sites as well as the cleavage on D421 and E391 are also drawn in this schematic presentation (it is found that caspase-3 mediates D421 cleavage, but the mechanism of E391 cleavage remains unclear)

## Animal models of tauopathies

Animal modeling is widely used in the study of tauopathy and is our main source for the mechanistic understanding of tau toxicity. Both tau knock-out and knock-in models have been generated. Some of the model organisms that have been used include invertebrates *Caenorhabditis elegans* and *Drosophila melanogaster*, and vertebrates such as mouse, rat, and, to a lesser extent, primates.

*Caenorhabditis elegans* and *Drosophila* are endowed with certain distinct advantages such as small body size, rapid propagation, and short life span. Most importantly, many biological processes and neuronal cellular functions are evolutionarily conserved from these organisms to vertebrates or human. Using the aex-3 promoter, a *C. elegans* tauopathy model was established expressing human P301L or V337M forms of tau [38]. These animals displayed some of the same important phenotypes observed in humans such as severe neurodegeneration and tau pathology, including abnormal tau phosphorylation and accumulation. P301L and V337M had strikingly more pronounced phenotypes, consistent with the idea that these mutations may exacerbate tau toxicity. Genetic screening in *C. elegans* identified some potential tau suppressors and enhancers, and furthered our understanding of tau pathology. Pan-neuronal expression of the wild-type tau and pseudo-hyperphosphorylated tau using the rgef-1 promoter revealed that *C. elegans* was capable of phosphorylating tau and caused similar conformational changes to tau as in AD patients [39, 40].

Both the wild-type and mutant tau have also been introduced into *Drosophila* to generate tauopathy models. Compared with *C. elegans* and other model animals, the UAS/Gal4 system routinely used in fruit flies facilitates the construction of *Drosophila* models. Once UAS-tau is introduced, various Gal4 lines like *elav-gal4* (pan-neuron expression), *gmr-gal4* (expression in fly eyes) and *cha-gal4* (cholinergic neuron expression) can be combined to generate tau expression in all or specific subsets of neurons or other types of cells [41]. *Drosophila* expression of both wild-type and FTDP-17 mutant tau displayed some similar aspects of tau pathology found in humans, specifically the adult onset progressive neurodegeneration and the accumulation of abnormal tau. In addition, the FTDP-17 mutant tau was found to be more toxic than the wild-type tau [37]. tau expression in *Drosophila* also causes neuronal dysfunctions, exemplified by abnormal axonal transport, synaptic dysfunctions and learning and memory defects [42, 43]. tau mutations on critical phosphorylation sites such as Ser262 and Ser356 lead to a reduction in both phosphorylation and toxicity in *Drosophila* [44]. Using fly tauopathy models, suppressors and enhancers of tau toxicity have been isolated. These include some previously known factors such as par-1 and PP2A, as well as some novel factors including Glypican, Filamin, MAP1b, cytoskeleton proteins, and metal ion homeostasis-related genes [45–48]. Moreover, Aβ and tau co-expression leads to more severe neurodegeneration and tau pathology in *Drosophila*, accompanied with higher tau hyperphosphorylation [49]. Compared with vertebrate models, *C. elegans* and *Drosophila* models provide more rapid screening of tau modifiers, and provide insights into disease mechanisms that enhance studies of tauopathy research in higher animals [39, 41].

Mice are widely used as vertebrate models in tauopathy studies. tau knock-out mice were viable and were initially reported to display no obvious abnormalities, except with increased MAP1A expression, likely a result of some compensatory effects [50]. Notably, additional detailed analyses revealed some deleterious effects in axons from tau null animals, including decreased microtubule stability and altered microtubule organization, which might underlie the motor and cognitive deficits observed in some aged cases [16, 51]. The introduction of an artificial chromosome expressing the six human tau forms into tau null mice resulted in the accumulation and aggregation of hyperphosphorylated tau, and also caused memory and behavior defects in aged animals [52, 53]. The FTDP-17-related tau mutants caused more severe tau pathology in mouse models in terms of filament formation [54], memory defects [55], behavioral disturbances and neurodegeneration [56]. Mouse studies have helped to clarify the roles of tau kinases and phosphatases (like GSK3β, CDK5 and PP2A), the truncated form of tau [57], and the abnormal modification of tau in tauopathy [58]. Overall, results from mouse models have greatly improved our understanding of the mechanism of tau toxicity, and drug testing on these animals also facilitated relevant drug development.

Besides mouse models, other vertebrates were also used in the study of tauopathy. The zebrafish is a small vertebrate that is sometimes adopted for rapid drug screening [59]. Using the zebrafish model, effects of drugs could be readily evaluated [59]. In rats, expressing the truncated tau form generated neurofibrillary tangles composed of endogenous rat tau and exogenic tau [60, 61], accompanied with tau hyperphosphorylation, behavior impairments, and decreased lifespan [62]. Recently, it was reported in rats that traumatic brain injury induced oligomerization and hyperphosphorylation of rat tau, mimicking some pathology features observed in TBI-related dementia [63]. Primates such as monkeys are evolutionarily closest to humans. Although no experimental transgenic tauopathy models of primates have been reported so far, some other related efforts have been undertaken. Immunocytochemistry studies of aged brains of cynomolgus monkeys revealed Aβ deposition in the blood vessels, and diffused

and compacted plaques similar to those in human brains [64]. Injection of fibrillar Aβ into the rhesus monkeys caused tau phosphorylation [65]. In aged rhesus monkeys, hyperphosphorylated tau was found in the hippocampus and entorhinal cortex, which is also one of the earlier affected regions for tangle spreading in Alzheimer's patients [66]. In 20-year-old cynomolgus monkeys, accumulation of phosphorylated tau was reported, and tau tangles were found in 36-year-old animals [67]. Notably, in baboons, age-dependent tau accumulation was reported. Among animals aged from 26 to 30 years, 91 % of them developed abnormal tau accumulations [68]; in addition, in a 41-year-old pan troglodyte, humanlike paired helical filaments tau pathology was reported [69]. The findings from non-human primates indicate that these animals, without transgenic human tau, could also potentially be used towards understanding the mechanisms of tauopathy and developing treatment therapies [70].

## tau toxicities and their regulations

### tau toxicities: what damages does tau cause?

In tauopathies, the disorganized tau proteins first lose their normal functions through release from the MT and mislocalization to other organelles. The potential damage accrues as the proteins begin to form aggregates. Thus, toxicity can arise from either or both of the two events: loss of normal functions and/or gain of new functions. tau aggregation/oligomerization-related toxicities, which will be discussed later, are accepted by many researchers in the field as the cause of tauopathy [71, 72]. We will start our discussion with the known early events of tauopathy.

At the initial stage, when tau gets hyperphosphorylated, especially on Ser262 and Thr231, its microtubule-binding affinity significantly declines [73, 74]. Subsequently, the tau proteins disassociate from the microtubules and the microtubules become depolymerized due to destabilization from the loss of MAPs [12, 75]. This destabilization of microtubules can distinctly inhibit the axonal anterograde and retrograde transport between the soma and synapse, which is vital to maintain the normal function and survival of mature neurons [76]. In the tauopathy mouse model, compounds that stabilize microtubules can, to some extent, counteract tau toxicities such as reversing synapse loss and improving cognition, without affecting the aggregation of NFTs [77, 78]. However, there are also reports showing that tau phosphorylation on Thr50 can promote the assembly of microtubules. It has also been observed that in the presence of TMAO (trimethylamine N-oxide), a natural occurring osmolyte, PKA and Gsk mediated tau phosphorylations can similarly promote the assembly process [79, 80]. Furthermore, it has been shown that tau phosphorylation can enhance the cargo trafficking in cultured cells and axons, while normal tau can inhibit cargo trafficking by blocking kinesin movement [81, 82]. These paradoxical observations demonstrate the complexity of the regulation and consequence of tau phosphorylation. The phosphorylation events vary not only in magnitude but can also lead to drastically different or even opposite outcomes.

When tau is released from the microtubule, it may missort to other subcellular compartments. In a tau transgenic mouse model, excessively phosphorylated tau proteins can be found in the somatodendritic compartment in the cerebral cortex, in contrast to normal tau protein, which should only localize in the axon [83]. This observation is consistent with symptoms found in human AD patients where abnormal tau proteins are also found to aggregate to NFTs in the somatodendritic compartment [84, 85]. Under normal physiological conditions, tau proteins are retained in the axon mainly due to the high affinity for axonal MT and low affinity for dendritic MT [86]. However, the phosphorylation status of tau can reverse this affinity [87]. tau proteins also become more diffusible under disease conditions where barriers of protein diffusion have been disrupted [88].

Intriguingly, mutations causing FTDP-17 may also induce tau missorting and lead to the development of diverse pathologies in different cell types. For example, the P301L/S mutation causes both neuronal and glial damage, while V337M and R406W mainly induce neuronal tau pathology in the somatodendritic compartment, similar to AD cases [89–91]. These observations have been recapitulated in rodent models as well. The P301L mutation increases tau levels in the dendritical spine and eventually causes spine loss [92]. Furthermore, a double mutation (K257T/P301S) reproduces human symptoms of FTDP-17 including tau missorting and protein aggregation [93]. Why different mutations can endow tau with different sorting abilities during pathogenesis is still a mystery. Phosphorylation differences are one possible reason, though others likely exist [34]. For example, mutations may produce additional binding sites on tau for new partner proteins, potentially leading to tau translocation and harmful consequences.

Pathological appearance of tau protein in the soma and dendrites is deleterious. A small quantity of missorted tau protein may induce more tau proteins to release from the microtubules, mislocalize and aggregate. This harmful positive feedback indicates a spreading mechanism of tauopathy, which can eventually and irreversibly kill the neurons [94]. Missorted tau can also interfere with other processes, including mitochondrial functions, mitochondria trafficking [95], motor-mediated cargo transport [96], and the trafficking of receptors in the post-synapses and the

synaptic transmission [97, 98]. tau can also interact with Fyn kinase and result in the alteration of post-synapse components like PSD-95 and NR2b. A similar process is also thought to be involved in Aβ-induced neuron excitotoxicity [99, 100]. These factors are thought to contribute to the pathogenesis and abnormalities caused by tau.

Although it is widely accepted that hyperphosphorylation corresponds to neuron loss in tauopathies, the precise mechanism of cell death in neurodegenerative diseases like AD has not yet been fully explained and might be more complicated than originally thought. Hyperphosphorylated truncated protein tau is found to induce a caspase-3 independent apoptosis-like pathway in the Alzheimer's disease cellular model [101]. However, in P301S mice, abundant tau hyperphosphorylation and NFTs, but no apoptosis, are detected [56]. The latter is consistent with the observation that in AD brains little apoptotic cell death is found. This implies that tau phosphorylation might connect with certain pathways, such as the nuclear, ER and mitochondrial pathways, to prevent neuronal apoptosis [102].

## tau toxicities: an effect of the oligomer form or the NFT?

Whether or not tau aggregates are toxic is still debated. Numerous correlative evidence suggests that NFTs might be damaging. An abundance of NFTs has been observed in AD brains, together with tau hyperphosphorylation, and the severity of NFT accumulation correlates with the neurodegeneration progression in AD patients. In addition, in the P301L mouse model, NFT bearing neurons undergo synapse loss and synaptic functional impairment [103–108]. Nevertheless, neurons with NFTs can survive for 20–30 years [109, 110], indicating that NFTs might not be the major toxic species in tauopathies [72]. Studies in animal models also argue against the possibility that NFTs are the major toxicity source. For example, in the fly tau model, severe neuronal loss and short lifespan were observed, but without NFTs accumulating in the brain [37]. In addition, in the P301L transgenic mouse, memory impairment was observed at 2.5 months, however, NFTs did not appear until 10 months [111]. Furthermore, in the same study, turning off P301L expression reduced memory impairment and neuron loss but not NFT formation. These studies suggest that NFTs may be an accompanying phenomenon or a protective mechanism insulating the cell from tau toxicities. Indeed, the current body of data implies that oligomerized forms of tau might be the true source of tau toxicity [72]. As discussed previously, studies in *Drosophila* and rodent models indicate that soluble forms of tau may behave as the toxic species [37, 111], due to the observation that no NFTs were detected in these animals, yet severe phenotypes still developed. Consistently, in a methylene blue treatment study, soluble tau levels correlated well with memory and other performance indexes of the tau mouse, while NFT levels were not changed during the treatment [112].

Using a plethora of antibodies generated against different forms and epitopes of tau, distinct tau species could be identified at different stages of tauopathy. tau antibody TOC1 (antibody against tau oligomers and aggregation intermediates, but not NFTs) staining revealed that tau oligomers appeared at early stages of AD, far before the formation of NFTs [113]. T22 (antibody against tau oligomers) staining confirmed this result in early stage tauopathy autopsy samples [114]. More importantly, tau oligomers were found to be much more toxic than fibrous forms in both in vivo and in vitro experiments [115, 116]. Blocking oligomer formation by expressing chaperone protein Hsp70 could restore the impaired axonal transport in isolated axon membranes [117]. Consistent with this, inhibiting oligomerization by curcumin could significantly improve the behavioral defects and rescue synaptic abnormalities in tau mouse models [118]. Notably, it was shown that external human tau oligomers, when infused into naïve mice, could work as a seed and propagate through release and aggregation of the endogenous tau protein, so that both human tau and murine tau could be found in the protein deposits [119].

Altogether, many aspects of tau abnormalities including disrupted MT equilibrium, tau missortment, hyperphosphorylation, oligomerization and fibrillization were found in tauopathy. Many of these can be damaging and, therefore, contribute to tau's toxicity. However, the relative importance of these aspects to tauopathy development has not yet been fully elucidated and further studies are clearly necessary.

## tau toxicity: what are the regulators?

Apart from phosphorylation, various other types of tau post-translational modifications have been reported (Fig. 1). Some post-translational modifications such as glycosylation, O-GlcNAcylation, acetylation and abnormal truncations [120–123] will not be covered in details here. This is not meant to imply that these aspects of tau modifications are unimportant. For example, O-GlcNAcylation, a recently discovered tau modification, shows cross-talk with phosphorylation in vitro. It competes with and negatively regulates tau phosphorylation, and irregular O-GlcNAcylation has been observed in AD brains [123, 124]. It has even been shown that upregulating O-GlcNAcylation level by small-molecule inhibitors like thiamet-G can efficiently mitigate tau toxicity in mouse models [124]. Nevertheless, these processes are not as well studied and understood. In this work, we will focus on

discussions of tau phosphorylation, the role of metals ions, folding and clearance control in tauopathy development (refer to Figs. 1, 2).

*Hyperphosphorylation and tau toxicity*

Phosphorylation of tau proteins is tightly regulated under both physiological and pathological conditions. In the pre-neurofibrillary tangle stage, tau is excessively phosphorylated on sites Ser262 and Thr231. However, in the post-neurofibrillary tangle stage, phosphorylation is prominent on sites Ser396, Ser404 and Ser422 [125]. This indicates a series of phosphorylating and dephosphorylating events occurring during neurofibrillary tangle formation. Although the eventual outcome of hyperphosphorylation is not completely understood, there is evidence implicating direct links between tau function and phosphorylation status. For example, phosphorylation at Ser262 and Ser214 sites greatly weakens the MT binding affinity of tau and causes the release of tau proteins into the cytosol, a factor that is thought to be an early event in tau pathology [73, 75, 87, 125]. As discussed previously, studies in animal models including *C. elegans* [40], *Drosophila* [126] and mice [127] indicate that hyperphosphorylation events correlate well with vesicular motion in axons, neurohormone release, synaptic loss, neural activity inhibition and lifespan reduction. Ser to Ala mutations at significant phosphorylation sites such as S262A and S356A can greatly reduce

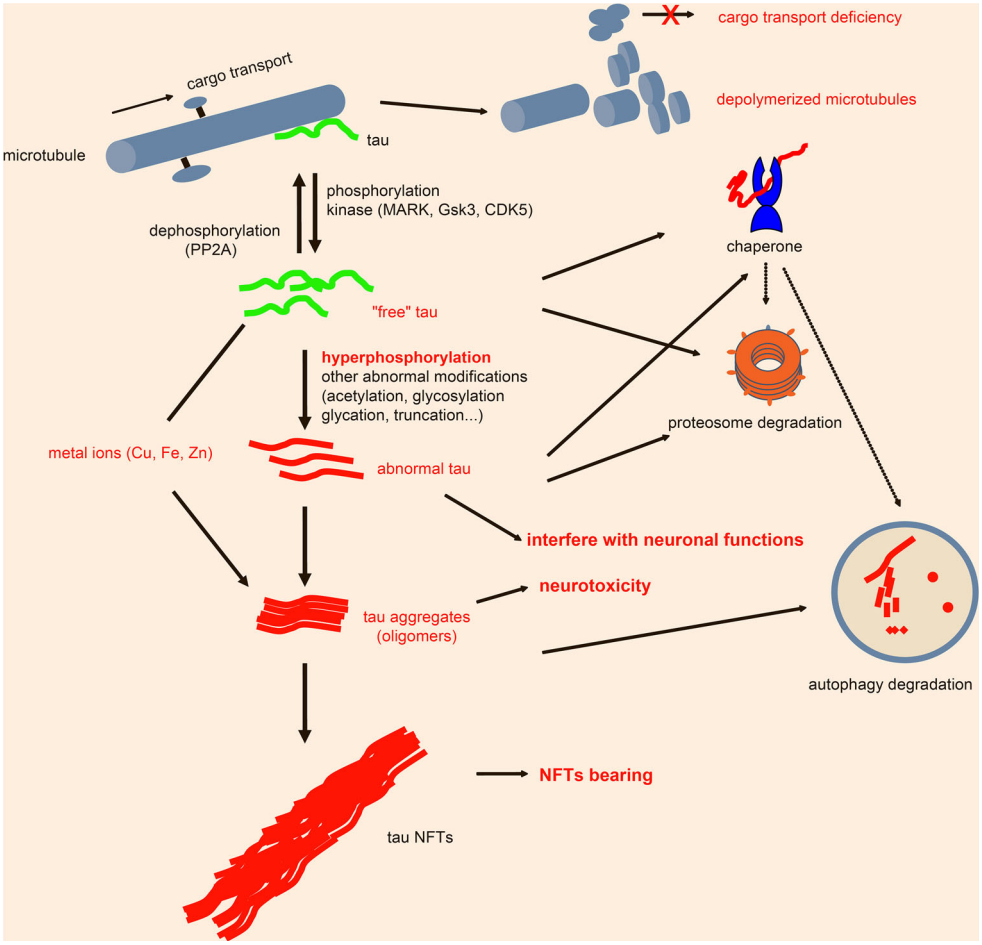


**Fig. 2** A model showing some of the mechanisms regulating tau toxicity. The physiological binding of tau to MTs (microtubules) is regulated by kinases (GSK3, CDK5 and MARK) and phosphatases (like PP2A). Under pathological conditions, tau is misregulated (such as abnormal phosphorylation and mutation) and dissociates from MTs, "freeing" tau and destabilizing the MTs (leading to MT depolymerization and cargo transporting defects). Abnormal modifications (hyperphosphorylation, metal ion, acetylation, glycosylation, glycation, prolyl-isomerization, cleavage or truncation, nitration and sumoylation) also result in tau aggregation, oligomer formation progressing to more advanced aggregates (like NFTs or neurofibrillary tangles). Metal ions, though also possibly affecting hyperphosphorylation, largely act to bind tau and work in parallel with phosphorylation to facilitate tau aggregation. The abnormally modified tau and tau aggregates can interfere with neuronal functions and lead to neuronal toxicity. To combat tau toxicity, molecular chaperons like Hsp70/Hsc70 can refold the abnormal tau, the proteasome system can degrade the abnormal tau species, and the autophagy apparatus can eliminate tau oligomers and higher order or insoluble aggregates. Dysfunction of these refolding and clearance systems can exacerbate tau pathology

tau toxicity [44]. In addition, inhibition of tau phosphorylation by kinase inhibitor K252a can prevent the movement impairment observed in tau moue models [128]. Conversely, the pseudo-hyperphosphorylated tau proteins (S198E, S199E, S202E, T231E, S235E, S396E, S404E, S409E and S413E, which mimic the hyperphosphorylation modifications in AD brains) can induce axonal abnormalities in *C. elegans* [40]. These mutations, as well as S422E, can also reduce the binding capability of tau and destabilize the MT network [129]. In addition, it has been shown that S396E and S404E tau show an increased propensity to form aggregates and fibrils [130], and these sites are exceedingly phosphorylated in AD [131, 132].

Among various kinases and phosphatases reportedly involved in AD-like tau hyperphosphorylation, MARK, GSK-3β, CDK5 and protein phosphatase-2A (PP2A) are the most important ones. MARK kinase is critical in initiating a phosphorylation cascade of tau [44]. It has been shown that MARK regulates tau phosphorylation in a cell culture model [133], and Par-1 (*Drosophila* homolog of MARK) contributes to tau toxicity in vivo by regulating Ser262 phosphorylation and may facilitate further phosphorylation events like the phosphorylation on Ser202 and Ser396/404 [44]. Phosphorylation by MARK dissociates tau from MT [82, 134], and consistent with this, MARK was found to be elevated in AD patients and co-deposited with NFTs [135].

GSK-3β is another key kinase that mediates phosphorylation at multiple sites of tau [136], and its expression is elevated in AD [137]. One of the sites receptive to GSK-3β phosphorylation is Thr231, and its phosphorylation state is an important factor in MT regulation [138]. In culture cells and animal models, GSK-3β expression enhances tau toxicity [139, 140]. Activation of GSK-3β inhibits long-term potentiation (LTP), while spatiotemporal inhibition of GSK-3β attenuates tau phosphorylation and protects LTP [141]. It has also been shown that inhibition of GSK-3β by lithium can rescue tau pathology [142, 143].

Besides MARK and GSK-3β, CDK5 is another important regulator of tau phosphorylation. CDK5 phosphorylates tau on Thr181, Thr231, Ser202 and Ser396/Ser404. CDK5 activation stimulates MT release of tau, as well as tau aggregation and NFT formation [144, 145]. In contrast to MARK, CDK5-dependent phosphorylation is not thought to be an initiating event in the phosphorylation cascade. The Ser396/Ser404 phosphorylations require prior modification on Ser262, a MARK phosphorylation site [44]. Activation of GSK-3β and inhibition of protein phosphatase 1 may be another aspect of CDK5′s functions [146, 147]. Importantly, both GSK-3β and CDK5 are vital mediators connecting β-amyloid toxicity with tau hyperphosphorylation [136, 148, 149].

Overall, the activities of tau phosphatases (PP1, PP2A and PP2B) are decreased in AD brains [150]. Among them, PP2A is an effective tau phosphatase that dephosphorylates tau on Ser199, Ser202, Thr205, Ser396 and Ser404 [151–153], and restores the normal activity of tau. Inhibition of PP2A by okadaic acid, homocysteine, or zinc further increase tau phosphorylation levels and toxicities [154–156]. In vivo, PP2A activity can be inhibited by I2PP2A (inhibitor 2 of PP2A), which is phosphorylated at Ser9 and accumulated in the cytoplasm, resulting in increased PP2A inhibition and tau hyperphosphorylation in AD brains [157]. Conversely, compounds that can activate PP2A, such as betaine and NMNAT2 (nicotinamide mononucleotide adenylyltransferase 2), can reduce tau hyperphosphorylation [158, 159]. The cross-talk that occurs between kinases and phosphatases can sometimes complicate analysis. For example, GSK-3β activation can inhibit PP2A by upregulating PTP1B (protein tyrosine phosphatase 1B), which can phosphorylate PP2A at Tyr307 [160, 161]. This mechanism suggests a potentially vicious cycle between activation of kinases and inhibition of phosphatases in AD brains.

### *Roles of metal ions in AD and tauopathy*

Metal elements such as zinc (Zn), copper (Cu) and iron (Fe) are indispensable in numerous fundamental biological processes. They can either function as the structural components of proteins or act as the critical co-factors for many enzymes [162]. Homeostasis of metal ions is important for the maintenance of normal cell functions, and dyshomeostasis can lead to various diseases states such as Wilson's disease, Menkes disease, and anemia [163]. The intricate balance of metal metabolism is regulated stringently by metal ion transporters, chaperons, and other metal ion homeostasis-related proteins [163]. In AD autopsy, ectopic accumulation of metal ions is found in the cortical and neuropil region, and co-deposits of metal ions with Aβ plaques have been described [164]. The expression level and distribution of metal-related genes like zinc ion transporters ZnT1 and ZnT4, and iron ion homeostasis-related genes like transferrin are correspondingly altered in AD [165–167], and their protein products are also found to co-exist in plaques or NFTs [168].

The detailed mechanism of metal ion dysregulation in AD and other neurodegenerative diseases is largely unclear. However, these observations strongly suggest that elevation or reduction of certain metal ions is relevant in these diseases. In vivo, the effects of metal ion regulation in AD were tested in mouse models [169–172], and it was found that chelator and genetic manipulations of metal homeostasis-related genes can reduce Aβ accumulation and lessen the symptoms. In vitro studies show that metal ions (Zn, Cu, Fe) can promote Aβ aggregation and fibrillation by directly binding with the peptide and changing its

biochemical properties [173–175]. Based on these results, it was proposed that decreasing metal ion concentration with the 8-OH quinolone class hydrophobic chelators Clioquinol (CQ) and its derivative PBT2 might be a promising treatment for AD. Indeed, treatment with these chelators in mouse models shows dramatic reduction of Aβ aggregation and restores the neural activity [169]. In fly Aβ models, genetic modulation of iron or zinc ion homeostasis also delays disease development [176, 177].

Changes in normal metal ion regulation are also found in tauopathies. NFTs bearing neurons are accompanied with expressional alterations of zinc ion or other metal ion transporters [166]. In addition, aluminum (Al) and iron (Fe) ions have been found to co-deposit with NFTs [178]. In most cases, the exact role of these metal ions in tau toxicity in many cases is still unknown. However, there is evidence that metal ions such as Fe, Cu and Zn ions can directly bind with tau proteins and induce protein aggregation in vitro [179–181]. Two cysteine (Cys) residues in the MT binding domain may mediate the interaction between tau and the above-mentioned metal ions (Fig. 1), while Cys to Ala changes could nearly eliminate the fibrillization induced by Zn and Cu ions in vitro [181]. In fly models, changes of Cys to Ala (C291A/C322A or tauC2A) strongly suppress tau toxicities and almost erase the zinc effect on tau [47], whereas changes back to zinc ion binding His residues (C291H or C322H) restore some of tau's toxicity and, importantly, zinc ion responsiveness. Based on our work, we found that zinc ion chelation, either through genetic or chemical chelating measures, is effective but does not deliver the dramatic effects achieved through zinc ion binding elimination as seen in tauC2A [47]. This discrepancy is likely due to the fact that metal ions are important for cell survival and their levels are strictly regulated; therefore, depleting metals to a negligible level without serious consequences is likely not feasible. tau binding to zinc ion occurs in the submicromolar range [47, 181], and it is hypothesized that zinc ion depletion by genetic interference or chelator treatments can only progress to a limited degree, one which cannot effectively remove zinc ion binding from tau. From this perspective, it is understandable that removal of zinc ion binding in tauC2A results in far less toxicity in the mutant tau compared to that achievable through zinc ion modulation.

In addition to direct tau binding, zinc ion has also been found to influence the phosphorylation process of tau by activating kinases, such as p70S6 kinase and Raf/mitogen-activated protein kinase, as well as inhibiting PP2A activity [155, 182–185]. Zinc ion's effect on phosphorylation, however, appears to be much less dramatic on tau toxicity as compared to its tau binding. Substitutions of two zinc ion binding Cys residues (tauC2A) greatly mitigate tau toxicity without significantly affecting the hyperphosphorylation status of tau. Furthermore, the tauC2A mutant is largely impervious to zinc ion alterations. It can be concluded that zinc ion contributes to tauopathy in two independent pathways: increasing tau phosphorylation and, more importantly, by directly binding with tau proteins [47]. Interestingly, copper ion's role in Huntington's disease has also been shown to be due to its physical binding to Huntingtin protein [186]. These studies reveal some clear and detailed mechanistic insight into the functional consequences of metal dyshomeostasis in certain neurodegenerative diseases.

Copper ion's effects on tau toxicity through CDK5 dysregulation have also been reported [187], suggesting the existence of additional pathways of metal toxicity in tauopathy. In tissue culture neurons and fly models, elevation of zinc and copper ions has been shown to greatly enhance tau pathology [47, 187]. Consistent with this, zinc ion treatment dramatically increases tau phosphorylation in a mouse model [188], yet CQ, a chelator of zinc ion and other divalent metal ions, reduces the phosphorylation of tau and neurodegenerations in the brain [47, 155].

*Molecular chaperons, tau clearance and tau toxicity*

Molecular chaperons play essential roles in degrading misfolded proteins [189]. In tauopathies, chaperons are also involved in tau toxicity regulation, although a full understanding of the underlining mechanism remains to be elucidated. Hsc70 and Hsp70 are two proteins that can regulate levels of tau species, including that of total tau, hyperphosphorylated tau and aggregated tau in both tissue culture cells and transgenic mice [190, 191]. Hsp70/Hsc70 cooperates with CHIP (carboxyl terminus of Hsc70 interacting protein) to ubiquitinate and degrade tau protein. Upregulation of Hsp70 can reduce the accumulation of insoluble tau and overexpression of CHIP highly increases tau ubiquitination and aggregation of insoluble tau in the COS-7 cell. Correspondingly, deletion of CHIP results in the decrease of Hsp70 mRNA and accumulation of phospho-tau protein [190, 192].

Hsp110 is one of the Hsp70/Hsc70-associated proteins, and Hsp110 KO mice develop severe accumulation of hyperphosphorylated tau and neurodegeneration phenotypes [193]. BAG-1 is another Hsp70/Hsc70-interacting protein that associates with tau pathology in vivo, and in AD mouse models high expression of BAG-1 always accompanies tau aggregation [194]. It has also been demonstrated that overexpressing BAG-1 up-regulates tau expression, while its knock-down decreases tau levels. This regulation may work through interfering with the 20S proteasome to slow down tau degradation [195]. All these pieces of evidence point to the existence of an important

protein recognition and degradation system around the Hsp70/Hsc70 complex for tau species.

Hsp90 is another regulatory protein known to work downstream of Hsp70/Hsc70, and tau, like the other target proteins, would be subsequently processed by Hsp90 [196, 197], after being targeted by Hsp70/Hsc70. However, Hsp90 as a regulator of tau fates is quite complicated [198]. While tau proteins with modifications of pS202/pT205 or pS396/pS404 and tau protein in the MC-1 conformation (a representative form in tauopathy recognized by MC-1 antibody [199]) are considered specific targets of Hsp90 [200], it is also thought that Hsp90 inhibition can promote degradation of several substrates, including hyperphosphorylated tau [197, 200, 201]. Recent studies suggest that inhibition of Hsp90 activates autophagy and proteasome pathways, thus promoting the degradation of tau or tau kinases [198, 202, 203]. Based on this evidence, inhibitors of Hsp90, such as EC102 and 17-AAG, have been developed that can pass the blood–brain barrier (BBB) and help to degrade tau proteins in the mouse model [197, 204, 205].

Two immunophilins, FKBP51 and FKBP52 (FK506-binding protein 51 and 52), are also reportedly involved in chaperon-mediated tau detoxification [206, 207], albeit in different ways. FKBP52 overexpression can prevent tau accumulation in tissue culture cells [206], whereas knocking it down via RNAi enhances tau pathology in *C. elegans* [208]. FKB51 may associate with Hsp90 and negatively modulate activity of 20S proteasome, preventing tau refolding and degradation. It has also been shown that increasing FKB51 levels exacerbated tau pathology in mouse models, while its depletion attenuated the symptoms. Interestingly, NFTs did not excessively form under FKB51 elevation, suggesting that other aggregated tau forms contributed more to the toxicity [209].

The ubiquitin–proteasome pathway and autophagy machinery constitute the main players in tau degradation [210]. In AD brains, tau monomers are removed by proteasomes and NFTs are removed through autophagy. However, it is difficult to determine which pathway deals with the oligomer, the most toxic form of tau (see review [210]). Under pathological conditions, tau aggregations like PHFs are highly ubiquitinated and co-deposit with proteasome components [211, 212]. The function of proteasomes, especially that of the 20S proteasome, is impaired in both AD patients and animal models [213]. Likewise, inhibition of proteasome activity by lactacystin significantly reduces tau degradation in cultured cells [214]. While the underlying mechanism for proteasome malfunction in AD is still largely unknown, evidence shows that phosphorylated tau protein may attenuate its activity, implying possible feedback inhibition [213, 215]. Furthermore, a trypsin-like but not chymotrypsin-like activity might be required for tau processing [216]. As the key E3 ubiquitin ligase for tau processing [210], CHIP connects the degradation pathway with the molecular chaperon function. Although the detailed mechanism remains unknown, a non-canonical, ubiquitin-independent degradation pathway is thought to be involved in tau clearance [214].

As mentioned previously, autophagy is another tau clearance pathway. Autophagosomes and autolysosomes are abundant in AD samples; nevertheless, autophagy activity is impaired, accompanied with lysosomal vesicle accumulation [217]. More importantly, trehalose, a natural alpha-linked disaccharide and stimulator of autophagy, can significantly decrease the levels of phosphorylated tau and abnormal tau accumulation in rodent models [218]. It has additionally been shown that in the P301S tau transgenic mouse, trehalose can lessen the filamentous inclusions in the cerebral cortex and brainstem, but not in the spinal cord. More experiments are needed to further explain this selectivity [219].

Additional evidence supports the involvement of autophagy in tau clearance. For instance, rapamycin can inhibit mTORC1 activity and in turn upregulate autophagy. In a P301S mouse model, rapamycin can encouragingly reduce most hallmarks of tau disease, including phosphorylated tau levels, NFT formation and locomotion defects [220, 221]. On the contrary, inhibition of autophagy through inhibitors such as 3-methylamphetamine (3-MA), or by genetic means such as removing ATG7 activity or upregulating mTORC1 activity, leads to a suppression of tau clearance [221–223]. Intriguingly, different subtypes of autophagy may target distinct tau species. Full-length tau is preferentially degraded by macroautophagy. However, certain truncated forms of tau proteins such as $tau_{RD}\Delta K280$ are processed through Hsc70-mediated autophagy (Chaperone-mediated autophagy, CMA) [222], indicating that the role of autophagy in regulating tau proteins is complex.

## Strategies for development of tauopathy treatments

No effective treatments or medicines exist to cure or prevent AD or tauopathies. Based on our current understanding of tauopathy, some clinical strategies directed to attack certain aspects of tauopathy are summarized here (Table 1). There is no question that better elucidation of the etiology of tau will lead to more effective treatment against tauopathy in the future.

### Microtubule stabilization

Paclitaxel (taxol), a mitotic inhibitor broadly used in cancer chemotherapy, can stabilize cellular microtubules and

**Table 1** Tauopathy treatment strategies under development

| Aspect related to tauopathy | Therapy strategies | Drugs |
|---|---|---|
| Binding and regulation of microtubules | Stabilize microtubules | Paclitaxel, epothilones D, BMS-241027, TPI-287, NAP (Davunetide), TMAO |
| Abnormal modification (phosphorylation, acetylation) | Reduce abnormal modifications on tau | Lithium, tideglusib, AZD1080, metformin, sphingoid, phenolic and anionic compounds |
| Heavy metals (Cu, Fe, Zn) | Regulate metal homeostasis | CQ, DP-109, PBT2 |
| Protein aggregation | Inhibit protein aggregation | Methylene blue, LMTX |
| Molecular chaperons, and tau clearance | Regulate tau protein level | Rapamycin, trehalose, IU1 |
| | Immunotherapies | PHF-1 antibody, tau peptide (tau379-408), phosphor-peptides (tau 195-213 (P-Ser202, 205), tau 207-220 [P-Thr212, Ser214), and tau 224-238 (P-Ser238)] |

partially reverse tau toxicities from MT disassembly. In tau transgenic mice, weekly injections of paclitaxel can help restore axon transport ability and ameliorate movement impairment [224]. Epothilones D, another MT stabilizer, is also able to re-establish axon transport and improve the cognitive performance of aged PS19 mice [225]. To improve the permeability of the BBB, new generation compounds such as BMS-241027 have been synthesized and shown to benefit tau mice and it is now undergoing Phase 1 clinical trials [226]. In addition to taxol derivatives (paclitaxel, BMS-241027, TPI-287), neuroprotective peptides such as Davunetide (NAP) can also protect MT in tauopathies [227, 228]. NAP has been evaluated in a Phase II/III clinical trial, but was reported to have failed in progressive supranuclear palsy (PSP) treatment [229, 230]. Unfortunately, compounds targeting MTs are beneficial when treating cancer and schizophrenia, but have not proved very effective towards tauopathy, suggesting human tauopathy is most likely a tau gain-of-function disease, and MTs' defects may not be the main contributor. Nevertheless, since MT stabilizers are beneficial to experimental subjects in vertebrate models and pre-clinical studies, it is still possible that under some circumstances these stabilizers may find use in supportive treatments for tauopathies.

### Reduction of abnormal tau modifications

Phosphorylation appears to be the most important post-translation modification of tau and several candidates have been developed to target tau phosphorylation. GSK3β is one of the most important kinases in tau pathogenesis and is one of the best targets currently under study [231, 232]. LiCl (lithium chloride) is a well-known GSK3β inhibitor, and in fly and mouse models, LiCl treatment significantly reduced levels of tau phosphorylation and aggregation, as well as axonal transportation [43, 233, 234].

It was also reported that long-term lithium treatment in certain human trials led to promising results, including reduction of CSF phosphorylated tau and better performance in cognitive tests [235, 236]. However, other clinical reports found LiCl failed to improve the cognition of patients, or to reduce the hyperphosphorylation of tau [237, 238]. Therefore, additional carefully designed long-term studies are needed to investigate and clarify the effects of LiCl. Another GSK3β inhibitor, Tideglusib, can effectively reduce tau toxicity and pathology, and improve memory in mice [239], and is now in Phase II trial [240, 241]. The potency of Tideglusib in humans remains an area of debate. A double blind Phase II study suggested that it failed in AD and PSP patients [240, 241]; however, a recent report from trial investigators claimed that Tideglusib can reduce patient brain atrophy rate as monitored by MRI [230] (http://www.noscira.com/media/docs/Nota_Prensa20072012_en.pdf). AZD1080 is another GSK3β inhibitor that is permeable to the BBB. In the pre-clinical test, this compound significantly attenuated GSK3β activity [242]. In addition to GSK3β, other kinases such as CDK5, MARK and Fyn are considered potential targets as well [243, 244].

Alternatively, augmenting the activities of tau phosphatases can reduce tau phosphorylation. Sodium selenate ($Na_2SeO_4$) reduces hyperphosphorylation, abrogates NFTs of tau and relieves symptoms in tau mice by activating PP2A [245]. Another PP2A activator, biguanide metformin, formerly used as an anti-diabetic drug, also decreases tau phosphorylation [246]. PP2A activators generally belong to sphingoid, phenolic and anionic classes, so more candidates from these categories may possibly be tested in future studies [247]. It is worthwhile to point out that targeting PP2A to compromise tau toxicity has potential limitations. Due to the broad range of PP2A substrates [248], using PP2A as a drug target should be very carefully conceived, taking activator specificity and

safety into consideration (for more discussion of this see review by Jeffry [247]). One solution is to modulate specific PP2A components or regulatory partners such as inhibitors 1 or 2 of PP2A [249], which are less universally involved in other biological processes. Drugs such as Memantine have been shown to effectively block tau phosphorylation by modulating I2PP2A [250].

### Regulation of metal ion homeostasis and/or tau aggregation

Aggregate forms of tau are considered toxic to neurons and greatly contribute to tau pathology [72]. For this reason, compounds that block protein aggregation may qualify as potential candidates to treat tauopathies [251]. Metal ions are often inducers of protein aggregation and one important factor for zinc ion's aggravating effect on tau is through its direct binding to the protein. Data from animal models suggest that genetic manipulation of metal ion homeostasis can significantly moderate AD pathogenesis and tauopathy. Metal ion chelation also enhances β-amyloid solubility in AD tissues. In mouse model studies, metal ion chelator clioquinol (iodochlorhydroxyquin) [252] and DP-109 (1,2-bis(2-aminophenyloxy)ethane-*N*,*N*,*N*′,*N*′-bis(2-octadecyloxyethyl)ester, *N*,*N*′-disodium salt) can reduce Aβ toxicity and levels of insoluble Aβ aggregation as well as plaque formation [169, 171]. In addition, an improved analog of clioquinol, PBT2, shows more effective rescue of AD symptoms in mouse models [253–255]. Although PBT2 and CQ acting as ionophores or metal chaperones are also proposed [256], the data strongly indicate that reuse and redistribution of metal ions are critical to AD progression. A small AD clinical study suggested clioquinol may be somewhat effective in treating the disease [257]; however, some of the results were also the subject of debate [258]. More encouragingly, PBT2 performs better in clinical trials, showing evidence of CSF Aβ reduction and improved cognition in patients [259, 260].

Because metal ions are absolutely necessary for cell survival and their homeostasis is strictly regulated, their universal removal would unlikely be achievable in vivo without severe consequences. An ideal strategy would be to target the key metal ions involved in neurodegenerations through more complete chelation of metal ions specifically from relevant pathologic proteins. However, this strategy remains a challenge as there is currently no method to specifically prevent or interfere with the binding of metal ions to tau without also affecting their binding to the other proteins. Alternatively, for practical purpose, targeting metal ion levels in relevant pathologic tissues may be considered.

Other compounds have also been shown to be effective in reducing tau aggregation. Methylene blue (methylthioninium chloride), a heterocyclic aromatic chemical compound, effectively retards the aggregation process by directly inserting into β-sheet structures in vitro [261]. In vivo, methylene blue improves cognition in tau mouse models [112], and is now in phase II trial evaluations [262]. LMTX, a derivative of methylene blue, is currently in a phase III clinical trials and might be an even more effective remedy for AD [263]. Since β-sheet conformation is not unique for aggregated Aβ and tau, but is also observed in other proteinopathies, it is hypothesized that methylene blue and LMTX might also be effective therapies in other protein aggregation diseases.

### Regulation of tau protein level and/or immunotherapy

Because the tau protein itself is considered the central cause of pathogenesis in tauopathies [264–267], regulating tau proteins might be the most direct and efficient way to ameliorate the disease symptoms. As previously discussed, reducing tau protein levels can mitigate the severity of neuropathology and neurodegeneration in AD mouse models [268–270]. One possible approach to regulating tau levels is through changing mRNA stability or translation efficiency either via siRNA interference or pharmacologic compounds [271]; another approach to depress tau levels is through increasing protein degradation by stimulating the autophagy and/or proteasome pathway. It has been shown that autophagy activation with the use of rapamycin and trehalose significantly reduces tau levels [218, 220]. Activating the proteasome to eliminate pathological tau proteins constitutes an alternative to autophagy. IU1 (inhibitor of USP14) has been shown to enhance proteasome (20S) activity and promote tau degradation in tissue culture cells [272].

Immunotherapies which target key proteins involved in the pathogenesis of neurodegenerative diseases are another method of approach. The concept of using immunotherapy against AD was first tested in an APP transgenic rodent model. β-Amyloid-immunized young mice developed plaques and neurodegeneration at a much slower rate than the control group [273]. Additional studies showed that both active and passive immunization could benefit the disease model by reducing Aβ levels [274–276]. Based on these results, clinical trials of β-amyloid Immunotherapy have been performed in human subjects. Although there are concerns regarding the safety of the treatment and uncertainties with the reproducibility, some patients receiving AN1972 (a synthetic full-length β-amyloid peptide with QS-21 adjuvant) have been reported to be absent of plaques after treatment [277].

Immunotherapies aimed at tau proteins have also been explored. A peptide (tau 379–408 aa) immunization in

P301L transgenic mice can dramatically reduce tau aggregation and slow the pathogenesis [278]. Additional tau peptide fragments, some with phosphorylation residues (tau 195–213 aa with pS202/pS205; tau 207–220 aa with pT212/pS214; tau 224–238 aa with pS238), have also been tested in animal models [279, 280]. Promisingly, several of these show reduction of tau aggregation and restore cognitive abilities in test animals. Passive immunization which targets PHF-1 by injecting anti-PHF-1 IgG (recognizing a PHF-1 epitope with two phosphorylation sites pS396/pS404) also significantly reduces tau toxicities in mouse models [281]. Although a number of successful cases have been reported, mechanistically it is more difficult to understand how immunotherapy works against tau, since, unlike extracellular Aβ plaques, tau is mostly an intracellular component.

## Conclusive remarks

Great strides have been made towards understanding the mystery of tau toxicities. In general, it is thought that hyperphosphorylation on tau can lead to the downstream toxic effects, which include "loss of function", such as loss of microtubule binding, and "gain of toxicity", such as formation of tau oligomers and aggregates (refer to Fig. 2). The exact mechanism of tau toxicity might be the result of the combination of these two events. Phosphorylation of tau is by itself a highly complex process, regulated by various kinases and phosphatases at multiple positions (refer to Fig. 1). Some phosphorylation events are key to tau toxicity while some others are less important and some may even play protective roles.

Besides phosphorylation, other protein modifications, such as glycosylation and ubiquitination, metal ions, and protein degradation systems, can all influence tau toxicity and disease progression. This level of complexity hinders a rapid and concrete understanding of tau toxicity and impedes the progress of drug development.

In the past few years, models for tau studies have expanded. In addition to the use of traditional cell culture, and in vivo models of fruit flies, worms and mice, other mammalian models have also been developed including rat and primate monkey models. This expanding repertoire of models may better facilitate our understanding of tauopathy.

Though the number of aged people and the people who suffer the risk of severe neurodegenerative diseases such as AD are rising, effective drugs and therapies for these diseases are still lacking. Currently, various drug candidates against different individual aspects of tau toxicity have been developed or are under active development. The complexity of tauopathy also suggests the consideration of a combination strategy to block tau toxicity, such as blockage of tau aggregation and promotion of its clearance, coupled with regeneration of the neurons in the pathological lesions. To obtain more effective therapies, it is imperative to acquire a better understanding of tauopathy. This no doubt requires finding potential connections between known impact factors and some other lesser known elements. Clarifying their roles on tau toxicity as well as the relative importance of these factors' contributions to tauopathy needs to be further established. Investigations need also to be carried out to understand the underlying mechanism of how different mutations of tau can lead to distinct neuropathologies. A better understanding of tau etiology will undoubtedly lead to improved therapies in the future.

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