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Development of tau phosphorylation-targeting therapies for the treatment of neurodegenerative diseases

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ABSTRACT

Hyperphosphorylation of microtubule-associated protein tau is a major driver in the etiology of multiple neurodegenerative diseases, such as Alzheimer's disease (AD) and other tauopathies. Intracellular accumulation of hyperphosphorylated tau (pTau) decreases microtubule stability, induces protein aggregation, and impairs neuronal plasticity. Increasing attention has been devoted to the development of targeted therapies for modulating tau phosphorylation, including conventional protein kinase inhibitors, phosphatase activators, immunotherapies, as well as a new collection of tau-targeted hetero-bifunctional chimeras such as dephosphorylation-targeting chimeras (DEPTACs), proteolysis targeting chimeras (PROTACs) for pTau, phosphorylation targeting chimeras (phosTACs), and affinity-directed phosphatase (AdPhosphatase) system. In this review, we briefly introduce tau and its role in neurodegenerative diseases, provide progress in the development of pTau targeting therapies, and discuss their advantages and limitations.

1. Introduction

Hyperphosphorylation of tau initiates the intracellular formation of neurofibrillary tangles, a hallmark of a collection of neurodegenerative diseases named tauopathies, including Alzheimer's disease (AD), frontotemporal dementia (FTD), Pick's disease, multiple system atrophy, etc. Therefore, downregulation or removal of hyperphosphorylated tau (pTau) holds promise for the therapy of these diseases.[1–3]

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However, there remains a great challenge in the development of pTau-targeted drugs. For example, direct application of either tau kinase inhibitors or phosphatase activators may induce unacceptable toxic side effects, because the majority of these enzymes concurrently modulate numerous signaling pathways other than tau.[4–6] Another way to eliminate pTau is immunotherapy, which employs tau-targeted antibodies to specifically facilitate tau degradation.[2,7–10] Although these antibody drugs have shown moderate efficacy for alleviating cognitive impairment in AD patients, they are usually high-cost and it is generally difficult for antibodies to penetrate into the cells to bind tau. A new kind of hetero-bifunctional molecule, namely targeting chimera, has attracted increasing attention in drug discovery in recent years for its ability to recognize and change the property of a certain protein of interest (POI), typically by enhancing the proximity between the POI and a specific effector,[11–19] such as ubiquitin ligases for proteolysis targeting chimeras (PROTACs),[11,12] and autophagosome protein LC3 for autophagy-tethering compounds (ATTECs).[13,14] Several pTau targeting TACs have been developed in recent years, including dephosphorylation-targeting chimeras (DEPTACs),[20–22] proteolysis targeting chimeras (PROTACs) for pTau,[23–25] phosphorylation targeting chimeras (phosTACs),[26] and affinity-directed phosphatase (AdPhosphatases) system.[27]

In this review, we give a brief overview of pTau pathology in neurodegenerative diseases. Then, we mainly discuss the conventional pTau targeting small-molecular drugs and immunotherapies, as well as newly appeared pTau-targeting chimeras.

2. Tau and neurodegenerative diseases

2.1. Tau protein

Human tau is encoded by the *MAPT* gene, consisting of 16 exons on chromosome 17q21.[28] Alternative splicing of exons E2, E3, and E10 produces six human tau isoforms with different lengths, including 0N3R (352 aa), 1N3R (381 aa), 2N3R (410 aa), 0N4R (383 aa), 1N4R (412 aa), and 2N4R (441 aa)[29] (Fig. 1A). The additional repeat domain R2 provides 4R tau which has a higher affinity to microtubules than 3R tau, therefore, more efficiently promotes microtubule assembly.[30]

The longest 2N4R tau protein isoform contains N-terminus, a proline-rich region (PRD), a microtubule-binding domain (MTBD) with four repeat regions (R1–R4), and C-terminus[31] (Fig. 1A). The N-terminus may regulate the subcellular localization of tau for its ability to interact with membrane-binding protein annexin 2,[32] and influence the attachment or spacing of microtubules with other cell components.[33] The PRD of tau includes seven PxxP motifs, which share a common feature with Src homology-3 (SH-3) ligands, indicating that tau may be involved in intraneuronal signal transduction.[33]

The MTBD helps tau bind to and stabilize microtubules through its conserved KXGS motifs in each repeated region (R1–R4) and the electrostatic interaction between tau's positively charged Lys residues and microtubules's negatively charged residues.[34] Most missense and intronic mutations present in tauopathies such as G272V, P301L, P301S, and V337M were all located in the MTBD of tau. These mutations decrease the affinity between tau and microtubules.[35] The MTBD is also an important area mediating the aggregation between tau proteins by forming the core of the PHFs.[36] Truncated tau containing only the MTBD, like K18, K19 and K32 fragments, can also induce tau aggregations and seedings (Fig. 1B). Moreover, two hexapeptide motifs located at the R2 and R3 repeat domain of tau, namely PHF6*($_{275}$VQIINK$_{280}$) and PHF6 ($_{306}$VQIVYK$_{311}$), have been also identified as one of the main drivers of tau aggregation for their high propensity to form β-sheet structures and stack into steric zippers[37,38] (Fig. 1C). Lastly, the function of C-terminal region in tau proteins is still unclear, but emerging evidences suggest that it impedes tau polymerization along with the N-domain.[39,40]

Normal tau is natively unfolded or intrinsically disordered. Under normal conditions, tau protein forms an ordered structure or a paperclip-like shape when it binds to partner proteins like tubulin. The development of the paperclip structure might keep tau from self-aggregation, while disruption of this structure such as abnormal tau modification or truncation might promote the formation of tau fibrils.[41]

2.2. Physiological function of tau

Tau plays an essential role in a variety of cellular processes, such as in the regulation of microtubule dynamics,[42] axon transport,[42,43] axonal elongation and neurite formation,[44] autophagy activity,[45] synaptic plasticity,[46] glycogen synthase kinase-3β (GSK3β) upregulation,[47] neuronal activity,[48–50] neurogenesis,[51] long-term depression (LTD),[52] network hyperexcitability,[53] and finally cognitive dysfunction.[48–50,54] These physiological roles of tau have been well summarized in recent reviews,[55,56] we here mainly focus on the pathological role and drug development of tau hyperphosphorylation.

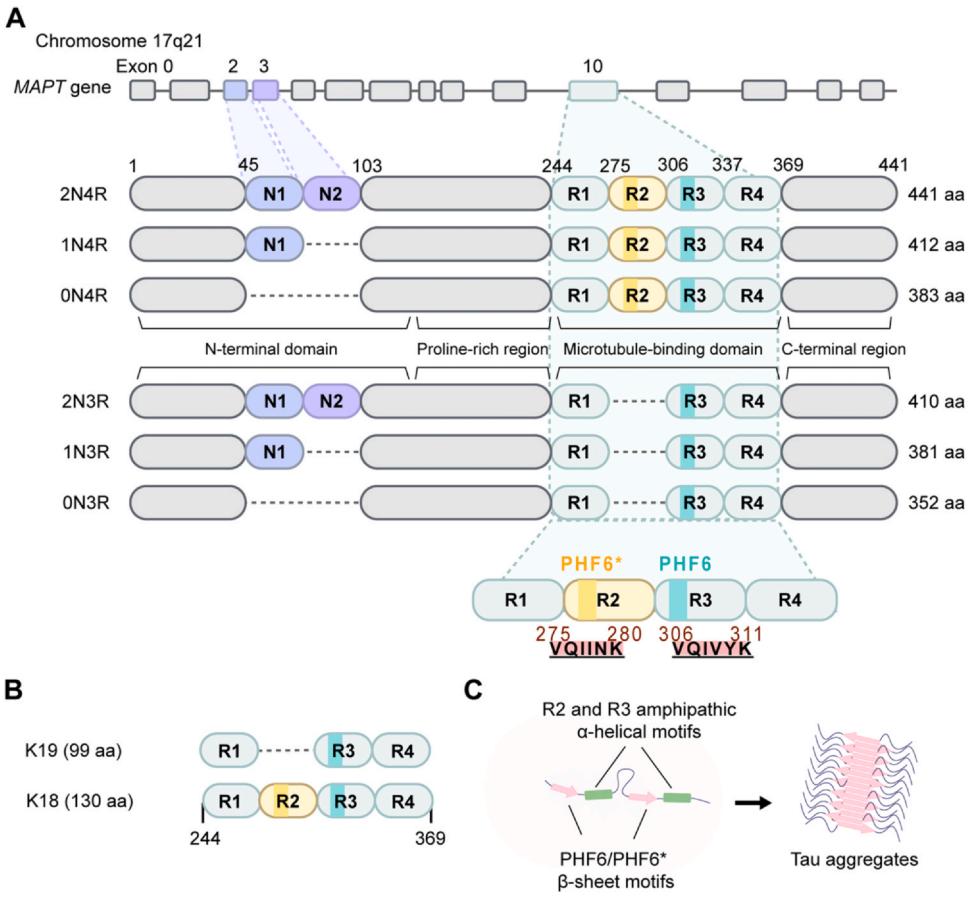

Fig. 1. Structural organization of tau isoforms. (A) Tau can be divided into an N-terminal domain, a proline-rich region (PRD), a microtubule-binding domain (MTBD) with four repeat regions (R1–R4), and a C-terminal domain. Alternative splicing of exons E2, E3, and E10 of the human *Mapt* gene mRNA generates six tau isoforms containing either 0, 1, or 2 N-terminal inserts (0N, 1N, and 2N) and 3 or 4 MTBR (3R and 4R), with lengths of 352 to 441 amino acids. The two hexapeptide motifs, PHF6*($_{275}$VQIINK$_{280}$) and PHF6 ($_{306}$VQIVYK$_{311}$), located respectively at the R2 and R3 repeat of the MTBD are the main drivers of tau aggregation and PHF formation. (B) K18, and K19 isoforms of truncated tau containing PHF6* and PHF6 in the MTBD are prone to be seeded for the formation of tau fibrils. (C) PHF6* and PHF6 promote tau aggregations by stacking into steric "zippers" with their β-sheet structures.

2.3. Tau pathology and targeting therapies

Tau shows different kinds of post-translational modifications[57] or aggregation forms[58] in different tauopathies. Various anti-tau therapeutics can be broadly classified into multiple mechanistic categories[7]: (1) Antisense oligonucleotides (ASOs), aiming to reduce tau level; (2) Enzyme regulators targeting posttranslational modifications (PTMs), such as acetylation (A), phosphorylation (P); (3) Tau aggregation inhibitors, such as methylene blue derivatives; (4) Immunotherapies (vaccines, anti-tau monoclonal antibodies) target intracellular and extracellular tau; (5) Microtubule stabilizers. The progress of clinical trials of these tau therapies has been summarized in Table 1.

Among these therapies, tau immunotherapies show great promise in addressing AD-related tau pathologies specifically. The tau targeting therapies may be ineffective for FTD because the FTD not only contains tau pathology but also includes TAR DNA binding protein-43 or fused in sarcoma pathology.[71]

2.3.1. Antisense oligonucleotides (ASOs)

ASOs, designed to reduce tau expression by targeting tau mRNA for degradation, represent another promising therapeutic approach. One notable example is BIIB080, which has shown promising results in early-phase trials. BIIB080 demonstrated a reduction of cerebrospinal fluid (CSF) pTau181 by up to 50% in its Phase 1 trial, and it is currently enrolling participants for its Phase 2 trial, with anticipated completion by 2027.[60] Additionally, NIO752, another ASO, is being explored in Phase 1 trials for its potential application in PSP and mild AD.[68] These trials are expected to provide crucial insights into the therapeutic potential of ASOs in targeting tauopathies.

Table 1
Current clinical trial stages of tau-targeting drugs.

Phase	Agent	Mechanism	Clinical trial	Start date	Estimated primary completion date	Reference
Phase 3	E2814	Passive immunotherapy	NCT01760005	Dec 2012	October 2027	[59]
			NCT05269394	Dec 2021	July 2027	
Phase 2	BIIB080	ASOs	NCT05399888	Aug 2022	November 2027	[60]
	LY3372689	O-GlcNAcase enzyme inhibitor	NCT05063539	Sep 2021	July 2024	[61]
	Methylene blue	Tau aggregation inhibitor	NCT02380573	Jul 2015	April 2022	[62]
	AADvac1	Active immunotherapy	NCT02579252	Mar 2016	June 2019	
	ACI-35	Active immunotherapy	NCT04445831	Jul 2019	September 2023	[63]
	Bepranemab	Passive immunotherapy	NCT04867616	Jun 2021	May 2024	[64]
	JNJ-63733657	Passive immunotherapy	NCT04619420	Jan 2021	March 2025	[65]
Phase 1	APNmAb005	Passive immunotherapy	NCT05344989	May 2022	March 2024	[66]
	MK-2214	Passive immunotherapy	NCT05466422	Sep 2022	May 2025	[67]
	NIO752	ASOs	NCT05469360	Feb 2023	October 2024	[68,69]
	OLX-07010	Tau aggregation inhibitor	NCT05696483	Jan 2023	December 2024	[70]

The above data come from ClinicalTrials.gov. ASOs, antisense oligonucleotides.

2.3.2. *Enzyme regulators targeting posttranslational modifications (PTMs)*

Therapeutic strategies targeting the enzymes involved in tau phosphorylation and acetylation have been explored, but with varying degrees of success. GSK-3β inhibitors, including lithium, valproate, and tideglusib, have aimed to mitigate tau hyperphosphorylation, yet their clinical outcomes have been underwhelming. Lithium initially showed promise in reducing the course of moderate cognitive impairment in patients., but its use in progressive supranuclear palsy (PSP) was discontinued due to significant side effects.[72] Similarly, valproate did not demonstrate efficacy in treating PSP,[73] and tideglusib, despite not showing significant benefits in AD and PSP, is still under investigation for other conditions such as amyotrophic lateral sclerosis (ALS) and myotonic dystrophy.[74]

In addition to GSK-3β inhibitors, other enzyme-targeting therapies include Fyn kinase inhibitors and O-GlcNAcase (OGA) inhibitors. AZD0530 (Saracatinib),[75] a Fyn kinase inhibitor, was repurposed for AD but showed no significant benefit and had notable gastrointestinal side effects. OGA inhibitors like LY3372689, currently in a Phase II trial for early AD (NCT05063539), are being investigated for their potential to reduce tau phosphorylation and aggregation by increasing tau O-GlcNAcylation.[61] Furthermore, acetylation inhibitors such as salsalate have been explored, but early-phase trials indicated poor tolerance in patients with PSP and AD, limiting their potential as viable therapeutic options.[76]

2.3.3. *Tau aggregation inhibitors*

Many efforts to inhibit tau aggregation have been pursued, most notably with TRx0237 (methylene blue). Despite being advanced to several phase III trials (NCT02380573), TRx0237 failed to produce positive outcomes.[77] Another tau aggregation inhibitor, OLX-07010, is currently in phase I trials (NCT05696483), representing a continued interest in this therapeutic approach despite past challenges.[70]

2.3.4. *Immunotherapies*

Immunotherapy includes active or passive immunotherapies which both show some progress through advanced clinical phases. Active immunotherapies, such as AADvac1[78] and ACI-35,[63] are vaccines designed to target N-terminal truncated tau fragments or p-tau396/404 epitopes. Both of them have entered phase II trials.

Passive immunotherapies have focused on humanized antibodies targeting various tau epitopes. Tau-specific antibodies are engineered to target pathological tau species, which include hyperphosphorylated tau, conformation-changed tau, or aggregated tau (such as neurofibrillary tangles). The antibodies can enter neurons through various mechanisms, such as binding to surface receptors and then entering in a clathrin-dependent manner,[79,80] via non-specific bulk endocytosis,[81–83] following disruption of the endosomal membrane or through translocation.[83–86] Once they enter the cells, they can specifically bind to intracellular pathological tau while sparing normal tau.

The binding of tau antibodies to tau proteins facilitates several key processes: (1) Antibody-mediated immunogenic clearance: Once bound, the tau-antibody complexes are recognized by the body's immune system, leading to the recruitment of microglia and other phagocytic cells that can engulf and degrade the tau-antibody complexes.[87–89] (2) Enhancement of proteolytic degradation: Antibodies can promote the degradation of tau through proteolytic pathways, such as the ubiquitin-proteasome system or autophagy-lysosome pathway. Once in the cytoplasm, antibody-bound tau can bind to tripartite-motif protein 21 and be ubiquitinated for proteasomal breakdown.[90] The binding of antibodies to tau may alter its conformation in a way that makes it more susceptible to proteolytic cleavage. By

binding to tau, the antibodies can facilitate the inclusion of tau into autophagosomes, which are then directed to lysosomes for degradation. The antibody-tau complex can also induce FcγRII/III-dependent endolysosomal degradation of tau.[91] By binding to endosomal tau, antibodies could stop tau-induced endosomal membrane disruption and help tau aggregates dissociate, thereby facilitating lysosomal enzyme access for digestion.[83,85,86] (3) Reduction of Tau seeding and propagation: The establishment of tau-antibody complexes blocked nearby neurons from absorbing the tau seeds and thus dampen pathological tau seeding.[92,93] In this way, the tau aggregates become smaller and easier to be cleared by the enter endosomal-lysosomal and proteasomal system.[79,81,94]

Antibodies targeting the proline-rich domain and microtubule-binding region (MTBR), including bepranemab,[64] E2814,[59] JNJ-63733657,[65] and APNmAb005,[66] are currently in various stages of clinical trials. E2814 has advanced to phase 3 trials, which are expected to be completed by 2027.[59]

2.3.5. Microtubule stabilizers

The therapeutic targeting of tau's role in microtubule stability has been explored through agents like davunetide,[95] and abeotaxane.[96] These agents were designed to stabilize microtubules, which are disrupted in tauopathies. However, they failed to demonstrate efficacy in clinical trials and were sometimes associated with severe side effects. For example, davunetide did not meet clinical endpoints in their respective trials, while abeotaxane caused significant adverse reactions in patients.[7–9]

3. Tau phosphorylation pathology

3.1. Tau phosphorylation

There are 85 potential phosphorylation sites including 45 serine residuals, 35 threonine residuals, and 5 tyrosine residuals within the longest tau 2N4R isoform, mostly found in PRDs (residues 172–251, approximately 22 of the 93 amino acids in the PRD are phosphorylatable) and the C-terminal tail region (residues 368–441).[29,33] Of these phosphorylation sites, around 45 have been observed experimentally.[97]

The phosphorylation pattern of tau shows temporal heterogeneity during the progression of neurodegenerative diseases. For example, in the brain of AD patients, tau phosphorylation at Thr231 increased significantly at Braak stages III–IV, and pTau at other residues like Ser199, Ser202/Thr205, and Ser422 increased only at Braak stages V–VI.[98] For pTau Ser396, it seems to be upregulated throughout the early and late stages in tauopathies.[99,100] It should be noted that the phosphorylation of tau at specific sites might happen in a stepwise way. For instance, Ser396 of tau tends to be phosphorylated after pre-phosphorylation of Ser404 and Ser400,[101] and prior phosphorylation of tau at Thr231 facilitates the subsequent phosphorylation at Ser235.[102] It remains to make clear the occurring sequence and mutual dependence of tau phosphorylation at different sites, which is important for the understanding of tau-related pathological mechanisms, as well as for identifying biomarkers for early diagnosis, like the recently identified CSF pTau 231, CSF, and plasma pTau 181 or pTau 217.[103–106]

Tau hyperphosphorylation also shows heterogeneity in function.[107,108] Although most pTau sites are reported to be deleterious, some phosphorylation sites are also reported to be protective. For instance, pTau at Ser198,[109] Ser199,[109] Ser202,[109] Ser214,[110] Ser262,[110] Ser305,[111] Ser400,[109] Thr403,[109] and Ser404[109] have been found to inhibit the aggregation or lower the seeding efficiency of tau. Notably, the exact effect of pTau may change according to different disease stages. For example, pTau Thr205 at the post-synaptic initially increases to protect neurons in AD[112] and reduce N-methyl-D-aspartic acid receptor-mediated Aβ excitotoxic,[112,113] but when the disease progresses, pTau Thr205 dissociates from the post-synaptic button and becomes a substrate for other tau kinases, which eventually exacerbates tau phosphorylation and related pathologies.[114] We have also found that pTau at multiple residuals promotes neurons to survive apoptotic attacks, while these neurons with tau hyperphosphorylation show at later stages slow but progressive retrograde degeneration.[108] The outcome of tau phosphorylation might depend on the number of phosphorylation residuals, namely, moderate phosphorylation of tau exerts protective roles by such as activating proteasome to clear toxic substrates, while excessive tau phosphorylation in turn inhibits the protease activity and leads to accumulation of pTau and other proteins.[107,108] Therefore, pTau shows a mutual face based on different phosphorylation sites, event stages, and levels. The imbalance between the protective and deleterious effects finally results in the happening of tauopathies.

3.2. Pathological role of tau hyperphosphorylation

Hyperphosphorylation of tau may induce a variety of pathologies (Fig. 2). Excessive phosphorylation of tau may change its conformation to show higher tendency of aggregation. For example, tau phosphorylation at the Thr231 can induce its transformation from the physiological trans isomer into a pathological cis isomer,[115] which has a higher

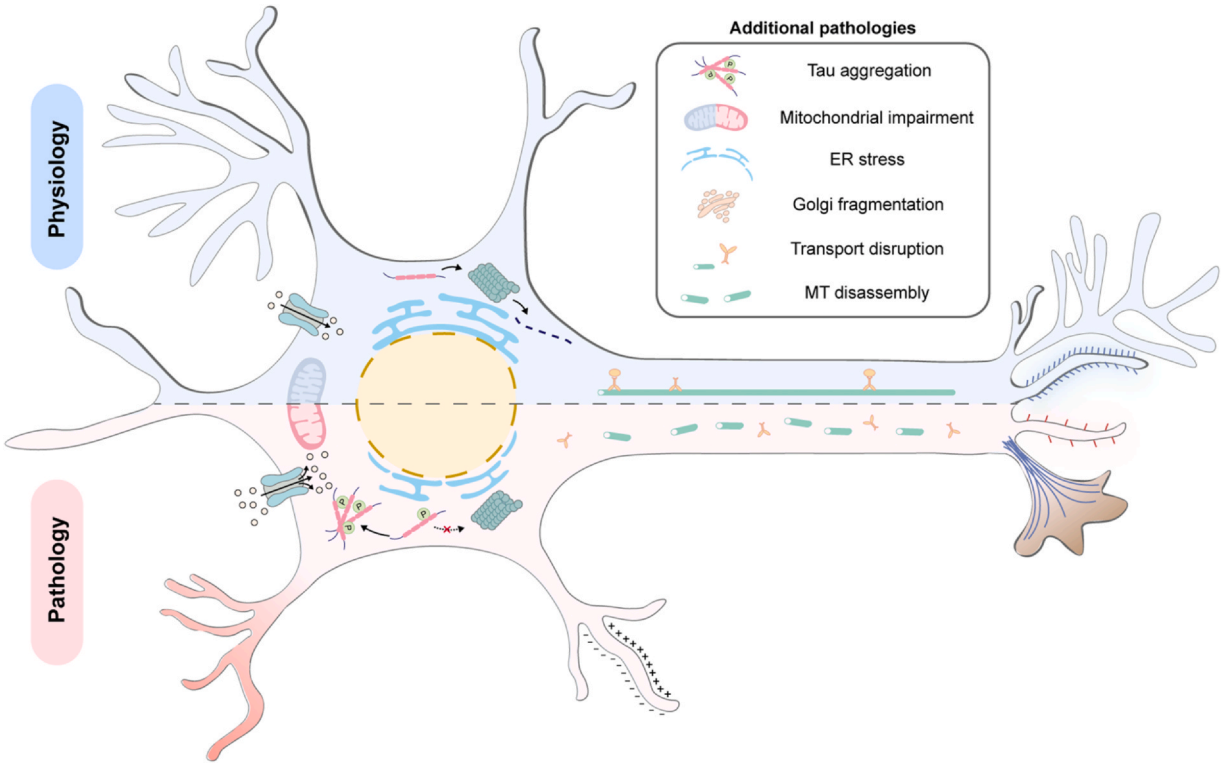

Fig. 2. Pathological mechanisms of the tau phosphorylation. Normal tau mainly distributes in axons and regulates microtubule stability, axonal transportation, and cellular homeostasis. Hyperphosphorylation of tau induces a series of pathologies, including microtubule disassembly, axon transport disruption, tau aggregation, mitochondrial impairment, ER stress, proteolysis deficit, etc. MT, microtubule.

tendency to accumulate. Besides, hyperphosphorylated tau, such as Ser214[97] and Thr231.[97] Ser235,[102] Ser262,[116] and 356[116] epitopes significantly reduce their affinity with α and β tubulins, which subsequently causes microtubule disassembly and a series of downstream pathologies such as impairments in axon transport.[117]

Moreover, tau detached from the microtubules would mislocalize to the cell body and dendrites, which can cause a chain of downstream reactions.[114] Hyperphosphorylation at many sites like Ser202/Thr205,[118,119] Thr231/Ser235, Ser262/Ser356, and Ser396/Ser404[120] on tau all promote its abnormal distribution in neuronal somatodendritic compartments, which may reduce glutamate receptor trafficking and result in synaptic dysfunctions,[121] as well as enhance protein interacting with C-kinase 1-mediated α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor downregulation during LTD.[122]

The aggregated tau caused by hyperphosphorylation is highly harmful to neurons by facilitating microtubule "clogging",[42] axonal transport disruption,[42,43] synaptic dysfunction,[123] mitochondrial impairment and mitophagy deficit,[124] autophagy flux repression,[125] endoplasmic reticulum stress,[126,127] Golgi fragment,[128] microglia activation and causing inflammation,[129,130] impairing γ-aminobutyric acidergic transmission and impeding adult hippocampal neurogenesis.[51] Specific phosphorylation sites making tau more prone to aggregate include Thr175,[131] Ser199,[132] Ser202,[132,133] Thr205,[132,133] Thr212,[134] Thr217,[123] Ser262,[134] Ser396,[132] Ser422,[135] Ser404,[132] combined Thr231/Ser235,[109] triple phosphorylation at Ser202/Thr205/Ser208,[136,137] etc.

4. Conventional pTau targeting therapies

An increasing number of strategies have been aimed at inhibiting tau hyperphosphorylation for the treatment of tauopathies, including activating protein phosphatases and inhibiting protein kinases. Other conventional tau-targeted therapies contain active or passive immunization to pathological tau species (Fig. 3). Here, we give a brief introduction to these conventional therapies, more information can be found in related reviews.[4–6,2,7–10]

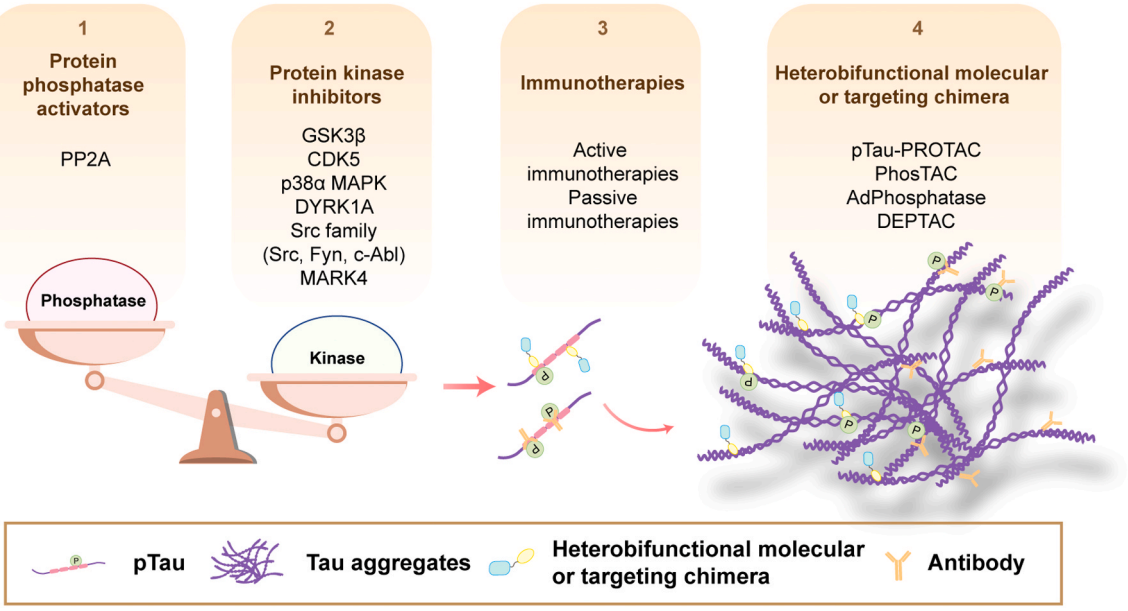

Fig. 3. Phosphorylated tau-targeting therapies. Conventional small-molecule drugs for reducing pTau include phosphatase activators of PP2A, and kinase inhibitors of GSK3β, CDK5, etc., as well as active or passive immunotherapies targeting pTau. Following DEPTAC, a series of heterobifunctional targeting chimeras for pTau including pTau-PROTACs, PhosTACs, and AdPhosphatase system have been developed. These targeting chimeras can reduce pTau and hold potential for the treatment of tauopathies. P, phosphorylation.

4.1. Protein phosphatase activators

The protein phosphatases (PPs) that have been implicated in tau dephosphorylation include PP1, PP2A, PP2B, PP2C, and PP5,[138] among which PP2A is the most active phosphatase for tau in the brain: It makes up around 70% of the tau phosphatase activity in the normal brain, but in the AD brain, its activity is decreased by about 20% in the grey matter and 40% in the white matter.[138] A specific PP2A activator named sodium selenate has shown reasonable effectiveness in reducing tau phosphorylation in animal models,[139,140] however, merely moderate benefits have been observed in clinical trials.[141]

4.2. Protein kinase inhibitors

Identified tau kinases to date include GSK-3β, Ca^{2+}/calmodulin-dependent protein kinase II, cyclin-dependent kinase 2 (CDK2), CDK5, protein kinase A (PKA), PKB (also named AKT), PKC, microtubule-affinity regulating kinase and tyrosine kinases including Src family kinases Src, Fyn, and c-Abl.[142] Among these kinases, GSK-3β can phosphorylate tau at most of the known AD sites (more than 30 phosphorylation sites), so it is currently a main target of drug discovery of tau kinase inhibitors.[143] A classic GSK-3β inhibitor lithium chloride has been found to produce beneficial cognitive effects in AD mouse models but again showed merely moderate beneficial effects in most clinical trials.[144,145]

4.3. Immunotherapies

Currently, the majority of tau-targeting drugs tested in clinical trials are immunotherapies. Active immunotherapies use synthetic peptides based on human p-tau as vaccines, such as ACI-35, a liposome-based tau fragment containing phosphorylation at the Ser396/404 epitope.[63] This strategy has the benefits of being low-cost, promoting a polyclonal antibody response, and having long-lasting efficacy. The disadvantage is potential irreversible adverse immune responses and antibodies generated from the tau vaccine might not target optimal phosphorylation epitopes.

By contrast, passive immunotherapy uses premade antibodies of tau. It is of high flexibility because the antibodies can be developed for specific pathological pTau epitopes that appeared in different disease stages and be further modified to enhance efficacy. The possible adverse effect of passive immune response is reversible once pTau antibodies are cleared up after the stop of treatment. Still, disadvantages of passive immunotherapy include high cost and long time of antibody

Table 2
The key information of p-Tau targeting chimeras.

	DEPTAC	P-tau-PROTAC	PhosTAC	AdPhosphatase
Form	Peptide	Molecular	Molecular	Retrovirus
Tau (POI) binder	Peptide sequence from Tau interactors	PET tracer (targeting endogenous pTau)	HaloTag (binding with HaloTag-fused tau protein) or PET tracer (targeting endogenous pTau)	GFP nanobody (bind with GFP-fused POI)
Effector or effector recruiter	Peptide sequence from PP interactors (recruit PP)	E3 ubiquitin Cereblon (CRBN) ligase	FKBP12(F36V) ligand (recruit FKBP12-fused PP2A A subunit)	Catalytic subunit of PP1 or PP2A
Effect	Dephosphorylation	Ubiquitination and proteasomal degradation	Dephosphorylation	Dephosphorylation
Advantage	Bind with endogenous tau and phosphatase; specific for dephosphorylation tau; promote tau degradation indirectly and mildly	Bind with endogenous tau	Bind with endogenous tau; promote tau degradation indirectly and mildly	High specificity to POI
Disadvantage	Easy to be proteolyzed; short half-life	Degrade not only abnormal tau but also normal tau	Fail to bind with endogenous PP	Fail to bind with endogenous POI and endogenous PP; hasn't been validated on tau protein; induce adverse immunogenic reaction
Reference	[20–22]	[23–25]	[26]	[27]

The structures of the targeting chimeras are provided in Table S1 (online). DEPTACs, dephosphorylation-targeting chimeras; GFP, green fluorescent protein; PET, positron emission tomography; phosTACs, phosphorylation targeting chimeras; POI, protein of interest; PP, phosphatase; PROTACs, proteolysis targeting chimeras.

administration, the latter increases the risks of anti-idiotypic response and delayed adverse effects.[7] Several tau antibodies have been tested in either preclinical or clinical tests, such as JNJ-63733657 targeting pThr217,[146] PNT001 recognizes neurotoxic cis pThr231,[115] Lu AF87908 against pSer396,[147,148] RO6926496 against pSer404,[149] MK-2214 against pSer413,[150,151] and RG7345 antibody against pSer422.[152] Aside from pTau, there are also some antibodies targeting a certain area of tau, like Tilavonemab (CN2–8E12),[153–155] Semorinemab (RO7105705),[156–158] and Gosuranemab against the N-terminal of tau,[159] BIIB076 against tau (125 − 131),[160] Bepranemab (formerly UCB0107) against tau (235 − 250),[161,162] PRX005,[163,164] and E2814[92] targeting the MTBR of 3R and 4R tau isoforms, respectively, as well as Zagotenemab (LY3303560) targeting both the N-terminal and the R3 area of tau.[165]

5. Phospho-tau targeting chimeras

In recent years, a new kind of heterobifunctional targeting chimera has been developed for selective modulation of tau phosphorylation and accumulation, including DEPTAC,[20] pTau-PROTAC,[23–25] phosTAC,[26] and AdPhosphatases system[27] (Fig. 4). The key information of p-Tau targeting chimeras are summarized in Table 2 and Table S1. All these targeting chimera s are currently in proof of concept or testing in pre-clinical experiments.

5.1. DEPTACs

To realize specific facilitation of pTau dephosphorylation *in vivo* and under natural conditions, we have conceptualized the construction of DEPTAC[20–22] (Fig. 4A), which is capable of simultaneously binding tau and recruiting phosphatases with specifical peptide motifs, respectively. DEPTACs could increase the proximity between tau protein and the phosphatases, thus promoting the dephosphorylation and subsequent clearance of pathological pTau. To our knowledge, the DEPTAC is the first chimera that functions both in culture cells and mouse models of AD and the related tauopathies.[20–22]

DEPTACs show many advantages including high selectivity on tau protein without affecting phosphatases activity and the phosphorylation level of other substrates, low cost in design and synthesis, the capability of directly targeting endogenous proteins, and high druggability for clinical treatment of AD and tauopathies.[20–22]

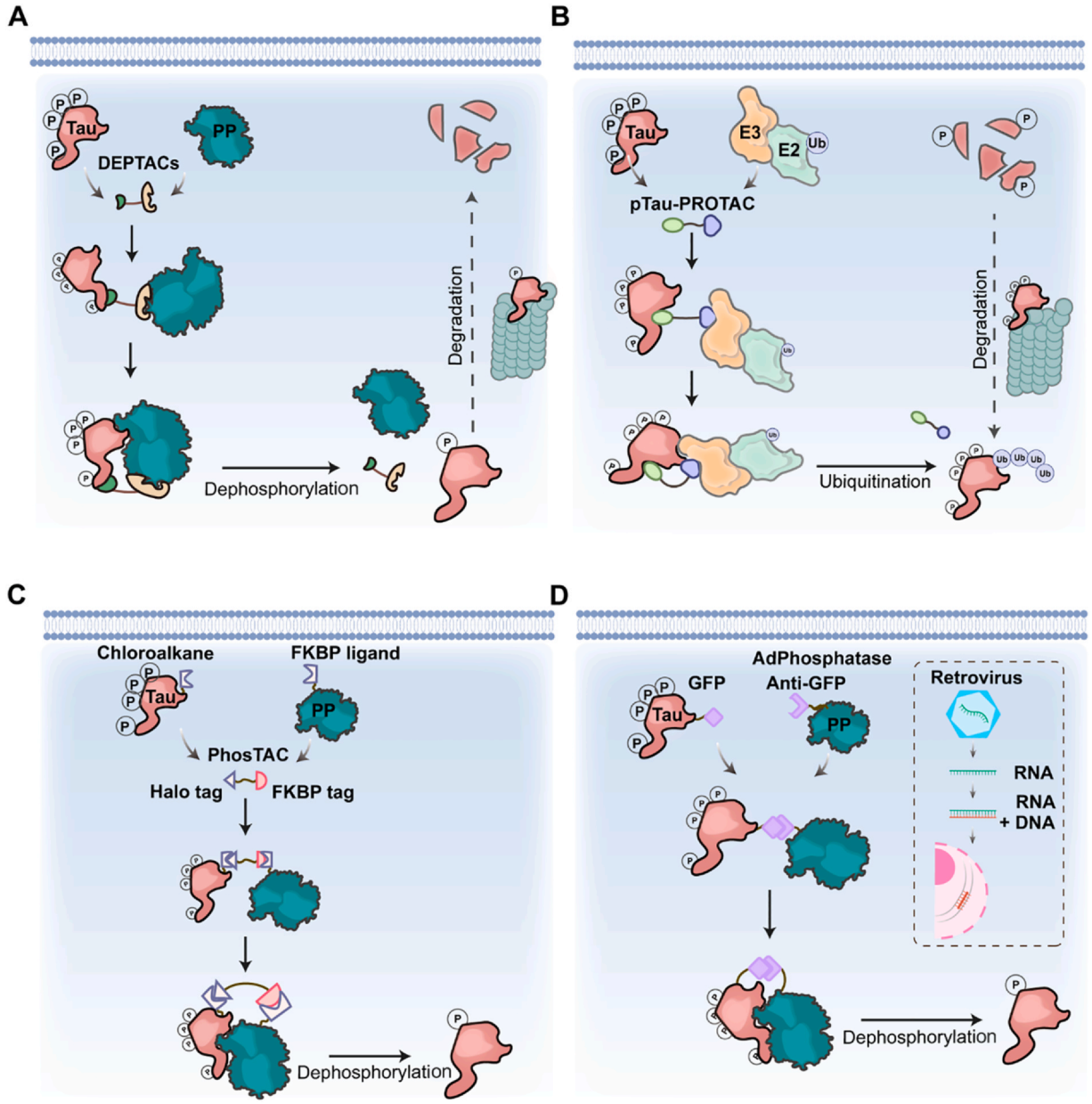

Fig. 4. The mechanism of pTau targeting chimeras. (A) DEPTAC uses tau binders to recruit endogenous tau and PPR to recruit endogenous phosphatases, thus strengthening the proximity between the pTau and phosphatase to facilitate the dephosphorylation and subsequent clearance of pathological tau. (B) pTau PROTAC uses a PET tracer targeting endogenous pTau at Ser285 (Ser23) and Tyr310 (Tyr44) as the tau binder, and contains an E3 ubiquitin cereblon (CRBN) ligase, thus, triggering tau ubiquitination and proteasomal degradation. (C) Tau PhosTAC is composed of an FKBP12(F36V) ligand aiming to recruit FKBP12-fused PP2A A subunit, and a chloroalkane aiming to bind with HaloTag-fused tau protein, thus enhancing the proximity between PP2A and tau and induce tau dephosphorylation. (D) The AdPhosphatase system might consist of a tau-binding GFP nanobody conjugated to the catalytic subunit of phosphatase (PP) and is expressed in cells via retroviral transduction. It can recognize and bind to GFP-tagged tau proteins, thus mediating tau dephosphorylation. This system has not been validated on tau. P, phosphorylation; Ub, ubiquitination.

5.2. Phospho-tau PROTACs

PROTAC is a chemical knockdown strategy to facilitate the degradation of target proteins through the ubiquitin-proteolysis system[23–25] (Fig. 4B). A pTau-targeting PROTAC named QC-01-175 is designed recently, which contains ^{18}F-T807 (or 18F-AV-1451),[166] one of the most advanced tau positron emission tomography (PET) tracers, to

recognize and bind pTau at Ser285 (S23) and Tyr310 (Y44), and an E3 ubiquitin cereblon (CRBN) ligase. These two ligands were linked by a polyethylene glycol (PEG) linker. The QC-01-175 has been evidenced effective in promoting the clearance of pTau and reducing stress vulnerability in FTD patient-derived neuron models, with minimal effect on tau in neurons from healthy controls. This work suggests that pTau PROTACs hold great potential for the removal of aberrant pTau accumulation in tauopathies.

5.3. PhosTACs

Craig Crews's team has developed several small molecule-based PhosTACs to specifically facilitate the binding of tau with PP2A thus enhancing tau dephosphorylation.[26] Given that there is currently no specific small molecule ligand of PP2A, they engineered an $FKBP12^{F36V}$-PP2A fusion protein in cultured cells, and subsequently constructed a series of PhosTACs consisting of PET tracer of tau PI2620, a PEG linker and an FKBP ligand to recruit fused $FKBP12^{F36V}$-PP2A (Fig. 4C). Their PhosTAC7 significantly promoted PP2A and tau interaction and induced tau dephosphorylation in Hela cell lines.

5.4. AdPhosphatase system

Simpson et al.[27] describe the generation of an AdPhosphatase system to facilitate the selective dephosphorylation of POIs (Fig. 4D), this system includes two parts: A green fluorescent protein (GFP) nanobody attached to a promiscuous phosphatase's catalytic subunit (PPP1CA/PPP2CA), and a GFP-tagged POI, both of which is expressed in cells via retrovirus tools. The natural high proximity between GFP nanobody and GFP helps to direct PPP1CA/PPP2CA to bind POIs such as family with sequence similarity 83 member D and UNC-51-like kinase 1, thus promoting their dephosphorylation. Their AdPhosphatase system indicated another possible strategy available for inducing tau dephosphorylation.

6. Perspectives of pTau targeting therapies

Conventional phosphatase activators and kinase inhibitors with acceptable toxic side effects still hold promise for the downregulation of tau hyperphosphorylation in related diseases thanks to their low cost, high stability, and simple pharmacokinetic characteristics. Immunotherapies are also a promising way because of their high specificity in reducing pTau, while it remains difficult for the pTau antibodies to penetrate the brain blood barrier (BBB) and enter the cytoplasm of neurons or glial cells in the brain.

Last but not least, the emerging pTau targeting chimeras, especially the DEPTAC, shed new light on the drug discovery of tauopathies. The pTau targeting chimeras have high specificity on pTau, low cost, reasonable druggability, and applicability under natural conditions. The concept of pTau targeting chimeras can be applied for the drug discovery of tauopathies, as well as for other proteinopathies with abnormal post-translational modification and accumulation of other proteins.

CRediT authorship contribution statement

Jingfen Su: Writing – original draft. **Yue Xiao:** Writing – original draft. **Xiaochuan Wang:** Writing – review & editing. **Jie Zheng:** Writing – review & editing. **Jian-Zhi Wang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.medp.2024.100060.

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