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Review

The Many Faces of Tau

Meaghan Morris,[1,2] Sumihiro Maeda,[1,3] Keith Vossel,[1,3] and Lennart Mucke[1,3,*]
[1]Gladstone Institute of Neurological Disease, San Francisco, CA 94158, USA
[2]Biochemistry, Cellular and Molecular Biology Graduate Program, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[3]Department of Neurology, University of California, San Francisco, San Francisco, CA 94158, USA
*Correspondence: lmucke@gladstone.ucsf.edu
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While the microtubule-binding capacity of the protein tau has been known for many years, new functions of tau in signaling and cytoskeletal organization have recently emerged. In this review, we highlight these functions and the potential roles of tau in neurodegenerative disease. We also discuss the therapeutic potential of drugs targeting various aspects of tau biology.

A Microtubule-Associated Protein Involved in Disease

The microtubule-associated protein tau was identified as a microtubule-assembly factor in the mid-1970s (Weingarten et al., 1975; Witman et al., 1976). Subsequently, hyperphosphorylated, insoluble, filamentous tau was shown to be the main component of neurofibrillary tangles (NFTs), a pathological hallmark of Alzheimer's disease (AD) (Grundke-Iqbal et al., 1986; Kondo et al., 1988; Lee et al., 1991; Nukina and Ihara, 1986; Wood et al., 1986). Neurodegenerative disorders with tau inclusions are referred to as tauopathies (Lee et al., 2001). These include AD; frontotemporal lobar degeneration with tau inclusions (FTLD-tau) such as Pick's disease, progressive supranuclear palsy, and corticobasal degeneration; agyrophillic grain disease; some prion diseases; amyotrophic lateral sclerosis/parkinsonism-dementia complex; chronic traumatic encephalopathy; and some genetic forms of Parkinson's disease (Lee et al., 2001; Omalu et al., 2011; Rajput et al., 2006; Santpere and Ferrer, 2009). Although associations per se cannot prove cause-effect relationships, tau inclusions are widely thought to contribute to the pathogenesis of these disorders because they occur in specific brain regions whose functions are altered by these conditions, and NFT formation correlates with the duration and progression of AD (Giannakopoulos et al., 2003; Ihara, 2001). Tau inclusions also appear to modulate the clinical features of other neurodegenerative diseases. In dementia with Lewy bodies, an α-synuclein disorder, accumulation of insoluble tau inclusions is associated with a more AD-like phenotype (Merdes et al., 2003).

Tau is expressed in the central and peripheral nervous system and, to a lesser extent, in kidney, lung, and testis (Gu et al., 1996). It is most abundant in neuronal axons (Lee et al., 2001; Trojanowski et al., 1989) but can also be found in neuronal somatodendritic compartments (Tashiro et al., 1997) and in oligodendrocytes (Klein et al., 2002). Tau can be subdivided into four regions: an N-terminal projection region, a proline-rich domain, a microtubule-binding domain (MBD), and a C-terminal region (Mandelkow et al., 1996). Alternative splicing around the N-terminal region and MBD generates six main isoforms in adult human brain (Goedert et al., 1989). Tau isoforms are named by how many microtubule binding repeat sequences are expressed (termed R) and by which N-terminal exons are included (termed N) (Figure 1). For example, 3R tau has three microtubule binding repeat sequences, while 4R tau has four due to inclusion of exon 10. 0N tau includes no N-terminal exons, 1N tau exon 2, and 2N tau exons 2 and 3 (Lee et al., 2001). Tau mutations are numbered by their location in 4R2N human tau (Lee et al., 2001). Six additional isoforms are formed by alternative splicing around exon 6, resulting in a total of 12 tau isoforms expressed in brain (Wei and Andreadis, 1998), although these additional splice variants have not yet been widely studied.

Physiological tau has an intrinsically disordered structure and is subject to a complex array of posttranslational modifications. Many serine and threonine residues on tau are phosphorylated by a variety of kinases in both physiological and pathological conditions (for a comprehensive table of tau phosphorylation and corresponding kinases, see http://cnr.iop.kcl.ac.uk/hangerlab/tautable). Tau is also posttranslationally modified by tyrosine phosphorylation (Lee et al., 2004), acetylation (Cohen et al., 2011; Min et al., 2010), cross-linking by transglutaminase (Wilhelmus et al., 2009), glycation (Ledesma et al., 1994), isomerization (Miyasaka et al., 2005b), nitration (Reyes et al., 2008), sumoylation (Dorval and Fraser, 2006), O-GlcNAcylation (Arnold et al., 1996), and ubiquitination (Cripps et al., 2006). The diversity of these modifications suggests that tau is highly regulated.

Tau can bind to the outside and, possibly, also the inside of microtubules, with its N- and C-terminal regions projecting outward (Kar et al., 2003; Santarella et al., 2004). Its N-terminal region can associate with the cell membrane, likely as part of a membrane-associated complex (Figure 1), and regulate the spacing between microtubules (Al-Bassam et al., 2002; Frappier et al., 1994; Maas et al., 2000). Its proline-rich domain includes many phosphorylation sites (Augustinack et al., 2002; Biernat et al., 1992) and can bind to SH3 domains of other proteins (Reynolds et al., 2008), including the tyrosine kinase Fyn (Lee et al., 1998). Tau's ability to bind microtubules depends on the MBD and on adjacent regions (Gustke et al., 1994). The tandem repeat sequences within the MBD are thought to directly bind microtubules through their positive net charge, which interacts with negatively charged residues in tubulin (Jho et al., 2010; Kar et al., 2003). Phosphorylation of tau regulates its binding to microtubules and is also associated with tau aggregation in disease. Phosphorylation of tau in and around the MBD may

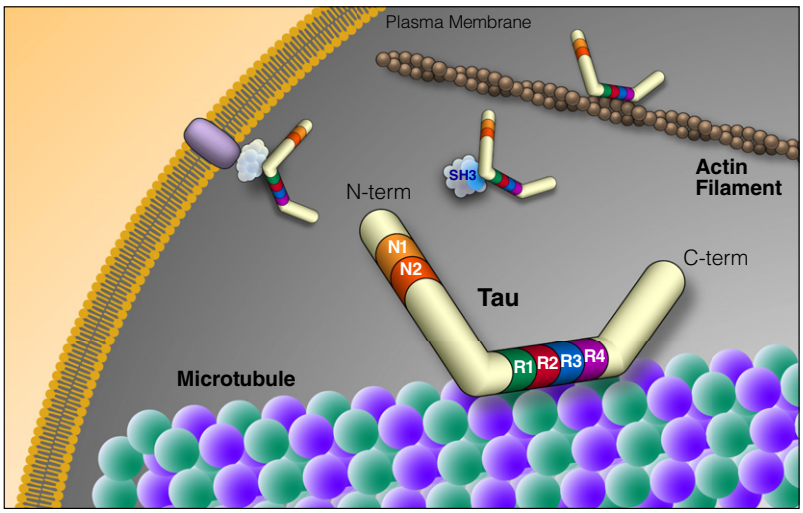

Figure 1. Tau Structure and Function
Tau is an intrinsically disordered protein that can be alternatively spliced at N terminal exons (N1, 2) and the microtubule repeat domains (R). The domains of tau bind many different types of molecules, suggesting a central role in signaling pathways and cytoskeletal organization. The diversity of tau binding partners is highlighted in Table 1. N-term, N terminus; C-term, C terminus; SH3, protein SH3 domain.

neutralize the positive charge (Jho et al., 2010) and alter the conformation of the MBD of tau (Fischer et al., 2009), detaching tau from microtubules. The detached tau accumulates in neuronal cell bodies and neurites, forming insoluble filaments and, ultimately, NFTs (Lee et al., 2001; von Bergen et al., 2005). The MBD also contains PHF6 (VQIVYK) and PHF6* (VQIINK), critical sequences that can assume the beta-sheet structures necessary for tau aggregation and formation of pathological inclusions (von Bergen et al., 2001, 2005).

Evidence for Multiple Functions of Tau

Although tau has been studied ever more intensely in recent years, its precise functions and roles have, if anything, become more mysterious. Some of its activities are known in great molecular detail but were established in rather reductionist paradigms, and their in vivo significance remains uncertain. Other functions were revealed by analysis of tau knockout mice, but the precise mechanisms are poorly understood. A major advantage of tau knockout models is that they can reveal unique functions of tau that are not redundant with the functions of other proteins. For example, tau reduction prevents behavioral deficits in several models of AD (see below), suggesting that unique functions of tau are important in the pathogenesis of this condition. It remains controversial whether animal models with high levels of tau overexpression can provide relevant insights into human conditions in which such overexpression does not occur. However, the accumulation and abnormal distribution of hyperphosphorylated and aggregated tau in these models does simulate key aspects of human tauopathies. Concerns may also be raised about the relevance of studies investigating tau in nonneuronal cells. Although neurons are probably the most relevant cell type to study in relation to tauopathies, some tauopathies are associated with tau pathology in glial cells (Higuchi et al., 2005), and the proteins that interact with tau in different cell types likely overlap.

Tau has numerous binding partners (Table 1), including signaling molecules, cytoskeletal elements and lipids, suggesting that it is a multifunctional protein. Indeed, tau can bind to and affect cytoskeletal components and regulate signaling pathways by acting as a protein scaffold for signaling complexes; tau binding also activates or inhibits several enzymes.

Cytoskeletal Binding Functions

The most extensively described activity of tau—binding to microtubules—occurs in vitro and in vivo. In fact, the majority of tau in the cell is bound to microtubules. In cell-free conditions, this microtubule binding activity promotes microtubule assembly and stability (Weingarten et al., 1975). However, in cell culture, tau colocalizes with those microtubules that are most dynamic and most susceptible to drug-induced depolymerization (Kempf et al., 1996). Moreover, the population of tau-bound microtubules has the highest basal turnover rate of any microtubule population, both in rat primary neuronal culture and in mouse hippocampus in vivo (Fanara et al., 2010), raising doubts about the essential role of tau in microtubule stabilization postulated on the basis of in vitro findings. In addition, knockdown of tau by siRNA is not lethal to primary neurons in culture and does not decrease the number of microtubules or their polymerization state (King et al., 2006; Qiang et al., 2006). Thus, microtubule stabilization may not be a critical function of tau in vivo.

The in vivo functions of tau appear to overlap with those of MAP1B, another microtubule-associated protein found in axons. Complete ablation of tau by homologous recombination does not significantly impair longevity or most critical brain functions, but clearly worsens the MAP1B knockout phenotype of premature mortality and brain dysgenesis (Takei et al., 2000). Knockout of both tau and MAP1B results in severe brain dysgenesis and is lethal within the first month of life. Assuming that this phenotype relates to the microtubule-binding activities of tau and MAP1B, which is uncertain, it is reasonable to speculate that MAP1B is more important for microtubule stabilization than tau and that their overlapping functions are critical for postnatal brain maturation. However, because of the early lethality, it is impossible to draw firm conclusions from the double-knockout phenotype on the functions of tau and MAP1B in the adult or aging brain.

In principle, tau's binding to microtubules could regulate axonal transport. Tau can interfere with the binding of motor proteins to microtubules (Dixit et al., 2008; Ebneth et al., 1998), and there is a gradient of tau along the axon; the highest levels are closest to the synapse (Mandell and Banker, 1996). This distribution might facilitate the detachment of motor proteins from their cargo near the presynaptic terminal, increasing axonal transport efficiency (Dixit et al., 2008). However, ablation of tau does not alter axonal transport in primary neuronal culture

Table 1. Partial List of Tau Binding Partners

Tau Binding Partners	Function of Binding Partner	References
ApoE3	Lipid carrier	Fleming et al., 1996; Strittmatter et al., 1994
Beta tubulin	Cytoskeleton	Kar et al., 2003
cSrc	Src-family kinase	Lee et al., 1998; Reynolds et al., 2008
F-actin	Cytoskeleton	Fulga et al., 2007
Fgr	Src-family kinase	Reynolds et al., 2008
Fyn	Src-family kinase	Lee et al., 1998; Reynolds et al., 2008
Growth factor receptor-bound protein 2 (Grb2)	Adaptor protein for growth factor signaling	Reynolds et al., 2008
Lck	Src-family kinase	Lee et al., 1998
p85α	Regulatory subunit of PI3K	Reynolds et al., 2008
Phosphatidylinositol	Signaling lipid	Surridge and Burns, 1994
Phosphatidylinositol bisphosphate	Signaling lipid	Flanagan et al., 1997
PLCγ	Cleaves phospholipids into signaling molecules	Hwang et al., 1996; Jenkins and Johnson, 1998; Reynolds et al., 2008

(Vossel et al., 2010) or in vivo (Yuan et al., 2008), making an essential role of tau in this physiological function less likely.

Tau can also bind to and bundle actin filaments (Fulga et al., 2007; He et al., 2009; Kotani et al., 1985), activities mediated primarily by its MBD (Farias et al., 2002; Yu and Rasenick, 2006) and assisted by the adjacent proline-rich domain (He et al., 2009; Figure 1). It is possible that tau connects microtubule and actin filament networks (Farias et al., 2002).

Modulation of Signaling Pathways through Scaffolding

Tau could also act as a protein scaffold, and regulation of its binding partners may alter signaling pathways. For example, tau modulates the activity of Src family kinases. In mouse brain tissues, tau coimmunoprecipitates with both the tyrosine kinase Fyn and the scaffolding protein PSD-95, and in the absence of tau, Fyn can no longer traffic into postsynaptic sites in dendrites (Figure 2; Ittner et al., 2010). The authors speculated that tau normally tethers Fyn to PSD-95/NMDA receptor signaling complexes. Although very little tau is normally present in dendrites, it may be enough to ensure proper localization of postsynaptic components (Ittner et al., 2010). Similarly, tau acts as a protein scaffold in oligodendrocytes, connecting Fyn and microtubules to enable process extension (Klein et al., 2002). In cell culture, tau binds to and activates both cSrc and Fyn and facilitates cSrc-mediated actin rearrangements following platelet-derived growth factor stimulation (Sharma et al., 2007).

Tau may also regulate signaling cascades that control neurite extension, although this is a somewhat controversial area. Some investigators have reported a defect in neurite extension in tau knockout neurons in vitro (Dawson et al., 2001), whereas others

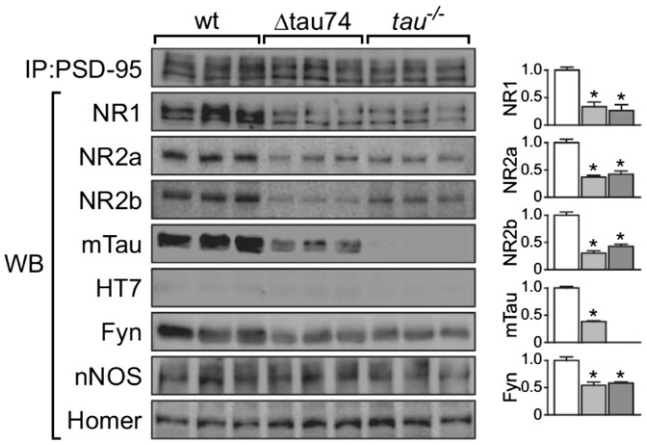

Figure 2. Endogenous Tau Exists in Dendritic Spines

Tau immunoprecipitates with PSD95, a postsynaptic protein, and regulates the association of PSD-95 with NMDAR subunits and the tyrosine kinase Fyn. Overexpression of a human N-terminal truncation of tau (Δtau74, light gray) or knocking out tau (*tau*$^{-/-}$, dark gray) reduced the amount of NR1, NR2a, NR2b, and Fyn within PSD95 complexes as compared with wild-type mice (white; *$p < 0.005$, Student's t test). Error bars represent standard error of the mean. From Ittner et al. (2010) with permission from Elsevier.

found no such defect despite observing decreased numbers of microtubules (Harada et al., 1994). Knockdown of tau by siRNA decreased the length of axons (Qiang et al., 2006) but not the number of microtubules (King et al., 2006; Qiang et al., 2006), and overexpression of tau promoted neurite extension in cell culture (Brandt et al., 1995). These effects may relate to tau's ability to thwart microtubule-severing proteins (Qiang et al., 2006) but could also involve facilitation of nerve growth factor (NGF) signaling.

In PC12 cells, overexpression of full-length tau was associated with normal neurite extension and an increased number of neurites per cell, whereas overexpression of the N terminus of tau suppressed NGF-induced neurite extension (Brandt et al., 1995). Thus, increased levels of tau may enhance NGF function, whereas the N terminus of tau may impair NGF signaling, possibly by a dominant-negative mechanism. Enhancement of NGF signaling by tau may involve increased association of tau with actin filaments, which occurs after stimulation with NGF and is mainly mediated by the MBD (Yu and Rasenick, 2006) rather than the N terminus. In PC12 cells, tau facilitates signaling through receptors for NGF and epidermal growth factor (EGF), thereby increasing activity in the mitogen-activated protein kinase (MAPK) pathway (Leugers and Lee, 2010). Stimulation of PC12 cells with NGF or EGF causes tau phosphorylation at T231, a modification necessary for the growth factor-induced activation of the Ras-MAPK pathway (Leugers and Lee, 2010), nicely illustrating the functional significance of a single tau phosphorylation site. As tau is not known to directly interact with growth factor receptors, it may facilitate signaling by binding to adaptor proteins such as Grb2 (Reynolds et al., 2008). The enhancement of growth factor signaling by increased tau expression may explain why several forms of chemotherapy-naive cancer cells overexpress tau (Rouzier et al., 2005; Souter and Lee, 2009).

Other Roles of Tau in Signaling Pathways

Tau binds phospholipase C (PLC) γ in human neuroblastoma (SH-SY5Y) cells (Jenkins and Johnson, 1998). Under cell-free conditions and in the presence of unsaturated fatty acids, tau activates PLCγ independently of the tyrosine phosphorylation usually required to activate this enzyme (Hwang et al., 1996). At high tau concentrations, this activation does not require fatty acids (Hwang et al., 1996) and may involve binding of tau to both the enzyme and the substrates phosphatidylinositol (Surridge and Burns, 1994) or phosphatidylinositol 4,5-bisphosphate (Flanagan et al., 1997), which could facilitate the phospholipid cleavage reaction. Activation of PLCγ by tau was particularly facilitated by arachidonic acid (Hwang et al., 1996). Arachidonic acid is released from phospholipids by cytosolic phospholipase A_2, whose activity in the brain is increased in AD patients and related mouse models (Sanchez-Mejia et al., 2008). Thus, increased levels of tau and arachidonic acid may jointly increase signaling through the PLCγ pathway in AD.

Tau can also act as a direct enzyme inhibitor. For example, it can bind to and inhibit histone deacetylase-6 (Perez et al., 2009), which deacetylates tubulin and may regulate microtubule stability (Perez et al., 2009). Thus, tau may affect microtubule stability by a mechanism independent of tubulin binding, although reports regarding the levels of acetylated tubulin in tau knockout mice vary (Perez et al., 2009; Rapoport et al., 2002).

Tau also appears to participate in the cellular response to heat shock. During heat shock of neurons, tau bound DNA and facilitated DNA repair, and tau knockout neurons showed increased DNA damage (Sultan et al., 2011). However, when cultured neurons were allowed to recover from heat shock, tau knockout actually protected against heat shock-induced cell damage, as determined by measurements of neurite length and lactate dehydrogenase release (Miao et al., 2010). Compared with wild-type neurons, tau knockout neurons showed a delayed and prolonged activation of Akt and less GSK3β activity during recovery from heat shock (Miao et al., 2010), suggesting that the protective effect of tau knockout may be upstream of Akt/GSK3β phosphorylation. In sensory neurons of *C. elegans*, overexpression of 4R0N tau decreased the response to touch, and this phenotype was exacerbated by heat shock when tested after a recovery period of 24 hr (Miyasaka et al., 2005a). These results suggest that tau has a role in the cellular response to heat shock, both during the insult and in the subsequent recovery phase.

Adult Neurogenesis

Tau affects adult neurogenesis. Three-repeat tau is expressed and highly phosphorylated in adult-born granule cells in the dentate gyrus (Bullmann et al., 2007; Hong et al., 2010). In one strain of tau knockout mice, adult neurogenesis was found to be severely reduced (Hong et al., 2010). However, tau does not appear to be needed for embryonic neurogenesis, as tau knockout mice have grossly normal brain anatomy. The functional significance of adult neurogenesis is a topic of intense study and debate (Zhao et al., 2008). Notably, adult tau knockout mice showed no deficits in a variety of learning and memory paradigms (Dawson et al., 2010; Ittner et al., 2010; Roberson et al., 2007, 2011).

Mechanisms by Which Tau Contributes to Disease

As mentioned above, tau probably fulfills multiple functions and may contribute to neuropathogenesis in multiple ways. In principle, this might include both gain- and loss-of-function effects, although the latter mechanism has recently been called into question by several lines of experimental evidence. Furthermore, tau does not act alone. For example, in AD it appears to enable the pathogenic effects of both Aβ and apolipoprotein E4 (apoE4) (Andrews-Zwilling et al., 2010; Ittner et al., 2010; Roberson et al., 2007, 2011).

Loss of Function

A prominent theory about tau pathology was that disease phenotypes were caused by loss of tau function due to hyperphosphorylation and sequestration of soluble tau (Zhang et al., 2005). In many tauopathies, tau is hyperphosphorylated, which releases tau from microtubules, apoE (Strittmatter et al., 1994), Src (Bhaskar et al., 2005), and possibly other binding partners. Although it is conceivable that this process results in loss of specific tau functions, increased phosphorylation of tau per se is probably not detrimental, as it occurs naturally during hibernation (Arendt et al., 2003) and fetal development (Yu et al., 2009). Phosphorylated tau from AD brains may seed the aggregation of control human tau (Alonso et al., 1996), but we are unaware of any evidence that tau aggregation actually lowers levels of soluble tau in vivo.

Recent experimental studies have shown more directly that loss of tau function is an unlikely cause of neurodegeneration and neuronal dysfunction. Longevity and behavioral functions are among the most compelling outcome measures for the evaluation of biologically meaningful processes affecting the central nervous system. Complete ablation of tau in knockout mice does not cause premature mortality or major neurological deficits (Dawson et al., 2001; Harada et al., 1994; Ikegami et al., 2000; Roberson et al., 2007, 2011; Yuan et al., 2008). Four independent tau knockout lines have been established and most of them have normal behavior throughout most of their lives (Table 2). Only one of these lines was reported to have motor deficits, hyperactivity in the open field test, and learning impairments in contextual fear conditioning at 10–11 weeks of age (Ikegami et al., 2000). Using fear conditioning to assess learning and memory in hyperactive mice is problematic because the hyperactivity confounds the interpretation of diminished freezing (Rudy et al., 2004). Because tau ablation has so little impact on neural functions, two of the tau knockout lines were actually generated as tools for neuron-specific expression of EGFP (Tucker et al., 2001) or Cre (Muramatsu et al., 2008). Although axonal abnormalities have been reported in the cingulate cortex and genu of the corpus callosum of 10- and 12-month-old tau knockout mice, these mice showed no behavioral deficits in the rotarod test, Morris water maze, or radial arm water maze (Dawson et al., 2010). To our knowledge, no deficits of any kind have been identified in hemizygous knockout mice, which have roughly half normal tau levels (Ikegami et al., 2000; Roberson et al., 2007).

Based on electrophysiological recordings in acute hippocampal slices, tau knockout mice and wild-type controls have similar NMDA/AMPA receptor currents, synaptic transmission strength, and short-term as well as long-term synaptic plasticity

Table 2. Behavioral Tests in Tau Knockout Mice

Mouse Line	Age	Behavioral Test	Result
Tau knockout line 1 (Harada et al., 1994)	10–11 weeks	Balance beam	Impaired motor function (Ikegami et al., 2000)
	10–11 weeks	Contextual fear conditioning	Impaired learning/memory (Ikegami et al., 2000)
	10–11 weeks	Open field	Hyperactive (Ikegami et al., 2000)
	10–11 weeks	Wire hang	Impaired motor function (Ikegami et al., 2000)
Tau knockout line 2 (Dawson et al., 2001)	4–7 months, 12–16 months	Elevated plus maze	Normal anxiety and exploration (Roberson et al., 2007)
	4–7 months, 10–12 months	Morris water maze	Normal learning/memory (Roberson et al., 2007) Normal learning/memory (Dawson et al., 2010)
	10–12 months	Rotarod	Normal motor function (Dawson et al., 2010)
	10–12 months	Radial arm water maze	Normal learning/memory (Dawson et al., 2010)
	4–7 months	Y maze	Normal activity (Roberson et al., 2007)
Tau knockout/EGFP knockin line (Tucker et al., 2001)	4.5–7.5 months	Elevated plus maze	Normal anxiety and exploration (Roberson et al., 2011)
	4.5–7.5 months	Novel object recognition	Normal learning/memory (Roberson et al., 2011)
	8 months	T maze	Normal learning/memory (Ittner et al., 2010)
Tau knockout/Cre knockin line (Muramatsu et al., 2008)	—	None	—

(Table 3; Roberson et al., 2011; Shipton et al., 2011). Surprisingly, tau knockout mice are more resistant to seizures caused by disinhibition, excitotoxins, or amyloid-β (Aβ) peptides than wild-type mice (Figure 3A; Ittner et al., 2010; Roberson et al., 2007, 2011). Compared with wild-type controls, neurons in hippocampal slices from tau knockout mice are more resistant to disinhibition-induced bursting activity (Figure 3B), which may be due, at least in part, to an increased frequency of spontaneous inhibitory postsynaptic currents in tau knockout mice (Roberson et al., 2011). These findings suggest that tau has a complex role in regulating neural network activity and that tau reduction could prevent aberrant neuronal excitability, network hypersynchrony or both.

The resistance of tau knockout mice to seizures may also relate to alterations in brain oscillatory patterns. Tau knockout mice have decreased peak frequency of theta waves in the hippocampus and decreased coherence of gamma waves in the frontal cortex (Cantero et al., 2010). The potential effects of these alterations on Aβ-induced dysrythmias and cognitive abnormalities remain to be determined.

In conventional tau knockout mice, other MAPs might compensate for tau loss, particularly MAP1A and MAP1B. However, no changes in MAP1A, MAP1B, or MAP2 protein levels were detected in 12-month-old adult tau knockout mice (Dawson et al., 2001). To evaluate the safety of tau reduction strategies for therapeutic purposes more conclusively, tau needs to be reduced in adult mice after brain development and maturation are complete, and such experiments are in progress. In cultured cells, acute knockdown of tau did not affect the stability or polymerization state of microtubules (King et al., 2006; Qiang et al., 2006), and reducing tau levels in brains of 3-month-old wild-type mice for 12 weeks by methylene blue administration caused no behavioral deficits in the rotarod test or Morris water maze (O'Leary et al., 2010). Thus, it is unlikely that loss of tau function is an important cause of neuronal dysfunction or degeneration in AD and related conditions. In fact, the findings summarized above suggest that partial reduction of tau may be well tolerated and could effectively protect the brain against Aβ, epileptogenesis, and excitotoxicity.

Tau Functions Enabling Pathogenesis

In transgenic mice, wild-type levels of tau are required for Aβ and apoE4 to cause neuronal, synaptic, and behavioral deficits (Andrews-Zwilling et al., 2010; Ittner et al., 2010; Roberson

Table 3. Electrophysiological Abnormalities of hAPP-J20 Mice Prevented by Tau Knockout (Roberson et al., 2011)[a]

Electrophysiological Measure[b]	*Tau*$^{-/-}$ Mice	hAPP/*Tau*$^{+/+}$ Mice	hAPP/*Tau*$^{-/-}$ Mice
Action potential-driven IPSC frequency	Normal	↓	Normal
Coastline burst index	↓	↑	↓
EEG epileptiform activity (cortex)	Normal	↑	Normal
eEPSC amplitude	Normal	↑	Normal
eIPSC amplitude	Normal	↓	Normal
Long-term potentiation	Normal	↓	Normal
mEPSC frequency	Normal	↓	Normal
mIPSC frequency	Normal	↑	Normal
NMDAR/AMPAR ratio	Normal	↓	Normal
Paired-pulse ratio	Normal	↓	Normal
sIPSC frequency	↑	↓	↑
Synaptic strength (CA1)	Normal	↓	Normal

Similar results were observed after exposure of hippocampal slices to recombinant Aβ aggregates (Shipton et al., 2011). EPSC, excitatory postsynaptic current; IPSC, inhibitory postsynaptic current; e, evoked; m, miniature; s, spontaneous.

[a] Relative to *Tau*$^{+/+}$ mice.

[b] Recorded at perforant path to granule cell synapse in dentate gyrus unless indicated otherwise. All recordings were obtained in acute hippocampal slices.

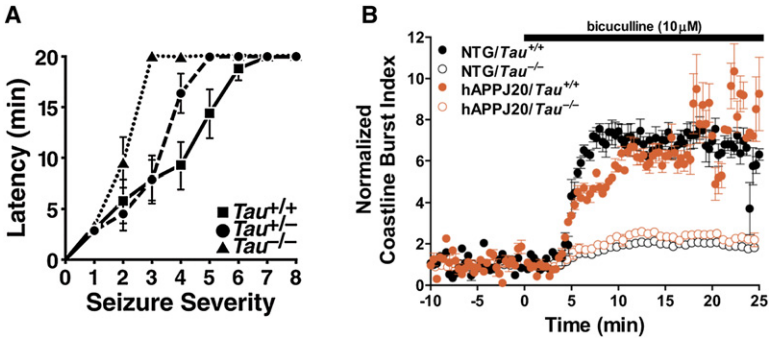

Figure 3. Tau Reduction Suppresses Drug-Induced Seizures in Mice and Neuronal Bursting Activity in Disinhibited Hippocampal Slices

(A) Partial or complete reduction of tau in mice without hAPP expression delayed the onset and reduced the severity of seizures induced by pentylenetetrazole, a $GABA_A$ receptor antagonist (Roberson et al., 2007).

(B) Tau knockout reduced aberrant neuronal discharges in acute hippocampal slices after disinhibition with the $GABA_A$ receptor antagonist bicuculline, as illustrated by measurements of the coastline burst index (Roberson et al., 2011). NTG, no hAPP expression; hAPPJ20, hAPP mice from line J20.

Error bars represent standard error of the mean.

et al., 2007, 2011). However, whether Aβ and apoE4 contribute to AD-related cognitive decline through the same or distinct tau-dependent mechanism(s) remains to be determined. Acute exposure of neuronal cultures to Aβ led to hyperphosphorylation (De Felice et al., 2008) and mislocalization of tau into dendritic spines (Zempel et al., 2010), which, at least in some dendrites, was associated with spine collapse and dendritic degeneration. As tau phosphorylation releases tau from many of its binding partners, it is tempting to speculate that tau is initially hyperphosphorylated in AD to reduce its function, in an effort to counteract Aβ-induced neuronal dysfunction. With time, though, this compensatory mechanism fails because hyperphosphorylated tau becomes detrimental at concentrations sufficient to form toxic tau aggregates.

While tau is abnormally phosphorylated in apoE4 transgenic mice (Brecht et al., 2004), we have so far found no evidence of abnormal phosphorylation or aggregation of tau in hAPP-J20 mice, whose robust Aβ-dependent neuronal and behavioral deficits were prevented by reduction of wild-type murine tau (Table 3 and Figure 4; Roberson et al., 2007, 2011). While we continue to search for a direct pathogenic tau mediator and a pathogenic mislocalization of tau in hAPP-J20 mice, the above findings raise the possibility that physiological functions of tau, rather than an abnormal tau gain of function (Figure 5), permit Aβ and other AD-related factors to elicit aberrant neuronal excitation (Ittner et al., 2010; Roberson et al., 2007, 2011), abnormalities in axonal transport (Vossel et al., 2010), and impairment of inhibitory interneurons (Andrews-Zwilling et al., 2010). Notably, even partial tau reduction improved longevity and cognitive functions in hAPP-J20 mice (Figure 4; Roberson et al., 2007). Tau knockout also improved longevity and cognitive functions in APP23 mice and in both lines markedly increased resistance to seizures in mice with or without hAPP (Ittner et al., 2010; Roberson et al., 2007, 2011). For unclear reasons, tau reduction was not beneficial in the Tg2576 hAPP mouse model (Dawson et al., 2010).

The tyrosine kinase Fyn appears to be important in the development of Aβ- and tau-dependent neuronal deficits. Neuronal overexpression of Fyn sensitizes hAPP mice to Aβ-induced neuronal, synaptic, and cognitive deficits (Chin et al., 2004, 2005) that are prevented by knocking out tau in hAPP-J9/FYN doubly transgenic lines (Roberson et al., 2011). Tau knockout prevented behavioral deficits in the Morris water maze and elevated plus maze of hAPP/FYN mice and premature mortality in two separate lines of hAPP/FYN mice (Roberson et al., 2011). In addition, tau knockout prevented spontaneous epileptic activity in hAPP/FYN mice and hAPP-J20 mice (Table 3; Roberson et al., 2011). This striking antiepileptic effect could result from the reduction of tau in axons, dendrites, or both. Although tau knockout did not affect axonal transport at baseline (Vossel et al., 2010; Yuan et al., 2008), it precluded Aβ-induced deficits in the axonal transport of cargoes that could affect neuronal excitability (Figure 4D; Vossel et al., 2010). Tau is also required for Fyn to gain access to and phosphorylate the NR2B subunit of dendritic NMDA receptors (Ittner et al., 2010). Consistent with our hypothesis that tau reduction protects against Aβ by preventing neuronal overexcitation (Roberson et al., 2007), targeted perturbation of the NR/PSD-95 interaction, which prevents excitotoxicity, also prevented premature mortality and memory deficits in APP23 mice (Ittner et al., 2010). These findings suggest that modulating tau, its interaction with Fyn, or key proteins involved in or affected by this interaction may be of therapeutic benefit in AD.

The interaction between Fyn and tau may also contribute to FTLD. Several forms of FTLD mutant tau and pseudohyperphosphorylated tau bind Fyn more tightly than wild-type tau (Bhaskar et al., 2005), which may increase neuronal Fyn activity. Furthermore, Fyn binds more tightly to 3R0N tau than 4R0N tau (Bhaskar et al., 2005), implying that FTLD mutations that alter tau splicing could also alter the activity or localization of Fyn. In mice overexpressing P301L 4R0N tau under the mouse prion promoter (JNPL3 model), phosphorylation of tau at Y18 by Fyn increases simultaneously with tau hyperphosphorylation on serine/threonine sites before the onset of behavioral deficits (Bhaskar et al., 2010).

The physiological actin-bundling function of tau may also contribute to pathology. Filamentous actin inclusions, closely resembling Hirano bodies in AD, were found in *Drosophila* models overexpressing wild-type or R406W 4R0N tau and in mice overexpressing P301L 4R0N tau under the TRE promoter with the tet-off element under the CaMKII promoter (rTg4510 model) (Fulga et al., 2007). Knocking out or destabilizing actin filaments in *Drosophila* models prevented tau-induced degeneration (Fulga et al., 2007), implicating alterations in actin dynamics as a mediator of tau toxicity.

Abnormal Gain of Function

The largest amount of work in this field has focused on tau phosphorylation and aggregation. Tau is highly phosphorylated

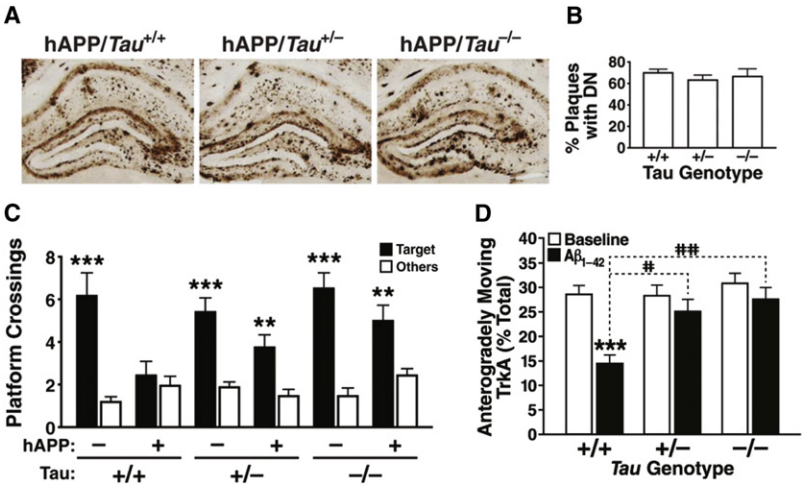

Figure 4. Tau Reduction Does Not Change Amyloid Pathology but Prevents Aβ-Dependent Cognitive and Functional Neuronal Deficits

Tau reduction did not affect (A) Aβ deposition or (B) the number of plaques with dystrophic neurites (DN) in hAPP-J20 mice. However, even partial tau reduction prevented (C) memory deficits in the Morris water maze (72 hr probe trial) in hAPP-J20 mice (**$p < 0.01$, ***$p < 0.001$ versus other quadrant; ANOVA, Tukey) (Roberson et al., 2007) and (D) Aβ oligomer-induced axonal transport deficits in primary hippocampal neurons, although it had no effect on axonal transport at baseline (***$p < 0.001$, indicates comparison to baseline within the genotype; paired t tests with Bonferroni correction. #$p < 0.05$, ##$p < 0.01$ versus Aβ-treated *Tau*$^{+/+}$; Kruskal-Wallis analysis of variance, Dunn) (Vossel et al., 2010). Error bars represent standard error of the mean.

in fetal brain without eliciting toxicity and is also phosphorylated on many sites in adult brain, albeit with lower frequency (Matsuo et al., 1994; Yu et al., 2009). Tau is transiently hyperphosphorylated during hibernation without long-term harm to neural networks (Arendt et al., 2003). Tau phosphorylation is also markedly increased in response to various stressors. In humans, tau becomes hyperphosphorylated and aggregated after head trauma, following earlier increases in APP expression, axonal swelling, and microtubule disruption (Gentleman et al., 1993; Omalu et al., 2011). Tau also becomes hyperphosphorylated in mouse brain in response to hypothermia and experimental insulin-dependent diabetes (Planel et al., 2007). In cell culture and brain slice models of neuronal injury, tau is hyperphosphorylated during recovery from heat shock (Miao et al., 2010), in response to Aβ oligomer treatment (De Felice et al., 2008; Zempel et al., 2010), hypoxia, and glucose deprivation (Burkhart et al., 1998). In cell culture, ATP, glutamate, hydrogen peroxide, serum deprivation and Aβ oligomer treatment all cause tau mislocalization into dendrites (Zempel et al., 2010), a process that is likely triggered by hyperphosphorylation-induced dissociation of tau from microtubules and cell membranes. Thus, increased phosphorylation and redistribution of tau may be common responses to neuronal stress.

Findings in *Drosophila* models suggest that tau phosphorylation may cause neurotoxicity in a combinatorial fashion rather than through the modification of individual phosphorylation sites and involves the folding of tau into an abnormal conformation resembling tau conformations found in AD (Steinhilb et al., 2007). Hyperphosphorylated tau has a tighter, more folded conformation and an increased propensity to aggregate (Jeganathan et al., 2008), as does tau with mutations found in FTLD (Lee et al., 2001). In *C. elegans*, overexpression of wild-type or mutant 4R1N tau causes axonal degeneration and an uncoordinated phenotype indicative of neuronal dysfunction (Kraemer et al., 2003). The extent of phosphorylation was similar across mutant and wild-type tau lines, but more insoluble tau was found in the former (Kraemer et al., 2003). Worms overexpressing mutant tau that formed aggregates had a more severe phenotype (Kraemer et al., 2003).

Although filamentous tau inclusions are a pathologic hallmark of tauopathies, experimental evidence suggests that filamentous tau may not be responsible for neuronal dysfunction. In a regulatable P301L 4R0N tau transgenic mouse (rTg4510 model), inhibiting tau production after filamentous tau inclusions formed reversed behavioral deficits in the Morris water maze, even though inclusion formation progressed (Santacruz et al., 2005). Acute tau reduction by methylene blue treatment in this model

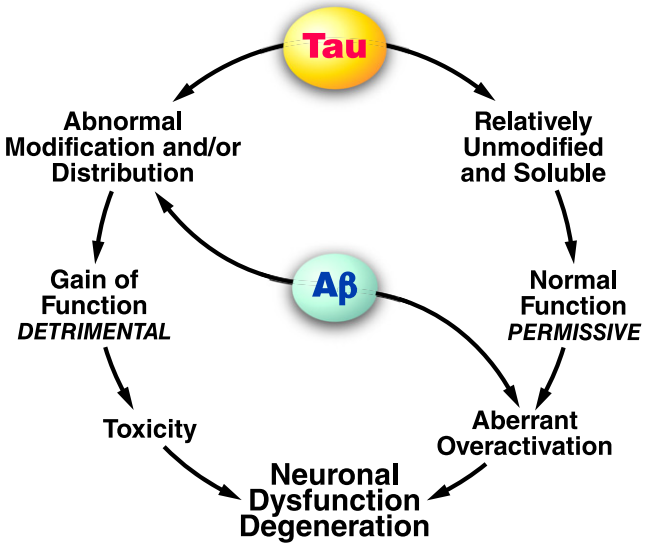

Figure 5. Potential Mechanisms of Tau-Dependent Aβ Toxicity

Experiments in which neurons were acutely exposed to Aβ suggest that Aβ can trigger tau-mediated neurotoxicity by enhancing tau phosphorylation, which in turn directs pathogenic tau species into dendritic spines where they exert adverse effects (left). In hAPP mice though, in which neurons are chronically exposed to elevated Aβ levels, it has so far been difficult to find clear evidence for a similar process. Nonetheless, Aβ-induced neuronal dysfunction in these models strictly depends on the presence of tau, raising the possibility that physiological functions of tau permit Aβ to cause neuronal dysfunction (right). Such functions may involve the intraneuronal trafficking of factors that regulate synaptic activity at the pre- or postsynaptic level.

improved memory scores in correlation with the reduction of soluble tau in the brain but did not alter the number or length of tau fibrils or the amount of Sarkosyl-insoluble tau compared to untreated transgenic mice (O'Leary et al., 2010). In other mouse lines, tet-off transgenes were regulated by the CaMKII promoter to express either the 4R microtubule repeat domain of human tau with a deletion of lysine 280 (termed Tau_{RD}), which is highly prone to aggregation, or Tau_{RD} with an additional two mutations (I277P/I308P) that prevent its aggregation (Mocanu et al., 2008). The proaggregation transgenic mouse, which formed hyperphosphorylated tau inclusions containing Tau_{RD} and endogenous mouse tau, developed synaptic loss (Mocanu et al., 2008), memory deficits and electrophysiological impairments (Sydow et al., 2011). In contrast, the antiaggregation transgenic mouse showed none of these abnormalities. Turning off the transgene in the proaggregation mouse reversed behavioral and electrophysiological alterations without eliminating insoluble tau aggregates, which were composed entirely of endogenous mouse tau after the transgene had been turned off for 4 months (Sydow et al., 2011). These data highlight that tau aggregation causes toxicity, possibly through the formation of tau oligomers.

This conclusion is supported by studies using in vivo two-photon microscopy demonstrating that very few neurons containing tau inclusions in regulatable rTg4510 transgenic mice have caspase activation or membrane disruption (de Calignon et al., 2009, 2010). Decreasing the levels of soluble tau reduced caspase activation in inclusion-positive neurons without affecting the number or size of tau inclusions (de Calignon et al., 2010), implicating soluble tau, not tau inclusions, in the activation of proapoptotic pathways. Neurons with or without tau inclusions in this regulatable P301L tau model showed similar electrophysiological deficits, relative to wild-type neurons (Rocher et al., 2010). Studies in young transgenic flies overexpressing wild-type or mutant 4R0N tau constructs also indicated that toxicity was conferred by soluble tau species, possibly dimers (Feuillette et al., 2010).

Collectively, these studies suggest that tau inclusions are not very toxic and that neuronal toxicity is caused by a smaller, soluble aggregate or a specific conformation of tau. Tau oligomers have been identified in in vitro and in vivo models as well as in AD brains (Berger et al., 2007; Maeda et al., 2007; Sahara et al., 2008). In regulatable P301L 4R0N transgenic mice (rTg4510 model), the extent of memory deficits correlated with the level of putative tau oligomers (Berger et al., 2007).

Tau can also be cleaved in various places by caspase-3, calpain, and cathepsin L, and several of the resulting fragments are thought to increase tau aggregation. In primary neurons exposed to Aβ, calpain generates a 17-kDa tau fragment (Park and Ferreira, 2005). However, the toxicity and in vivo relevance of this fragment are debated; its presence is variable in both control and AD brains (Garg et al., 2011) and it appears to be absent from brains of hAPP-J20 mice (Roberson et al., 2007). Aβ treatment of cortical neurons causes caspase cleavage of tau at Asp421, and cleavage at this site facilitates the formation of tau aggregates in cell-free conditions (Gamblin et al., 2003). Caspase activation precedes formation of filamentous tau inclusions in P301L 4R0N tau transgenic mice (rTg4510 model), raising the possibility that caspase cleavage is important for aggregation of FTLD mutant tau in vivo (de Calignon et al., 2010). In an inducible cell culture model overexpressing the microtubule repeat domain of tau missing K280, cytosolic cleavage by unknown proteases generated putative tau oligomers associated with lysosomal membranes and inhibited chaperone-mediated autophagy; smaller fragments produced by cathepsin L seeded tau aggregation (Wang et al., 2009).

Tau may also exert toxic effects from the extracellular milieu (Frost et al., 2009; Gómez-Ramos et al., 2006). The death of degenerating neurons or extrusion of tau from living cells containing tau aggregates may result in the release of pathogenic tau species into the extracellular space, where they may adversely affect neighboring cells. For example, a peptide in the C terminus of tau (amino acids 391–407) increased intracellular calcium concentrations by activating the muscarinic receptors M1 and M3 (Gómez-Ramos et al., 2008). Tau aggregates released from cells were taken up by and triggered tau aggregation within co-cultured cells that had no pre-existing tau aggregates (Frost et al., 2009).

Injection of insoluble P301S human 4R0N tau from transgenic mouse brainstem extracts into the hippocampus of transgenic mice expressing wild-type 4R2N human tau under the mouse Thy1.2 promoter caused intraneuronal formation of wild-type 4R2N human tau inclusions in the hippocampus that spread along synaptic connections to distant brain regions (Clavaguera et al., 2009). However, we are unaware of any evidence that this transfer of tau aggregation causes neuronal dysfunction or neurodegeneration. Injecting soluble P301S tau into the transgenic mice or insoluble P301S tau into nontransgenic mice failed to cause extensive pathology.

Thus, injection of insoluble tau into the brain parenchyma triggers propagation of tau pathology along neuronal pathways, but only in the presence of the correct tau template. It is unknown whether the potential progression of AD from one brain region to another (Braak and Braak, 1997) depends on similar processes, the presynaptic release of Aβ (Harris et al., 2010), or other mechanisms.

Do FTLD Mutations Simulate Tau Pathology in AD?

Interestingly, mutations in the tau gene cause FTLD disorders such as progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia, but never AD. While the clinical spectrum associated with the many rare tau mutations varies, most FTLD disorders differ from AD both in the types of tau inclusions and in the brain regions affected (Mann et al., 2001), indicating a possible divergence in the roles of tau in these conditions. The trigger for increased phosphorylation and aggregation of tau is also likely different in AD and FTLD.

Two recent studies set out to compare the consequences of overexpressing wild-type human 4R2N tau versus P301L mutant human 4R2N tau in transgenic mice. Each group generated two mouse lines with approximately matched tau expression levels and patterns directed by the Thy1 promoter (Terwel et al., 2005) or the CaMKII promoter (Kimura et al., 2010). In both studies, P301L tau mice differed from wild-type tau mice in tau phosphorylation patterns and in that P301L tau aggregated more readily than wild-type tau, consistent with previous

Table 4. Tau-Targeted Therapies in Clinical Trials[a]

Target	Drug	Diagnosis	Goal Sample Size	Treatment Duration	Stage	Status/Outcome
Tau phosphorylation by GSK3β	Lithium ± Divalproex	AD	35	6 weeks	Phase II	Completed, not yet published
	Lithium	MCI	80	2 years	Phase II	Ongoing
	Lithium	PSP, CBD	17	28 weeks	Phase I/II	Completed, not yet published
	Lithium	Mild AD	71	10 weeks	RCT	Completed. No effect on lymphocyte GSK3 activity or CSF p-tau (Hampel et al., 2009)
Microtubule stabilization/ tau phosphorylation	NAP, AL-108	MCI	120	12 weeks	Phase II	Completed, not yet published
		PSP	300	52 weeks	Phase II/III	Recruiting
		FTLD-tau, PSP, CBD	12	12 weeks (pilot)	Phase II	Ongoing
Tau aggregation	Methylene blue[b]	AD	321	50 weeks	Phase II	Completed, not yet published

CBD, corticobasal degeneration; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; PSP, progressive supranuclear palsy; p-tau, phosphorylated tau; RCT, randomized placebo-controlled trial.
[a] Based on www.clinicaltrials.gov and PubMed search.
[b] May also reduce soluble tau levels.

findings (von Bergen et al., 2001). Remarkably, in both studies, behavior was impaired earlier in wild-type tau transgenic mice than in P301L tau transgenic mice, even though tau was similarly expressed under the same promoter and only P301L mutant tau transgenic mice had tau aggregates. Thy1-wild-type tau mice had early motor impairments and axonopathy, whereas Thy1-P301L tau mice had late motor impairments, insoluble tau inclusions, and no axonopathy (Terwel et al., 2005). CaMKII-wild-type tau mice had earlier memory deficits in the Morris water maze and synaptic loss than CaMKII-P301L tau mice, but tau inclusions and neuronal loss were observed only in CaMKII-P301L tau mice (Kimura et al., 2010). These findings suggest that P301L tau causes neuronal loss through its propensity to aggregate and, possibly, to form toxic oligomeric tau species, whereas wild-type human tau may cause early neuronal and cognitive dysfunction without neuronal cell death, possibly by enhancing normal functions of tau or altering the regulation of endogenous tau.

Another recent study compared transgenic mice expressing P301L human 4R0N tau (rTg4510 model) or wild-type human 4R0N tau directed by the TRE promoter and tTA (tet-off) directed by the CaMKII promoter. Both lines showed deficits in the Morris water maze; however, the deficits worsened with aging in the P301L tau line but not in the wild-type tau line (Hoover et al., 2010). As in the lines described above, neurodegeneration was identified only in the P301L tau line but not in the wild-type tau line. In primary cultures, neurons expressing P301L tau showed tau in dendritic spines more frequently and had greater reductions of miniature excitatory postsynaptic potentials (mEPSCs) and of dendritic GluR1, GluR2/3, and NR1 levels than neurons expressing wild-type tau (Hoover et al., 2010). Tau phosphorylation was required for tau to enter into dendritic spines and to impair mEPSCs in transfected primary rat neurons (Hoover et al., 2010).

In slice cultures, wild-type human 3R0N tau and R406W human 4R2N tau each enhanced Aβ-induced neuronal cell death in the hippocampal CA3 region, whereas P301L human 4R2N tau did not (Tackenberg and Brandt, 2009). A 3R0N tau mutant that prevents phosphorylation and a 3R0N tau mutant that mimics hyperphosphorylation showed no synergistic neurotoxic effect with Aβ, implying that dynamic tau phosphorylation may be required for the enhancement of Aβ toxicity by tau (Tackenberg and Brandt, 2009). These results are consistent with findings indicating that tau requires phosphorylation to enter the dendritic spine in order to affect synaptic function (Hoover et al., 2010) and that Aβ oligomers acutely increase phosphorylation and pathogenic enrichment of wild-type tau in dendritic spines (Zempel et al., 2010).

It may be that P301L tau is already phosphorylated and present in dendritic spines, and therefore no further toxicity is seen in the presence of Aβ oligomers. Interestingly, the adverse effects of wild-type tau were dependent on the activity of both NMDAR and GSK3β, whereas the effects of R406W tau were dependent only on NMDAR activity, suggesting partly distinct mechanisms of toxicity (Tackenberg and Brandt, 2009).

These results imply that AD-relevant pathogenic mechanisms and therapeutic opportunities might be missed in FTLD mutant transgenic mice due to overriding effects of the FTLD mutations.

Treatments Targeting Tau

Treatments targeting various aspects of tau biology are under intense investigation. Inhibitors of tau phosphorylation and aggregation and microtubule stabilizers are already in clinical trials for people with mild cognitive impairment, AD, or FTLD (Table 4), while tau reduction strategies are still in preclinical stages of development. As we learn more about how tau expression is regulated and about tau's involvement in cell signaling and cytoskeletal organization, additional approaches are likely to emerge.

Tau Phosphorylation Inhibitors

The function and aggregation of tau appear to be regulated by phosphorylation, as reviewed above. Of the numerous tau kinases implicated in AD pathogenesis, the most widely studied are GSK-3β, CDK5, MARK, and MAPK (Augustinack et al., 2002;

Mi and Johnson, 2006). Lithium, which inhibits GSK-3β and is used to treat bipolar disorder, improved behavior and reduced tau pathology in transgenic mice overexpressing P301L human 4R0N tau (JNPL3 model) (Noble et al., 2005). However, because lithium has multiple targets, the rescue observed may not have been solely due to a reduction in GSK3β activity. Lithium also has a narrow safety margin (Grandjean and Aubry, 2009). In addition, reduction of GSK-3β impairs NMDAR-mediated long-term depression (Peineau et al., 2007) and memory consolidation (Kimura et al., 2008), raising concerns about potential side effects of GSK-3β inhibitors. In a similar vein, CDK5 inhibitors prevent Aβ-induced hyperphosphorylation of tau and cell death in culture (Alvarez et al., 1999; Zheng et al., 2005), but CDK5 is essential for multiple cell signaling pathways and adult neurogenesis, limiting its appeal as a tau-targeting approach in AD. However, CDK5 and p25, a truncated form of the CDK5 subunit p35, also promote neurodegeneration through mechanisms that are independent of tau phosphorylation, involving inhibition of histone deacetylase 1 (HDAC1) and aberrant expression of cell cycle genes (Kim et al., 2008), raising possibilities for additional therapeutic intervention.

Tau Aggregation Inhibitors

In vitro, tau aggregation is induced by polyanionic compounds such as RNA (Kampers et al., 1996), heparin (Crowe et al., 2007; Goedert et al., 1996; Pérez et al., 1996), and lipid micelles (Chirita et al., 2003). Many of the drugs that block the aggregation of tau also block the pathological aggregation of other proteins under cell-free conditions, including Aβ and α-synuclein (Masuda et al., 2006), suggesting that they might be of benefit in diverse proteinopathies. Some tau aggregation inhibitors are effective in Neuro2A cell lines overexpressing a 4R tau microtubule repeat domain fragment with a K280 deletion, which promotes its aggregation (Pickhardt et al., 2005). In human AD patients, the phenothiazine methylene blue showed some promise for slowing disease progression in a phase II clinical trial conducted for 1 year (Gura, 2008). Methylene blue was originally thought to inhibit tau-tau interactions (Wischik et al., 1996), but it may also reduce soluble tau through other mechanisms (O'Leary et al., 2010) as it is known to have many targets (Schirmer et al., 2011). Phase III trials with a newer formulation of methylene blue (LMTX) are planned (Wischik, 2002).

The immunosuppressant FK506 reduces microgliosis and tau aggregation in transgenic mice overexpressing P301S human 4R1N tau under the mouse prion promoter (PS19 model) (Yoshiyama et al., 2007). Since FK506 affects diverse signaling pathways in many cell types, it may act directly on neurons or influence the neuronal environment by modulating glial activation. Inhibition of tau aggregation may also be mediated by direct binding of tau to the FK506 binding protein 52 (Chambraud et al., 2010).

As discussed above, it is far from certain that filamentous tau is actually toxic. Indeed, it is not known which tau assembly or conformation is responsible for tau-dependent neuronal dysfunction and degeneration. Not surprisingly, it is equally uncertain whether the abundance of this entity is lowered by any of the available tau aggregation blockers. In fact, some tau aggregation inhibitors enhance the formation of potentially toxic tau oligomers (Taniguchi et al., 2005). This scenario is reminiscent of the current state of anti-Aβ treatment, where it is also unclear whether any of the anti-Aβ strategies that have undergone or are currently in clinical trials significantly reduce the abundance of Aβ oligomers in human brain tissues, which are suspected to be the main mediators of Aβ-induced neuronal dysfunction (Ashe and Zahs, 2010; Cheng et al., 2007; Sakono and Zako, 2010; Shankar et al., 2008).

Reduction of Overall Tau Levels

In mice, partial reduction of tau during early development is well tolerated, increases resistance to chemically induced seizures, and markedly diminishes Aβ- and ApoE4-induced neuronal and cognitive impairments in vivo (Andrews-Zwilling et al., 2010; Ittner et al., 2010; Roberson et al., 2007, 2011). Assuming ongoing experiments confirm that reduction of overall tau levels is efficacious and safe also when initiated in adult and old animals with AD-related pathologies, tau could be targeted directly with RNAi approaches in patients with AD. Alternatively, tau levels could be reduced indirectly by targeting molecules that regulate the expression or clearance of tau.

Tau is thought to be degraded via the ubiquitin-proteasome and lysosomal pathways. The ubiquitin ligase for tau was identified as the C terminus of HSP70-interacting protein (CHIP) (Hatakeyama et al., 2004; Petrucelli et al., 2004; Shimura et al., 2004). Reduction of CHIP levels increased the accumulation of tau aggregates in P301L human 4R0N tau mice (JNPL3 model), and CHIP levels are reduced in AD brains (Sahara et al., 2005). Furthermore, as its name suggests, CHIP works in combination with heat shock proteins to regulate tau degradation (Dickey et al., 2007); levels of heat shock protein 90 (Hsp90) correlate inversely with the levels of soluble tau and tau oligomers (Sahara et al., 2007b).

In AD brains, tau is hyperacetylated, which should increase its half-life (Min et al., 2010), alter its microtubule binding and enhance aggregation (Cohen et al., 2011). Because both acetylation and ubiquitination modify lysine residues, acetylation of tau by the acetyl transferase p300 inhibits ubiquitination and stabilizes tau (Min et al., 2010). In addition, acetylation of the 6 amino acid motif VQIINK (PHF6*) inhibits the binding of tau to microtubules and enhances tau aggregation (Cohen et al., 2011). This motif is critical for the formation of tau oligomers and filaments (Sahara et al., 2007a; von Bergen et al., 2001). Thus, the combination of a tau acetylation inhibitor and a ubiquitination-proteasome enhancer might synergize to lower the level of pathogenic tau species.

Larger aggregates of tau are not likely to be accessible to the proteasome but can be degraded by the lysosomal pathway, in which autophagosomes engulf the aggregates and fuse with lysosomes. In cells overexpressing the microtubule repeat domain of tau with a deletion of K280, aggregated tau is removed by the lysosomal pathway (Wang et al., 2009). In slice culture, inhibition of the lysosomal pathway produces NFT-like tau deposition (Bi et al., 1999). The lysosomal pathway of tau degradation is also involved in Niemann-Pick type C (NPC) disease, an autosomal recessive disorder associated with neurological symptoms and NFT formation in the brain (Auer et al., 1995). NPC disease is caused by a loss of function of NPC1, a lysosomal trafficking protein (Pacheco and Lieberman, 2008), suggesting that tau is degraded in lysosomes and that lysosomal dysfunction

leads to tau accumulation. Consistent with this notion, phosphorylated tau is increased in the brains of NPC1-deficient mice and of NPC patients (Bu et al., 2002). However, cross-breeding of NPC1-deficient mice with tau knockout mice worsened the phenotype (Pacheco et al., 2009), suggesting that the role of tau in this disease is complex. The autophagic-lysosomal pathway has also been interrogated in a Park2-deficient tauopathy model with parkinsonism overexpressing mutant human 4R2N tau under the mouse Thy1 promoter ($PK^{-/-}/Tau^{VLW}$) (Rodríguez-Navarro et al., 2010). Treatment of 3-month-old $PK^{-/-}/Tau^{VLW}$ mice with trehalose, an mTOR-independent autophagy activator, for 2.5 months prevented dopaminergic neuron loss in the ventral midbrain, reduced phosphorylated tau and total tau in the striatum and limbic system, prevented brain astrogliosis, and improved motor and cognitive behavior. Biochemical and electron microscopy data suggested that the protective effects of trehalose were mediated, at least in part, by autophagy activation (Rodríguez-Navarro et al., 2010).

Tau degradation can also be enhanced by specific activation of the immune system. Active immunization targeting phosphorylated tau reduced filamentous tau inclusions and neuronal dysfunction in transgenic mice overexpressing K257T/P301S human 4R0N tau under the rat tau promoter or P301L human 4R0N tau (JNPL3 model) (Asuni et al., 2007; Boimel et al., 2010). The mechanism by which intracellular proteins, including tau and α-synuclein, are cleared by immunization is not known but may involve lysosomal degradation (Masliah et al., 2005; Sigurdsson, 2008, 2009). Once antibodies enter into brain, they could be taken up by receptor-mediated endocytosis and activate autophagy (Sigurdsson, 2009) or interact with tau in the extracellular matrix. Extracellular tau in cerebrospinal fluid (CSF) is used in combination with other biomarkers to diagnose AD (Trojanowski et al., 2010); phosphorylated tau and total tau levels in the CSF can also predict disease severity (Wallin et al., 2010). Extracellular tau could come from the death of neurons or be released from live cells (Kim et al., 2010). If there is an equilibrium between intracellular and extracellular tau, clearance of tau/antibody complexes from the extracellular space may ultimately lower intracellular tau levels (Brody and Holtzman, 2008; Sigurdsson, 2009).

Microtubule Stabilizers

Microtubule disruption has been observed in several models of AD and FTLD, including transgenic mice overexpressing wild-type human 0N3R tau under the mouse prion promoter (T44 model) (Zhang et al., 2005) or P301S human 4R1N tau (PS19 model) (Yoshiyama et al., 2007) and wild-type neuronal cultures exposed to Aβ oligomers (King et al., 2006; Zempel et al., 2010). Some FTDP-17 tau mutations (Hong et al., 1998) and tau hyperphosphorylation (Alonso et al., 1994; Merrick et al., 1997) reduce the binding of tau to microtubules. Although tau overexpression seems to be associated with destabilization of microtubules, it is unclear whether this phenomenon is always pathogenic and whether it results from a loss- or gain-of-function of tau. Indeed, tau is necessary for Aβ-induced microtubule disassembly in vitro (King et al., 2006), suggesting that tau is actually required for microtubule destabilization. A loss-of-function mechanism seems also unlikely because tau knockout mice have a rather benign phenotype, and tau reduction protects neurons from Aβ-induced impairments in vitro (King et al., 2006; Rapoport et al., 2002; Vossel et al., 2010), ex vivo (Shipton et al., 2011), and in vivo (Ittner et al., 2010; Roberson et al., 2007, 2011).

Despite these caveats regarding underlying mechanism, microtubule stabilizers have shown promise in preclinical and clinical trials for AD. For example, paclitaxel prevented Aβ-induced toxicity in vitro (Zempel et al., 2010) as well as axonal transport deficits and motor impairments in transgenic mice overexpressing wild-type human 0N3R tau (T44 model) (Zhang et al., 2005). Epothilone D, which has better blood-brain barrier permeability, improved microtubule density and cognition in P301S human 4R1N tau mice (PS19 model) (Brunden et al., 2010). The peptide NAP stabilizes microtubules (Divinski et al., 2006) and reduces tau hyperphosphorylation (Vulih-Shultzman et al., 2007), suggesting that microtubule-stabilizing compounds can have more than one mechanism of action. NAP can be administered intranasally and showed some promise in a phase II clinical trial (Gozes et al., 2009).

Conclusions

While tau has long been implicated in neurodegenerative conditions, its functions in the adult brain and the precise mechanisms by which it contributes to neuronal dysfunction and degeneration in these disorders remain to be elucidated. A flurry of recent publications has challenged major dogmas in this field, including the notion that filamentous tau aggregates are the most pernicious forms of tau, that loss of tau function plays a major role in the pathogenesis of tauopathies, that tau enters dendritic spines only under pathological circumstances, and that the adverse activities of tau aggregates are restricted to intracellular compartments. Provocative discoveries suggest that tau regulates neuronal excitability and that it is required for Aβ and other excitotoxins to cause neuronal deficits, aberrant network activity and cognitive decline. Indeed, tau has "graduated" from a putative microtubule stabilizer to a multifunctional protein with many interacting signaling networks and to a master regulator of the intracellular trafficking of organelles and molecules involved in synaptic functions at the pre- and postsynaptic level. The hunt has also been intensified for the most pathogenic forms of tau, some of which have been traced into dendritic spines, and more has been learned about the complex posttranslational modification of tau, particularly acetylation, which appears to regulate the ubiquitination, turnover, and aggregation of tau. These and other findings are providing critical guidance in the development of better treatments for tauopathies aimed at tau itself, tau regulators or factors mediating its putative functions. Identifying the functions and precise roles of tau in neurodegenerative disorders will likely require the analysis of conditional knockout models and the clinical evaluation of pertinent drugs with well defined modes of action.

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