Published in final edited form as:

Biochem Soc Trans. 2012 August 1; 40(4): 661–666. doi:10.1042/BST20120010.

Brain-penetrant microtubule-stabilizing compounds as potential therapeutic agents for tauopathies

Kurt R. Brunden^{*,1}, Carlo Ballatore^{*,†}, Virginia M.-Y. Lee^{*}, Amos B. Smith III[†], and John Q. Trojanowski^{*}

^{*}Center for Neurodegenerative Disease Research, Institute on Aging and Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

[†]Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

Abstract

Neurons within the brains of those with AD (Alzheimer's disease) and related neurodegenerative disorders, collectively termed 'tauopathies', contain fibrillar inclusions composed of hyperphosphorylated tau protein. Tau is normally enriched in axons, where it binds and stabilizes MTs (microtubules). Tau hyperphosphorylation and aggregation probably result in reduced MT binding that could affect axonal transport and neuronal function. A possible therapeutic strategy to overcome a loss of tau function in tauopathies is administration of MT-stabilizing agents, such as those used in the treatment of cancer. However, these drugs elicit severe side effects, and most existing MT-stabilizing compounds have poor BBB (blood–brain barrier) permeability, which renders them unsuitable for tauopathy treatment. We identified EpoD (epothilone D) as a brain-penetrant MT-stabilizing agent with preferred pharmacokinetic and pharmacodynamic properties. EpoD was evaluated for its ability to compensate for tau loss-of-function in an established Tg (transgenic) mouse model, using both preventative and interventional dosing paradigms. EpoD at doses much lower than previously used in human cancer patients caused improved axonal MT density and decreased axonal dystrophy in the tau Tg mice, leading to an alleviation of cognitive deficits. Moreover, EpoD reduced the extent of tau pathology in aged tau Tg mice. Importantly, no adverse side effects were observed in the EpoD-treated mice. These results suggest that EpoD might be a viable drug candidate for the treatment of AD and related tauopathies.

Keywords

Alzheimer's disease; axon; microtubule; tauopathy; therapeutic agent

Introduction

The neurons within the brains of patients with many neurodegenerative diseases, including AD (Alzheimer's disease), FTL (frontotemporal lobar degeneration), PSP (progressive supranuclear palsy), Pick's disease and CBD (corticobasal degeneration), contain insoluble fibrillar aggregates of hyperphosphorylated tau protein [1,2]. The tau inclusions in these various 'tauopathies' reside either within the neuronal soma as NFTs (neurofibrillary tangles) or within neuritic processes as neurofibrillary threads. As discussed in greater detail

below, tau is normally a highly soluble protein that is enriched within axons, where it binds and stabilizes MTs (microtubules) [3–6]. The common occurrence of intracellular tau deposits in the various tauopathies suggests that misfolded tau causes neuronal dysfunction and damage. In fact, there is a strong correlation between NFT burden in the brain and cognitive deterioration in AD [7–9], and a familial form of FTLD is caused by mutations in the tau gene [10,11]. More recent genome-wide association studies reveal that SNPs (single nucleotide polymorphisms) within the tau gene are associated with an increased risk of Parkinson's disease [12,13], a condition where tau inclusions can be found in addition to the more common Lewy bodies [14,15]. Tau-mediated neurodegeneration probably results from a gain-of-function toxicity associated with one or more misfolded tau species [2,16], and/or a loss-of-function as a consequence of a decrease in tau interaction with MTs due to its hyperphosphorylation and sequestration in aggregates [17]. In the present paper, we examine the evidence supporting a tau loss-of-function hypothesis, and review recent data which suggest that a viable pharmacological approach might be compensation of impaired tau function with MT-stabilizing agents.

Tau functions

There are six isoforms of tau in adult humans that are generated through alternative splicing of three exons within the tau transcript [18–20]. The inclusion or exclusion of exon 10 results in tau with four (4-R tau) or three (3-R tau) MT-binding motifs, respectively, with the ratio of 4-R/3-R tau being ~1 in healthy humans [11]. The additional MT-binding domain of the 4-R tau isoforms results in greater avidity for MTs than is observed with 3-R tau species [21], and thus an imbalance of the normal 4-R/3-R tau ratio could affect MT dynamics. In AD, the insoluble tau found within NFTs consists of both 4-R and 3-R tau. Interestingly, in Pick's disease, the tau inclusions are composed nearly exclusively of 3-R tau, whereas the NFTs in CBD and PSP contain predominantly 4-R tau [22]. The reason for the differences of tau composition in inclusions from these various tauopathies is not understood, but may provide clues to unique aetiologies among these neurodegenerative diseases.

Although tau isoform composition can differ within NFTs from the different tauopathies, the tau within these inclusions is invariably hyperphosphorylated [23–25]. This increased phosphorylation generally decreases the binding of tau to MTs [26–28], and could contribute to a loss of tau function. In addition, tau hyperphosphorylation at some sites can enhance fibrillization [29,30], and thus could increase the rate of NFT and neuropil thread formation. As the primary function attributed to tau is MT stabilization within axons, a loss of tau function could result in axonal MT destabilization and a consequent impairment of the axonal transport that is critical for the proper trafficking of cellular materials. This has been demonstrated in a neuronal culture system, where the induction of tau hyperphosphorylation resulted in a diminution of stable MTs [27], whereas *in vitro* studies show that dephosphorylation of pathological tau isolated from AD brains restores the ability of tau to bind to MTs [31]. In addition to stabilizing MTs, tau may also modulate FAT (fast axonal transport) along MTs, as overexpression of tau in neuronal culture systems decreased the binding of motor proteins to MTs [32–34], thereby affecting FAT. It has been suggested that a distal axonal localization of MT-bound tau normally facilitates kinesin disengagement, so that cargo undergoing anterograde transport is released at axon terminals. Thus the somato-dendritic relocation of tau that is observed in tauopathies might cause an increase in tau bound to MTs in the proximal axon, such that kinesin cannot initially engage with MTs [33]. Although this possibility merits further investigation, it should be noted that the studies supporting a role of tau in regulating MT motor function have only been conducted *in vitro* under conditions of tau overexpression, and thus it remains to be determined whether tau affects kinesin-mediated FAT under the normal levels of expression that typically occur in human tauopathies.

In addition to interacting with MTs in axons, it has been suggested that tau has a potential dendritic role in which it assists in the postsynaptic targeting of the tyrosine kinase fyn [35]. This tau–fyn interaction could be disrupted by an N-terminal fragment of tau, resulting in reduced postsynaptic fyn localization. One consequence of this diminished fyn trafficking was decreased phosphorylation of the NR2b subunit of the NMDA (*N*-methyl-D-aspartate) receptor, with a resulting reduction of NR2b interaction with the PSD-95 (postsynaptic density 95) protein that led to diminished NR2b anchoring at the postsynaptic density [35]. Interestingly, a diminution of NR2b-PSD-95 binding appeared to prevent memory deficits and lethality associated with APP (amyloid precursor protein) overexpression and A β (amyloid β -peptide) production in Tg (transgenic) mice. This proposed dendritic function of tau thus provides a potential explanation for the observation that reducing endogenous tau levels led to an improvement of A β -induced memory deficits in an APP Tg mouse model [36].

Tau loss-of-function

The tau loss-of-function hypothesis is supported by studies which have examined MTs in AD brain. For example, there is a reduction in MT number and MT length in AD pyramidal neurons [37], and although this was not correlated with the presence of NFTs, the presence of tau pathology at sites distal to the perikarya in the axons and dendrites of affected pyramidal neurons could account for these MT abnormalities. Alternatively, the suggested absence of a direct relationship between NFT deposition and MT alterations in AD might suggest that it is predominantly tau hyperphosphorylation that affects MT engagement and stabilization, rather than tau sequestration into NFTs. This interpretation is consistent with the observation that an induction of tau hyperphosphorylation in NT2N cells, through the inhibition of protein phosphatases, led to an impairment of tau binding to MTs with resulting MT depolymerization and axonal degeneration [27]. However, another study revealed a decrease in stable MTs that correlated with the presence of NFTs within neurons in AD brain [38], and the accumulation of pathological tau in the AD brain is associated with a reciprocal depletion of normal tau when affected versus unaffected regions of the AD brain are compared [39]. Thus it is possible and perhaps likely that both tau hyperphosphorylation and sequestration within insoluble inclusions contribute to a loss of MT stabilization in AD brain.

Similar reductions of MT density have been observed in Tg mouse models of tauopathy. For example, 12-month-old T44 tau Tg mice, which express the smallest human isoform of 3-R tau and accumulate insoluble, hyperphosphorylated tau inclusions primarily within the spinal cord and brainstem, have decreased MT density in spinal ventral root axons [40]. These same mice also demonstrate impaired FAT, which is consistent with a loss of MT function [41]. Similarly, PS19 Tg mice expressing 4-R tau with the P301S mutation found in inherited FTLT have reduced MT density in the optic nerve that coincides with the development of forebrain tau pathology [42], and recent data reveal that these mice also have deficient FAT [43]. Finally, it has been demonstrated that human 3-R tau expressed in *Drosophila* motor neurons is hyperphosphorylated and has reduced MT-binding. Interestingly, this hyperphosphorylated human tau also appears to sequester the endogenous *Drosophila* tau, further contributing to MT destabilization [44].

Compensating for tau loss-of-function

The idea that it might be possible to compensate for diminished tau binding to MTs with existing MT-stabilizing drugs, such as paclitaxel (Figure 1), was first hypothesized nearly two decades ago [17]. However, this concept was not tested until 2005, when the T44 3-R tau Tg mice described above, which develop tau inclusions in the spinal cord and brainstem

[40], were treated weekly with paclitaxel for a 3-month period [41]. The Tg mice dosed with paclitaxel showed improvement of MT density and FAT in ventral root axons, as well as improved motor performance, relative to Tg mice that received vehicle only. These data provided the initial proof-of-principle that it is possible to overcome a loss of tau function with a small molecule. However, paclitaxel does not readily cross the BBB (blood–brain barrier) and thus is not suitable for the treatment of human tauopathies, where tau pathology is found primarily in the higher learning centres of the telencephalon. Although our laboratory and others have attempted to develop taxanes with improved BBB permeability [45–47], these efforts have been largely unsuccessful. More recently, we have identified the epothilone class of MT-stabilizing agents as being generally brain-penetrant, and among this family of compounds, EpoD (epothilone D; Figure 1) was found to have preferred pharmacokinetic and pharmacodynamic properties [48]. This led to the evaluation of EpoD in the previously mentioned PS19 tau Tg mice, which develop tau pathology in the forebrain with age. Asymptomatic 3-month-old PS19 mice received weekly intraperitoneal administrations of 1 or 3 mg/kg EpoD until 6 months of age [42], a time point at which these mice normally begin to develop detectable tau pathology. Whereas vehicle-treated PS19 mice had reduced MT density and increased axonal dystrophy relative to non-Tg littermates, those PS19 mice that received either the high or low dose of EpoD showed a normalization of these endpoints. Moreover, spatial learning deficits that were observed in the vehicle-treated 6-month-old PS19 mice were largely corrected by EpoD treatment. Importantly, the beneficial effects of EpoD were achieved at doses that were 1/30 and 1/10 of the dose utilized in a Phase II clinical trial with cancer patients [49]. There was an absence of any observable side effects in mice that received these low doses of EpoD, including neutropenia and sensory nerve neuropathy, which are the primary side effects observed in patients treated with MT-stabilizing agents [50,51].

The ability of EpoD to safely attenuate axonal deficits and related cognitive impairments in PS19 mice that are developing tau pathology suggests that this compound might have potential as a primary or secondary prevention treatment for AD and related tauopathies. However, tauopathy patients will almost certainly have existing tau pathology in their brains by the time they present with clinical symptoms, and thus a more rigorous proof that EpoD has potential as an interventional therapeutic for patients with clinically manifest disease required demonstration of efficacy in a tauopathy model with pre-existing pathology. To this end, we recently completed an evaluation of EpoD in PS19 mice in which the animals received weekly dosing of 0.3 or 1 mg/kg of EpoD from 9 to 12 months of age [43], as 9-month-old PS19 mice have appreciable tau pathology. Notably, both doses of the MT-stabilizing compound reduced axonal dystrophy in these older Tg mice, with a resulting normalization of impaired FAT. Moreover, the EpoD-treated 12-month-old PS19 mice showed an improvement of both working and spatial memory performance, with the higher dose of the compound eliciting the greatest change. Somewhat unexpectedly, the extent of tau pathology in the brains of the aged PS19 mice was reduced by EpoD, as is evident from a reduction in forebrain phospho-tau and misfolded tau as detected by immunohistochemical staining. Moreover, the EpoD-treated PS19 mice had a lower amount of insoluble tau within the brain as determined by ELISA quantification. Importantly, EpoD treatment resulted in diminution of the hippocampal neuron and synapse loss that is normally observed in the aged PS19 mice as they accumulate increasing amounts of tau pathology [52]. Notably, no adverse effects of EpoD were observed in the interventional study with aged PS19 mice, which is highly significant for AD and related tauopathies since intervention therapy with EpoD or a related MT-stabilizing agent will probably require chronic administration of the drug for months to years. Thus these recent results provide encouragement that EpoD might be suitable for testing in human tauopathy patients.

Conclusions and future directions

The aforementioned studies which demonstrated that paclitaxel improved axonal and motor outcomes in a tau Tg mouse model with spinal cord pathology [41], and that the brain-penetrant MT-stabilizing agent EpoD was safe and efficacious in prevention [42] and intervention [43] studies in PS19 tau Tg mice with forebrain pathology, provide compelling evidence that this therapeutic approach holds promise for the treatment of tauopathies. In addition to these small-molecule MT-stabilizing agents, there is also evidence that the peptidic agent NAP, which has been suggested to act via stabilization of MTs, improved outcomes in AD-like Tg models [53,54]. The most parsimonious explanation for the beneficial effects of MT-stabilizing agents in these models is that they compensated for a deficiency of tau-mediated stabilization of MTs. However, it is also possible that the improvement of FAT and cognitive performance observed with the small-molecule compounds resulted, in part, from displacement of mislocalized tau from axonal MTs. There is evidence that paclitaxel can compete with tau binding to MTs [55,56], and EpoD and paclitaxel have a shared binding site on MTs [57]. Thus these agents could inhibit tau interaction with MTs, perhaps resulting in greater kinesin binding to proximal MTs in neurons with somato-dendritic tau, leading to improved FAT [33].

The hypothesis that a known MT-stabilizing agent such as EpoD might overcome a loss of tau function is based on the role that tau normally plays in facilitating MT stabilization. However, the observed reduction of tau pathology after EpoD treatment of aged PS19 tau Tg mice was unexpected, as it is not obvious that an improvement of MT stabilization should result in a decrease in insoluble and hyperphosphorylated tau. Although we do not have a mechanistic explanation for the EpoD-mediated reduction of misfolded tau, it has been demonstrated that tau pathology can be exacerbated when tau Tg mice are genetically crossed with mice that have altered FAT due to knockout of the kinesin light chain [58]. This observation would suggest that a deficiency in anterograde FAT leads to increased tau phosphorylation and misfolding [59]. Consequently, a vicious cycle may ensue, whereby the initial development of hyperphosphorylated and aggregated tau leads to diminished FAT, which then causes further development of tau pathology. This feed-forward mechanism could be ameliorated by an agent such as EpoD that normalizes FAT, which could then prevent development of tau pathology.

Although our results reveal that EpoD proved to be both safe and efficacious when administered to PS19 tau Tg mice, either in a preventative study design or in an interventional paradigm, the establishment of a reasonable projected therapeutic index (i.e., efficacy dose relative to the dose at which side effects are observed) will be paramount to the advancement of such compounds into clinical trials with tauopathy patients. As noted, MT-stabilizing drugs are known to elicit fairly severe side effects at the doses that are utilized for the treatment of cancer [49,51]. These dose-limiting side effects will probably be more noticeable in an aged population, such as AD patients. Moreover, whereas MT-stabilizing drugs are used for a fairly short period in an oncology setting, it is possible that tauopathy patients would require regular dosing for years. Thus it will be important to examine how well EpoD or comparable compounds are tolerated in humans upon repeated administration at doses similar to those that were efficacious in tau Tg mice (e.g. 1/30–1/100 of the doses used in cancer patients).

Finally, although EpoD appears to have a unique set of pharmacokinetic properties, including prolonged brain retention [42,48], that may make it a potential drug candidate for the treatment of tauopathies, it would be desirable to identify additional MT-stabilizing agents that have similar or improved features. To date, nearly all MT-stabilizing small molecules have been identified from natural products, and have relatively complex

structures that are difficult to synthesize. The identification of novel, brain-penetrant MT-stabilizing agents that are more amenable to synthetic chemistry would thus be of significant interest.

Acknowledgments

We thank our colleagues for their contributions to the work summarized here and the Marian S. Ware Alzheimer Program. V.M.-Y.L. is the John H. Ware 3rd Professor for Alzheimer's Disease Research and J.Q.T. is the William Maul Measey-Truman G. Schnabel, Jr, M.D. Professor of Geriatric Medicine and Gerontology. Finally, we thank our patients and their families whose commitment to research has made our work possible.

Funding

This work was supported by the National Institutes of Health [grant numbers P01 AG09215, P30 AG10124, P01 AG11542, P01 AG14382, P01 AG14449, P01 AG17586, P01 AG19724, P01 NS-044233 and U01 AG24904].

Abbreviations used

Aβ	amyloid β -peptide
AD	Alzheimer's disease
APP	amyloid precursor protein
BBB	blood–brain barrier
CBD	corticobasal degeneration
EpoD	epothilone D
FAT	fast axonal transport
FTLD	frontotemporal lobar degeneration
MT	microtubule
NFT	neurofibrillary tangle
PSD-95	postsynaptic density 95
PSP	progressive supranuclear palsy
Tg	transgenic

References

1. Lee VMY, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* 2001; 24:1121–1159. [PubMed: 11520930]
2. Ballatore C, Lee VMY, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* 2007; 8:663–672. [PubMed: 17684513]
3. Cleveland DW, Hwo SY, Kirschner MW. Physical and chemical properties of purified tau factor and role of tau in microtubule assembly. *J. Mol. Biol.* 1977; 116:227–247. [PubMed: 146092]
4. Cleveland DW, Connolly JA, Kalnins VI, Spiegelman BM, Kirschner MW. Physical properties and cellular localization of tau, a microtubule-associated protein which induces assembly of purified tubulin. *J. Cell Biol.* 1977; 75:A283.
5. Drechsel DN, Hyman AA, Cobb MH, Kirschner MW. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. *Mol. Biol. Cell.* 1992; 3:1141–1154. [PubMed: 1421571]
6. Gustke N, Trinczek B, Biernat J, Mandelkow EM, Mandelkow E. Domains of tau protein and interactions with microtubules. *Biochemistry.* 1994; 33:9511–9522. [PubMed: 8068626]

7. Arriagada PV, Growdon JH, Hedleywhyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology*. 1992; 42:631–639. [PubMed: 1549228]
8. Wilcock GK, Esiri MM. Plaques, tangles and dementia: a quantitative study. *J. Neurol. Sci.* 1982; 56:343–356. [PubMed: 7175555]
9. Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, Hyman BT. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann. Neurol.* 1997; 41:17–24. [PubMed: 9005861]
10. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. 1998; 393:702–705. [PubMed: 9641683]
11. Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L, Miller BI, Geschwind DH, Bird TD, McKeel D, Goate A, et al. Mutation-specific functional impairments in distinct Tau isoforms of hereditary FTDP-17. *Science*. 1998; 282:1914–1917. [PubMed: 9836646]
12. Edwards TL, Scott WK, Almonte C, Burt A, Powell EH, Beecham GW, Wang LY, Zuchner S, Konidari I, Wang GF, et al. Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. *Ann. Hum. Genet.* 2010; 74:97–109. [PubMed: 20070850]
13. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* 2009; 41:1308–1312. [PubMed: 19915575]
14. Kotzbauer PT, Giasson BI, Kravitz AV, Golbe LI, Mark MH, Trojanowski JQ, Lee VMY. Fibrillization of α -synuclein and tau in familial Parkinson's disease caused by the A53T α -synuclein mutation. *Exp. Neurol.* 2004; 187:279–288. [PubMed: 15144854]
15. Winton MJ, Joyce S, Zhukareva V, Practico D, Perl DP, Galasko D, Craig U, Trojanowski JQ, Lee VMY. Characterization of tau pathologies in gray and white matter of Guam parkinsonism–dementia complex. *Acta Neuropathol.* 2006; 111:401–412. [PubMed: 16609851]
16. Brunden K, Trojanowski JQ, Lee VMY. Evidence that non-fibrillar tau causes pathology linked to neurodegeneration and behavioral impairments. *J. Alzheimer's Dis.* 2008; 14:393–399.
17. Lee VMY, Daughenbaugh R, Trojanowski JQ. Microtubule stabilizing drugs for the treatment of Alzheimer's disease. *Neurobiol. Aging*. 1994; 15:S87–S89. [PubMed: 7700471]
18. Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron*. 1989; 3:519–526. [PubMed: 2484340]
19. Goedert M, Jakes R. Expression of separate isoforms of human tau protein: correlation with the tau-pattern in brain and effects on tubulin polymerization. *EMBO. J.* 1990; 9:4225–4230. [PubMed: 2124967]
20. Andreadis A, Brown WM, Kosik KS. Structure and novel exons of the human tau gene. *Biochemistry*. 1992; 31:10626–10633. [PubMed: 1420178]
21. Panda D, Samuel JC, Massie M, Feinstein SC, Wilson L. Differential regulation of microtubule dynamics by three- and four-repeat tau: implications for the onset of neurodegenerative disease. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:9548–9553. [PubMed: 12886013]
22. Hasegawa M. Biochemistry and molecular biology of tauopathies. *Neuropathology*. 2006; 26:484–490. [PubMed: 17080729]
23. Ksiezak-Reding H, Liu WK, Yen SH. Phosphate analysis and dephosphorylation of modified tau associated with paired helical filaments. *Brain Res.* 1992; 597:209–219. [PubMed: 1472994]
24. Kopke E, Tung YC, Shaikh S, Alonso AD, Iqbal K, Grundke-Iqbal I. Microtubule-associated protein tau: abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J. Biol. Chem.* 1993; 268:24374–24384. [PubMed: 8226987]
25. Matsuo ES, Shin RW, Billingsley ML, Vandevoorde A, Oconnor M, Trojanowski JQ, Lee VMY. Biopsy-derived adult human brain tau is phosphorylated at many of the same sites as Alzheimer's disease paired helical filament-tau. *Neuron*. 1994; 13:989–1002. [PubMed: 7946342]
26. Alonso AD, Grundke-Iqbal I, Iqbal K. Abnormally phosphorylated tau from Alzheimer disease brain depolymerizes microtubules. *Neurobiol. Aging*. 1994; 15:S37. [PubMed: 7700457]

27. Merrick SE, Trojanowski JQ, Lee VMY. Selective destruction of stable microtubules and axons by inhibitors of protein serine/threonine phosphatases in cultured human neurons (NT2N cells). *J. Neurosci.* 1997; 17:5726–5737. [PubMed: 9221771]
28. Wagner U, Utton M, Gallo JM, Miller CCJ. Cellular phosphorylation of tau by GSK-3 β influences tau binding to microtubules and microtubule organisation. *J. Cell Sci.* 1996; 109:1537–1543. [PubMed: 8799840]
29. Alonso AD, Grundke-Iqbal I, Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat. Med.* 1996; 2:783–787. [PubMed: 8673924]
30. Necula M, Kuret J. Pseudophosphorylation and glycation of tau protein enhance but do not trigger fibrillization *in vitro*. *J. Biol. Chem.* 2004; 279:49694–49703. [PubMed: 15364924]
31. Bramblett GT, Goedert M, Jakes R, Merrick SE, Trojanowski JQ, Lee VMY. Abnormal tau phosphorylation at Ser³⁹⁶ in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. *Neuron.* 1993; 10:1089–1099. [PubMed: 8318230]
32. Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J. Cell Biol.* 2002; 156:1051–1063. [PubMed: 11901170]
33. Dixit R, Ross JL, Goldman YE, Holzbaur ELF. Differential regulation of dynein and kinesin motor proteins by tau. *Science.* 2008; 319:1086–1089. [PubMed: 18202255]
34. Vershinin M, Carter BC, Razafsky DS, King SJ, Gross SP. Multiple-motor based transport and its regulation by Tau. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:87–92. [PubMed: 17190808]
35. Ittner LM, Ke YD, Delerue F, Bi MA, Gladbach A, van Eersel J, Wolfing H, Chieng BC, Christie MJ, Napier IA, et al. Dendritic function of tau mediates amyloid- β toxicity in Alzheimer's disease mouse models. *Cell.* 2010; 142:387–397. [PubMed: 20655099]
36. Roberson ED, Scarce-Levie K, Palop JJ, Yan FR, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L. Reducing endogenous tau ameliorates amyloid β -induced deficits in an Alzheimer's disease mouse model. *Science.* 2007; 316:750–754. [PubMed: 17478722]
37. Cash AD, Aliev G, Siedlak SL, Nunomura A, Fujioka H, Zhu XW, Raina AK, Vinters HV, Tabaton M, Johnson AB, et al. Microtubule reduction in Alzheimer's disease and aging is independent of tau filament formation. *Am. J. Pathol.* 2003; 162:1623–1627. [PubMed: 12707046]
38. Hempen B, Brion JP. Reduction of acetylated α -tubulin immunoreactivity in neurofibrillary tangle-bearing neurons in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* 1996; 55:964–972. [PubMed: 8800092]
39. Bramblett GT, Trojanowski JQ, Lee VMY. Regions with abundant neurofibrillary pathology in human brain exhibit a selective reduction in levels of binding-competent tau and accumulation of abnormal tau isoforms (A68 proteins). *Lab. Invest.* 1992; 66:212–222. [PubMed: 1735956]
40. Ishihara T, Hong M, Zhang B, Nakagawa Y, Lee MK, Trojanowski JQ, Lee VMY. Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron.* 1999; 24:751–762. [PubMed: 10595524]
41. Zhang B, Maiti A, Shively S, Lakhani F, McDonald-Jones G, Bruce J, Lee EB, Xie SX, Joyce S, Li C, et al. Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:227–231. [PubMed: 15615853]
42. Brunden KR, Zhang B, Carroll J, Yao Y, Potuzak JS, Hogan A-ML, Iba M, James MJ, Xie SX, Ballatore C, et al. Epothilone D improves microtubule density, axonal integrity and cognition in a transgenic mouse model of tauopathy. *J. Neurosci.* 2010; 30:13861–13866. [PubMed: 20943926]
43. Zhang B, Carroll J, Trojanowski JQ, Yao Y, Iba M, Potuzak JS, Hogan AL, Xie SX, Ballatore C, Smith A II, et al. The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits and Alzheimer-like pathology in an interventional study with aged tau transgenic mice. *J. Neurosci.* 2011; 32:3601–3611. [PubMed: 22423084]
44. Cowan CM, Bossing T, Page A, Shepherd D, Mudher A. Soluble hyper-phosphorylated tau causes microtubule breakdown and functionally compromises normal tau *in vivo*. *Acta Neuropathol.* 2010; 120:593–604. [PubMed: 20617325]

45. Ballatore C, Hyde E, Deiches RF, Lee VMY, Trojanowski JQ, Hurn D, Smith AB. Paclitaxel C-10 carbamates: potential candidates for the treatment of neurodegenerative tauopathies. *Bioorg. Med. Chem. Lett.* 2007; 17:3642–3646. [PubMed: 17485207]
46. Rice A, Liu YB, Michaelis ML, Himes RH, Georg GI, Audus KL. Chemical modification of paclitaxel (taxol) reduces P-glycoprotein interactions and increases permeation across the blood–brain barrier *in vitro* and *in situ*. *J. Med. Chem.* 2005; 48:832–838. [PubMed: 15689167]
47. Ojima I, Chen J, Sun L, Borella CP, Wang T, Miller ML, Lin SN, Geng XD, Kuznetsova LR, Qu CX, et al. Design, synthesis, and biological evaluation of new-generation taxoids. *J. Med. Chem.* 2008; 51:3203–3221. [PubMed: 18465846]
48. Brunden KR, Yao Y, Potuzak JS, Ferrar NI, Ballatore C, James MJ, Hogan AL, Trojanowski JQ, Smith AB 3rd, Lee VMY. The characterization of microtubule-stabilizing drugs as possible therapeutic agents for Alzheimer's disease and related tauopathies. *Pharmacol. Res.* 2011; 63:341–351. [PubMed: 21163349]
49. Beer TM, Higano CS, Saleh M, Dreicer R, Hudes G, Picus J, Rarick M, Fehrenbacher L, Hannah AL. Phase II study of KOS-862 in patients with metastatic androgen independent prostate cancer previously treated with docetaxel. *Invest. New Drugs.* 2007; 25:565–570. [PubMed: 17618407]
50. Cheng KL, Bradley T, Budman DR. Novel microtubule-targeting agents: the epothilones. *Biol. Targets Ther.* 2008; 2:789–811.
51. Cortes J, Baselga J. Targeting the microtubules in breast cancer beyond taxanes: the epothilones. *Oncologist.* 2007; 12:271–280. [PubMed: 17405891]
52. Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, Maeda J, Suhara T, Trojanowski JQ, Lee VMY. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* 2007; 53:337–351. [PubMed: 17270732]
53. Matsuoka Y, Gray AJ, Hirata-Fukae C, Minami SS, Waterhouse EG, Mattson MP, LaFerla FM, Gozes I, Aisen PS. Intranasal NAP administration reduces accumulation of amyloid peptide and tau hyperphosphorylation in a transgenic mouse model of Alzheimer's disease at early pathological stage. *J. Mol. Neurosci.* 2007; 31:165–170. [PubMed: 17478890]
54. Matsuoka Y, Jouroukhin Y, Gray AJ, Ma L, Hirata-Fukae C, Li HF, Feng L, Lecanu L, Walker BR, Planel E, et al. A neuronal microtubule-interacting agent, NAPVSIPQ, reduces tau pathology and enhances cognitive function in a mouse model of Alzheimer's disease. *J. Pharmacol. Exp. Ther.* 2008; 325:146–153. [PubMed: 18199809]
55. Kar S, Fan J, Smith MJ, Goedert M, Amos LA. Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. *EMBO. J.* 2003; 22:70–77. [PubMed: 12505985]
56. Samsonov A, Yu JZ, Rasenick M, Popov SV. Tau interaction with microtubules *in vivo*. *J. Cell Sci.* 2004; 117:6129–6141. [PubMed: 15564376]
57. Bollag DM, McQueney PA, Zhu J, Hensens O, Koupal L, Liesch J, Goetz M, Lazarides E, Woods CM. Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res.* 1995; 55:2325–2333. [PubMed: 7757983]
58. Falzone TL, Gunawardena S, McCleary D, Reis GF, Goldstein LSB. Kinesin-1 transport reductions enhance human tau hyperphosphorylation, aggregation and neurodegeneration in animal models of tauopathies. *Hum. Mol. Genet.* 2010; 19:4399–4408. [PubMed: 20817925]
59. Falzone TL, Stokin GB, Lillo C, Rodrigues EM, Westerman EL, Williams DS, Goldstein LSB. Axonal stress kinase activation and tau misbehavior induced by kinesin-1 transport defects. *J. Neurosci.* 2009; 29:5758–5767. [PubMed: 19420244]

Epothilone D

Paclitaxel

Figure 1.
Structures of EpoD and paclitaxel