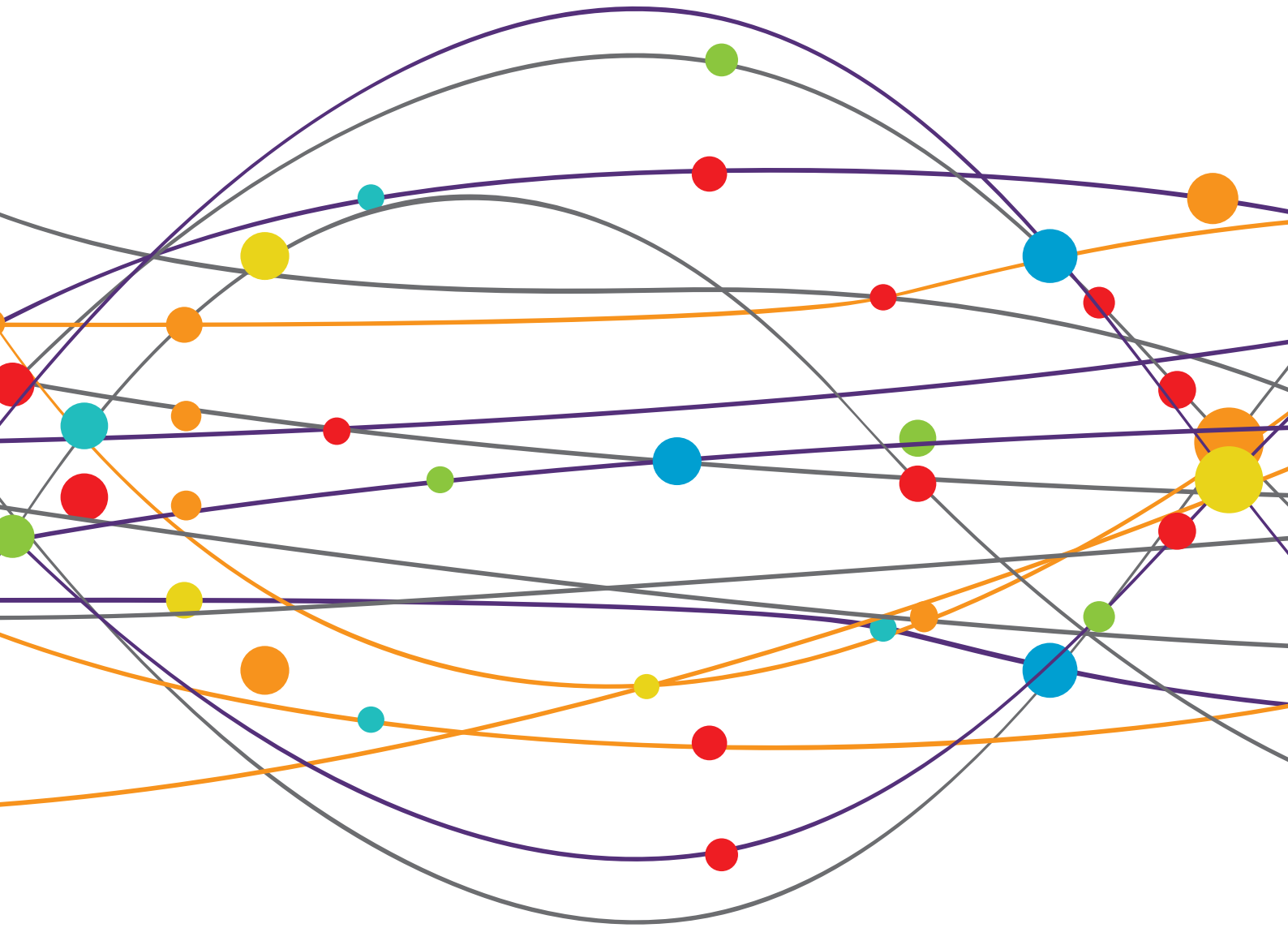
TAU PATHOLOGY IN NEUROLOGICAL DISORDERS

EDITED BY: Sonia Do Carmo, A. Claudio Cuello and Maria Grazia Spillantini
PUBLISHED IN: Frontiers in Neurology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88971-625-8
DOI 10.3389/978-2-88971-625-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

TAU PATHOLOGY IN NEUROLOGICAL DISORDERS

Topic Editors:
Sonia Do Carmo, McGill University, Canada
A. Claudio Cuello, McGill University, Canada
Maria Grazia Spillantini, University of Cambridge, United Kingdom

Citation: Do Carmo, S., Cuello, A. C., Spillantini, M. G., eds. (2021). Tau Pathology in Neurological Disorders. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88971-625-8

Table of Contents

frontiers in Neurology

EDITORIAL
published: 24 September 2021
doi: 10.3389/fneur.2021.754669

Editorial: Tau Pathology in Neurological Disorders

Sonia Do Carmo[1], *Maria Grazia Spillantini*[2] *and A. Claudio Cuello*[1,3,4,5]*

[1] Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada, [2] Department of Clinical Neurosciences, Clifford Allbutt Building, University of Cambridge, Cambridge, United Kingdom, [3] Department of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada, [4] Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada, [5] Department of Pharmacology, Oxford University, Oxford, United Kingdom

Keywords: tau pathology, tauopathies, Alzheimer's disease, neurodegeneration, dementia, tau therapeutics

Editorial on the Research Topic

Tau Pathology in Neurological Disorders

The histological alterations now known as neurofibrillary tangles (NFTs) were first described in 1906 by Alois Alzheimer using light microscopy and silver staining (1); in the late 1960's it was shown by electron microscopy that they were made of paired helical filaments (PHFs) and a small percentage of straight filaments (SFs) (2, 3). Around this time, it was also shown that abundant NFTs were associated with cognitive decline (4, 5). Between 1985 and 1991 it was shown by immunohistochemistry, biochemical approaches and molecular cloning that PHFs and SFs are made of hyperphosphorylated tau protein (6–11) a microtubule-associated protein first described in 1975 (12). Tau filaments from Alzheimer's disease were shown to be made of a structured core and the less structured fuzzy coat (10, 13, 14). These pioneering accomplishments paved the way to further recognize the complexity of tau translation and splicing leading to the identification of the different tau isoforms in the healthy brain and modification such as phosphorylation, truncation and conformational changes in neurodegenerative diseases (7, 15–18). In normal human brain, six tau isoforms are expressed from a single gene by alternative mRNA splicing (19, 20). They differ by the presence or absence of two amino-terminal inserts and an extra repeat of 31 amino acids in the C-terminal region. Depending on its presence, one can divide tau isoforms into two groups, three isoforms with three repeats and three isoforms with four repeats. Both groups of isoforms are expressed at similar levels in normal brain. The repeats and some adjoining sequences make up the microtubule-binding region of tau. They also form part of the filament core, suggesting that physiological function and pathological assembly are mutually incompatible. In Alzheimer's disease, all six tau isoforms are present in PHFs and SFs (15). Electron microscopy and image reconstruction showed that tau filaments from Alzheimer's disease are made of two identical C-shaped protofilaments (21). However, the resolution was insufficient to see individual amino acids. This changed in 2017, when the structure of the Alzheimer tau fold was obtained at near-atomic resolution using electron cryo-microscopy (cryo-EM) (22). The same tau fold has been described in multiple individuals with Alzheimer's disease (23), as well as in some cases with prion protein amyloidosis (24) and in primary age-related tauopathy (PART) (25). Different tau folds have been described in Pick's disease (23), chronic traumatic encephalopathy (26), and corticobasal degeneration (27).

OPEN ACCESS

Edited and reviewed by:
Bruce Miller,
University of California, San Francisco, United States

***Correspondence:**
A. Claudio Cuello
claudio.cuello@mcgill.ca

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *06 August 2021*
Accepted: *26 August 2021*
Published: *24 September 2021*

Citation:
Do Carmo S, Spillantini MG and Cuello AC (2021) Editorial: Tau Pathology in Neurological Disorders. Front. Neurol. 12:754669. doi: 10.3389/fneur.2021.754669

These findings tell us that Alzheimer NFTs are made of filamentous tau, but they do not say anything about the relevance of tau assembly for the disease process. This required human genetics. In June 1998, three publications reported mutations in *MAPT*, the tau gene, in familial forms of frontotemporal dementia (28–30). Even though *MAPT* mutations do not cause Alzheimer's disease, these findings proved that dysfunction of tau protein is sufficient to cause neurodegeneration and dementia. To date, 65 missense, deletion and intronic mutations in *MAPT* have been shown to cause disease. Gene dosage mutations have also been reported (31). Importantly, filamentous tau assemblies were present in all cases. Depending on the mutations, assemblies are made of either 3R, 4R, or 3R+4R tau. Similar findings have been described for idiopathic human tauopathies. Thus, Pick's disease is a 3R tauopathy, whereas progressive supranuclear palsy, globular glial tauopathy, corticobasal degeneration, and argyrophilic grain disease are 4R tauopathies. Alzheimer's disease, familial British dementia, familial Danish dementia, chronic traumatic encephalopathy and Guam-Parkinsonism-Dementia are 3R+4R tauopathies.

In 2009, it was shown that assembled tau exhibits prion-like properties in experimental systems (32, 33). It is now well-established that a tau seed can induce the assembly of monomeric protein, but it remains to be established what role the prion-like spreading of assembled tau plays in human brain. Although the staging of tau pathology is associated with development of dementia (34–37) and is consistent with prion-like spreading, it cannot prove its existence. Perhaps the best evidence for prion-like spreading in human brain comes from a study showing the absence of assembled tau in denervated frontal cortex from Alzheimer's disease (38). Using sucrose gradient fractionation of the brains from mice transgenic for human mutant P301S tau and a cellular assay, it has been shown that small tau filaments are the most potent seeds (39). It remains to be determined which species of assembled tau cause neurodegeneration. It has been suggested that tau oligomers may play an important role (40–42). Current knowledge also suggests that the intercellular transfer of tau aggregates is dependent on the tau species (43) and is likely mediated through mechanisms such as the release of tau seeds into the extracellular space in a free form or within vesicles, such as exosomes, which enter recipient neighboring cells by fluid-phase or receptor-mediated endocytosis or by vesicle fusion. The transfer of tau seeds to recipient cells can also occur through nanotubes [reviewed in (44)]. The recipient cells can be either neurons or other brain cell types such as microglia, astrocytes, oligodendrocytes and endothelial cells as highlighted in this Research Topic.

Notwithstanding that the presence and importance of tau and its modifications in neuropathology were first studied in Alzheimer's disease, tau inclusions are also represented in other neurodegenerative diseases grouped under the umbrella term "tauopathies." The strong association between NFTs and cognitive capabilities in the continuum of Alzheimer's disease, and observed also in Down syndrome, was described in a wealth of literature (34, 45–57). These observations were also corroborated by the recent report of an autosomal dominant Alzheimer's disease case who presented mild cognitive impairment at the age of 70, 3 decades later of what expected in the family and who presented with extreme amyloid burden but minimal tau pathology (58). In addition to Alzheimer's disease, tau pathology has been described in other neurodegenerative diseases which lack significant Aβ pathology including Pick's disease, progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia and parkinsonism linked to chromosome 17, among others (30, 59–62) [reviewed in (63, 64)]. In consequence the term "tauopathy" used to describe the pathology in a family with MAPT mutation (61) encompassing now all the above neurodegenerative diseases.

Still, despite the colossal amount of knowledge on tau biology gathered so far, significant questions related to how tau transitions from the homeostatic into the disease state remain at least partially unanswered: (1) How do the differential levels of tau post-translational modifications and aggregation affect tau propagation and its biological activity? (2) What is the contribution of tau regions outside the microtubule binding domain to tau physiology and pathology? (3) Which factors define the phenotypic heterogeneity of tauopathies; is miRNA-mediated regulation of tau involved? (4) What are the functional consequences of tau interaction with cellular organelles such as the nucleus and mitochondria? (5) How and how much does the transmission of tau to non-neuronal cells contribute to tauopathy progression and what is the functional consequence of such transmission in the recipient cells? (6) What is the contribution of tau pathology to the malfunction of the brain vasculature and neurovascular unit in Alzheimer's disease? (7) To which extent does tau intersect with other pathologies to drive neurodegeneration and cognitive dysfunction? (8) How does the current knowledge of tau biology translate into tau-targeted therapies, and can these be of benefit in tauopathies other than Alzheimer's?

We present this Research Topic and e-book to provide an insight into some of these questions and highlights the most recent advances in understanding the molecular and cellular mechanisms underlying the evolution of tau pathology in Alzheimer's disease and in other tauopathies.

In this Research Topic, the extent of tau post-translational modifications, the assembly states and conformations of tau, the distribution of tau in different cell types, the spreading of tau to cells and tissues and the toxic effects of abnormal tau are comprehensively reviewed by Kang et al. who conclude that "although heterogeneity [of tau biology] in the here and now is an inconvenient truth, embracing this effect, defining its origins and then adjusting approaches may pave the way for more sophisticated testing and more realistic interventions."

The extent of tau post-translational modifications across tauopathies, their frequency, and their effect on tau function, aggregation and degradation are further reviewed by Alquezar et al. It is noteworthy that the vast number of sites on the tau protein that can be targeted by specific modifications, often on the same amino acid residue, opens the door for a competition between post-translational modifications that defines a dynamic code regulating tau function, aggregation and degradation and could therefore explain the heterogeneity of

tauopathies. The significance of tau phosphorylation is further emphasized by Duquette et al. who discuss that the pattern of tau hyperphosphorylation varies greatly in physiological and pathological conditions. Tau hyperphosphorylation appears mainly protective and reversible in brain development and in hibernating animals. However, in pathological conditions the pattern of tau hyperphosphorylation is disease-specific in the same way as the atomic structure of their intrinsic tau filaments as revealed by cryo-EM.

The disease-specificity of tau phosphorylation is further highlighted in amyotrophic lateral sclerosis (ALS) and in ALS frontotemporal spectrum disorder (ALS-FTSD) as reviewed by Strong et al. where the distinctive pathological phosphorylation at Thr175 promotes exposure of the phosphatase activating domain in the tau N-terminus thereby activating GSK3β-mediated phosphorylation at Thr231 leading to the formation of tau oligomers. Indeed, it has been shown that tau phosphorylation and aggregation are intimately linked and tau hyperphosphorylation by proline-directed stress kinases, such as GSK3β favors tau oligomerization (65). Novel information regarding the reason for which oligomeric tau might be more neurotoxic than fibrillar tau is provided by Jiang et al. Using a seeding assay in primary neuron cultures expressing human 4R0N tau Jiang et al. demonstrate that oligomeric seeds of tau show a greater co-localization with RNA binding proteins associated with stress granules, an element that could contribute to increased pathology.

The molecular organization of the tau protein in relation to the functional interactions of diverse tau regions, including the particular features of the tau non-microtubule binding region are eloquently reviewed by Brandt et al. Of relevance, tau knockout in mouse models results in a several of effects, with only some of them related to microtubule-dependent processes. This highlights the role of the N-terminal region of tau in the organization and function of membrane organelles, such as the plasma membrane (66, 67) and synaptic vesicles as well as its involvement in tauopathies.

Tau aggregates are mostly found within neurons where they disrupt synaptic plasticity, cell signaling, and DNA integrity eventually leading to cell death. Indeed, stress and pathological conditions promote tau accumulation in cellular compartments other than axons which are considered their main sub-cellular localisation. As such, tau can also be found in the soma, the dendrites, and the nucleus where it can interact with nuclear components. As reviewed by Diez and Wegmann, the dual localisation of tau in cytoplasm and nucleus suggests the transport of tau between the two compartments by mechanisms still to be defined given the absence of nuclear localisation signals. The nuclear tau interaction with DNA appears to be regulated by its phosphorylation and it has been suggested as protective of double stranded DNA breaks and participant of regulation of gene expression.

Such nuclear localization may also promote chromosome stability. On the other hand, cytoplasmic tau could alter the structure of the nuclear envelope and promote the aggregation of nucleoporins in the cytoplasm. This would alter the nucleocytoplasmic protein transport and therefore impacting major cellular signaling pathways. In response to stress nuclear tau can also translocate to the nucleolus and be found in stress granules in the cytoplasm as revealed in ALS and ALS-FTSD and discussed by Strong et al. Moreover, tau pathology impacts negatively adult hippocampal neurogenesis in both Alzheimer's disease and tau mouse models as summarized by Houben et al. This provides a new insight on a less documented tau-related pathological pathway leading to cognitive deficits. However, the studies carried so far have produced conflicting results.

The presence of tau aggregates is being increasingly reported in non-neuronal brain cells. Whether such aggregates derive directly from the intracellular microtubule tau detachment of glial expressed tau or from the glial uptake of neuronal tau is still a matter of debate (68–70). It is well-established that astrocytes play a critical role in supporting neuronal function and that pathological astrocytic changes are associated with and precede neuronal loss in Alzheimer's disease and overt tau aggregation in mouse models of tau aggregation (71). Interestingly, while tau deposition in astrocytes is mostly seen in aging, it can also be found in tauopathies, although with varying frequency according with the disease condition, as reviewed by Reid et al. This deposition of tau impacts the function of astrocytes through mechanisms which includes disruption of calcium signaling, gliotransmitters release, and immune responses consequentially affecting neuronal function. In addition, astrocytes may participate in the spreading of tau *via* the astrocytic water channel aquaporin-4 (AQP4) involved in the elimination of aberrant proteins.

The disruption of astrocytic and microglial function by tau may also participate in vascular dysfunction, an early characteristic of Alzheimer's disease (72). Canepa and Fossati discuss studies *in vitro*, in animal and human supporting the view that tau has deleterious effects on cellular and molecular pathways in vascular and immune cells which lead to the dysregulation of the neurovascular unit. These authors also propose mechanisms mediating the effects of tau on the cerebrovasculature, including tau-induced mitochondrial dysfunction which would lead to increased reactive oxygen species and decreased ATP production and caspase activation. Further to it, Bryant et al. provide novel data showing that microvessels isolated from the dorsolateral prefrontal cortex of Alzheimer's subjects display extensive tau pathology (Braak V/VI, B3) and a strong upregulation of endothelial senescence and leukocyte adhesion-related genes.

The disease-specificity of tau accumulation in glia and the role of oligodendrocytes in tau spreading is further highlighted by Zareba-Paslawska et al. Using a humanized tau mouse line overexpressing all six human tau isoforms in a murine tau knockout background inoculated with insoluble tau extracts from corticobasal degeneration brain homogenates, they show a 4R-tau dependent spreading primarily mediated by oligodendrocytes.

Given the critical role of tau in Alzheimer's and other tauopathies, understanding how tau expression, splicing, post-translational modifications and aggregation are regulated is

essential. Boscher et al. describe the involvement of microRNAs (miRNAs) in such regulation. In particular, loss of the miR-132/212 cluster is strongly associated with memory decline and increased tau pathology. Intriguingly, Boscher et al. show that deletion of the miR-132/212 in PS19 mice, a model of tauopathy, had little effects on disease phenotypes with divergent effects on tau biology. They also discuss potential pitfalls explaining these observations including the lack of adequate tools and animal models to study the involvement of miRNAs in tau pathology.

As evidenced in this Research Topic, because of its key function and its dysregulation and aggregation in Alzheimer's and other tauopathies, tau has become an attractive target for therapy. Existing tau-targeting approaches, their advantages and limitations were elegantly summarized by Masnata et al., with an emphasis on their potential in treating Huntington's disease, a secondary tauopathy. Some approaches target tau phosphorylation using kinase inhibitors, phosphatase activators or immunotherapy targeted at tau phosphorylation at sites that are either hyperphosphorylated or exclusively phosphorylated in Alzheimer's. In the particular case of immunotherapy, a few factors must be considered when designing antibodies including the differential toxicity of tau seeds and the disease-specificity of tau hyperphosphorylation, which precludes the use of immunotherapy targets across tauopathies as discussed by Duquette et al.

The disease-specific characteristics of tau aggregates across tauopathies as revealed by cryo-EM, their trans-cellular propagation and the information gathered from models used to study tau aggregation further prompted the development of approaches targeting tau self-assembly as discussed by Oakley et al. and by Masnata et al. The present most promising avenues include tau aggregation inhibitors, and active or passive immunisation against either post-translational modifications facilitating tau aggregation and conformationally altered forms of tau or aggregated tau, thus preventing the formation of PHFs.

Tau spreading could also be targeted by inhibiting tau receptors favouring spreading. A recent study by Rauch et al. (73) showed that neuronal surface low-density-lipoprotein-receptor-related protein-1 (LRP1) mediates internalization and spreading of both physiological tau and pathogenic tau oligomers, suggesting that LRP1 could be a suitable target for intervention. Through their commentary on the paper by Rauch et al., Fearon and Lynch highlight the potential and limitations of LRP1 as a key player in tau physiology and its potential as a therapeutic target for tauopathies. As previously suggested in Fearon and Lynch's the *in vitro* and animal data do not always translate to human studies.

Other approaches to mitigate the deleterious effects of pathological tau are being examined such as modulating *MAPT* gene expression. As revealed in preclinical studies, the restoration of physiological miRNA levels could also provide an attractive alternative.

As emphasized in several contributions to this Research Topic, models mimicking tau biology in physiological and pathological conditions, while allowing testing potential therapeutics they present considerable limitations. To overcome these limitations Shamir et al. introduce a neuron-like *in vitro* model of human origin which shows the expected toxicity of human-derived PHF-enriched tau and enables studies on the internalization and interaction of tau antibodies with pathological tau.

A significant limitation of mouse tauopathy models is that while mouse models provide invaluable insight into tau biology and pathology, they do not recapitulate the human tau splicing into six isoforms and the human 3R/4R ratio [reviewed in (74, 75)]. There are a few mouse models which mimic human tau pathology leading to brain atrophy such as the PS19 and the P301S tau (76, 77). More recently, P301S tau transgenic mice with targeted replacement of endogenous ApoE with human ApoE revealed the important role of ApoE in regulating tau-mediated neurodegeneration. In this model, the expression of the human ApoE4 protein caused the most severe tauopathy leading to human-like brain atrophy (78). In this regard rat models of tauopathy display significant advantages (74). For example, the McGill-R962-hTau rat model of tauopathy, as similar models under development, overexpresses the longest isoform of human tau (2N4R) with the P301S mutation causative of FTDP-17 under the control of the CaMKII alpha gene promoter. These rats progressively develop human-like tau pathology and related phenotype with tau hyperphosphorylation and conformationally-altered tau, resulting in NFT-like inclusions and neuroinflammation followed by neuronal loss, marked by brain atrophy, ventricular dilation, demyelination, and cognitive impairment (79). Of relevance to the study of the human-like tauopathy and therapeutics rats are genetically and physiologically closer to humans than mice and unlike mice they display all six human tau isoforms, although not at the same ratio. Furthermore, the rat endogenous ApoE protein has a high homology with the human ApoE4 protein.

CONCLUSION

This collection of reviews covers a wide variety of progresses regarding the participation of the tau protein in a diversity of neurodegenerative conditions. These contributions bring new ideas regarding physiological and pathological molecular mechanisms involving this multi-faceted microtubule-associated protein. These contributions offer an excellent background to further define the differential pathological aspects of diverse tauopathies. It supports the analysis of tau-mediated glia-neuron interactions and their impact in the homeostatic maintenance of the brain vascular bed. The main future challenge in the field will remain the development of effective therapeutics able to halt or delay the devastating consequences of brain tau pathology. This e-book should be of much assistance to conceive and investigate novel therapeutic approaches of eventual clinical value.

AUTHOR CONTRIBUTIONS

SD, MS, and AC have participated in writing the Editorial and approved it for publication. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Alzheimer A. Über einen eigenartigen schweren ErkrankungsprozeB der Hirnrinde. *Neurologisches Centralblatt*. (1906) 23:1129–36.
2. KIDD M. Paired helical filaments in electron microscopy of Alzheimer's disease. *Nature*. (1963) 197:192–3. doi: 10.1038/197192b0
3. Hirano A, Dembitzer HM, Kurland LT, Zimmerman HM. The fine structure of some intraganglionic alterations. Neurofibrillary tangles, granulovacuolar bodies and "rod-like" structures as seen in Guam amyotrophic lateral sclerosis and parkinsonism-dementia complex. *J Neuropathol Exp Neurol.* (1968) 27:167–82. doi: 10.1097/00005072-196804000-00001
4. Blessed G, Tomlinson BE, Roth M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *Br J Psychiatry*. (1968) 114:797–811. doi: 10.1192/bjp.114.512.797
5. Tomlinson BE, Blessed G, Roth M. Observations on the brains of demented old people. *J Neurol Sci*. (1970) 11:205–42. doi: 10.1016/0022-510X(70)90063-8
6. Brion JP, Passareiro H, Nunez J, Flament-Durand J. Mise en évidence immunologique de la protéine tau au niveau des lésions de dégénérescence neurofibrillaire de la maladie d'Alzheimer. *Arch Biol.* (1985) 95:229–35.
7. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA*. (1986) 83:4913–7. doi: 10.1073/pnas.83.13.4913
8. Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proc Natl Acad Sci USA*. (1986) 83:4044–8. doi: 10.1073/pnas.83.11.4044
9. Wood JG, Mirra SS, Pollock NJ, Binder LI. Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau). *Proc Natl Acad Sci USA*. (1986) 83:4040–3. doi: 10.1073/pnas.83.11.4040
10. Goedert M, Wischik CM, Crowther RA, Walker JE, Klug A. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proc Natl Acad Sci USA*. (1988) 85:4051–5. doi: 10.1073/pnas.85.11.4051
11. Lee VM, Balin BJ, Otvos L Jr, Trojanowski JQ. A68: a major subunit of paired helical filaments and derivatized forms of normal Tau. *Science*. (1991) 251:675–8. doi: 10.1126/science.1899488
12. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci USA*. (1975) 72:1858–62. doi: 10.1073/pnas.72.5.1858
13. Wischik CM, Novak M, Edwards PC, Klug A, Tichelaar W, Crowther RA. Structural characterization of the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci USA*. (1988) 85:4884–8. doi: 10.1073/pnas.85.13.4884
14. Wischik CM, Novak M, Thøgersen HC, Edwards PC, Runswick MJ, Jakes R, et al. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci USA*. (1988) 85:4506–10. doi: 10.1073/pnas.85.12.4506
15. Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron*. (1992) 8:159–68. doi: 10.1016/0896-6273(92)90117-V
16. Ihara Y, Nukina N, Miura R, Ogawara M. Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease. *J Biochem*. (1986) 99:1807–10. doi: 10.1093/oxfordjournals.jbchem.a135662
17. Zilka N, Filipcik P, Koson P, Fialova L, Skrabana R, Zilkova M, et al. Truncated tau from sporadic Alzheimer's disease suffices to drive neurofibrillary degeneration *in vivo*. *FEBS Lett*. (2006) 580:3582–8. doi: 10.1016/j.febslet.2006.05.029
18. Weaver CL, Espinoza M, Kress Y, Davies P. Conformational change as one of the earliest alterations of tau in Alzheimer's disease. *Neurobiol Aging*. (2000) 21:719–27. doi: 10.1016/S0197-4580(00)00157-3
19. 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) 3:519–26. doi: 10.1016/0896-6273(89)90210-9
20. Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO J*. (1989) 8:393–9. doi: 10.1002/j.1460-2075.1989.tb03390.x
21. Crowther RA. Straight and paired helical filaments in Alzheimer disease have a common structural unit. *Proc Natl Acad Sci USA*. (1991) 88:2288–92. doi: 10.1073/pnas.88.6.2288
22. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature*. (2017) 547:185–90. doi: 10.1038/nature23002
23. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature*. (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y
24. Hallinan GI, Hoq MR, Ghosh M, Vago FS, Fernandez A, Garringer HJ, et al. Structure of Tau filaments in Prion protein amyloidoses. *Acta Neuropathol*. (2021) 142:227–41. doi: 10.1007/s00401-021-02336-w
25. Shi Y, Murzin AG, Falcon B, Epstein A, Machin J, Tempest P, et al. Cryo-EM structures of tau filaments from Alzheimer's disease with PET ligand APN-1607. *Acta Neuropathol*. (2021) 141:697–708. doi: 10.1007/s00401-021-02294-3
26. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature*. (2019) 568:420–3. doi: 10.1038/s41586-019-1026-5
27. Zhang W, Tarutani A, Newell KL, Murzin AG, Matsubara T, Falcon B, et al. Novel tau filament fold in corticobasal degeneration. *Nature*. (2020) 580:283–7. doi: 10.1038/s41586-020-2043-0
28. Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol*. (1998) 43:815–25. doi: 10.1002/ana.410430617
29. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. (1998) 393:702–5. doi: 10.1038/31508
30. Spillantini MG, Crowther RA, Kamphorst W, Heutink P, van Swieten JC. Tau pathology in two Dutch families with mutations in the microtubule-binding region of tau. *Am J Pathol*. (1998) 153:1359–63. doi: 10.1016/S0002-9440(10)65721-5
31. Wallon D, Boluda S, Rovelet-Lecrux A, Thierry M, Lagarde J, Miguel L, et al. Clinical and neuropathological diversity of tauopathy in MAPT duplication carriers. *Acta Neuropathol*. (2021) 142:259–78. doi: 10.1007/s00401-021-02320-4
32. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem*. (2009) 284:12845–52. doi: 10.1074/jbc.M808759200
33. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol*. (2009) 11:909–13. doi: 10.1038/ncb1901
34. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. (1991) 82:239–59. doi: 10.1007/BF00308809
35. Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW. The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb Cortex*. (1991) 1:103–16. doi: 10.1093/cercor/1.1.103
36. Mena R, Wischik CM, Novak M, Milstein C, Cuello AC. A progressive deposition of paired helical filaments (PHF) in the brain characterizes the evolution of dementia in Alzheimer's disease. An immunocytochemical study with a monoclonal antibody against the PHF core. *J Neuropathol Exp Neurol*. (1991) 50:474–90. doi: 10.1097/00005072-199107000-00008
37. Duyckaerts C, Bennecib M, Grignon Y, Uchihara T, He Y, Piette F, et al. Modeling the relation between neurofibrillary tangles and intellectual status. *Neurobiol Aging*. (1997) 18:267–73. doi: 10.1016/S0197-4580(97)80306-5
38. Duyckaerts C, Uchihara T, Seilhean D, He Y, Hauw JJ. Dissociation of Alzheimer type pathology in a disconnected piece of cortex. *Acta Neuropathol*. (1997) 93:501–7. doi: 10.1007/s004010050645
39. Jackson SJ, Kerridge C, Cooper J, Cavallini A, Falcon B, Cella CV, et al. Short fibrils constitute the major species of seed-competent tau in the brains of mice transgenic for human P301S tau. *J Neurosci*. (2016) 36:762–72. doi: 10.1523/JNEUROSCI.3542-15.2016

40. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener.* (2011) 6:39. doi: 10.1186/1750-1326-6-39
41. Fá M, Puzzo D, Piacentini R, Staniszewski A, Zhang H, Baltrons MA, et al. Extracellular tau oligomers produce an immediate impairment of LTP and memory. *Sci Rep.* (2016) 6:19393. doi: 10.1038/srep19393
42. Shafiei SS, Guerrero-Muñoz MJ, Castillo-Carranza DL. Tau oligomers: cytotoxicity, propagation, and mitochondrial damage. *Front Aging Neurosci.* (2017) 9:83. doi: 10.3389/fnagi.2017.00083
43. Dujardin S, Bégard S, Caillierez R, Lachaud C, Carrier S, Lieger S, et al. Different tau species lead to heterogeneous tau pathology propagation and misfolding. *Acta Neuropathol Commun.* (2018) 6:132. doi: 10.1186/s40478-018-0637-7
44. Goedert M, Eisenberg DS, Crowther RA. Propagation of tau aggregates and neurodegeneration. *Annu Rev Neurosci.* (2017) 40:189–210. doi: 10.1146/annurev-neuro-072116-031153
45. Haroutunian V, Purohit DP, Perl DP, Marin D, Khan K, Lantz M, et al. Neurofibrillary tangles in non-demented elderly subjects and mild Alzheimer disease. *Arch Neurol.* (1999) 56:713–8. doi: 10.1001/archneur.56.6.713
46. Nelson PT, Jicha GA, Schmitt FA, Liu H, Davis DG, Mendiondo MS, et al. Clinicopathologic correlations in a large Alzheimer disease center autopsy cohort: neuritic plaques and neurofibrillary tangles "do count" when staging disease severity. *J Neuropathol Exp Neurol.* (2007) 66:1136–46. doi: 10.1097/nen.0b013e31815c5efb
47. Robinson JL, Geser F, Corrada MM, Berlau DJ, Arnold SE, Lee VM, et al. Neocortical and hippocampal amyloid-β and tau measures associate with dementia in the oldest-old. *Brain.* (2011) 134:3708–15. doi: 10.1093/brain/awr308
48. Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. *Arch Neurol.* (2004) 61:378–84. doi: 10.1001/archneur.61.3.378
49. Hanseeuw BJ, Betensky RA, Jacobs HIL, Schultz AP, Sepulcre J, Becker JA, et al. Association of amyloid and tau with cognition in preclinical Alzheimer disease: a longitudinal study. *J Am Med Assoc Neurol.* (2019) 76:915–24. doi: 10.1001/jamaneurol.2019.1424
50. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology.* (1992) 42:631–9. doi: 10.1212/WNL.42.3.631
51. Ghoshal N, García-Sierra F, Wuu J, Leurgans S, Bennett DA, Berry RW, et al. Tau conformational changes correspond to impairments of episodic memory in mild cognitive impairment and Alzheimer's disease. *Exp Neurol.* (2002) 177:475–93. doi: 10.1006/exnr.2002.8014
52. Murray ME, Lowe VJ, Graff-Radford NR, Liesinger AM, Cannon A, Przybelski SA, et al. Clinicopathologic and 11C-Pittsburgh compound B implications of Thal amyloid phase across the Alzheimer's disease spectrum. *Brain.* (2015) 138:1370–81. doi: 10.1093/brain/awv050
53. Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch Neurol.* (2003) 60:729–36. doi: 10.1001/archneur.60.5.729
54. Giannakopoulos P, Herrmann FR, Bussière T, Bouras C, Kövari E, Perl DP, et al. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology.* (2003) 60:1495–500. doi: 10.1212/01.WNL.0000063311.58879.01
55. Digma LA, Madsen JR, Reas ET, Dale AM, Brewer JB, Banks SJ. Alzheimer's disease neuroimaging initiative. Tau and atrophy: domain-specific relationships with cognition. *Alzheimers Res Ther.* (2019) 11:65. doi: 10.1186/s13195-019-0518-8
56. Gordon BA, Blazey TM, Christensen J, Dincer A, Flores S, Keefe S, et al. Tau PET in autosomal dominant Alzheimer's disease: relationship with cognition, dementia and other biomarkers. *Brain.* (2019) 142:1063–76. doi: 10.1093/brain/awz019
57. Rafii MS, Lukic AS, Andrews RD, Brewer J, Rissman RA, Strother SC, et al. Down syndrome biomarker initiative and the Alzheimer's disease neuroimaging initiative. PET imaging of tau pathology and relationship to amyloid, longitudinal MRI, and cognitive change in down syndrome: results from the down syndrome biomarker initiative (DSBI). *J Alzheimers Dis.* (2017) 60:439–50. doi: 10.3233/JAD-170390
58. Arboleda-Velasquez JF, Lopera F, O'Hare M, Delgado-Tirado S, Marino C, Chmielewska N, et al. Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. *Nat Med.* (2019) 25:1680–3. doi: 10.1038/s41591-019-0611-3
59. Spillantini MG, Bird TD, Ghetti B. Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies. *Brain Pathol.* (1998) 8:387–402. doi: 10.1111/j.1750-3639.1998.tb00162.x
60. Spillantini MG, Crowther RA, Goedert M. Comparison of the neurofibrillary pathology in Alzheimer's disease and familial presenile dementia with tangles. *Acta Neuropathol.* (1996) 92:42–8. doi: 10.1007/s004010050487
61. Spillantini MG, Goedert M, Crowther RA, Murrell JR, Farlow MR, Ghetti B. Familial multiple system tauopathy with presenile dementia: a disease with abundant neuronal and glial tau filaments. *Proc Natl Acad Sci USA.* (1997) 94:4113–8. doi: 10.1073/pnas.94.8.4113
62. Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci USA.* (1998) 95:7737–41. doi: 10.1073/pnas.95.13.7737
63. Goedert M, Spillantini MG. Ordered assembly of tau protein and neurodegeneration. *Adv Exp Med Biol.* (2019) 1184:3–21. doi: 10.1007/978-981-32-9358-8_1
64. Arendt T, Stieler JT, Holzer M. Tau and tauopathies. *Brain Res Bull.* (2016) 126:238–92. doi: 10.1016/j.brainresbull.2016.08.018
65. Nuebling GS, Plesch E, Ruf VC, Högen T, Lorenzl S, Kamp F, et al. Binding of metal-ion-induced tau oligomers to lipid surfaces is enhanced by GSK-3β-mediated phosphorylation. *ACS Chem Neurosci.* (2020) 11:880–7. doi: 10.1021/acschemneuro.9b00459
66. Brandt R, Léger J, Lee G. Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. *J Cell Biol.* (1995) 131:1327–40. doi: 10.1083/jcb.131.5.1327
67. Pooler AM, Hanger DP. Functional implications of the association of tau with the plasma membrane. *Biochem Soc Trans.* (2010) 38:1012–5. doi: 10.1042/BST0381012
68. Brelstaff J, Tolkovsky AM, Ghetti B, Goedert M, Spillantini MG. Living neurons with tau filaments aberrantly expose phosphatidylserine and are phagocytosed by microglia. *Cell Rep.* (2018) 24:1939–48.e4. doi: 10.1016/j.celrep.2018.07.072
69. Hallmann AL, Araúzo-Bravo MJ, Mavrommatis L, Ehrlich M, Röpke A, Brockhaus J, et al. Astrocyte pathology in a human neural stem cell model of frontotemporal dementia caused by mutant TAU protein. *Sci Rep.* (2017) 7:42991. doi: 10.1038/srep42991
70. Walker LC. Glial tauopathy: neurons optional? *J Exp Med.* (2020) 217:e20191915. doi: 10.1084/jem.20191915
71. Sidoryk-Wegrzynowicz M, Gerber YN, Ries M, Sastre M, Tolkovsky AM, Spillantini MG. Astrocytes in mouse models of tauopathies acquire early deficits and lose neurosupportive functions. *Acta Neuropathol Commun.* (2017) 5:89. doi: 10.1186/s40478-017-0478-9
72. Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Pérez JM, Evans AC. Alzheimer's disease neuroimaging initiative. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat Commun.* (2016) 7:11934. doi: 10.1038/ncomms11934
73. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, et al. LRP1 is a master regulator of tau uptake and spread. *Nature.* (2020) 580:381–5. doi: 10.1038/s41586-020-2156-5
74. Do Carmo S, Cuello AC. Modeling Alzheimer's disease in transgenic rats. *Mol Neurodegener.* (2013) 8:37. doi: 10.1186/1750-1326-8-37
75. Jankowsky JL, Zheng H. Practical considerations for choosing a mouse model of Alzheimer's disease. *Mol Neurodegener.* (2017) 12:89. doi: 10.1186/s13024-017-0231-7
76. Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* (2007) 53:337–51. doi: 10.1016/j.neuron.2007.01.010

77. Hampton DW, Webber DJ, Bilican B, Goedert M, Spillantini MG, Chandran S. Cell-mediated neuroprotection in a mouse model of human tauopathy. *J Neurosci.* (2010) 30:9973–83. doi: 10.1523/JNEUROSCI.0834-10.2010
78. Shi Y, Yamada K, Liddelow SA, Smith ST, Zhao L, Luo W, et al. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature.* (2017) 549:523–7. doi: 10.1038/nature24016
79. Malcolm JC, Breuillaud L, Do Carmo S, Hall H, Welikovitch LA, Macdonald JA, et al. Neuropathological changes and cognitive deficits in rats transgenic for human mutant tau recapitulate human tauopathy. *Neurobiol Dis.* (2019) 127:323–38. doi: 10.1016/j.nbd.2019.03.018

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neurology

ORIGINAL RESEARCH
published: 16 September 2020
doi: 10.3389/fneur.2020.575953

Cerebrovascular Senescence Is Associated With Tau Pathology in Alzheimer's Disease

*Annie G. Bryant[1], Miwei Hu[1], Becky C. Carlyle[1], Steven E. Arnold[1], Matthew P. Frosch[1,2], Sudeshna Das[1], Bradley T. Hyman[1] and Rachel E. Bennett[1]**

[1] Department of Neurology, Harvard Medical School, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Charlestown, MA, United States, [2] Department of Pathology, Harvard Medical School, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Charlestown, MA, United States

Alzheimer's Disease (AD) is associated with neuropathological changes, including aggregation of tau neurofibrillary tangles (NFTs) and amyloid-beta plaques. Mounting evidence indicates that vascular dysfunction also plays a key role in the pathogenesis and progression of AD, in part through endothelial dysfunction. Based on findings in animal models that tau pathology induces vascular abnormalities and cellular senescence, we hypothesized that tau pathology in the human AD brain leads to vascular senescence. To explore this hypothesis, we isolated intact microvessels from the dorsolateral prefrontal cortex (PFC, BA9) from 16 subjects with advanced Braak stages (Braak V/VI, B3) and 12 control subjects (Braak 0/I/II, B1), and quantified expression of 42 genes associated with senescence, cell adhesion, and various endothelial cell functions. Genes associated with endothelial senescence and leukocyte adhesion, including SERPINE1 (PAI-1), CXCL8 (IL8), CXCL1, CXCL2, ICAM-2, and TIE1, were significantly upregulated in B3 microvessels after adjusting for sex and cerebrovascular pathology. In particular, the senescence-associated secretory phenotype genes SERPINE1 and CXCL8 were upregulated by more than 2-fold in B3 microvessels after adjusting for sex, cerebrovascular pathology, and age at death. Protein quantification data from longitudinal plasma samples for a subset of 13 ($n = 9$ B3, $n = 4$ B1) subjects showed no significant differences in plasma senescence or adhesion-associated protein levels, suggesting that these changes were not associated with systemic vascular alterations. Future investigations of senescence biomarkers in both the peripheral and cortical vasculature could further elucidate links between tau pathology and vascular changes in human AD.

Keywords: Alzheimer's disease, tau pathology, neurofibrillary tangles, vascular dysfunction, endothelial senescence, gene expression, plasma biomarkers

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Andrej Kovac, Slovak Academy of Sciences (SAS), Slovakia
Antonio Giuliano Zippo, National Research Council, Italy

***Correspondence:**
Rachel E. Bennett
rebennett@mgh.harvard.edu

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *24 June 2020*
Accepted: *12 August 2020*
Published: *16 September 2020*

Citation:
Bryant AG, Hu M, Carlyle BC, Arnold SE, Frosch MP, Das S, Hyman BT and Bennett RE (2020) Cerebrovascular Senescence Is Associated With Tau Pathology in Alzheimer's Disease. Front. Neurol. 11:575953. doi: 10.3389/fneur.2020.575953

INTRODUCTION

Vascular dysfunction has become increasingly implicated in the pathogenesis of Alzheimer's Disease (AD) (1, 2). Cerebrovascular diseases including cerebral amyloid angiopathy (CAA) and atherosclerosis often co-present with AD (3–5), and reduced cerebral blood flow in the human AD brain is associated with both cognitive decline (6, 7) and tau accumulation (8–10). Proper function of the neurovascular unit, which is comprised primarily of endothelial cells, mural cells, and

astrocytic endfeet, is essential for nutrient supply and protection from peripheral blood molecules via the blood-brain barrier (BBB). Impaired cerebral blood flow represents a key mechanism in neurodegeneration as it contributes to neuronal injury (11) and the accumulation of amyloid beta aggregates (12).

One of the neuropathological hallmarks of AD is the aggregation of phosphorylated tau protein into neurofibrillary tangles (NFTs). Several lines of research indicate that tau pathology may interact with endothelial cell changes to drive vascular impairment in AD. Senescence is a complex process in which cells irreversibly cease proliferating, change morphology, and present the senescence-associated secretory phenotype (SASP) (13). In neurons, cellular senescence and tau NFT formation have been reported to exhibit a positive feedback relationship (14, 15), and suppression of senescence prevents tau aggregation *in vivo* (15). Moreover, endothelial senescence is associated with vascular dysfunction via BBB disruption (16, 17), increased vascular stiffness (18, 19), and aberrant neurovascular coupling (10, 20–22).

The SASP profile includes pro-inflammatory cytokines, cell cycle regulators, and pro-angiogenic factors (13, 23, 24), which propagate senescence via autocrine and paracrine signaling (25, 26). This profile phenocopies, to some extent, changes we have observed in the microvasculature of tau-overexpressing transgenic (Tg4510) mice (27). We therefore examined whether endothelial senescence contributes to microvascular alterations in tau-related neurodegenerative disease. To investigate this hypothesis, we isolated intact microvessels from the dorsolateral prefrontal cortex (PFC, Brodmann area 9) of 16 AD and 12 control cases and measured expression of 42 genes related to senescence, cell adhesion, and general endothelial cell functions. Since many of the senescence-associated biomarkers are secreted, we also investigated whether changes in plasma protein levels could be detected in AD subjects in the years before death.

MATERIALS AND METHODS

Subject Information

Twenty-eight cases were selected from the Neuropathology Core of the Massachusetts Alzheimer's Disease Research Center. Thirteen of the subjects also had longitudinal plasma sample collection over several years; these cases are indicated with an asterisk in the Plasma column in **Table 1**. All cases were assessed by a neuropathologist and scored for Alzheimer's Disease (AD) neuropathology burden according to the NIA-AA guidelines (28). Control cases ($n = 12$) were defined as subjects with a Braak neurofibrillary tangle (NFT) score of 0/I/II (B1) and were classified as low AD probability. One of the B1 subjects exhibited Lewy Body Disease with Primary Age-Related Tauopathy and one exhibited Progressive Supranuclear Palsy pathology, as labeled in **Table 1**. AD cases ($n = 16$) were defined as subjects with a Braak NFT score of V/VI (B3) indicating extensive tau pathology in the neocortex, with NIA-AA classification as intermediate or high AD probability. B3 subjects did not exhibit Lewy bodies, though 6 out of 16 had evidence of TDP-43 inclusions in the amygdala and hippocampus.

Cerebral Microvessel Isolation

We adapted this protocol for intact cortical microvessel isolation from Boulay et al. (29) and Hartz et al. (30), which is designed to protect the intercellular connections between endothelial cells and mural cells. All steps for microvessel isolation were performed on ice under RNAse-free conditions. Approximately 200 mg of frozen postmortem dorsolateral prefrontal cortex (PFC, Brodmann area 9) tissue was measured by a histologist for each case. Meninges were removed from the cortical surface and the cortical tissue was sliced into ∼2 mm sections using a sterile razor blade in Hank's Balanced Salt Solution (HBSS) and HEPES buffer. The tissue sections were manually dissociated with a dounce homogenizer and centrifuged for 10 min at 2,000 g in 4°C. The pellet was resuspended in a dextran solution and centrifuged at 4,400 g for 15 min at 4°C. A myelin layer formed atop the supernatant, which was removed by inverting the centrifuge tube and carefully blotting the inside walls. The pellet was resuspended in the HBSS-HEPES buffer with RNAse-free bovine serum albumin (BSA) and filtered over a 20 μm mesh filter (Millipore) to collect microvessels. Microvessels were collected from the filter by gently stirring the filter in HBSS-HEPES-BSA buffer. The collected microvessels were washed and spun twice at 2,000 g for 5 min at 4°C. The supernatant was discarded and the pellet containing isolated microvessels was stored at −80°C prior to RNA isolation.

RNA Isolation and RT-qPCR

Frozen microvessel pellets or 25 mg of whole tissue was resuspended in RLT buffer (Qiagen) and sonicated for 20 pulses at 10% amplitude to lyse cells. The lysed cells were then centrifuged at 13,000 rpm for 3 min to pellet out residual cellular debris. RNA was extracted using the RNeasy Mini Kit (Qiagen) and was eluted in 30 μl of nuclease-free water. RNA was assessed using the NanoDrop spectrophotometer (ThermoFisher) and diluted to a standard concentration of 20 ng/μl for each sample. cDNA was synthesized using the RT^2 HT First Strand kit (Qiagen) and was combined with RT^2 SYBR Green Mastermix fluorescent dye (Qiagen). The cDNA reaction mix was loaded into a custom 96-well RT^2 Profiler Array with pre-loaded primers. Each plate contained 42 genes of interest (**Table 2**), three housekeeping genes, a Human Genomic DNA Control (HGDC), a Reverse Transcription Control (RTC), and a Positive PCR Control (PPC). Two samples were run per plate. The plate was covered with a plastic seal to prevent sample evaporation and briefly spun at ∼400 rpm to remove bubbles. The qPCR reactions were performed using the BioRad CFX96 Real-Time Detection System. The sequence began with a 10-min incubation at 95°C to activate the DNA polymerase enzyme. Fluorescence data collection then commenced with 40 cycles of alternating 15 s at 95°C and 60 s at 60°C.

The ΔΔCT relative quantification method was used to calculate gene expression (59). First, positive controls for reverse transcription and PCR (RTC and PPC) were analyzed for each sample according to manufacturer instructions. Gene Ct (cycle threshold) results were quality filtered to remove any data points with a Ct value greater than the sample Ct for the genomic DNA (HGDC) control primer. Any reaction with no

TABLE 1 | Description of the 28 subjects included in this study.

Subject ID	Plasma	ABC-Amyloid	ABC-Braak	ABC-CERAD	ABC-burden	Age at death	ApoE	Neuropathology notes	Cerebrovascular pathology
AD01		2	3	2	Intermediate	80–90	e2/e3		
AD02		2	3	2	Intermediate	80–90	e3/e3		
AD03		3	3	1	Intermediate	>90	e4/e3		
AD04		2	3	2	Intermediate	80–90	e3/e3		CVD, INF
AD05		2	3	2	Intermediate	80–90	e3/e3		
AD06		2	3	2	Intermediate	80–90	e3/e3		
AD07	*	3	3	3	High	70–80	e4/e4		
AD08	*	3	3	3	High	60–70	e4/e3		
AD09	*	3	3	2	High	>90	e4/e3		CVD, CAA
AD10	*	3	3	2	High	80–90	e4/e3	Hippocampal Sclerosis	
AD11	*	3	3	3	High	80–90	e4/e4		
AD12		3	3	3	High	70–80	e4/e3		CVD
AD13	*	3	3	3	High	80–90	e4/e3		
AD14	*	3	3	2	High	80–90	e4/e3		CVD, CAA
AD15	*	3	3	3	High	60–70	e4/e3		CAA
AD16	*	3	3	2	High	>90	e2/e3		CVD, CAA
CTRL01		0	0	0	Not AD	50–60	e3/e3		
CTRL02	*	0	0	0	Not AD	>90	e3/e3	PSP	
CTRL03		0	1	0	Not AD	80–90	e3/e3		
CTRL04		0	1	1	Not AD	>90	e2/e3		
CTRL05		0	1	1	Not AD	>90	e3/e3		CVD
CTRL06	*	0	1	0	Not AD	>90	e3/e3		CVD, INF
CTRL07		0	1	0	Not AD	>90	e3/e3		
CTRL08		0	1	0	Not AD	70–80	e3/e3		
CTRL09	*	0	1	0	Not AD	>90	e3/e3		INF
CTRL10		2	1	1	Low	>90	e3/e3		CVD
CTRL11		1	1	1	Low	70–80	e3/e3		CAA
CTRL12	*	1	1	0	Low	>90	e3/e3	LBD, PART	CVD, Arteriolosclerosis

These 28 subjects were selected for microvascular gene expression analysis. Age at death is binned by decade per journal policy. An asterisk in the "Plasma" column indicates the subject also had longitudinal plasma collection for protein quantification. PSP, progressive supranuclear palsy; LBD, Lewy-body dementia; PART, primary age-related tauopathy; CVD, cerebrovascular disease; INF, infarcts; CAA, cerebral amyloid angiopathy.

fluorescence detection by 40 qPCR cycles was reported as not detected, and any gene with undetectable expression in more than 25% of samples in total was excluded. Three reference genes were included in the RT^2-Profiler array: ACTB, GAPDH, and PGK1. The geometric mean of the reference gene Ct values was calculated for each subject and subtracted from the Ct value of each target gene, yielding ΔCT values. For each gene, the average ΔCT value in B1 subjects was then subtracted from each sample's ΔCT value to obtain ΔΔCT values. The relative quantification (RQ) value was calculated as $2^{-\Delta\Delta CT}$, which was then log2-transformed to yield fold changes.

Cell-type specific genes were included to verify the presence of vascular cells in microvessel isolates and compared to bulk RNA measures from total cortex. Using the endothelial cell marker PECAM1 (CD31) and the vascular smooth muscle cell marker ACTA2, we confirmed these cells are significantly enriched in isolated microvessels compared to total cortex homogenate in 16 representative samples (**Supplementary Figure 1A**). Astrocytes (ALDH1L1) were not enriched in isolated microvessels compared with total cortex, and microglial (ITGAM) cell composition was not statistically different. There was also no statistical difference in cell composition between B1 vs. B3 microvessels when examining all $n = 28$ microvessel samples (**Supplementary Figure 1B**). Overall, endothelial cell and vascular smooth muscle cell expression was greater than astrocyte and microglia expression in isolated microvessels.

Plasma Proteomics

For 13 of the subjects ($n = 4$ B1, $n = 9$ B3), plasma was collected at two or three time points spaced at ∼1-year intervals

TABLE 2 | Gene and protein biomarkers analyzed in this study.

Hypothesis-driven functional group	Description	References	Genes ($n = 42$)	Proteins ($n = 22$)
Senescence-associated	The endothelial senescence-associated secretory phenotype (SASP), a suite of inflammation- and angiogenesis-associated genes upregulated in endothelial senescence.	(13, 23, 24, 31)	CDKN1A	
			CDKN2A	
			CSF2	
			CXCL1	CXCL1
			CXCL2	
			CXCL8	IL8
			IL6	IL6
			SERPINE1	PAI-1
Cell adhesion molecules	These genes encode cell adhesion proteins active in the cerebrovascular endothelium to mediate endothelial-leukocyte adhesion, a process central to the inflammatory response.	(32–34)	EMCN	
			ICAM1	ICAM-1
			ICAM2	ICAM-2
			MAdCAM1	
			SELE	SELE
			SELP	SELP
			VCAM1	VCAM1
Endothelial cell markers	These genes are specifically expressed in endothelial cells and facilitate various endothelial cell functions.	(35–42)	NOSTRIN	
			PECAM1	PECAM1
			SLC2A1	
			TEK	TIE2
			TIE1	TIE1
			VWF	VWF
Junction proteins	These genes encode proteins that are integral to the formation of endothelial gap junctions and tight junctions, which mediate vasodilation and enable strict regulation of molecular transport across the BBB.	(43–45)	CDH5	CDH5
			CLDN5	
			ESAM	
			GJA1	
			OCLN	
			TJP1	
VEGF/Notch pathway	These genes encode part of an angiogenesis-regulating protein network that exhibits aberrant expression in the AD cerebral vasculature.	(46–50)	ADGRL4	
			DLL4	
			ENG	ENG
			FLT1	
			KDR	VEGFR-2
			VEGFA	VEGFA
Cell stress	HMOX1 and NOS3 are upregulated in endothelial cells in response to cell stressors including oxidative stress and hypoxia.	(51, 52)	HMOX1	HO-1
			NOS3	NOS3
Plasmin/APOE pathways	In this pathway, LRP1 binds the plasmin activators tPA (encoded by PLAT) and uPA (encoded by PLAU), as well as APOE, for internalization and proteolysis. The regulation of this system is implicated in BBB integrity and proper clearance of amyloid-beta.	(53–55)	APOE	
			LRP1	
			PLAT	tPA
			PLAU	uPA
Other cell markers	ALDH1L1, ACTA2, and ITGAM (aka CD11b) are putative markers of astrocytes, smooth muscle cells, and microglia, respectively.	(56–58)	ALDH1L1	
			ITGAM	ITGAM
			ACTA2	

The roles of these genes and proteins were classified into hypothesized functional groups according to potential contributions to vascular dysfunction in the human AD brain, with corresponding references. Gene and corresponding protein names are listed.

(**Table 1**, labeled with asterisks). Plasma was sent to Olink Proteomics for protein quantification using 92-plex antibody labeling with a proximity extension assay. Quantification results were normalized and log-2 transformed and were reported as Normalized Protein eXpression (NPX) values. Twenty-two of the biomarkers analyzed via RT-qPCR were also included in the Olink protein panels (**Table 2**). Quality control was applied by eliminating any data points with an NPX value below the manufacturer-specified protein limit of detection (LOD) threshold.

Statistical Analysis

All statistical analyses and data visualizations were performed with R (v4.0.0). A significance level was set at $p < 0.05$, and correction for multiple comparisons was applied where necessary using the Benjamini-Hochberg (BH) False Discovery Rate (FDR) (60). This was calculated with the p.adjust() function in R using the "stats" package (v4.0.0).

Univariate comparisons were conducted using Welch's two-way unpaired *t*-test using the t.test() function from the R "stats" package. To test the robustness of a given result, bootstrapped iterations were performed in which 75% (12/16) of B3 and 83% (10/12) of B1 subjects were randomly sampled 1,000 times, and the sample estimates were computed (61). The average fold change difference and proportion of significant iterations ($p < 0.05$) were reported to summarize bootstrapped iteration results. Regression models were implemented using the lm() function from the R "stats" package and results were reported using the tidy.lm() function from the "broom" R package (v0.5.6). ANOVA and Tukey's *post-hoc* HSD test were implemented using the aov() functions from the R "stats" package and the tukey_hsd() function from "rstatix" (v0.6.0). Note: Missing data were excluded on a gene-wise basis for *t*-test and regression analyses, though imputation with the highest Ct value per gene was also examined (62).

Principal component analysis (PCA) was implemented using the "FactoMineR" (v2.3) and "factoextra" (v1.0.7) packages in R. As PCA requires complete data, missing data were imputed on a gene-wise basis with the highest observed Ct value for the corresponding gene (62). Fold change data were centered to have a mean of zero before PCA was applied using the prcomp() function in FactoMineR. The proportion of variance per principal component (PC) as well as cumulative variance were examined with a Scree plot to determine how many components would be further analyzed.

RESULTS

Senescence-Associated Genes Are Upregulated in B3 PFC Microvessels

We isolated intact microvessels from the dorsolateral prefrontal cortex (PFC, BA9) to examine gene expression changes related to the extent of AD-related tau pathology. Enrichment of vascular transcripts in microvessel preparations was first verified by RT-qPCR (see Methods, **Supplementary Figure 1**). The PFC was selected as a Braak III/IV (B2) region, meaning it does not generally exhibit tau NFT pathology in early-stage AD (63). Braak 0/I/II (B1) subjects are therefore unlikely to present any PFC tau pathology, while Braak V/VI (B3) subjects are likely to have a high tau NFT burden in the PFC (64).

To assess gene expression associated with senescence, cell adhesion, and endothelial cell function, we measured expression of 42 genes classified into eight hypothesis-driven functional groups (**Table 2**). These eight classes were proposed based on literature reviewed in **Table 2**. Of the 42 genes measured, CDKN2A and SELP were excluded due to undetectable expression in more than 25% of samples (defined as no fluorescence detection by 40 cycles or a Ct value above the Human Genomic DNA Contamination Ct value) (**Supplementary Figure 2**). For the remaining 40 genes, heatmap visualization of log2 fold changes showed an observable hotspot of senescence-associated gene upregulation in B3 PFC microvessels (**Figure 1**). The average functional group fold change value was calculated per subject (excluding the non-endothelial cell markers) which confirmed a 2.5-fold increase in senescence-associated gene mRNA in B3 microvessels (**Figure 2A**; $p = 0.0030$, BH-FDR $= 0.0208$, 95% CI: [0.5019, 2.1442]). Additionally, expression of cell adhesion genes was increased by 1.9-fold in B3 microvessels compared to B1 microvessels (**Figure 2A;** $p = 0.0161$, BH-FDR=0.0562, 95% CI: [0.1945, 1.7123]). Missing values were omitted from these mean fold change calculations, though very similar results were obtained by imputing missing values with the highest Ct value of the gene. The other functional groups examined did not exhibit differential expression in B1 vs. B3 PFC microvessels (**Figure 2B**).

Sex, cerebrovascular pathology, and age can contribute to AD neurodegeneration, both directly and via tau pathology (65). To adjust for these important and potentially confounding covariates, stepwise multiple regression models were implemented to compare senescence-associated and cell adhesion mean fold changes between B3 vs. B1 samples (**Figure 2C**). We created the binary variable "Cerebrovascular pathology" to indicate presence or absence of at least one of cerebral amyloid angiopathy (CAA), cerebrovascular disease (CVD), and infarcts in the subject's neuropathology report (**Table 1**). Senescence-associated gene expression was still significantly increased in B3 microvessels compared to B1 after adjusting for sex, cerebrovascular pathology, and age. Cell adhesion gene expression was significantly increased in B3 microvessels after adjusting for sex and cerebrovascular pathology but not age at death, indicating that microvascular adhesion gene expression may be related to age. We note there was no statistical difference in the age at death between B1 and B3 subjects (**Supplementary Figure 3**).

IL-8, PAI-1, and TIE1 Demonstrate Robust Upregulation in B3 PFC Microvessels Independent of Age, Sex, and Cerebrovascular Pathology

After observing the general upregulation of senescence-associated and cell adhesion functional group genes in B3 PFC microvessels, we next investigated changes in individual

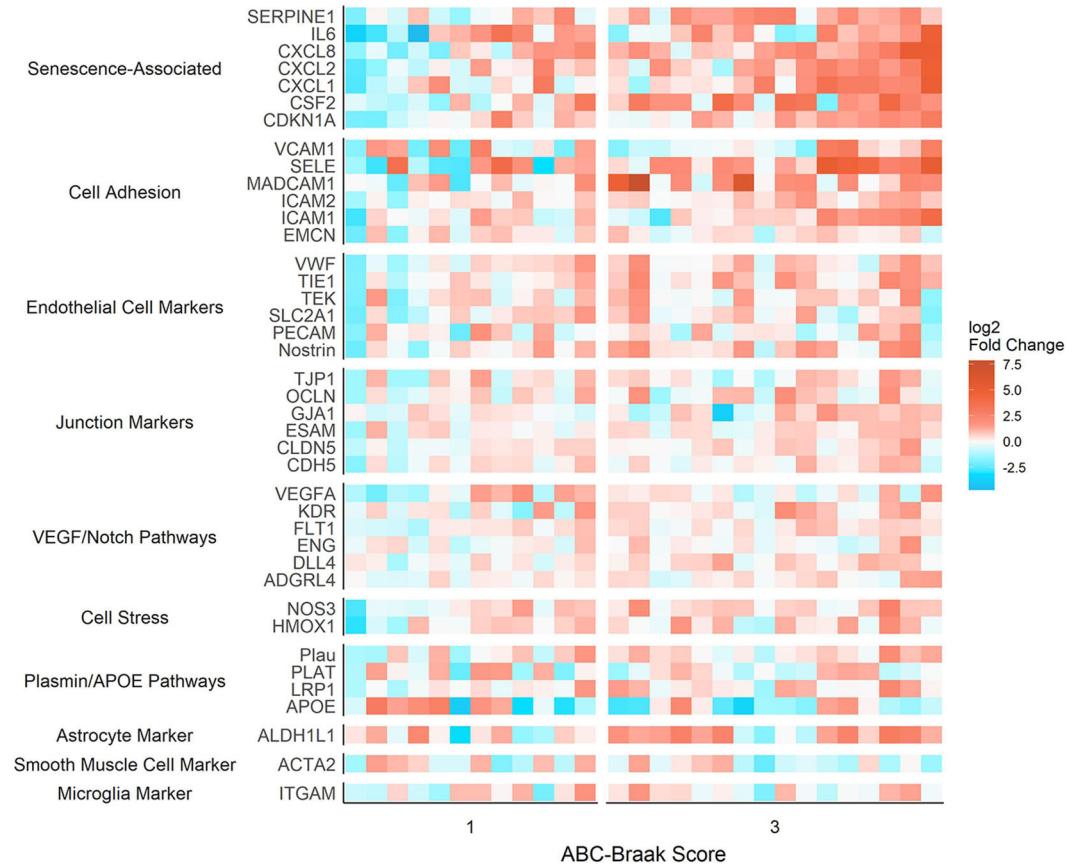


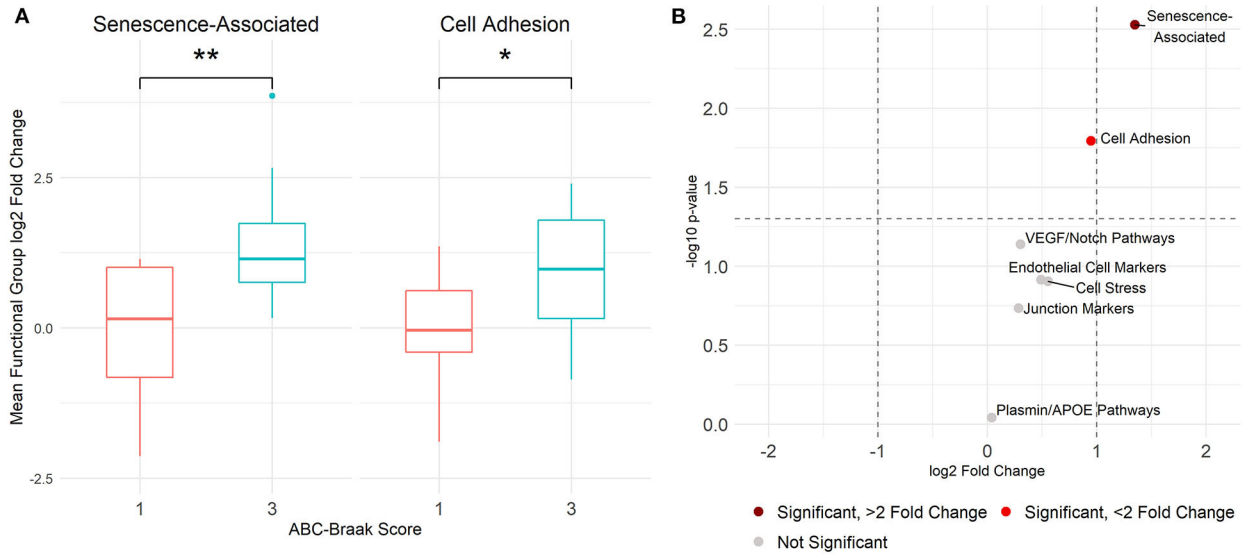
FIGURE 1 | Senescence-associated genes form a fold change hotspot in prefrontal cortex microvessels isolated from high-Braak (B3) vs. low-Braak (B1) samples. Differential gene expression between microvessels isolated from B3 vs. B1 samples, grouped by hypothesis-driven gene function groups. Values reflect log2-transformed fold change values relative to the B1 subject mean expression per gene. Relative quantification was performed such that the mean of B1 fold changes is zero for each gene. For this visualization, missing data were imputed with the ABC-Braak group mean fold change for the gene.

genes. Of the 40 genes that we measured in PFC microvessels which passed quality control filtering, 11 were significantly upregulated (with $p < 0.05$, BH-FDR<0.20): CXCL8 (IL8), SERPINE1 (PAI-1), CXCL1, CXCL2, CSF2, CDKN1A, ICAM-2, MAdCAM-1, SELE, TIE1, and DLL4 (**Figure 3A**). With the exception of CDKN1A, all of the senescence-associated genes in this group exhibited greater than a 2-fold average increase in mRNA expression in B3 microvessels. Further, these results were robust for CXCL8 (IL-8), SERPINE1 (PAI-1), CXCL1, CXCL2, TIE1, and ICAM-2, which were significantly upregulated ($p < 0.05$) in 62–94% of 1,000 bootstrapped iterations (**Supplementary Figure 4**). Missing values were excluded on a gene-wise basis for these comparisons, though these six genes were still significantly upregulated after imputing missing values with the highest Ct value of the gene.

For each of these six robustly upregulated genes, individual linear models were fit to regress gene fold change on ABC-Braak score, with stepwise inclusion of sex, cerebrovascular pathology, and age as covariates. The increased expression in B3 samples remained significant for all six genes after adjusting for sex and cerebrovascular pathology, indicating their upregulation was independent of these factors (**Figure 3B**). However, with the inclusion of age at death in the regression model, only CXCL8 (IL-8), SERPINE1 (PAI-1), and TIE1 were still significantly upregulated in B3 microvessels, suggesting their upregulation was age-independent. For the other three genes (ICAM-2, CXCL1, CXCL2), elevated expression in B3 PFC microvessels may be associated with age.

Tau Pathology and Cerebrovascular Pathology Interact to Increase Microvessel MAdCAM-1 Expression

Approximately half of both the B1 and B3 subjects exhibited at least one form of cerebrovascular pathology (**Table 1**), which is consistent with population-based studies in elderly demented and non-demented individuals (66, 67). Given the documented association between tau pathology and cerebrovascular pathology (68–70), we investigated whether the two interact in association with microvessel gene expression. Two-way ANOVA revealed an interaction between tau pathology and cerebrovascular pathology in MAdCAM-1 expression in PFC microvessels, which was significant prior to adjusting for multiple comparisons (**Supplementary Figure 5A**). *Post-hoc*



C

	ABC-Braak			ABC-Braak + Sex			ABC-Braak + Sex + Cerebrovascular			ABC-Braak + Sex + Cerebrovascular + Age		
	Braak β_1	p-value	BH-FDR	**Braak β_1**	p-value	BH-FDR	**Braak β_1**	p-value	BH-FDR	**Braak β_1**	p-value	BH-FDR
Senescence-Associated	**1.323****	0.0021	0.0042	**1.205****	0.0039	0.0077	**1.144****	0.0063	0.0126	**0.930***	0.0249	0.0498
Cell Adhesion	**0.953***	0.0142	0.0142	**0.788***	0.0232	0.0232	**0.801***	0.0254	0.0254	0.4901	0.1159	0.1159

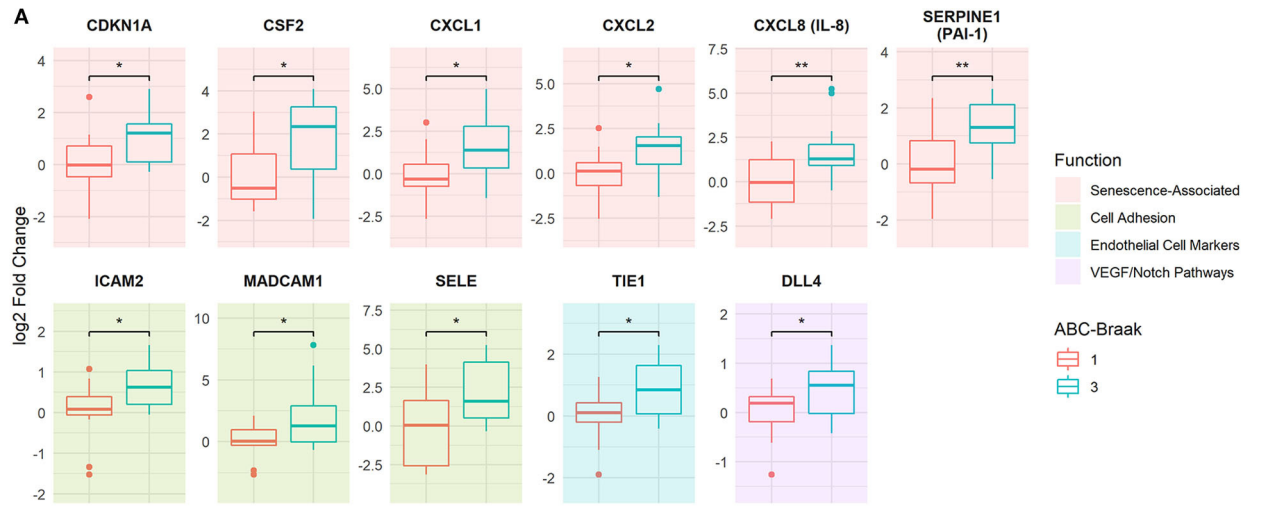
FIGURE 2 | Senescence- and adhesion-associated genes are significantly upregulated in prefrontal cortex microvessels isolated from B3 vs. B1 subjects. **(A)** Senescence-associated genes are significantly upregulated in B3 cerebral microvessels with a log2 fold change of 1.323; Welch's unpaired *t*-test, $p = 0.0030$ (BH-corrected FDR 0.0208), 95% CI for B3 log2 fold change: 0.5019, 2.1442. Cell adhesion genes are also significantly upregulated with a log2 fold change of 0.953; Welch's unpaired *t*-test, $p = 0.0161$ (BH-FDR 0.0562), 95% CI for B3 log2 fold change: 0.1945, 1.7123. **(B)** Volcano plot showing *t*-test results. Dashed vertical lines denote magnitudes of 2-fold change and the dashed horizontal line denotes the cutoff for $p < 0.05$. **(C)** Stepwise multiple regression models were applied to senescence-associated and cell adhesion functional groups to measure the association between Braak stage and functional group average fold change. The first row indicates the independent variables upon which the average fold change was regressed per model for each functional group. Within each model, the ABC-Braak term regression coefficient (β_1), *p*-value, and BH-FDR are reported. Significant ABC-Braak β1 terms are denoted with $^{**}p < 0.01$, $^{*}p < 0.05$.

analysis via Tukey's HSD test revealed that MAdCAM-1 mRNA expression was upregulated by more than 16-fold (4 log2-fold) in B3 subjects with cerebrovascular pathology compared to all other subjects (**Figure 4**, **Supplementary Figure 5B).** Of note, the top three MAdCAM-1 expression values correspond to B3 subjects with concomitant cerebral amyloid angiopathy (CAA) and general cerebrovascular disease (CVD). However, we note that the two-way ANOVA was not significant for MAdCAM-1 when missing values were imputed with the highest MAdCAM-1 Ct value.

Endothelial Senescence-Associated and Cell Adhesion Gene-Driven Principal Component Composite Score Distinguishes B3 vs. B1 Microvessels

To explore patterns of gene covariance in B1 vs. B3 cortical microvessels, principal component (PC) analysis was applied to centered fold change values for all 40 genes. The first two PCs collectively explained 51.2% of variance across the samples (**Supplementary Figure 6**). The top contributors to the first principal component (PC1), defined as those with more than one standard deviation above the mean contribution, included senescence-associated genes (IL6, CXCL8, CSF2) and cell adhesion genes (SELE and MAdCAM-1) (**Figure 5A**). As these genes are associated with endothelial dysfunction and/or senescence (23, 24, 31, 71–73), we hypothesized that PC-derived composite gene expression scores (a.k.a. PC composite score) would distinguish B3 from B1 microvessels. Indeed, the PC scores from the first and second principal components show partial separation of the B1 and B3 sample clusters, primarily along the PC1 axis (**Figure 5B**). This difference was statistically significant for the PC1 composite score, with B3 samples exhibiting significantly larger composite scores (Welch's unpaired *t*-test, $p = 0.0064$, 95% CI: [1.477, 7.956]; **Figure 5C**).

Four of the five genes driving fold change composite scores—CXCL8 (IL8), CSF2, SELE, and MAdCAM-1—were



B

Function	Gene	ABC-Braak			ABC-Braak + Sex			ABC-Braak + Sex + Cerebrovascular			ABC-Braak + Sex + Cerebrovascular + Age		
		Braak β_1	p-value	BH-FDR	**Braak β_1**	p-value	BH-FDR	**Braak β_1**	p-value	BH-FDR	Braak β_1	p-value	BH-FDR
Senescence-Associated	CXCL8 (IL8)	**1.775****	0.0055	0.0177	**1.640****	0.0099	0.0277	**1.562***	0.0152	0.0422	**1.489***	0.0297	0.0836
	SERPINE1 (PAI-1)	**1.356****	0.0077	0.0177	**1.262***	0.0117	0.0277	**1.207***	0.0217	0.0422	**1.222***	0.0418	0.0836
	CXCL1	**1.529***	0.0177	0.0177	**1.450***	0.0275	0.0277	**1.323***	0.0422	0.0422	1.104	0.1022	0.1022
	CXCL2	**1.410***	0.0139	0.0177	**1.223***	0.0248	0.0277	**1.060***	0.0374	0.0422	1.000	0.0655	0.0982
Cell Adhesion	ICAM2	**0.649***	0.0131	0.0177	**0.588***	0.0231	0.0277	**0.572***	0.0312	0.0422	0.456	0.0912	0.1022
Endothelial Function	TIE1	**0.860***	0.0156	0.0177	**0.763***	0.0277	0.0277	**0.856***	0.0115	0.0422	**0.717***	0.0375	0.0836

FIGURE 3 | Eleven genes related to senescence, adhesion, endothelial cell function, and VEGF/NOTCH pathways are significantly upregulated in PFC capillaries from B3 samples relative to B1 samples. **(A)** 11 genes show significant upregulation in B3 vs. B1 microvessels. Welch's unpaired *t*-test results are shown: $^{**}p < 0.01$, $^{*}p < 0.05$, all BH-FDR < 0.2. Plots are shaded by hypothesis-driven functional group. **(B)** Stepwise multiple regression models were applied to each of the 6 most-consistently upregulated genes to measure the association between Braak change and log2 fold change, adjusting for potential confounders. The first row indicates the independent variables upon which the log2 fold change was regressed per model for each gene. Within each model, the ABC-Braak term regression coefficient (β_1), *p*-value, and BH-FDR are reported. Significant ABC-Braak β1 terms are marked in bold with $^{**}p < 0.01$, $^{*}p < 0.05$.

identified as upregulated in B3 microvessels, with CXCL8 (IL8) upregulation particularly pronounced after adjusting for age, sex, and cerebrovascular pathology (**Figure 3**). By contrast, IL6 upregulation did not reach significance in B3 microvessels. Its strong contribution to the gene composite score that separated B1 vs. B3 microvessels may indicate that IL-6 is associated with ABC-Braak differences in the context of other senescence-associated and cell adhesion genes. Of note, IL6 expression differences were not driven by any other neuropathological variable, sex, APOE genotype, or cerebrovascular pathology (**Supplementary Figure 7**). Furthermore, PCA composite scores did not improve separation B1 vs. B3 microvessels based on these other covariates relative to ABC-Braak scores (**Supplementary Figure 8**).

NOS3 Antemortem Plasma Protein Expression Is Associated With Postmortem Cortical Microvessel Gene Expression

The cerebral vasculature interacts with the peripheral vasculature via plasma proteins and soluble blood factors (74). Therefore, we compared postmortem PFC microvessel gene expression with antemortem plasma protein expression in a subset of 13 subjects with both metrics available (**Table 1**, subjects with asterisks). In total, 22 proteins overlapped with cognate genes measured in our qPCR assay (**Table 2**), though SELP was not analyzed as it was excluded from qPCR analysis due to a high proportion of missing data. Protein expression was quantified as Normalized Protein eXpression (NPX), a log2-transformed unit that enables inter-subject comparison within a given protein. Of note, two ITGAM values were omitted as they were below the

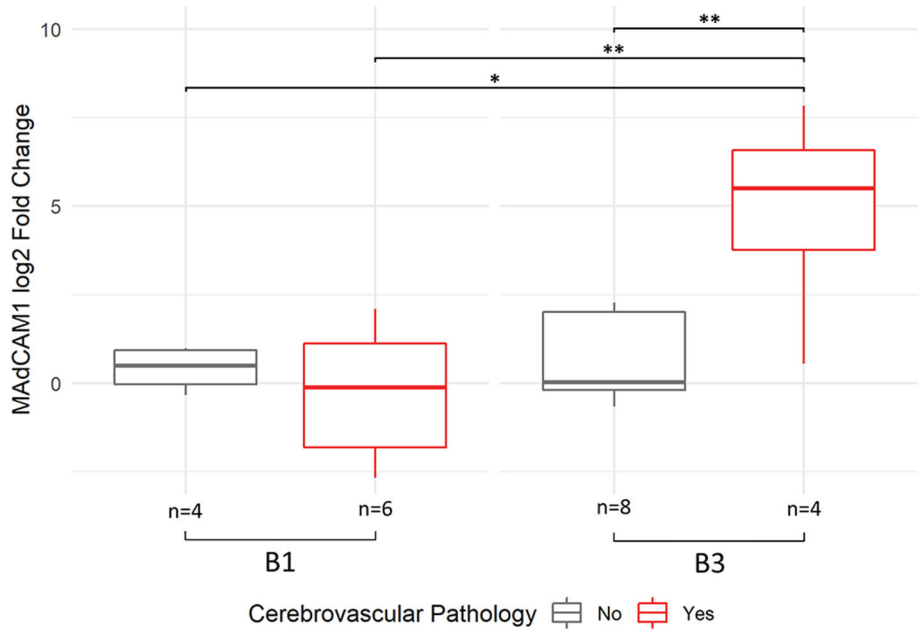


FIGURE 4 | MAdCAM-1 upregulation demonstrates interaction between Braak stage and presence of cerebrovascular pathology. ANOVA with interaction demonstrated significant interaction between a high Braak score (B3) and presence of cerebrovascular pathology in MAdCAM1 expression out of all 40 genes quantified. Data shown in the boxplot reflect Tukey's HSD *post-hoc* test results, with $^{**}p < 0.01$, $^{*}p < 0.05$. A total of 6 samples did not have detectable expression, with an even distribution of missing data across the four groups. The number of samples with detected MAdCAM-1 expression per group are indicated at the bottom of the boxes.

manufacturer-specified limit of detection (see Methods). Protein expression was measured longitudinally in two or three plasma samples per subject, though we focused here on the final plasma samples to minimize pre-mortem intervals.

Univariate regression revealed that NOS3 postmortem microvessel expression was significantly associated with antemortem plasma expression (**Figure 6A**; NPX $\beta_1 = 1.022$, $p = 0.0024$, BH-FDR $= 0.0511$, $R^2 = 0.58$). However, the pre-mortem interval ranged from 1 month to 6.7 years among samples. To account for this variability, we included pre-mortem interval as a covariate in a subsequent multiple regression. This still yielded a significant linear relationship between NOS3 plasma NPX and PFC microvessel gene fold change (NPX $\beta_1 = 1.070$, $p = 0.0031$, BH-FDR $= 0.0660$). For visualization, the gene fold change values were adjusted for the partial residual of the model, which demonstrated a strong linear association between antemortem plasma NPX and postmortem gene expression ($R^2 = 0.94$, **Figure 6B**).

Plasma Senescence-Associated Protein Levels Are Not Associated With Postmortem Severity of AD-Related Neuropathological Changes

Six of the genes that were upregulated in B3 microvessels were also quantified in plasma as secreted proteins: IL8 (CXCL8), PAI-1 (SERPINE1), CXCL1, TIE1, ICAM-2, and SELE. We reasoned that the changes seen in the CNS microvessels might reflect systemic vascular factors, which might then be observed in the plasma; alternatively, CNS-only microvascular changes may be difficult to detect in the systemic circulation. We therefore investigated whether these biomarkers exhibited protein expression differences in B1 vs. B3 antemortem plasma. Univariate analysis comparing the protein NPX from the final plasma sample between B1 vs. B3 samples failed to yield any significant results (**Figure 7A**) as measured via *t*-test and linear regression. Similarly, multivariate analysis adjusting for sex, age at visit, and pre-mortem interval yielded no significant differences in protein NPX by Braak score. While the magnitude of NPX measurements cannot be directly compared between proteins, their relative changes from baseline can be compared to assess temporal stability. Plasma time points differed between subjects, with an average of 1.30 years (±0.38) between plasma samples per subject. To that end, PAI-1 levels exhibited the greatest fluctuations from baseline across all subjects, followed by CXCL1 (**Figures 7B,C**). By contrast, TIE1 plasma levels remained the most stable over time across all subjects.

None of the other 15 plasma proteins assayed in this study showed statistically different plasma levels in B1 vs. B3 subjects. Further descriptive and exploratory analyses were carried out to examine effects of age and pre-mortem interval duration on plasma protein levels. In a multiple regression of plasma protein NPX on ABC-Braak, age at visit, and pre-mortem interval duration, IL6, IL8, and ITGAM exhibited negative slopes between pre-mortem interval vs. plasma NPX before adjusting for multiple comparisons (**Table 3**). This indicates that subjects

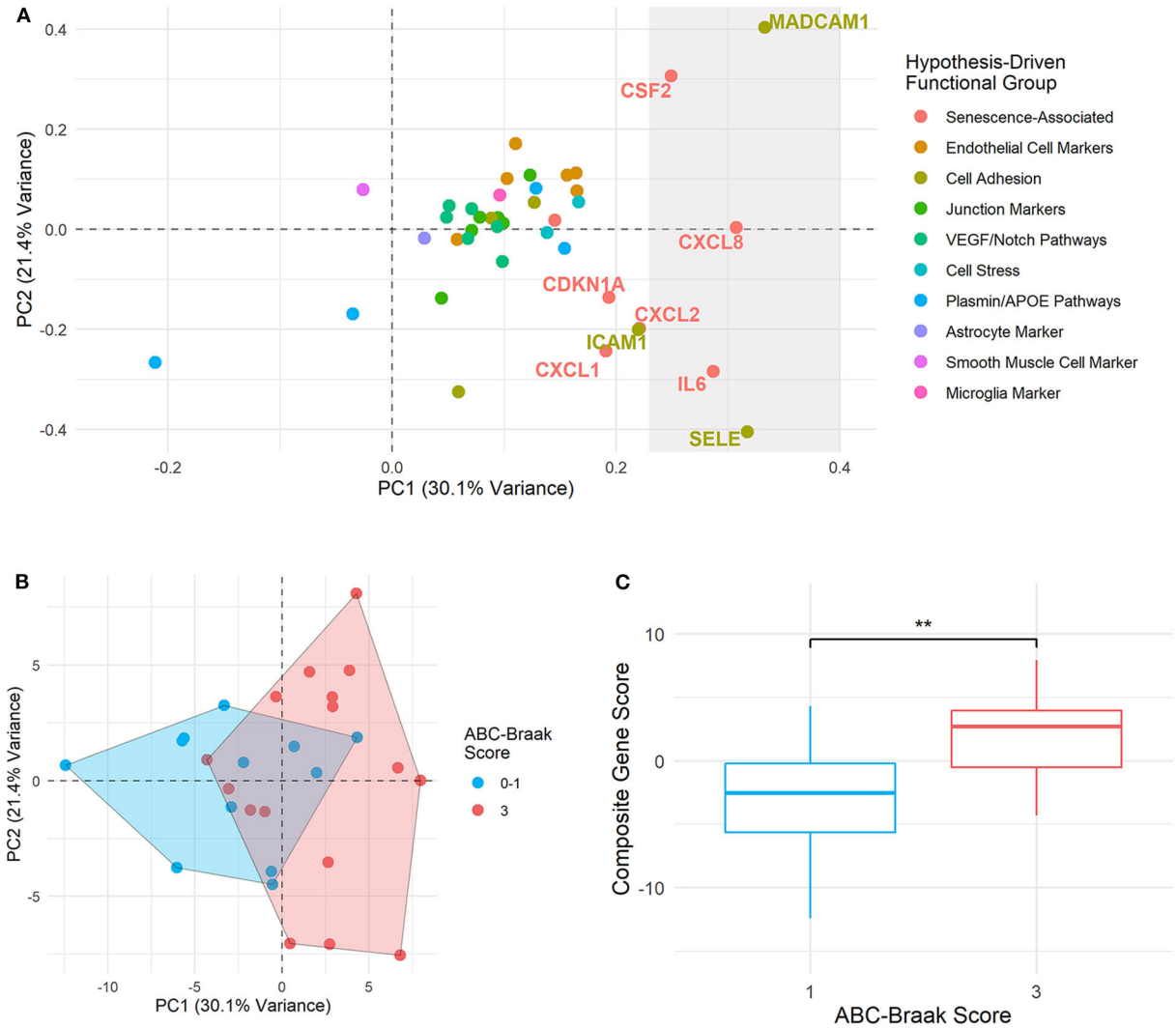


FIGURE 5 | Principal component composite gene scores in B3 vs. B1 microvessels show variance driven by senescence and adhesion genes. **(A)** PC loadings are plotted for each gene in PC1 (x-axis) and PC2 (y-axis). The rightmost shaded region contains the genes considered to be top contributors to PC1, with their percent contribution at least one standard deviation above the mean contribution. Genes immediately adjacent to this top contributor subset are also labeled. Point and text colors correspond to each gene's hypothesis-driven functional group, listed on the right. **(B)** Samples are plotted by PC1 and PC2 composite gene fold change scores, with geometric encircling to outline B1 vs. B3 group boundaries. **(C)** Boxplots show PC1 composite gene fold change scores in B1 vs. B3 PFC microvessels. Welch's unpaired *t*-test was applied to compare B1 and B3 composite scores, with ** indicating $p < 0.01$. Composite gene fold change scores are significantly larger in B3 vs. B1 microvessels ($p = 0.0064$, 95% CI: [1.477, 7.956]).

with longer pre-mortem intervals generally had lower IL6, IL8, and ITGAM plasma protein levels. IL6 also exhibited a positive slope between age vs. plasma NPX, as did CXCL1, suggesting that IL6 and CXCL1 plasma secretion may increase with age.

DISCUSSION

Tau pathology leads to senescence-associated transcriptomic changes (14, 15, 27) and subsequent vascular alterations in the mouse cortex, including transient capillary occlusion (27). Such intermittent episodes of capillary blockage arise from leukocyte adhesion in the endothelium, a phenomenon also reported in an APP-PS1 mutant mouse model of AD (75), as well as in chronic hypoperfusion (76) and traumatic brain injury (77, 78). This may be related to imbalanced capillary transit in AD, which can impair neuronal oxygenation even in the absence of gross reductions in cerebral blood flow (79, 80). Senescent endothelial cells secrete factors that attract peripheral leukocytes (81, 82) and upregulate surface leukocyte adhesion molecules (24, 73, 83, 84). Moreover, transcriptomic (85) and proteomic (86) analyses of human AD cortical microvessels have demonstrated upregulation of pathways related to senescence, including inflammation, leukocyte migration, and cell adhesion. However, this is the first study to directly examine the relationship between AD-related tau pathology and vascular senescence in both the cerebral vasculature and peripheral blood.

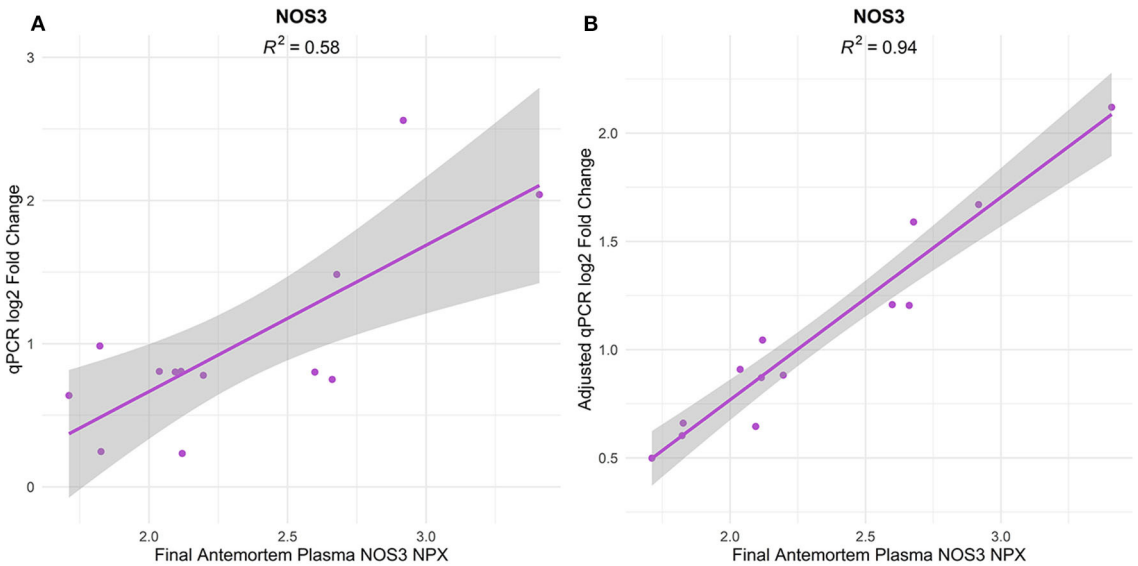


FIGURE 6 | NOS3 antemortem plasma expression is significantly associated with postmortem NOS3 gene expression in PFC capillaries of both B1 and B3 subjects. **(A)** Univariate regression of NOS3 plasma NPX (from final plasma sample per subject) vs. postmortem microvessel gene log2 fold change demonstrated a significant association between NPX and log2 fold change (β1 =1.022, $p = 0.0024$, BH-FDR = 0.0511). The shaded region indicates the 95% confidence interval of the regression slope. **(B)** After adjusting for years until death in multiple regression, there is still a significant association between NOS3 plasma NPX and postmortem capillary gene log2 fold change (β1 = 1.070, $p = 0.0031$, BH-FDR = 0.0660). For visualization, the adjusted log2 fold change values were calculated as the NPX β1 coefficient plus the partial residual associated between plasma NPX and years until death.

We report robust upregulation of the senescence- and leukocyte adhesion-associated genes CXCL8 (IL8), SERPINE1 (PAI-1), CXCL1, CXCL2, ICAM-2, and TIE1 in B3 cortical microvasculature. Interestingly, these genes are also involved in DNA damage response (DDR) signaling, a pathway that potently mediates cellular senescence (87–89). The pronounced upregulation of CXCL8 and SERPINE1 in B3 microvessels is notable, given their reported upregulation in senescent endothelial cells (23, 24, 90–92) and synergistic roles in leukocyte-endothelial adhesion (93). ICAM-2 also mediates leukocyte-endothelial adhesion in cerebral vasculature (94), and neurons upregulate ICAM-2 with the formation of tau NFTs (95). Tau burden and cerebrovascular pathology also appeared to additively interact with regard to microvascular expression of MAdCAM-1, a leukocyte adhesion molecule that is upregulated in the CNS following chronic vascular inflammation (96–98). Since MAdCAM-1 detection was variable across samples, future studies in a larger cohort are warranted to clarify the relationship between MAdCAM-1, tau pathology, and cerebrovascular pathologies.

One marker of cerebrovascular dysfunction is impaired blood-brain barrier (BBB) integrity, a phenomenon that has been linked to tau misfolding and aggregation (16, 99, 100). However, tau-independent BBB dysfunction is also observed with cognitive decline (101), possibly in relation to APOE ε4-associated BBB impairment in the hippocampus and medial temporal lobes (102). The BBB is comprised largely of endothelial cell tight junctions, supported by tight junction proteins such as OCLN, CLDN5, CDH5, and TJP1. We report here no change in gene expression of these four tight junction markers with tau progression in the AD cortical vasculature. This is a departure from previous reports of reduced cerebrovascular tight junction protein expression in AD (103) as well as the Parkinsonism–dementia complex of Guam (Guam PDC) tauopathy (104). However, Yamazaki et al. (17) showed that senescent endothelial cells exhibit aberrant tight junction protein localization without differences in overall tight junction protein expression, possibly reconciling this discrepancy. Furthermore, the assembly and localization of such tight junction proteins is largely influenced by post-transcriptional modifications (105).

Antemortem plasma protein expression did not associate with postmortem brain microvascular gene expression in this small sample, with the exception of NOS3. NOS3 is the endothelial source of nitric oxide (NO), a potent vasodilator that mediates vascular homeostasis and cerebral blood flow (106). While the association between gene and protein expression is generally tenuous, even within the same CNS cell type (107), future analysis in a larger cohort could determine the utility of NOS3 as a peripheral readout of cerebrovascular health. Of note, the temporal stability of a peripheral biomarker is an important consideration for its utility in studying the pathogenesis and prediction of AD. This is particularly relevant for PAI-1, which greatly fluctuated over time in both B1 and B3 plasma, possibly owing to its reported circadian variations (108) and cell surface binding after secretion (109). Furthermore, observed discrepancies in senescence-associated secretory phenotype (SASP) biomarker expression in cerebral vasculature vs. peripheral blood may be attributable to mismatches in transcriptional and translational upregulation.

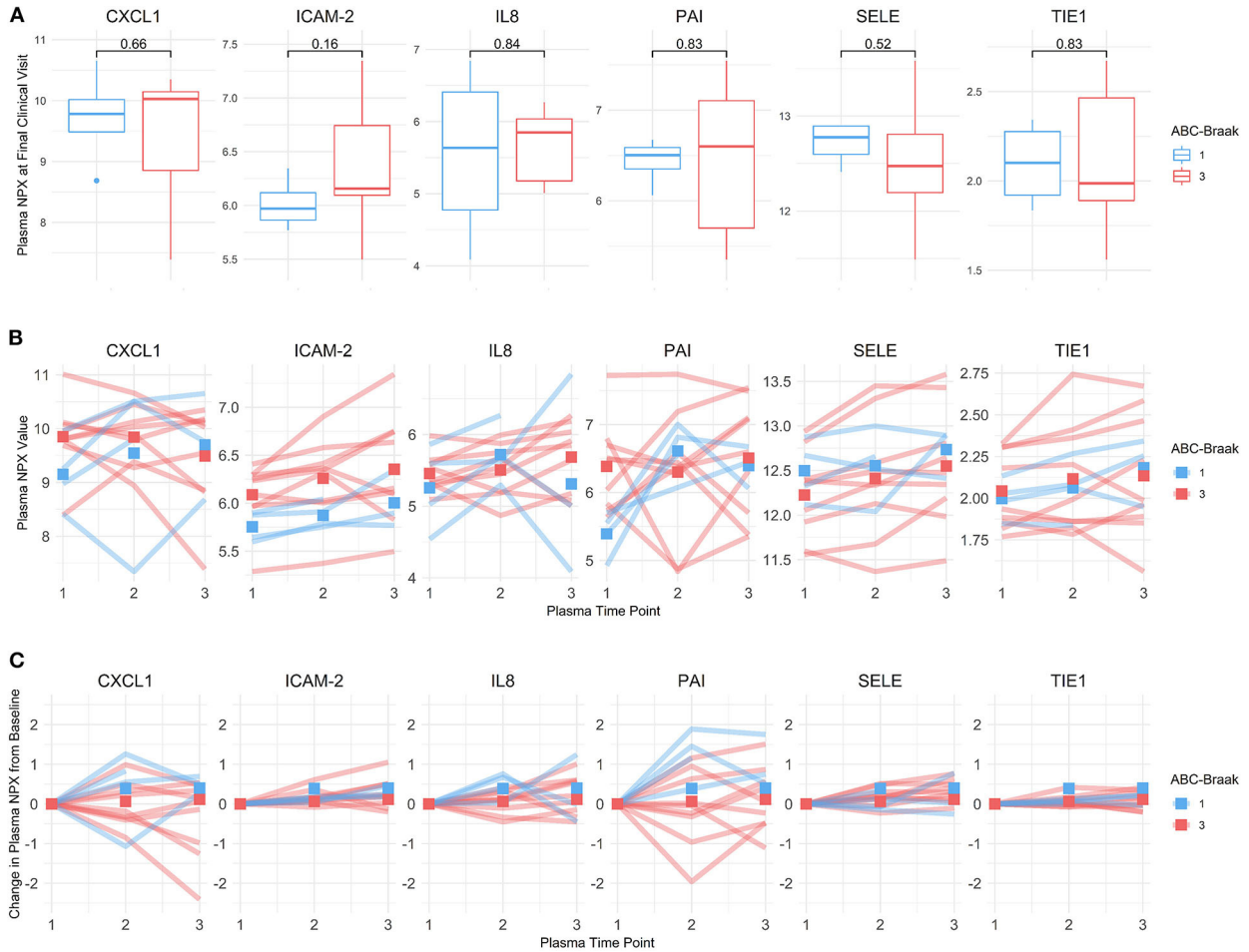


FIGURE 7 | B1 vs. B3 subject plasma expression of protein biomarkers corresponding to genes upregulated in B3 cortical PFC microvessels. **(A)** NPX values from each subject's last plasma sample for CXCL1, ICAM-2, IL8, PAI-1, SELE, and TIE1. B1 samples are shown in blue and B3 samples are shown in red. Welch's unpaired *t*-test *p*-values are shown above the boxes. **(B)** Plasma NPX values across three longitudinal samples per subject for CXCL1, ICAM-2, IL8, PAI-1, SELE, and TIE1. B1 samples are shown in blue and B3 samples are shown in red. Squares represent the ABC-Braak group mean NPX value at the corresponding plasma sample time point (which is an arbitrary unit, relative to each subject). **(C)** Relative plasma NPX change from baseline for CXCL1, ICAM-2, IL8, PAI-1, SELE, and TIE1. Values on the y-axis represent net change in plasma NPX from the first plasma sample, which is set to zero for all subjects for visualization. Squares represent the ABC-Braak group mean change in NPX at the corresponding plasma sample time point. Scales are fixed for all six proteins to compare relative temporal stability.

TABLE 3 | Age and pre-mortem interval significantly influence plasma NPX expression.

Protein	Regression term	β_1	*p*-value	BH-FDR
CXCL1	Age at visit	0.0716	0.0475	0.5990
IL6	Age at visit	0.1015	0.0292	0.4604
IL6	Pre-mortem interval	−0.5339	0.0059	0.3745
IL8	Pre-mortem interval	−0.2469	0.0257	0.4604
ITGAM	Pre-mortem interval	0.1047	0.0253	0.4604

Results shown are significant terms in multiple regression of plasma protein NPX on ABC-Braak score, age at visit, and pre-mortem interval. FDR calculations were based on n = 63 comparisons (21 proteins with 3 regression terms in model: NPX, age, and pre-mortem interval).

One advantage of this study includes the preservation of the intercellular milieu in the neurovascular unit by isolating intact cortical microvessels. Additionally, the use of tau pathology scores, microvascular gene expression, and plasma protein levels from the same subjects enabled direct comparison across modalities. However, this study is limited by its small sample size, particularly in the plasma protein subgroup, with a bias toward AD samples. We included potential confounders such as sex, age, and cerebrovascular pathology in statistical models, though their roles in tau pathology and vascular dysfunction cannot be excluded. Additionally, AD commonly presents with other co-morbidities, including TDP-43 proteinopathy and Lewy body disease (110–112); future studies are warranted to compare tau-related vascular changes with and without such co-morbidities.

Due to insufficient statistical power and a high degree of multicollinearity in this dataset, we did not adjust for APOE genotype nor for amyloid-beta burden, the effects of which also cannot be ruled out here. However, we note that the transcriptional changes shown in human AD cortical vasculature here are similar to those observed in the Tg4510 mouse model (15, 27), which exclusively over-expresses pathological tau. Future studies investigating individuals with high NFT burden but low amyloid accumulation, as seen in Primary Age Related Tauopathy (PART) (113), will be needed to confirm the specificity of these changes.

Looking forward, a larger sample size with evenly distributed pre-mortem intervals may reveal changes in the systemic vasculature that associate with cortical microvessel gene expression and tau pathology. Alternatively, rapidly advancing tau-PET neuroimaging represents a viable technique to track peripheral biomarker changes with tau accumulation in the AD brain. Tau tracer uptake correlates with the Braak stages of tau pathology progression in AD (114–116), and Ashton et al. (117) recently showed that tau-PET can be used to compare antemortem plasma protein expression and antemortem tau pathology. Real-time investigations of senescence-associated biomarker secretion in plasma and cerebral tau accumulation could better elucidate their relationship to vascular dysfunction in AD.

In conclusion, microvessels isolated from the human AD prefrontal cortex with extensive tau pathology upregulate genes involved in endothelial senescence and in recruiting leukocytes to the endothelium, which may contribute to AD-related vascular dysfunction and impaired cerebral blood flow. Future studies could identify peripheral biomarkers that are associated with vascular senescence and its relation to tau pathology in the human AD brain.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Partners Healthcare, Massachusetts General Hospital, Boston, MA. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB, SA, BH, and RB contributed to study concepts and experimental design. AB, MH, BC, and RB collected data. AB performed statistical analyses, with interpretation guided by input from BC, SD, and RB. MF provided access to biomaterials in the Massachusetts Alzheimer's Disease Research Center. AB drafted the manuscript, with critical revisions from BC, SD, BH, and RB for intellectual content. All co-authors reviewed the final manuscript and approved it for submission.

FUNDING

This work was supported by the NIH/NIA K99 AG061259 and by a New Vision Investigator Award. We would also like to thank the donors and their families for their contribution to the Massachusetts ADRC (NIH/NIA P30AG062421).

ACKNOWLEDGMENTS

We thank Dr. Pia K. Webb for coordination of the longitudinal plasma sample collection and analysis. We thank Dr. Lori Chibnik for guidance and review of statistical analyses employed in this study. We also thank Patrick M. Dooley and Theresa R. Connors for identifying and procuring postmortem tissue used in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.575953/full#supplementary-material

REFERENCES

1. de la Torre JC, Mussivan T. Can disturbed brain microcirculation cause Alzheimer's disease? *Neurol Res.* (1993) 15:146–53. doi: 10.1080/01616412.1993.11740127
2. de la Torre JC. The vascular hypothesis of Alzheimer's disease: bench to bedside and beyond. *Neurodegener Dis.* (2010) 7:116–21. doi: 10.1159/000285520
3. Roher AE, Debbins JP, Malek-Ahmadi M, Chen K, Pipe JG, Maze S, et al. Cerebral blood flow in Alzheimer's disease. *Vasc Health Risk Manag.* (2012) 8:599–611. doi: 10.2147/VHRM.S34874
4. Yarchoan M, Xie SX, Kling MA, Toledo JB, Wolk DA, Lee EB, et al. Cerebrovascular atherosclerosis correlates with Alzheimer pathology in neurodegenerative dementias. *Brain.* (2012) 135:3749–56. doi: 10.1093/brain/aws271
5. Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, Grossman M, et al. Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. *Brain.* (2013) 136:2697–706. doi: 10.1093/brain/awt188
6. Nagahama Y, Nabatame H, Okina T, Yamauchi H, Narita M, Fujimoto N, et al. Cerebral correlates of the progression rate of the cognitive decline in probable Alzheimer's disease. *Eur Neurol.* (2003) 50:1–9. doi: 10.1159/000070851
7. Benedictus MR, Leeuwis AE, Binnewijzend MAA, Kuijer JPA, Scheltens P, Barkhof F, et al. Lower cerebral blood flow is associated with faster cognitive decline in Alzheimer's disease. *Eur Radiol.* (2017) 27:1169–75. doi: 10.1007/s00330-016-4450-z
8. Bradley KM, O'Sullivan VT, Soper NDW, Nagy Z, King EM-F, Smith D, et al. Cerebral perfusion SPET correlated with Braak pathological stage in Alzheimer's disease. *Brain.* (2002) 125:1772–81. doi: 10.1093/brain/awf185
9. Binnewijzend MAA, Benedictus MR, Kuijer JPA, van der Flier WM, Teunissen CE, Prins ND, et al. Cerebral perfusion in the predementia stages of Alzheimer's disease. *Eur Radiol.* (2016) 26:506–14. doi: 10.1007/s00330-015-3834-9
10. Nedelska Z, Senjem ML, Przybelski SA, Lesnick TG, Lowe VJ, Boeve BF, et al. Regional cortical perfusion on arterial spin labeling MRI in dementia with Lewy bodies: associations with clinical severity, glucose metabolism and tau PET. *NeuroImage Clin.* (2018) 19:939–47. doi: 10.1016/j.nicl.2018.06.020

11. Matsumoto M, Hatakeyama T, Morimoto K, Yanagihara T. Cerebral blood flow and neuronal damage during progressive cerebral ischemia in gerbils. *Stroke.* (1990) 21:1470–7. doi: 10.1161/01.STR.21.10.1470
12. Sagare AP, Bell RD, Zlokovic BV. Neurovascular defects and faulty amyloid-β vascular clearance in Alzheimer's disease. *J Alzheimer's Dis.* (2012) 33:S87–S100. doi: 10.3233/JAD-2012-129037
13. Coppé J-P, Desprez P-Y, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol Mech Dis.* (2010) 5:99–118. doi: 10.1146/annurev-pathol-121808-102144
14. Bussian TJ, Aziz A, Meyer CF, Swenson BL, van Deursen JM, Baker DJ. Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. *Nature.* (2018) 562:578–82. doi: 10.1038/s41586-018-0543-y
15. Musi N, Valentine JM, Sickora KR, Baeuerle E, Thompson CS, Shen Q, et al. Tau protein aggregation is associated with cellular senescence in the brain. *Aging Cell.* (2018) 17:e12840. doi: 10.1111/acel.12840
16. Blair LJ, Frauen HD, Zhang B, Nordhues BA, Bijan S, Lin Y-C, et al. Tau depletion prevents progressive blood-brain barrier damage in a mouse model of tauopathy. *Acta Neuropathol Commun.* (2015) 3:8. doi: 10.1186/s40478-015-0186-2
17. Yamazaki Y, Baker DJ, Tachibana M, Liu C-C, van Deursen JM, Brott TG, et al. Vascular cell senescence contributes to blood–brain barrier breakdown. *Stroke.* (2016) 47:1068–77. doi: 10.1161/STROKEAHA.115.010835
18. Tian X-L, Li Y. Endothelial cell senescence and age-related vascular diseases. *J Genet Genomics.* (2014) 41:485–95. doi: 10.1016/j.jgg.2014.08.001
19. Merlini M, Wanner D, Nitsch RM. Tau pathology-dependent remodelling of cerebral arteries precedes Alzheimer's disease-related microvascular cerebral amyloid angiopathy. *Acta Neuropathol.* (2016) 131:737–52. doi: 10.1007/s00401-016-1560-2
20. Holmes HE, Colgan N, Ismail O, Ma D, Powell NM, O'Callaghan JM, et al. Imaging the accumulation and suppression of tau pathology using multiparametric MRI. *Neurobiol Aging.* (2016) 39:184–94. doi: 10.1016/j.neurobiolaging.2015.12.001
21. Qiu L, Ng G, Tan EK, Liao P, Kandiah N, Zeng L. Chronic cerebral hypoperfusion enhances Tau hyperphosphorylation and reduces autophagy in Alzheimer's disease mice. *Sci Rep.* (2016) 6:23964. doi: 10.1038/srep23964
22. Graves SI, Baker DJ. Implicating endothelial cell senescence to dysfunction in the ageing and diseased brain. *Basic Clin Pharmacol Toxicol.* (2020) 127:102–10. doi: 10.1111/bcpt.13403
23. Khan SY, Awad EM, Oszwald A, Mayr M, Yin X, Waltenberger B, et al. Premature senescence of endothelial cells upon chronic exposure to TNFα can be prevented by N-acetyl cysteine and plumericin. *Sci Rep.* (2017) 7:39501. doi: 10.1038/srep39501
24. Prattichizzo F, De Nigris V, Mancuso E, Spiga R, Giuliani A, Matacchione G, et al. Short-term sustained hyperglycaemia fosters an archetypal senescence-associated secretory phenotype in endothelial cells and macrophages. *Redox Biol.* (2018) 15:170–81. doi: 10.1016/j.redox.2017.12.001
25. Kuilman T, Michaloglou C, Vredeveld LCW, Douma S, van Doorn R, Desmet CJ, et al. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell.* (2008) 133:1019–31. doi: 10.1016/j.cell.2008.03.039
26. Hubackova S, Krejcikova K, Bartek J, Hodny Z. IL1- and TGFβ-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine 'Bystander senescence.' *Aging.* (2012) 4:932–51. doi: 10.18632/aging.100520
27. Bennett RE, Robbins AB, Hu M, Cao X, Betensky RA, Clark T, et al. Tau induces blood vessel abnormalities and angiogenesis-related gene expression in P301L transgenic mice and human Alzheimer's disease. *Proc Natl Acad Sci USA.* (2018) 115:E1289–98. doi: 10.1073/pnas.1710329115
28. Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement.* (2012) 8:1–13. doi: 10.1016/j.jalz.2011.10.007
29. Boulay AC, Saubaméa B, Declèves X, Cohen-Salmon M. Purification of mouse brain vessels. *J Vis Exp.* (2015) e53208. doi: 10.3791/53208
30. Hartz AMS, Schulz JA, Sokola BS, Edelmann SE, Shen AN, Rempe RG, et al. Isolation of cerebral capillaries from fresh human brain tissue. *J Vis Exp.* (2018) 57346. doi: 10.3791/57346
31. Kiss T, Nyúl-Tóth Á, Balasubramanian P, Tarantini S, Ahire C, DelFavero J, et al. Single-cell RNA sequencing identifies senescent cerebromicrovascular endothelial cells in the aged mouse brain. *GeroScience.* (2020) 42:429–44. doi: 10.1007/s11357-020-00177-1
32. Cotran R, Mayadas-Norton T. Endothelial adhesion molecules in health and disease. *Pathol Biol.* (1998) 46:164–70.
33. Reglero-Real N, Colom B, Bodkin JV, Nourshargh S. Endothelial cell junctional adhesion molecules. *Arteriosc Thromb Vasc Biol.* (2016) 36:2048–57. doi: 10.1161/ATVBAHA.116.307610
34. Zahr A, Alcaide P, Yang J, Jones A, Gregory M, dela Paz NG, et al. Endomucin prevents leukocyte–endothelial cell adhesion and has a critical role under resting and inflammatory conditions. *Nat Commun.* (2016) 7:10363. doi: 10.1038/ncomms10363
35. Piovella F, Nalli G, Malamani GD, Majolino I, Frassoni F, Sitar GM, et al. The ultrastructural localization of factor VIII-antigen in human platelets, megakaryocytes and endothelial cells utilizing a ferritin-labelled antibody. *Br J Haematol.* (1978) 39:209–13. doi: 10.1111/j.1365-2141.1978.tb01090.x
36. Nakhaei-Nejad M, Farhan M, Mojiri A, Jabbari H, Murray AG, Jahroudi N. Regulation of von willebrand factor gene in endothelial cells that are programmed to pluripotency and differentiated back to endothelial cells. *Stem Cells.* (2019) 37:542–54. doi: 10.1002/stem.2978
37. Partanen J, Armstrong E, Mäkelä TP, Korhonen J, Sandberg M, Renkonen R, et al. A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains. *Mol Cell Biol.* (1992) 12:1698–707. doi: 10.1128/MCB.12.4.1698
38. Dumont DJ, Yamaguchi TP, Conion RA, Rossant J, Breitman ML. Tek, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells and their presumptive precursors. *Oncogene.* (1992) 7:1471.
39. Maher F, Vannucci SJ, Simpson IA. Glucose transporter isoforms in brain: absence of GLUT3 from the blood—brain barrier. *J Cereb Blood Flow Metab.* (1993) 13:342–5. doi: 10.1038/jcbfm.1993.43
40. Zimmermann K, Opitz N, Dedio J, Renne C, Muller-Esterl W, Oess S. NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase. *Proc Natl Acad Sci USA.* (2002) 99:17167–72. doi: 10.1073/pnas.252345399
41. DeLisser HM, Newman PJ, Albelda SM. Molecular and functional aspects of PECAM-1/CD31. *Immunol Today.* (1994) 15:490–5. doi: 10.1016/0167-5699(94)90195-3
42. Guo X, Geng M, Du G. Glucose transporter 1, distribution in the brain and in neural disorders: its relationship with transport of neuroactive drugs through the blood-brain barrier. *Biochem Genet.* (2005) 43:175–87. doi: 10.1007/s10528-005-1510-5
43. Cuevas P, Gutierrez-Diaz JA, Reimers D, Dujovny M, Diaz FG, Ausman JI. Pericyte endothelial gap junctions in human cerebral capillaries. *Anat Embryol.* (1984) 170:155–9. doi: 10.1007/BF00319000
44. Wallez Y, Huber P. Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis. *Biochim Biophys Acta Biomembr.* (2008) 1778:794–809. doi: 10.1016/j.bbamem.2007.09.003
45. Dejana E, Orsenigo F, Molendini C, Baluk P, McDonald DM. Organization and signaling of endothelial cell-to-cell junctions in various regions of the blood and lymphatic vascular trees. *Cell Tissue Res.* (2009) 335:17–25. doi: 10.1007/s00441-008-0694-5
46. Thurston G, Kitajewski J. VEGF and Delta-Notch: interacting signalling pathways in tumour angiogenesis. *Br J Cancer.* (2008) 99:1204–9. doi: 10.1038/sj.bjc.6604484
47. Favara DM, Zois CE, Haider S, Pires E, Sheldon H, McCullagh J, et al. ADGRL4/ELTD1 silencing in endothelial cells induces ACLY and SLC25A1 and alters the cellular metabolic profile. *Metabolites.* (2019) 9:287. doi: 10.3390/metabo9120287
48. Tarkowski E, Issa R, Sjögren M, Wallin A, Blennow K, Tarkowski A, et al. Increased intrathecal levels of the angiogenic factors VEGF and TGF-beta in Alzheimer's disease and vascular dementia. *Neurobiol Aging.* (2002) 23:237–43. doi: 10.1016/S0197-4580(01)00285-8
49. Cho S-J, Park MH, Han C, Yoon K, Koh YH. VEGFR2 alteration in Alzheimer's disease. *Sci Rep.* (2017) 7:17713. doi: 10.1038/s41598-017-18042-1

50. Cho S-J, Yun S-M, Jo C, Jeong J, Park MH, Han C, et al. Altered expression of Notch1 in Alzheimer's disease. *PLoS ONE.* (2019) 14:e0224941. doi: 10.1371/journal.pone.0224941
51. Poss KD, Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci USA.* (1997) 94:10925–30. doi: 10.1073/pnas.94.20.10925
52. Love S. Oxidative stress in brain ischemia. *Brain Pathol.* (1999) 9:119–31.
53. Herz J, Strickland DK. LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest.* (2001) 108:779–84. doi: 10.1172/JCI200113992
54. Lillis AP, Van Duyn LB, Murphy-Ullrich JE, Strickland DK. LDL receptor-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies. *Physiol Rev.* (2008) 88:887–918. doi: 10.1152/physrev.00033.2007
55. Kanekiyo T, Bu G. The low-density lipoprotein receptor-related protein 1 and amyloid-Î[2] clearance in Alzheimer's disease. *Front Aging Neurosci.* (2014) 6:93. doi: 10.3389/fnagi.2014.00093
56. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J Neurosci.* (2008) 28:264–78. doi: 10.1523/JNEUROSCI.4178-07.2008
57. Yuan S-M. α-smooth muscle actin and ACTA2 gene expressions in vasculopathies. *Rev Bras Cir Cardiovasc.* (2015) 30:644–9. doi: 10.5935/1678-9741.20150081
58. Grabert K, Michoel T, Karavolos MH, Clohisey S, Baillie JK, Stevens MP, et al. Microglial brain region–dependent diversity and selective regional sensitivities to aging. *Nat Neurosci.* (2016) 19:504–16. doi: 10.1038/nn.4222
59. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* (2001) 25:402–8. doi: 10.1006/meth.2001.126
60. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B.* (1995) 57:289–300.
61. Kulesa A, Krzywinski M, Blainey P, Altman N. Sampling distributions and the bootstrap: the bootstrap can be used to assess uncertainty of sample estimates. *Nat Methods.* (2015) 12:477. doi: 10.1038/nmeth.3414
62. Carlyle BC, Kitchen RR, Kanyo JE, Voss EZ, Pletikos M, Sousa AMM, et al. A multiregional proteomic survey of the postnatal human brain. *Nat Neurosci.* (2017) 20:1787–95. doi: 10.1038/s41593-017-0011-2
63. Braak H, Del Tredici K. The preclinical phase of the pathological process underlying sporadic Alzheimer's disease. *Brain.* (2015) 138:2814–33. doi: 10.1093/brain/awv236
64. Bussire T, Gold G, Kvari E, Giannakopoulos P, Bouras C, Perl D, et al. Stereologic analysis of neurofibrillary tangle formation in prefrontal cortex area 9 in aging and Alzheimer's disease. *Neuroscience.* (2003) 117:577–92. doi: 10.1016/S0306-4522(02)00942-9
65. Vemuri P, Lesnick TG, Przybelski SA, Knopman DS, Lowe VJ, Graff-Radford J, et al. Age, vascular health, and Alzheimer disease biomarkers in an elderly sample. *Ann Neurol.* (2017) 82:706–18. doi: 10.1002/ana.25071
66. Fernando MS, Ince PG. Vascular pathologies and cognition in a population-based cohort of elderly people. *J Neurol Sci.* (2004) 226:13–7. doi: 10.1016/j.jns.2004.09.004
67. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology.* (2007) 69:2197–204. doi: 10.1212/01.wnl.0000271090.28148.24
68. Williams S, Chalmers K, Wilcock GK, Love S. Relationship of neurofibrillary pathology to cerebral amyloid angiopathy in Alzheimer's disease. *Neuropathol Appl Neurobiol.* (2005) 31:414–21. doi: 10.1111/j.1365-2990.2005.00663.x
69. Castillo-Carranza DL, Nilson AN, Van Skike CE, Jahrling JB, Patel K, Garach P, et al. Cerebral microvascular accumulation of Tau oligomers in Alzheimer's disease and related tauopathies. *Aging Dis.* (2017) 8:257–66. doi: 10.14336/AD.2017.0112
70. Kim HJ, Park S, Cho H, Jang YK, San Lee J, Jang H, et al. Assessment of extent and role of Tau in subcortical vascular cognitive impairment using [18] F-AV1451 positron emission tomography imaging. *JAMA Neurol.* (2018) 75:999. doi: 10.1001/jamaneurol.2018.0975
71. Camós S, Mallolas J. Experimental models for assaying microvascular endothelial cell pathophysiology in stroke. *Molecules.* (2010) 15:9104–34. doi: 10.3390/molecules15129104
72. Wilhelmsen K, Mesa KR, Prakash A, Xu F, Hellman J. Activation of endothelial TLR2 by bacterial lipoprotein upregulates proteins specific for the neutrophil response. *Innate Immun.* (2012) 18:602–16. doi: 10.1177/1753425911429336
73. Coleman PR, Chang G, Hutas G, Grimshaw M, Vadas MA, Gamble JR. Age-associated stresses induce an anti-inflammatory senescent phenotype in endothelial cells. *Aging.* (2013) 5:913–24. doi: 10.18632/aging.100622
74. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. *Neurobiol Dis.* (2017) 107:41–56. doi: 10.1016/j.nbd.2016.07.007
75. Cruz Hernández JC, Bracko O, Kersbergen CJ, Muse V, Haft-Javaherian M, Berg M, et al. Neutrophil adhesion in brain capillaries reduces cortical blood flow and impairs memory function in Alzheimer's disease mouse models. *Nat Neurosci.* (2019) 22:413–20. doi: 10.1038/s41593-018-0329-4
76. Yata K, Nishimura Y, Unekawa M, Tomita Y, Suzuki N, Tanaka T, et al. *In vivo* imaging of the mouse neurovascular unit under chronic cerebral hypoperfusion. *Stroke.* (2014) 45:3698–703. doi: 10.1161/STROKEAHA.114.005891
77. Bullock R, Maxwell WL, Graham DI, Teasdale GM, Adams JH. Glial swelling following human cerebral contusion: an ultrastructural study. *J Neurol Neurosurg Psychiatry.* (1991) 54:427–34. doi: 10.1136/jnnp.54.5.427
78. Mazzoni MC, Schmid-Schonbein GW. Mechanisms and consequences of cell activation in the microcirculation. *Cardiovasc Res.* (1996) 32:709–19. doi: 10.1016/S0008-6363(96)00146-0
79. Jespersen SN, Ostergaard L. The roles of cerebral blood flow, capillary transit time heterogeneity, and oxygen tension in brain oxygenation and metabolism. *J Cereb Blood Flow Metab.* (2012) 32:264–77. doi: 10.1038/jcbfm.2011.153
80. Østergaard L, Aamand R, Gutiérrez-Jiménez E, Ho Y-CL, Blicher JU, Madsen SM, et al. The capillary dysfunction hypothesis of Alzheimer's disease. *Neurobiol Aging.* (2013) 34:1018–31. doi: 10.1016/j.neurobiolaging.2012.09.011
81. Davalos AR, Coppe J-P, Campisi J, Desprez P-Y. Senescent cells as a source of inflammatory factors for tumor progression. *Cancer Metastasis Rev.* (2010) 29:273–83. doi: 10.1007/s10555-010-9220-9
82. Lasry A, Ben-Neriah Y. Senescence-associated inflammatory responses: aging and cancer perspectives. *Trends Immunol.* (2015) 36:217–28. doi: 10.1016/j.it.2015.02.009
83. Gorgoulis VG, Pratsinis H, Zacharatos P, Demoliou C, Sigala F, Asimacopoulos PJ, et al. p53-Dependent ICAM-1 overexpression in senescent human cells identified in atherosclerotic lesions. *Lab Investig.* (2005) 85:502–11. doi: 10.1038/labinvest.3700241
84. Pietronigro E, Zenaro E, Constantin G. Imaging of leukocyte trafficking in Alzheimer's disease. *Front Immunol.* (2016) 7:33. doi: 10.3389/fimmu.2016.00033
85. Wang S, Qaisar U, Yin X, Grammas P. Gene expression profiling in Alzheimer's disease brain microvessels. *J Alzheimer Dis.* (2012) 31:193–205. doi: 10.3233/JAD-2012-120454
86. Grammas P, Ovase R. Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. *Neurobiol Aging.* (2001) 22:837–42. doi: 10.1016/S0197-4580(01)00276-7
87. Di Leonardo A, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev.* (1994) 8:2540–51. doi: 10.1101/gad.8.21.2540
88. Nakamura AJ, Chiang YJ, Hathcock KS, Horikawa I, Sedelnikova OA, Hodes RJ, et al. Both telomeric and non-telomeric DNA damage are determinants of mammalian cellular senescence. *Epigenetics Chromatin.* (2008) 1:6. doi: 10.1186/1756-8935-1-6
89. Bielak-Zmijewska A, Mosieniak G, Sikora E. Is DNA damage indispensable for stress-induced senescence? *Mech Ageing Dev.* (2018) 170:13–21. doi: 10.1016/j.mad.2017.08.004
90. Hampel B, Fortschegger K, Ressler S, Chang M, Unterluggauer H, Breitwieser A, et al. Increased expression of extracellular proteins as a hallmark of

human endothelial cell *in vitro* senescence. *Exp Gerontol.* (2006) 41:474–81. doi: 10.1016/j.exger.2006.03.001
91. Kortlever RM, Higgins PJ, Bernards R. Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat Cell Biol.* (2006) 8:877–84. doi: 10.1038/ncb1448
92. Vaughan DE, Rai R, Khan SS, Eren M, Ghosh AK. Plasminogen activator inhibitor-1 is a marker and a mediator of senescence. *Arterioscler Thromb Vasc Biol.* (2017) 37:1446–52. doi: 10.1161/ATVBAHA.117.309451
93. Marshall LJ, Ramdin LSP, Brooks T, DPhil PC, Shute JK. Plasminogen activator inhibitor-1 supports IL-8-mediated neutrophil transendothelial migration by inhibition of the constitutive shedding of endothelial IL-8/heparan sulfate/syndecan-1 complexes. *J Immunol.* (2003) 171:2057–65. doi: 10.4049/jimmunol.171.4.2057
94. Halai K, Whiteford J, Ma B, Nourshargh S, Woodfin A. ICAM-2 facilitates luminal interactions between neutrophils and endothelial cells *in vivo*. *J Cell Sci.* (2014) 127:620–9. doi: 10.1242/jcs.137463
95. Dunckley T, Beach TG, Ramsey KE, Grover A, Mastroeni D, Walker DG, et al. Gene expression correlates of neurofibrillary tangles in Alzheimer's disease. *Neurobiol Aging.* (2006) 27:1359–71. doi: 10.1016/j.neurobiolaging.2005.08.013
96. Hänninen A, Taylor C, Streeter PR, Stark LS, Sarte JM, Shizuru JA, et al. Vascular addressins are induced on islet vessels during insulitis in nonobese diabetic mice and are involved in lymphoid cell binding to islet endothelium. *J Clin Invest.* (1993) 92:2509–15. doi: 10.1172/JCI116859
97. Farkas S, Hornung M, Sattler C, Edtinger K, Steinbauer M, Anthuber M, et al. Blocking MAdCAM-1 *in vivo* reduces leukocyte extravasation and reverses chronic inflammation in experimental colitis. *Int J Colorectal Dis.* (2006) 21:71–8. doi: 10.1007/s00384-004-0709-y
98. Kuhbandner K, Hammer A, Haase S, Terbrack E, Hoffmann A, Schippers A, et al. MAdCAM-1-mediated intestinal lymphocyte homing is critical for the development of active experimental autoimmune encephalomyelitis. *Front Immunol.* (2019) 10:903. doi: 10.3389/fimmu.2019.00903
99. Kovac A, Zilkova M, Deli MA, Zilka N, Novak M. Human truncated tau is using a different mechanism from amyloid-β to damage the blood-brain barrier. *J Alzheimer Dis.* (2009) 18:897–906. doi: 10.3233/JAD-2009-1197
100. Majerova P, Michalicova A, Cente M, Hanes J, Vegh J, Kittel A, et al. Trafficking of immune cells across the blood-brain barrier is modulated by neurofibrillary pathology in tauopathies. *PLoS ONE.* (2019) 14:e0217216. doi: 10.1371/journal.pone.0217216
101. Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Pachicano M, et al. Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat Med.* (2019) 25:270–6. doi: 10.1038/s41591-018-0297-y
102. Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. APOE4 leads to blood–brain barrier dysfunction predicting cognitive decline. *Nature.* (2020) 581:71–6. doi: 10.1038/s41586-020-2247-3
103. Yamazaki Y, Shinohara M, Shinohara M, Yamazaki A, Murray ME, Liesinger AM, et al. Selective loss of cortical endothelial tight junction proteins during Alzheimer's disease progression. *Brain.* (2019) 142:1077–92. doi: 10.1093/brain/awz011
104. Majerova P, Garruto RM, Kovac A. Cerebrovascular inflammation is associated with tau pathology in Guam parkinsonism dementia. *J Neural Trans.* (2018) 125:1013–25. doi: 10.1007/s00702-018-1883-3
105. Huang SF, Othman A, Koshkin A, Fischer S, Fischer D, Zamboni N, et al. Astrocyte glutathione maintains endothelial barrier stability. *Redox Biol.* (2020). 34:101576. doi: 10.1016/j.redox.2020.101576
106. Yang Y-M, Huang A, Kaley G, Sun D. eNOS uncoupling and endothelial dysfunction in aged vessels. *Am J Physiol Circ Physiol.* (2009) 297:H1829–36. doi: 10.1152/ajpheart.00230.2009
107. Sharma K, Schmitt S, Bergner CG, Tyanova S, Kannaiyan N, Manrique-Hoyos N, et al. Cell type– and brain region–resolved mouse brain proteome. *Nat Neurosci.* (2015) 18:1819–31. doi: 10.1038/nn.4160
108. Vaughan DE. PAI-1 and atherothrombosis. *J Thromb Haemost.* (2005) 3:1879–83. doi: 10.1111/j.1538-7836.2005.01420.x
109. Gerenu G, Martisova E, Ferrero H, Carracedo M, Rantamäki T, Ramirez MJ, et al. Modulation of BDNF cleavage by plasminogen-activator inhibitor-1 contributes to Alzheimer's neuropathology and cognitive deficits. *Biochim Biophys Acta.* (2017) 1863:991–1001. doi: 10.1016/j.bbadis.2017.01.023
110. Rahimi J, Kovacs GG. Prevalence of mixed pathologies in the aging brain. *Alzheimer Res Ther.* (2014) 6:82. doi: 10.1186/s13195-014-0082-1
111. James BD, Wilson RS, Boyle PA, Trojanowski JQ, Bennett DA, Schneider JA. TDP-43 stage, mixed pathologies, and clinical Alzheimer's-type dementia. *Brain.* (2016) 139:2983–93. doi: 10.1093/brain/aww224
112. Robinson JL, Lee EB, Xie SX, Rennert L, Suh E, Bredenberg C, et al. Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated. *Brain.* (2018) 141:2181–93. doi: 10.1093/brain/awy146
113. Crary JF, Trojanowski JQ, Schneider JA, Abisambra JF, Abner EL, Alafuzoff I, et al. Primary age-related tauopathy (PART): a common pathology associated with human aging. *Acta Neuropathol.* (2014) 128:755–66. doi: 10.1007/s00401-014-1349-0
114. Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimer Dement.* (2018) 14:989–97. doi: 10.1016/j.jalz.2018.02.013
115. Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med.* (2020) 26:379–86. doi: 10.1038/s41591-020-0755-1
116. Thijssen EH, La Joie R, Wolf A, Strom A, Wang P, Iaccarino L, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med.* (2020) 26:387–97. doi: 10.1038/s41591-020-0762-2
117. Ashton NJ, Leuzy A, Lim YM, Troakes C, Hortobágyi T, Höglund K, et al. Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. *Acta Neuropathol Commun.* (2019) 7:5. doi: 10.1186/s40478-018-0649-3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

MINI REVIEW
published: 24 September 2020
doi: 10.3389/fneur.2020.572850

Astrocytes in Tauopathies

*Matthew J. Reid, Paula Beltran-Lobo, Louisa Johnson, Beatriz Gomez Perez-Nievas and Wendy Noble**

Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

Tauopathies are a group of neurodegenerative diseases characterized by the progressive accumulation across the brain of hyperphosphorylated aggregates of the microtubule-associated protein tau that vary in isoform composition, structural conformation and localization. Tau aggregates are most commonly deposited within neurons but can show differential association with astrocytes, depending on the disease. Astrocytes, the most abundant neural cells in the brain, play a major role in synapse and neuronal function, and are a key component of the glymphatic system and blood brain barrier. However, their contribution to tauopathy progression is not fully understood. Here we present a brief overview of the association of tau with astrocytes in tauopathies. We discuss findings that support a role for astrocytes in the uptake and spread of pathological tau, and we describe how alterations to astrocyte phenotype in tauopathies may cause functional alterations that impedes their ability to support neurons and/or cause neurotoxicity. The research reviewed here further highlights the importance of considering non-neuronal cells in neurodegeneration and suggests that astrocyte-directed targets that may have utility for therapeutic intervention in tauopathies.

Keywords: tau, astrocyte, tauopathy, prion-like propagation, Alzheimer's disease, glia

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Margaret Ellen Flanagan, Northwestern Medicine, United States
Anthoula Charalampos Tsolaki, Aristotle University of Thessaloniki, Greece
Todd Cohen, University of North Carolina at Chapel Hill, United States

****Correspondence:***
Wendy Noble
wendy.noble@kcl.ac.uk

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *15 June 2020*
Accepted: *24 August 2020*
Published: *24 September 2020*

Citation:
Reid MJ, Beltran-Lobo P, Johnson L, Perez-Nievas BG and Noble W (2020) Astrocytes in Tauopathies. Front. Neurol. 11:572850. doi: 10.3389/fneur.2020.572850

INTRODUCTION

Tauopathies are a heterogeneous group of neurodegenerative diseases in which the deposition of hyperphosphorylated tau aggregates in affected brain regions accompanies synapse and neuron loss (1, 2). Primary tauopathies exhibit tau aggregates as the predominant pathological hallmark and include a diverse family of frontal-temporal lobar dementia (FTLD) subtypes referred to as FTLD-tau, and progressive supranuclear palsy (PSP) and Pick's disease (PiD). Alzheimer's disease (AD) is considered a secondary tauopathy owing to the presence of extracellular amyloid-beta (Aß) plaques, and is the most common cause of dementia (1).

Tau proteins undergo several post-translational and other modifications in disease (2). Modified forms of tau spreads from the original site of deposition to anatomically connected regions by a "prion-like" mechanism, whereby tau proteopathic seeds passively recruit tau monomers (3). The mechanisms underlying tau release, uptake and spread are not fully understood. It has long been acknowledged that in some tauopathies astrocytes accumulate tau leading to characteristic disease neuropathology. Accumulating evidence now suggests that astrocytes may actively participate in tau spread and/or clearance mechanisms by actively internalizing tau. This review summarizes the association of tau with astrocytes in tauopathies, and discusses the evidence implicating astrocytes in tau spread, as well as the impact of tauopathy brain environments on physiological astrocytic functions.

TAU PROTEIN

Human tau is encoded by the *MAPT* gene on chromosome 17 which comprises 16 exons. Exons 2, 3, and 10 undergo alternative splicing to produce the six main tau isoforms present in the adult human central nervous system (CNS) (4). Alternative splicing of exon 10 gives rise to tau isoforms containing either three or four microtubule binding repeats (referred to as 3R or 4R tau) in the C-terminal region, and alternative splicing of exons 2 and 3 produces tau proteins with zero, one or two inserts in the N-terminal tail (0N, 1N, or 2N tau, respectively). A conserved proline-rich domain is found between these two spliced regions and is known to be important for tau interactions with other proteins, including actin (5). Tau isoforms are developmentally regulated; the shortest 0N3R isoform is expressed in the fetal brain whereas in the adult human brain 3R and 4R isoforms are equally represented (6). Tau has a number of key functions, the most recognized of which is stabilizing microtubules in the axons of neurons, however tau roles in other important physiological functions such as axonal transport, DNA protection, cell signaling at the membrane, and synaptic vesicle release, have been described (2, 7). Tau is primarily expressed in neurons (8), but is known to be expressed to a lesser extent in glial cells (9–12).

Monomeric tau is water soluble and resists aggregation (7). In tauopathies, tau undergoes extensive post-translational and other modifications including, but not limited to, phosphorylation, acetylation, nitration, SUMOylation, glycosylation, ubiquitination, cleavage, and aggregation (2). The best studied of these is phosphorylation. There are 85 potential phosphorylation sites in 2N4R tau (13) and increased phosphorylation of tau, alongside other tau modifications, can reduce tau affinity for microtubules, increase cytoplasmic tau concentrations and promote tau oligomerisation and aggregation (2). Differential extents of tau modifications lead to the accumulation of heterogeneous pools of modified tau between, and within, different tauopathies. Recently, Dujardin et al. (14) found variations in the relative abundance of soluble, oligomeric and seed-competent species of hyperphosphorylated tau in tauopathy brain. Specific post-translational modifications were found to influence tau seeding capacity, and tau seeding potential strongly correlated with the rate of clinical symptoms/disease progression.

The isoform composition of tau aggregates, as well as the structure of tau filaments, also differs between tauopathies. In AD, both paired helical and straight filaments contain identical protofilament cores comprising residues 306–378 that define the aggregatory seed/core (15). This structure differs from the folds of tau filaments observed in Pick's disease (16) and tau filaments of chronic traumatic encephalopathy (CTE) have a unique hydrophobic core (17). A novel fold in corticobasal degeneration (CBD) tau has now also been discovered (18). These features may be important for the tau lesions that arise in different tauopathies (**Table 1**).

ASTROCYTES IN HEALTH AND DISEASE

Astrocytes are organized into distinct domains, and each astrocyte can connect with thousands of neurons, allowing them to coordinate synaptic activity in the CNS (45, 46). Astrocytes were long considered as supporting cells in the brain, providing metabolic and nutritional support for neurons. However, astrocytes are critical for neuronal function due to their ability to sense changes in neuronal activity through their complement of cell surface receptors, and to modulate neuronal activity by releasing gliotransmitters and gliomodulators, as well as controlling the availability of glutamate, GABA, and energy substrates (45, 47, 48). Hence, astrocytes are now known to be actively involved in synaptic transmission (49), neural circuit maintenance (50) and long-term potentiation (51). In addition, astrocytic end-feet are a structural component of the blood-brain barrier (BBB), and together with endothelial cells and pericytes have a central role in the regulation of blood flow (52). Furthermore, astrocyte end-feet are crucial for the glymphatic system of the brain, a perivascular network that allows for exchange of interstitial and cerebrospinal fluid (CSF), providing a route for clearance of molecules and proteins including Aβ (53, 54).

In neurodegenerative disease brain, astrocytes undergo pathological changes in responses to changes in the local brain environment that precede neuronal loss (55). These morphologically and functionally modified astrocytes are often termed "reactive." Reactive astrocytes show considerable heterogeneity related to their localization in the brain and the severity and length of injury/insult to their local environment (56). Reactive astrocytes are traditionally characterized by increased levels of glial fibrillary acidic protein (GFAP), which allows cytoskeletal and morphological arrangements as astrocytes alter their function (57, 58). The accumulation of GFAP-immunopositive astrocytes is common in neurodegenerative diseases. For example, reactive astrocytes are often found surrounding plaques in AD (59, 60). Indeed, levels of GFAP-reactive astrocytes are closely associated with dementia in AD (61). While increased GFAP is also found in aged brain (62), new evidence suggests that there are subgroups of astrocytes, with varying levels of GFAP expression, that distinguish aging from AD, at least in mice (63). Alterations in GFAP expression have also been noted in primary tauopathies including PSP, PiD and corticobasal degeneration (CBD) (24).

Functional changes in reactive astrocytes are well-documented and include impaired gliotransmitter release (64), alterations in calcium signaling (65), deficient ability to regulate glutamate levels at neuronal synapses and aberrant GABA release (58). In addition, astrocytes are now recognized to contribute to neuroinflammatory responses that accelerate the progression of neurodegenerative diseases (59, 66, 67). For example, reactive astrocytes increase their production and release of pro-inflammatory cytokines, complement components, and reactive oxygen species, alongside downregulating anti-inflammatory, and repair proteins to induce neurotoxicity in diseased environments (59, 68–70). Recent seminal findings proposed that astrocytes respond to their local environment by

TABLE 1 | Overview of the main clinical, genetic, molecular, and pathological features of tauopathies, including description of astrocyte abnormalities.

Disease	PiD	PSP	CBD	AGD	GGT	ARTAG	AD	PART	CTE
Common clinical symptoms	Aphasia, several behavioral changes including and personality changes, cognitive changes at later stages of disease.	Balance and motor deficits, dysphagia and aphagia.	Motor problems (often one-sided), aphagia, dysphagia.	Amnestic mild cognitive impairment often accompanied by neuropsychiatric symptoms.	Behavioral changes, mood swings, short-term memory loss.	Often no cognitive impairment or dementia related symptoms. Focal pathology may correlate with specific deficits, especially in the presence of co-pathology.	Dementia; progressive episodic memory deficits; navigational and multi-tasking difficulties; diverse behavioral and personality changes.	Associated with cognitive impairment and mild AD-like symptoms.	Behavioral changes, mood swings, short-term memory loss.
MAPT cause/risk	Mostly sporadic; MAPT mutations (exon 9, 10, 11, 12, 13 and intron 9, 10).	Mostly sporadic, H1/H1c MAPT haplotype increases risk; MAPT mutations (exon 1, 10, and intron 10);	Mostly sporadic; H1 MAPT haplotype increases risk; MAPT mutations (exon 10, 13 & intron 10);	H1 MAPT haplotype may increase risk; MAPT mutations (exon 10)	H1 MAPT haplotype; MAPT mutations (exons 1, 10, 11, intron 10).	*Depending on sub-type and classification*	Mostly sporadic; APP, PSEN1, PSEN2; No MAPT mutations	*Depending on sub-type and classification*	*Unknown* (external causes)
Primary tau isoforms that accumulate in lesions	3R	4R	4R	4R	4R	4R	3R & 4R	3R & 4R	3R & 4R
Affected brain regions	Frontal and temporal cortices.	Precentral cortex, subcortex (globus pallidus, substantia nigra, pontine nuclei, subthalamic nuclei).	Frontal and temporal cortices.	Medial temporal lobe.	Frontal, precentral and/or temporal cortices.	Gray and/or white matter, perivascular, subpial, subependymal.	Entorhinal cortex and hippocampus, spreading to most regions except the cerebellum.	Entorhinal cortex, hippocampus.	Begins focally at depths of cerebral sulci, spreads widely to frontal temporal lobes.
Hallmark astrocytic tau pathology	Ramified	Tufted	Astrocytic plaques	Thorn-shaped & granular fuzzy/bush-like	Globular inclusions	Thorn-shaped & granular fuzzy	*None*	*None*	Astrocytic tangles and some thorn-shaped astrocytes.
Cellular localization of astrocytic tau inclusions	Asymmetric 3R (predominant) or 4R tau inclusions in cell bodies & proximal processes.	Symmetric 4R tau inclusions in proximal processes.	4R tau in distal processes and end feet; thread-like processes are also common.	4R tau inclusions and diffuse staining in cell bodies & proximal-distal processes.	4R globular tau in cell bodies & proximal processes.	4R tau inclusions and diffuse staining in cell bodies & proximal processes.	*n/a*	*n/a*	Irregular p-tau lesions (around small vessels).
References	Forrest et al. (19, 20); Dickson et al. (21); Dickson (22); Josephs et al. (23); Ferrer et al. (24).	Forrest et al. (19, 20); Cairns et al. (25); Kovacs and Budka (26).	Forrest et al. (19, 20); Dickson et al. (21); Ling et al. (27).	Forrest et al. (19, 20); Botez et al. (28); Rodriguez and Grinberg (29); Saito et al. (30).	Forrest et al. (19, 20); Ahmed et al. (31).	Forrest et al. (19, 20); Kovacs et al. (32); Kovacs (33, 34); Kovacs et al. (35);	Guerreiro et al. (36); Braak and Braak (37); Braak et al. (38); Lane et al. (39).	Forrest et al. (19, 20); Crary et al. (40); Jellinger et al. (41).	Forrest et al. (19, 20); Stein et al. (42); McKee et al. (43, 44).

PiD, Pick's disease; PSP, progressive supranuclear palsy; CBD, corticobasal degeneration; AGD, argyrophilic grain disease; GGT, globular glial tauopathy; ARTAG, age-related tau astrogliopathy; AD, Alzheimer's disease; PART, primary age-related tauopathy; CTE, chronic traumatic encephalopathy; 3R, 3-repeat tau; 4R, 4-repeat tau.

adopting "A1-neurotoxic" or "A2-neuroprotective" phenotypes (71). Secretion of Il-1α, TNFα, and C1q by microglia in response to damage, induces astrocytes to upregulate their expression of a specific cluster of "A1" genes, lose their trophic and synaptic support for neurons, and induce neuron death (71). Markers of A1 astrocytes are upregulated in AD and other neurodegenerative diseases (71), strongly implicating microglia-astrocyte communications in neurodegeneration. However, it is likely that there is a spectrum of reactive astrocyte states in different brain regions, throughout aging and disease progression (63, 72), similar to dynamic microglial responses in disease (25).

THE ASSOCIATION OF ASTROCYTES WITH TAUOPATHY

Tau aggregates accumulate in both neurons and astrocytes in different tauopathies. In AD, tau aggregates containing both 3R and 4R tau deposit as intraneuronal neurofibrillary tangles and there is scant evidence of astrocytic tau inclusions (73). In contrast, astrocytic tau pathology is the defining feature of several FTLD-tau subtypes (**Table 1**). In PSP, a neuropathological diagnosis criterion is "tufted" astrocytes that show 4R tau aggregates in their proximal processes (26, 74). CBD has extensive clinical overlap with PSP. In CBD, astrocytic plaques containing 4R tau deposits that mark distal and end processes are an exclusive feature in most (19), but not all cases (75). Thread-like tau-positive astrocytic processes are also common in CBD (21, 27). Argyrophilic grain disease (AGD) is a rare tauopathy that is characterized by 4R tau-immunopositive astrocytes, described as thorn-shaped and fuzzy/bush-like, in the medial temporal lobe (19, 28, 30). In contrast, PiD is typically characterized by neuronal 3R tau inclusions, predominantly in granular neurons in the hippocampus, frontal and temporal cortices (22, 23). "Ramified" astrocytes immunopositive for tau have also been reported in PiD, but they are not considered a major pathological hallmark of the disease (21, 24). Several rarer tauopathy subtypes that show 4R tau-immunopositive globular inclusions, predominantly in oligodendrocytes, and more rarely in the cytoplasm, and proximal processes of astrocytes, are collectively termed globular glial tauopathy (GGT) (31).

A spectrum of FTLD-tau subtypes that accumulate both 3R and 3R tau in neurofibrillary tangles (NFTs), typically occurring in cognitively normal aged individuals, is referred as primary age-related tauopathy (PART) (40, 41). Depending on the co-occurrence of Aß pathology, PART can be histologically classified as "definite PART" in the absence of Aß deposits, or "possible PART" when a limited number of Aß deposits are present (40). Although the neuropathological characteristics of PART can overlap with other tauopathies, particularly AD, PART shows a lower threshold of amyloid load, and appears to have a more limited impact on cognition (40, 76). Tau pathology in PART is predominantly neuronal and found in the CA2 hippocampal subfield, with little evidence of astrocytic tau deposits (40, 77). In contrast, age-related tau astrogliopathy (ARTAG) describes a spectrum of abnormal tau pathology, predominantly in the aged brain, that is characterized by thorn-shaped and granular or fuzzy astrocytes containing phosphorylated tau (32, 33). ARTAG can present alongside more typical tau pathology in tauopathies such as CBD (33, 34), but is not always linked with dementia (78). In a recent detailed review, Kovacs (34) describe two distinct distribution patterns of ARTAG. They describe ARTAG as a consequence of repeated mechanical damage (related to CTE), or chronic damage such as blood-brain barrier dysfunction. Furthermore, they propose that the location and type (white vs. gray matter) of ARTAG pathology may result in decompensation of cognitive functions, the rate of which may be influenced by co-existing pathologies (34). It is important to note that the presence of astrocytic tau accumulations in the absence of dementia may suggest that tau-containing astrocytes are not damaging in tau-associated neurodegeneration, or at least in ARTAG, and may internalize tau aggregates as a means of clearing damaging protein species.

Finally, chronic traumatic encephalopathy (CTE) is caused by mild repetitive head injuries. 3R and 4R tau-positive aggregates are common in CTE, however the tau aggregates that accumulate in astrocytes are predominantly 4R and localize in astrocytes near small vessels in the cerebral sulci of the frontal and temporal cortices (42, 43, 79). Thorn-shaped astrocytes are also observed subpial and periventricular regions, an interesting link to ARTAG (34, 44).

DO ASTROCYTES CONTRIBUTE TO TAU PATHOLOGY SPREAD?

Neurofibrillary tangles have long been acknowledged to follow a stereotypical temporospatial pattern of spread from the entorhinal cortex as AD progresses (38). Recent evidence indicates that differences in the tau species that deposit in characteristic tau lesions may confer specific neuronal vulnerabilities and/or prion-like spread of tau (14, 80). Mouse models that express wild-type 3R and 4R human tau isoforms in appropriate ratios recapitulate the same cell type vulnerabilities that typify human tauopathies when injected with human tau extracts, including the development of tufted astrocytes in PSP tau-injected mice, and astroglial plaques in CBD tau-injected mice (81). These data raise the possibility that astrocytes actively contribute to the spread of pathological forms of tau, particularly in PSP and CBD. That tau spreads in a prion-like manner trans-synaptically along anatomical connections was elegantly shown in transgenic mice in which mutant human (P301L) FTLD-causing tau expression was restricted to layer II neurons in the entorhinal cortex. Following local tau aggregation, tau "seeds" were found to spread to the hippocampus and onwards as mice aged (82, 83). Notably, PHF1-positive tau was detected in GFAP-positive astrocytes in the hippocampus of older mice, suggesting that astrocytes internalize and may contribute to tau spread (82) (**Figure 1**).

Heparan sulfate proteoglycans (HSPGs) are a well-conserved group of proteoglycans expressed on the cell surface of astrocytes and neurons (84, 85) that mediate targeted endocytosis (84), including that of purified prion proteins *in vitro* (86, 87). HSPGs were recently shown to interact with protein aggregates including

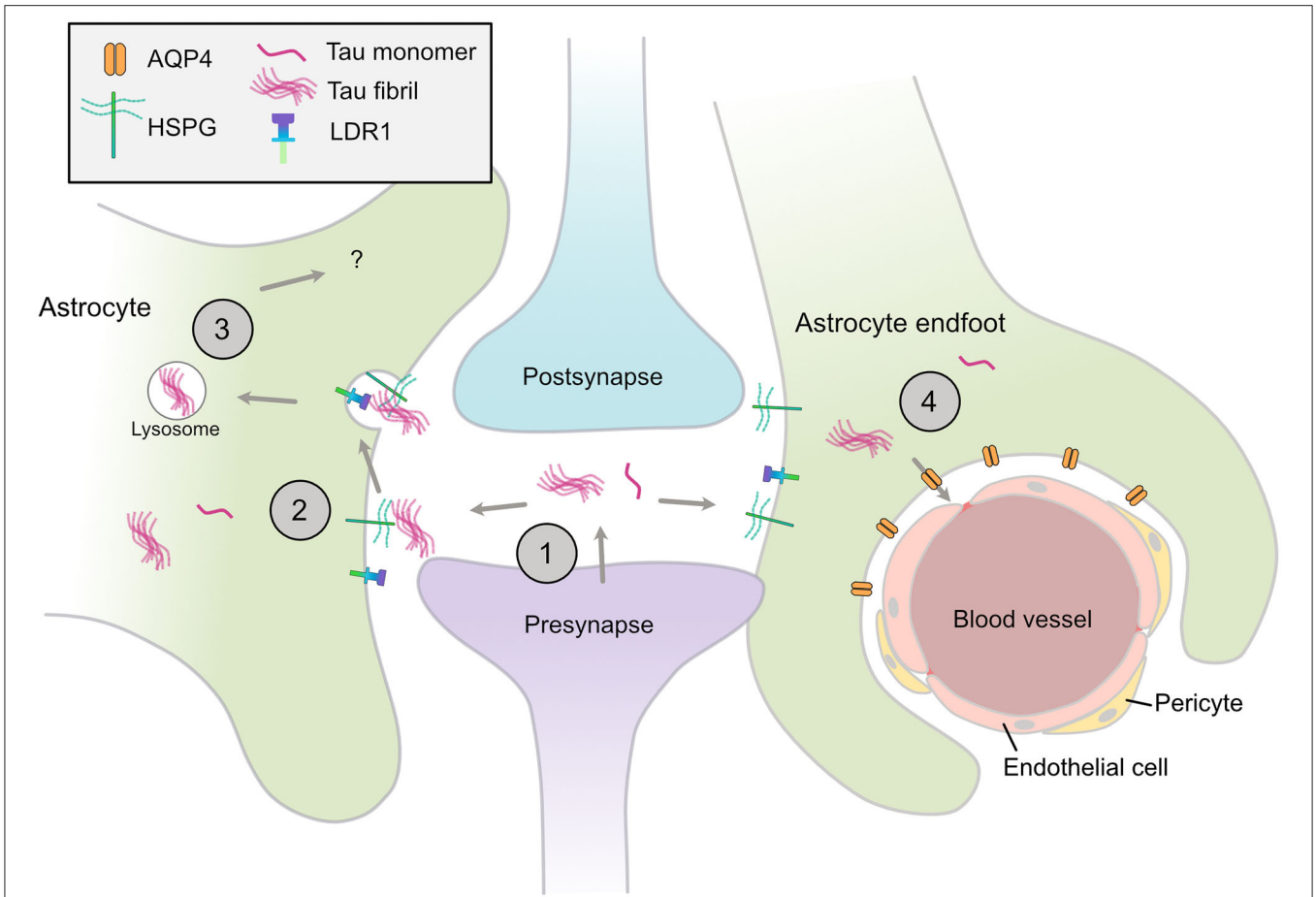


FIGURE 1 | Astrocytic mechanisms that may contribute to spread of tau pathology. (1) Tau monomers and aggregates are released from neurons *via* various mechanisms, including from the pre-synapse, (2) Astrocytes have specific HSPGs and receptors such as LDR1 that may mediate the uptake of tau aggregates, (3) These aggregates may be internalized and processed by various mechanisms, include lysosomal degradation, (4) Disruption of AQP4 in perivascular astrocytic end-feet may contribute to the disrupted tau clearance and the accumulation of tau aggregates in the CNS. HSPG, heparin sulfate proteoglycan; LDR1, low density lipoprotein receptor-related protein 1; AQP4, aquaporin-4.

α-synuclein, Aβ and tau (88–90). HSPGs regulate the uptake of synthetic tau fibrils (89) and human brain-derived tau (91) in human immortalized cell lines and mouse primary neuronal cultures. HSPGs vary in the length of their glycosaminoglycan chains and sulfation patterns, properties that are important for tau uptake in human embryonic kidney cells (92) and human iPSC derived neurons (93). Interestingly, tau fibrils are efficiently internalized in a HSPG-dependent manner by primary astrocytes exogenously expressing transcription factor EB (TFEB), a master regulator of lysosomal biogenesis (94). In contrast, monomeric tau appears to be taken up by astrocytes using an HSPG-independent mechanism (95). Together this suggests that multiple mechanisms are involved in tau uptake by astrocytes, that may be specific to tau aggregation state or conformation, as well as the HSPG profile of the cell type (96).

HSPGs can also partner with cell surface receptors to mediate the intake of protein aggregates. For example, HSPGs interact with members of the low-density lipoprotein receptor (LDLR) such as LRP1, to facilitate Aβ uptake and degradation by astrocytes (97, 98). Knockdown of LRP1 was recently shown to block the uptake of monomeric and oligomeric tau in a human neuroglioma cell line, and partially inhibit uptake of sonicated tau fibrils (99), warranting further investigation into how astrocytic LRP1 may mediate tau uptake and spread in tauopathies.

Astrocytes are an integral part of the glymphatic system of the brain, a clearance system of soluble proteins and solutes. The astrocytic water channel aquaporin-4 (AQP4), expressed at the astrocyte end feet, facilitates this process and is important for Aβ clearance (53, 100). Disruption to AQP4 may also contribute to tauopathy progression. In a mouse model of CTE, knockout of AQP4 exacerbated neurofibrillary tau pathology and neurodegeneration (101). Distinct phosphorylation marks in AQP4 have been reported in human post-mortem ARTAG samples relative to controls (102) that are suggested to increase water permeability of AQP4. However, the functional implications of these modifications in ARTAG remain to be explored (103, 104). A recent

transcriptional analysis of cognitively-impaired subjects and controls showed that components of the dystrophin-associated complex, which anchors AQP4 at the perivascular astrocytic end foot, are associated with phosphorylated tau levels in the temporal cortex (54). This analysis also revealed other astrocyte endfoot candidate genes that significantly correlate with temporal cortex tau pathology. The authors speculate that endfoot functions of astrocytes may play a role in the accumulation of tau aggregates throughout the brain. Although AQP4 might contribute to the clearance of aberrant proteins early in the disease process, this function could become impaired at later stages, hindering the clearance of pathogenic tau.

TAU EFFECTS ON ASTROCYTE FUNCTION

In addition to potential roles in tau spread, internalization of pathological forms of tau has been shown to disrupt a myriad of astrocytic functions, central for the maintenance and support of neurons. Oligomeric tau uptake alters calcium signaling and gliotransmitter release (e.g. ATP) *via* Ca^{2+}-dependant mechanisms, to disrupt post-synaptic currents and downregulate pre- and post-synaptic markers in neuronal-astrocyte co-cultures (64), together suggesting that tau-induced changes to astrocyte function are toxic to neighboring neurons, at least *in vitro*. Astrocytes isolated from a transgenic tauopathy model (P301S) expressing a 4R mutant tau isoform also acquired early functional deficiencies that impaired their ability to support neurons in culture (105). Astrocytes from mouse models of tauopathies also show altered expression of neuronally regulated genes (106), indicating that the accumulation of abnormal tau species is sufficient to drive transcriptional and likely functional changes in astrocytes, *via* altered neuron-astrocyte interactions. In addition, human astrocytes differentiated from iPSCs harboring FTD-causing *MAPT* mutations display an increased vulnerability to oxidative stress and elevated protein ubiquitination, alongside disease-associated transcriptomic alterations (107).

The immune-related functions of astrocytes are a major contributor to neuroinflammatory response that directly alter neuronal integrity in neurodegenerative diseases (52). In particular, the complement cascade, which also involves microglia, has an important role in the accumulation of beta-amyloid pathology (108, 109). C3 is a major component of the complement cascade and is highly expressed in reactive astrocytes (71). C3, as well as its downstream receptor C3aR1, that is mainly expressed by microglia, (9), is upregulated in postmortem tauopathy brain and correlates with cognitive decline during disease progression (110). Levels of C3 also correlate with tau amounts in AD CSF (111). Ablation of C3aR or C3 in mouse models of tauopathy reversed neuronal loss and neurodegeneration (110, 111), alongside reduced numbers of GFAP-reactive hypertrophied astrocytes being apparent upon C3aR knockout (110). These data indicate that complement activation downstream of astrocyte reactivity may be an important driver of tauopathy.

Astrocytes, together with microglia, are also hypothesized to induce synaptic loss and neurotoxicity in tauopathies, as they do during development (112), through dysregulated synaptic pruning (113). Sleep deprivation is common in AD (114), where it is believed to be both a cause and consequence of neurodegenerative changes (114). Sleep deprivation leads to enhanced tau release and spread (115), alongside astrocyte-mediated synapse elimination (116). It is therefore possible that astrocyte engulfment of tau-containing synapses may be one route by which astrocytes contribute to tau spread in AD.

Ultimately, cross-talk between astrocytes and microglia forms part of a complex innate immune response that may be exacerbated during tauopathies in response to protein aggregates. Deeper investigation of these pathways may reveal novel targets that can be exploited to slow or halt disease progression.

DISCUSSION

Recent evidence has highlighted that altered astrocyte functions have detrimental consequences for neurons and may be a driver of neurodegenerative diseases. Astrocytes are closely associated with the accumulation of pathological forms of tau in tauopathies. There is some evidence that astrocytes internalize tau aggregates, *via* mechanisms that are not yet fully understood, and contribute to tau pathology spread across the brain and tau aggregate clearance *via* the glymphatic system. However, astrocytes show significant regional heterogeneity and more work is needed to better understand the contribution of different astrocyte subtypes in affected brain regions at different disease stages. Such understanding may aid in the development of astrocyte-targeted therapies for tauopathies. Astrocyte-targeted therapeutic approaches have been well-described elsewhere including by Sadik and Liddelow (70), and could include antagonists that prevent tau uptake by astrocytes to reduce tau spread, agents that prevent the release of neurotoxic astrocyte secretions or their uptake by neurons, or therapies that restore physiological astrocyte functions including their trophic support for neurons and synapses, maintenance of the blood brain barrier, and roles in the glymphatic clearance of protein aggregates.

AUTHOR CONTRIBUTIONS

MR, PB-L, LJ, BP-N, and WN wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Work in our laboratories is supported by funding from Alzheimer's Research UK; ARUK-PHD2018-002 to WN, BP-N, and PB-L and ARUK-RF2014-2 to BP-N Medical Research Council, British Biotechnology and Biological Sciences Research Council, and the van Geest Charitable Foundation.

REFERENCES

1. Prince M, Albanese E, Guerchert M, Prina M, Ferri C, Mazzotti DR, et al. *World Alzheimer Report 2014: Dementia and Risk Reduction, an Analysis of Protective and Modifiable Factors*. Alzheimer's Disease International. (2014). Available at: https://www.alz.co.uk/research/WorldAlzheimerReport2014.pdf.
2. Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. *Acta Neuropathol.* (2017) 133:665–704. doi: 10.1007/s00401-017-1707-9
3. Jucker M, Walker LC. Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases. *Nat Neurosci.* (2018) 21:1341–9. doi: 10.1038/s41593-018-0238-6
4. Andreadis A. Tau gene alternative splicing: expression patterns, regulation and modulation of function in normal brain and neurodegenerative diseases. *Biochim Biophys Acta Mol Basis Dis.* (2005) 1739:91–103. doi: 10.1016/j.bbadis.2004.08.010
5. He HJ, Wang XS, Pan R, Wang DL, Liu MN, He RQ. The proline-rich domain of tau plays a role in interactions with actin. *BMC Cell Biol.* (2009) 10:81. doi: 10.1186/1471-2121-10-81
6. 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) 9:4225–30. doi: 10.1002/j.1460-2075.1990.tb07870.x
7. Wang Y, Mandelkow E. Tau in physiology and pathology. *Nat Rev Neurosci.* (2016) 17:5–21. doi: 10.1038/nrn.2015.1
8. Binder LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol.* (1985) 101:1371–8. doi: 10.1083/jcb.101.4.1371
9. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci.* (2014) 34:11929–47. doi: 10.1523/JNEUROSCI.1860-14.2014
10. Darmanis S, Sloan SA, Zhang Y, Enge M, Caneda C, Shuer LM, et al. A survey of human brain transcriptome diversity at the single cell level. *Proc Natl Acad Sci USA.* (2015) 112:7285–90. doi: 10.1073/pnas.1507125112
11. Seiberlich V, Bauer NG, Schwarz L, Ffrench-Constant C, Goldbaum O, Richter-Landsberg C. Downregulation of the microtubule associated protein Tau impairs process outgrowth and myelin basic protein mRNA transport in oligodendrocytes. *Glia*. (2015) 63:1621–35. doi: 10.1002/glia.22832
12. McKenzie AT, Wang M, Hauberg ME, Fullard JF, Kozlenkov A, Keenan A, et al. Brain cell type specific gene expression and co-expression network architectures. *Sci Rep.* (2018) 8:1–19. doi: 10.1038/s41598-018-27293-5
13. Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med.* (2009) 15:112–9. doi: 10.1016/j.molmed.2009.01.003
14. Dujardin, S., Commins, C., Lathuiliere, A., Beerepoot, P., Fernandes, A. R., Kamath, T. V., et al. (2020). Tau molecular diversity contributes to clinical heterogeneity in Alzheimer's disease. *Nat Med.* (2020) 26:1256–63. doi: 10.1038/s41591-020-0938-9
15. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature*. (2017) 547:185–90. doi: 10.1038/nature23002
16. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature*. (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y
17. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature.* (2019) 568:420–3. doi: 10.1038/s41586-019-1026-5
18. Zhang W, Tarutani A, Newell KL, Murzin AG, Matsubara T, Falcon B, et al. Novel tau filament fold in corticobasal degeneration. *Nature.* (2020) 580:283–7. doi: 10.1038/s41586-020-2043-0
19. Forrest SL, Kril JJ, Halliday GM. Cellular and regional vulnerability in frontotemporal tauopathies. *Acta Neuropathol.* (2019) 138:705–27. doi: 10.1007/s00401-019-02035-7
20. Forrest SL, Kril JJ, Stevens CH, Kwok JB, Hallupp M, Kim WS, et al. Retiring the term FTDP-17 as MAPT mutations are genetic forms of sporadic frontotemporal tauopathies. *Brain*. (2018) 141:521–34. doi: 10.1093/brain/awx328
21. Dickson DW, Kouri N, Murray ME, Josephs KA. Neuropathology of frontotemporal lobar degeneration-Tau (FTLD-Tau). *J Mol Neurosci.* (2011) 45:384–9. doi: 10.1007/s12031-011-9589-0
22. Dickson DW. Neuropathology of Pick's disease. *Neurology*. (2001) 56:S16–S20. doi: 10.1212/WNL.56.suppl_4.S16
23. Josephs KA, Hodges JR, Snowden JS, MacKenzie IR, Neumann M, Mann DM, et al. Neuropathological background of phenotypical variability in frontotemporal dementia. *Acta Neuropathol.* (2011) 122:137–53. doi: 10.1007/s00401-011-0839-6
24. Ferrer I, López-González I, Carmona M, Arregui L, Dalfó E, Torrejón-Escribano B, et al. Glial and neuronal tau pathology in tauopathies: characterization of disease-specific phenotypes and tau pathology progression. *J Neuropathol Exp Neurol.* (2014) 73:81–97. doi: 10.1097/NEN.0000000000000030
25. Cairns NJ, Bigio EH, Mackenzie IRA, Neumann M, Lee VMY, Hatanpaa KJ, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the consortium for frontotemporal lobar degeneration. *Acta Neuropathol.* (2007) 114:5–22. doi: 10.1007/s00401-007-0237-2
26. Kovacs GG, Budka H. Current concepts of neuropathological diagnostics in practice: neurodegenerative diseases. *Clin Neuropathol.* (2010) 29:271–88. doi: 10.5414/NPP29271
27. Ling H, Kovacs GG, Vonsattel JPG, Davey K, Mok KY, Hardy J, et al. Astrogliopathy predominates the earliest stage of corticobasal degeneration pathology. *Brain*. (2016) 139:3237–52. doi: 10.1093/brain/aww256
28. Botez G, Probst A, Ipsen S, Tolnay M. Astrocytes expressing hyperphosphorylated tau protein without glial fibrillary tangles in argyrophilic grain disease. *Acta Neuropathol.* (1999) 98:251–6. doi: 10.1007/s004010051077
29. Rodriguez RD, Grinberg LT. Argyrophilic grain disease: an underestimated tauopathy. *Dement Neuropsychol.* (2015) 9:2–8. doi: 10.1590/S1980-57642015DN91000002
30. Saito Y, Ruberu NN, Sawabe M, Arai T, Tanaka N, Kakuta Y, et al. Staging of argyrophilic grains: an age-associated tauopathy. *J Neuropathol Exp Neurol.* (2004) 63:911–8. doi: 10.1093/jnen/63.9.911
31. Ahmed Z, Bigio EH, Budka H, Dickson DW, Ferrer I, Ghetti B, et al. Globular glial tauopathies (GGT): consensus recommendations. *Acta Neuropathol.* (2013) 126:537–44. doi: 10.1007/s00401-013-1171-0
32. Kovacs GG, Ferrer I, Grinberg LT, Alafuzoff I, Attems J, Budka H, et al. Aging-related tau astrogliopathy (ARTAG): harmonized evaluation strategy. *Acta Neuropathol.* (2016) 131:87–102. doi: 10.1007/s00401-015-1509-x
33. Kovacs G. Understanding the Relevance of Aging-Related Tau Astrogliopathy (ARTAG). *Neuroglia*. (2018) 1:339–50. doi: 10.3390/neuroglia1020023
34. Kovacs GG. Astroglia and tau: new perspectives. *Front Aging Neurosci.* (2020) 12:1–14. doi: 10.3389/fnagi.2020.00096
35. Kovacs GG, Robinson GL, Xie SX, Lee EB, Grossman M, Wolk DA, et al. Evaluating the patterns of aging-related tau astrogliopathy unravels novel insights into brain aging and neurodegenerative diseases. *J Neuropathol Exp Neurol.* (2017) 76:270–88. doi: 10.1093/jnen/nlx007
36. Guerreiro RJ, Gustafson DR, Hardy J. The genetic architecture of Alzheimer's disease: beyond APP, PSENS and APOE. *Neurobiol Aging*. (2012) 33:437–56. doi: 10.1016/j.neurobiolaging.2010.03.025
37. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* (1991) 82:239–59. doi: 10.1007/BF00308809
38. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol.* (2011) 70:960–9. doi: 10.1097/NEN.0b013e318232a379
39. Lane CA, Hardy J, Schott JM. Alzheimer's disease. *Eur J Neurol.* (2018). 25:59–70. doi: 10.1111/ene.13439
40. Crary JF, Trojanowski JQ, Schneider JA, Abisambra JF, Abner EL, Alafuzoff I, et al. Primary age-related tauopathy (PART): a common pathology associated with human aging. *Acta Neuropathol.* (2014) 128:755–66. doi: 10.1007/s00401-014-1349-0
41. Jellinger KA, Alafuzoff I, Attems J, Beach TG, Cairns NJ, Crary JF, et al. PART, a distinct tauopathy, different from classical sporadic Alzheimer disease. *Acta Neuropathol.* (2015) 129:757–62. doi: 10.1007/s00401-015-1407-2

42. Stein TD, Alvarez VE, McKee AC. Chronic traumatic encephalopathy: a spectrum of neuropathological changes following repetitive brain trauma in athletes and military personnel. *Alzheimers Res Ther.* (2014) 6:4. doi: 10.1186/alzrt234
43. McKee AC, Stein TD, Kiernan PT, Alvarez VE. The neuropathology of chronic traumatic encephalopathy. *Brain Pathol.* (2015) 25:350–64. doi: 10.1111/bpa.12248
44. McKee AC, Cairns NJ, Dickson DW, Folkerth RD, Dirk Keene C, Litvan I, et al. The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. *Acta Neuropathol.* (2016) 131:75–86. doi: 10.1007/s00401-015-1515-z
45. Parpura V, Basarsky TA, Liu F, Jeftinijatt K. Glutamate-mediated astrocyte-neuron signalling. *Nature.* (1994) 369:744–7. doi: 10.1038/369744a0
46. Oberheim NA, Wang X, Goldman S, Nedergaard M. Astrocytic complexity distinguishes the human brain. *Trends Neurosci.* (2006) 29:547–53. doi: 10.1016/j.tins.2006.08.004
47. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci.* (2005) 6:626–40. doi: 10.1038/nrn1722
48. Choi SS, Lee HJ, Lim I, Satoh JI, Kim SU. Human astrocytes: secretome profiles of cytokines and chemokines. *PLoS ONE.* (2014) 9:e92325. doi: 10.1371/journal.pone.0092325
49. Santello M, Toni N, Volterra A. Astrocyte function from information processing to cognition and cognitive impairment. *Nat Neurosci.* (2019) 22:154–66. doi: 10.1038/s41593-018-0325-8
50. Mederos S, González-Arias C, Perea G. Astrocyte–neuron networks: a multilane highway of signaling for homeostatic brain function. *Front Synaptic Neurosci.* (2018) 10:45. doi: 10.3389/fnsyn.2018.00045
51. Lushnikova I, Skibo G, Muller D, Nikonenko I. Synaptic potentiation induces increased glial coverage of excitatory synapses in CA1 *Hippocampus*. (2009) 762:753–62. doi: 10.1002/hipo.20551
52. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol.* (2010) 119:7–35. doi: 10.1007/s00401-009-0619-8
53. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci Transl Med.* (2012) 4:147ra111. doi: 10.1126/scitranslmed.3003748
54. Simon MJ, Wang MX, Murchison CF, Roese NE, Boespflug EL, Woltjer RL, et al. Transcriptional network analysis of human astrocytic endfoot genes reveals region-specific associations with dementia status and tau pathology. *Sci Rep.* (2018) 8:1–16. doi: 10.1038/s41598-018-30779-x
55. Kersaitis C, Halliday GM, Kril JJ. Regional and cellular pathology in frontotemporal dementia: relationship to stage of disease in cases with and without Pick bodies. *Acta Neuropathol.* (2004) 108:515–23. doi: 10.1007/s00401-004-0917-0
56. Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, et al. Genomic analysis of reactive astrogliosis. *J Neurosci.* (2012) 32:6391–410. doi: 10.1523/JNEUROSCI.6221-11.2012
57. Kamphuis W, Kooijman L, Orre M, Stassen O, Pekny M, Hol EM. GFAP and vimentin deficiency alters gene expression in astrocytes and microglia in wild-type mice and changes the transcriptional response of reactive glia in mouse model for Alzheimer's disease. *Glia.* (2015) 63:1036–56. doi: 10.1002/glia.22800
58. Acosta C, Anderson HD, Anderson CM. Astrocyte dysfunction in Alzheimer disease. *J Neurosci Res.* (2017) 95:2430–47. doi: 10.1002/jnr.24075
59. Bouvier DS, Jones EV, Quesseveur G, Davoli MA, Ferreira TA, Quirion R, et al. High resolution dissection of reactive glial nets in Alzheimer's Disease. *Sci Rep.* (2016) 6:1–15. doi: 10.1038/srep24544
60. Osborn LM, Kamphuis W, Wadman WJ, Hol EM. Astrogliosis: an integral player in the pathogenesis of Alzheimer's disease. *Prog Neurobiol.* (2016) 144:121–41. doi: 10.1016/j.pneurobio.2016.01.001
61. Perez-Nievas BG, Stein TD, Tai HC, Dols-Icardo O, Scotton TC, Barroeta-Espar I, et al. Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. *Brain.* (2013) 136:2510–26. doi: 10.1093/brain/awt171
62. Wruck W, Adjaye J. Meta-analysis of human prefrontal cortex reveals activation of GFAP and decline of synaptic transmission in the aging brain. *Acta Neuropathol Commun.* (2020) 8:1–18. doi: 10.1186/s40478-020-00907-8
63. Habib N, McCabe C, Medina S, Varshavsky M, Kitsberg D, Dvir-Szternfeld R, et al. Disease-associated astrocytes in Alzheimer's disease and aging. *Nat Neurosci.* (2020) 23:701–6. doi: 10.1038/s41593-020-0624-8
64. Piacentini R, Li Puma DD, Mainardi M, Lazzarino G, Tavazzi B, Arancio O, et al. Reduced gliotransmitter release from astrocytes mediates tau-induced synaptic dysfunction in cultured hippocampal neurons. *Glia.* (2017) 65:1302–16. doi: 10.1002/glia.23163
65. Shigetomi E, Saito K, Sano F, Koizumi SC. Aberrant calcium signals in reactive astrocytes: a key process in neurological disorders. *Int J Mol Sci.* (2019) 20:996. doi: 10.3390/ijms20040996
66. Phillips EC, Croft CL, Kurbatskaya K, O'Neill MJ, Hutton ML, Hanger DP, et al. (2013). Astrocytes and neuroinflammation in Alzheimer's disease. *Biochem Soc Trans.* 42:1321–5. doi: 10.1042/BST20140155
67. Bright F, Ittner LM, Halliday GM. Neuroinflammation in frontotemporal dementia. *Nat Rev Neurol.* (2019) 15:540–55. doi: 10.1038/s41582-019-0231-z
68. Lian H, Yang L, Cole A, Sun L, Chiang ACA, Fowler SW, et al. NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's Disease. *Neuron.* (2015) 85:101–15. doi: 10.1016/j.neuron.2014.11.018
69. Leyns CEG, Holtzman DM. Glial contributions to neurodegeneration in tauopathies. *Mol Neurodegener.* (2017) 12:1–16. doi: 10.1186/s13024-017-0192-x
70. Sadick JS, Liddelow SA. Don't forget astrocytes when targeting Alzheimer's disease. *Br J Pharmacol.* (2019) 176:3585–98. doi: 10.1111/bph.14568
71. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature.* (2017) 541:481–7. doi: 10.1038/nature21029
72. Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ. The aging astrocyte transcriptome from multiple regions of the mouse brain. *Cell Rep.* (2018) 22:269–85. doi: 10.1016/j.celrep.2017.12.039
73. Garwood CJ, Ratcliffe LE, Simpson JE, Heath PR, Ince PG, Wharton SB. Review: astrocytes in Alzheimer's disease and other age-associated dementias: a supporting player with a central role. *Neuropathol Appl Neurobiol.* (2017) 43:281–98. doi: 10.1111/nan.12338
74. Vainchtein ID, Molofsky AV. Astrocytes and microglia: in sickness and in health. *Trends Neurosci.* (2020) 43:144–54. doi: 10.1016/j.tins.2020.01.003
75. Ling H, Gelpi E, Davey K, Jaunmuktane Z, Mok KY, Jabbari E, et al. Fulminant corticobasal degeneration: a distinct variant with predominant neuronal tau aggregates. *Acta Neuropathol.* (2020) 139:717–34. doi: 10.1007/s00401-019-02119-4
76. Besser LM, Crary JF, Mock C, Kukull WA. Comparison of symptomatic and asymptomatic persons with primary age-related tauopathy. *Neurology.* (2017) 89:1707–15. doi: 10.1212/WNL.0000000000004521
77. Jellinger KA. Different patterns of hippocampal tau pathology in Alzheimer's disease and PART. *Acta Neuropathol.* (2018) 136:811–3. doi: 10.1007/s00401-018-1894-z
78. Lace G, Ince PG, Brayne C, Savva GM, Matthews FE, de Silva R, et al. Mesial temporal astrocyte tau pathology in the MRC-CFAS ageing brain cohort. *Dement Geriatr Cogn Disord.* (2012) 34:15–24. doi: 10.1159/000341581
79. McKee AC, Stein TD, Nowinski CJ, Stern RA, Daneshvar DH, Alvarez VE, et al. The spectrum of disease in chronic traumatic encephalopathy. *Brain.* (2013) 136:43–64. doi: 10.1093/brain/aws307
80. Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. *Proc Natl Acad Sci USA.* (2013) 110:9535–40. doi: 10.1073/pnas.1301175110
81. He Z, McBride JD, Xu H, Changolkar L, Kim SJ, Zhang B, et al. Transmission of tauopathy strains is independent of their isoform composition. *Nat Commun.* (2020) 11:7. doi: 10.1038/s41467-019-13787-x
82. De Calignon A, Polydoro M, Suárez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron.* (2012) 73:685–97. doi: 10.1016/j.neuron.2011.11.033
83. Liu L, Drouet V, Wu JW, Witter MP, Small SA, Clelland C, et al. Trans-synaptic spread of tau pathology *in vivo*. *PLoS ONE.* (2012) 7:1–9. doi: 10.1371/journal.pone.0031302

84. Turnbull J, Powell A, Guimond S. Heparan sulfate: decoding a dynamic multifunctional cell regulator. *Trends Cell Biol.* (2001) 11:75–82. doi: 10.1016/S0962-8924(00)01897-3
85. Sarrazin S, Lamanna WC, Esko JD. Heparan Sulfate Proteoglycans. *Cold Spring Harb Perspect Biol.* (2011) 3:a004952. doi: 10.1101/cshperspect.a004952
86. Schonberger O, Horonchik L, Gabizon R, Papy-Garcia D, Barritault D, Taraboulos A. Novel heparan mimetics potently inhibit the scrapie prion protein and its endocytosis. *Biochem Biophys Res Commun.* (2003) 312:473–9. doi: 10.1016/j.bbrc.2003.10.150
87. Horonchik L, Tzaban S, Ben-Zaken O, Yedidia Y, Rouvinski A, Papy-Garcia D, et al. Heparan sulfate is a cellular receptor for purified infectious prions. *J Biol Chem.* (2005) 280:17062–7. doi: 10.1074/jbc.M500122200
88. Kanekiyo T, Zhang J, Liu Q, Liu CC, Zhang L, Bu G. Heparan sulphate proteoglycan and the low-density lipoprotein receptor-related protein 1 constitute major pathways for neuronal amyloid-β uptake. *J Neurosci.* (2011) 31:1644–51. doi: 10.1523/JNEUROSCI.5491-10.2011
89. Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, et al. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci USA.* (2013) 110:E3138–E3147. doi: 10.1073/pnas.1301440110
90. Ihse E, Yamakado H, Wijk XM, Van Lawrence R, Esko JD. Cellular internalization of alpha- synuclein aggregates by cell surface heparan sulfate depends on aggregate conformation and cell type. *Sci Rep*. (2017) 7:9008. doi: 10.1038/s41598-017-08720-5
91. Puangmalai N, Bhatt N, Montalbano M, Sengupta U, Gaikwad S, Mcallen S, et al. Internalization mechanisms of brain-derived tau oligomers from patients with Alzheimer ' s disease, progressive supranuclear palsy and dementia with Lewy bodies. *Cell Death Dis.* (2020) 11:314. doi: 10.1038/s41419-020-2503-3
92. Stopschinski BE, Holmes BB, Miller GM, Manon VA, Vaquer-Alicea J, Prueitt WL, et al. Specific glycosaminoglycan chain length and sulfation patterns are required for cell uptake of tau versus -synuclein and -amyloid aggregates. *J Biol Chem.* (2018) 293:10826–40. doi: 10.1074/jbc.RA117.000378
93. Rauch JN, Chen JJ, Sorum AW, Miller GM, Sharf T, See SK, et al. Tau Internalization is Regulated by 6-O Sulfation on Heparan Sulfate Proteoglycans (HSPGs). *Sci Rep.* (2018) 8:1–10. doi: 10.1038/s41598-018-24904-z
94. Martini-Stoica H, Cole AL, Swartzlander DB, Chen F, Wan YW, Bajaj L, et al. TFEB enhances astroglial uptake of extracellular tau species and reduces tau spreading. *J Exp Med.* (2018) 215:2355–77. doi: 10.1084/jem.20172158
95. Perea JR, López E, Díez-Ballesteros JC, Ávila J, Hernández F, Bolós M. Extracellular monomeric tau is internalized by astrocytes. *Front Neurosci.* (2019) 13:442. doi: 10.3389/fnins.2019.00442
96. Tselnicker IF, Boisvert MM, Allen NJ. The role of neuronal versus astrocyte-derived heparan sulfate proteoglycans in brain development and injury. *Biochem Soc Trans.* (2014) 42:1263–9. doi: 10.1042/BST 20140166
97. Kanekiyo T, Bu G. The low-density lipoprotein receptor-related protein 1 and amyloid-β clearance in Alzheimer's disease. *Front Aging Neurosci.* (2014) 6:93. doi: 10.3389/fnagi.2014.00093
98. Liu C, Hu J, Zhao N, Wang J, Wang N, Cirrito JR, et al. Astrocytic LRP1 mediates brain Aβ clearance and impacts amyloid deposition. *J Neurosci.* (2017) 37:4023–31. doi: 10.1523/JNEUROSCI.3442-16.2017
99. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, et al. LRP1 is a master regulator of tau uptake and spread. *Nature*. (2020) 1:1–5. doi: 10.1038/s41586-020-2156-5
100. Benarroch EE. Aquaporin-4, homeostasis, and neurologic disease. *Neurology*. (2007) 69:2266–2268. doi: 10.1212/01.wnl.0000286385.59836.e2
101. Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, Yang L, et al. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *J Neurosci.* (2014) 34:16180–93. doi: 10.1523/JNEUROSCI.3020-14.2014
102. Ferrer I, García MA, González IL, Lucena DD, Villalonga AR, Tech MC, et al. Aging-related tau astrogliopathy (ARTAG): not only tau phosphorylation in astrocytes. *Brain Pathol*. (2018) 28:965–85. doi: 10.1111/bpa.12593
103. Han Z, Wax MB, Patil RV. Regulation of aquaporin-4 water channels by phorbol ester-dependent protein phosphorylation. *J Biol Chem.* (1998) 273:6001–4. doi: 10.1074/jbc.273.11.6001
104. Kitchen P, Day RE, Taylor LHJ, Salman MM, Bill RM, Conner MT, et al. Identification and molecular mechanisms of the rapid tonicity-induced relocalization of the aquaporin 4 channel. *J Biol Chem.* (2015) 290:16873–81. doi: 10.1074/jbc.M115.646034
105. Sidoryk-Wegrzynowicz M, Gerber YN, Ries M, Sastre M, Tolkovsky AM, Spillantini MG. Astrocytes in mouse models of tauopathies acquire early deficits and lose neurosupportive functions. *Acta Neuropathol Commun.* (2017) 5:89. doi: 10.1186/s40478-017-0478-9
106. Hasel P, Dando O, Jiwaji Z, Baxter P, Todd AC, Heron S, et al. Neurons and neuronal activity control gene expression in astrocytes to regulate their development and metabolism. *Nat Commun*. (2017)8:15132. doi: 10.1038/ncomms15132
107. Hallmann AL, Araúzo-Bravo MJ, Mavrommatis L, Ehrlich M, Röpke A, Brockhaus J, et al. Astrocyte pathology in a human neural stem cell model of frontotemporal dementia caused by mutant TAU protein. *Sci Rep.* (2017) 7:1–10. doi: 10.1038/srep42991
108. Veerhuis R, Nielsen HM, Tenner AJ. Complement in the brain. *Mol Immunol.* (2011) 48:1592–603. doi: 10.1016/j.molimm.2011.04.003
109. Lian H, Litvinchuk A, Chiang ACA, Aithmitti N, Jankowsky JL, Zheng H. Astrocyte-microglia cross talk through complement activation modulates amyloid pathology in mouse models of alzheimer's disease. *J Neurosci.* (2016) 36:577–89. doi: 10.1523/JNEUROSCI.2117-15.2016
110. Litvinchuk A, Wan YW, Swartzlander DB, Chen F, Cole A, Propson NE, et al. Complement C3aR inactivation attenuates tau pathology and reverses an immune network deregulated in tauopathy models and Alzheimer's disease. *Neuron*. (2018) 100:1337–53.e5. doi: 10.1016/j.neuron.2018.10.031
111. Wu T, Dejanovic B, Gandham VD, Carano RAD, Sheng M, Hanson JE, et al. Complement C3 is activated in human AD brain and is required for neurodegeneration in mouse models of amyloidosis and tauopathy article. *Cell Rep.* (2019) 28:2111–23.e6. doi: 10.1016/j.celrep.2019.07.060
112. Chung WS, Clarke LE, Wang GX, Stafford BK, Sher A, Chakraborty C, et al. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature*. (2013) 504:394–400. doi: 10.1038/nature12776
113. Henstridge CM, Tzioras M, Paolicelli RC. Glial contribution to excitatory and inhibitory synapse loss in neurodegeneration. *Front Cell Neurosci.* (2019) 13:63. doi: 10.3389/fncel.2019.00063
114. Noble W, Spires-Jones TL. Sleep well to slow Alzheimer's progression? *Science*. (2019) 363:813–4. doi: 10.1126/science.aaw5583
115. Holth JK, Fritschi SK, Wang C, Pedersen NP, Cirrito JR, Mahan TE, et al. The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. *Science*. (2019) 363:880–4. doi: 10.1126/science.aav2546
116. Bellesi M, de Vivo L, Chini M, Gilli F, Tononi G, Cirelli C. Sleep loss promotes astrocytic phagocytosis and microglial activation in mouse cerebral cortex. *J Neurosci*. (2017) 37:5263–73. doi: 10.1523/JNEUROSCI.3981-16.2017

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 25 September 2020
doi: 10.3389/fneur.2020.01056

Nuclear Transport Deficits in Tau-Related Neurodegenerative Diseases

*Lisa Diez and Susanne Wegmann**

German Center for Neurodegenerative Diseases, Berlin, Germany

Tau is a cytosolic microtubule binding protein that is highly abundant in the axons of the central nervous system. However, alternative functions of tau also in other cellular compartments are suggested, for example, in the nucleus, where interactions of tau with specific nuclear entities such as DNA, the nucleolus, and the nuclear envelope have been reported. We would like to review the current knowledge about tau–nucleus interactions and lay out possible neurotoxic mechanisms that are based on the (pathological) interactions of tau with the nucleus.

Keywords: tau protein, Alzheimer's disease, neurodegenerative diseases, nuclear pore complex, nucleocytoplasmic transport

OPEN ACCESS

Edited by:
Sonia Do Carmo,
McGill University, Canada

Reviewed by:
Senthilvelrajan Kaniyappan,
University of Bonn, Germany
Jesus Avila,
Severo Ochoa Molecular Biology Center (CSIC-UAM), Spain
Paolo Paganetti,
Ente Ospedaliero Cantonale (EOC), Switzerland

****Correspondence:***
Susanne Wegmann
susanne.wegmann@dzne.de

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *12 July 2020*
Accepted: *11 August 2020*
Published: *25 September 2020*

Citation:
Diez L and Wegmann S (2020) Nuclear Transport Deficits in Tau-Related Neurodegenerative Diseases. Front. Neurol. 11:1056. doi: 10.3389/fneur.2020.01056

INTRODUCTION

Tau is a predominantly neuronal protein and, as a representative of the microtubule-associated protein family (1), contributes to the stabilization of microtubules (MT) and the modulation of their dynamics (2, 3). In neurodegenerative diseases such as Alzheimer's disease (AD), frontotemporal dementia with Parkinsonism on chromosome 17, Pick's disease, and others, intraneuronal aggregates of hyperphosphorylated tau are a hallmark pathological feature (4, 5), and their appearance correlates well with neuronal loss in these diseases (6–8). The filamentous tau aggregates found in human brain are amyloid-like and have a high β-sheet content; however, the architecture of their fibril core differs to some degree between aggregates from different tauopathies (9–12). *In vitro* aggregation of tau into filamentous aggregates can efficiently be induced by polyanionic co-factors such as heparin (13, 14), RNA (15), and arachidonic acid (16). However, small soluble oligomeric tau species also appear to contribute to synaptic dysfunction and cell death in tauopathies (17, 18) and are considered to mediate neurotoxicity before neurofibrillary tangle (NFT) formation (19, 20).

Monomeric tau is a highly soluble, intrinsically disordered protein that comprises four different major domains: the acidic N-terminal half (projection domain) projects from the MT surface and the proline-rich domain, which harbors a SH3-protein binding site (21, 22); the function(s) of these parts of the tau protein are rather uncertain, and they thus may play a role in alternative tau functions. The basic repeat domain containing four ∼30-amino-acid-long pseudorepeats is responsible for MT binding (23–26) and aggregation of tau (25, 27). The role of the shorter C-terminal end is unknown. In the human central nervous system (CNS), tau exists in six isoforms, which carry three or four pseudo-repeats in the repeat domain (3R and 4R isoforms) and zero, one, or two repeats in the N-terminal half (0N, 1N, or 2N isoforms) and are generated by the alternative splicing of exon 2, exon 3, and exon 10 in a 6-kb mRNA transcript.

The amino acid sequence of tau harbors 85 putative phosphorylation sites (28, 29) and various sites for other post-translational modifications (PTMs) such as acetylation, methylation, and

glycosylation (30, 31), which enable a complex regulation of tau's binding to MTs and its other functions (32). Phosphorylation is by far the most studied PTM of tau, also for nuclear tau.

Tau is highly abundant in axons of the CNS (33), but under stress and in pathological conditions, it can also be found in the soma, the dendrites, and the nucleus (34). This unusual cellular distribution of tau enables condition- and subcellular environment-dependent interactions (35, 36), for example, with the nucleus.

The first indication of nuclear tau, in the form of short paired helical filaments, came from transmission electron microscopy of AD frontal lobe sections by Metuzals et al. (37), and until today neither a physiological nor a pathological role of nuclear tau is clearly established. Interactions of tau with the outer neuronal envelope (NE) were recently suggested to induce deficits in RNA and protein transport in and out of the nucleus (38, 39). Regulated nucleocytoplasmic transport (NCT) of cellular biomolecules—such as transcription factors, mRNA and ribosomal RNA, and nuclear and cytosolic proteins—is essential for major principles of cell survival and function, for example, signal transduction, stress response, and proteostasis (40–42). In the recent years, defective neuronal NCT has been described in different neurodegenerative disorders (NDDs) like amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) (43, 44), Huntington's disease (HD) (45, 46), and AD (38).

In this review, we summarize the interactions of tau with the nucleus and discuss their potential role in pathology. After introducing known and conceivable interactions of tau with the nucleus—both of intranuclear as well as extranuclear tau, either direct or indirect—we will summarize the findings of NCT impairments in other NDDs and normal aging, aiming to gain an overall mechanistic insight for NCT disruptions as a potential culprit and therapeutic target in neurodegenerative diseases.

HOW DOES TAU INTERACT WITH THE NUCLEUS?—KNOWN AND CONCEIVABLE POINTS OF INTERACTION

Nuclear Tau Isoforms and Post-translational Modifications

In the last three decades, several interactions of tau with the nucleus were reported, and potential nuclear functions of tau were suggested. Early indications of tau in the nucleus came from Binder and colleagues who showed, by immunohistology, that tau can be found in neuronal nuclei in the human brain—both in healthy controls and in AD patients (47). Following this observation, a number of studies showed tau in the nucleus of neuronal cells [e.g., human neuroblastoma (48–50) and rat cells (51)] in primary mouse neurons (52), in the mouse brain (53–55), and also in non-neuronal cell lines (e.g., fibroblasts and lymphocytes) (56, 57). In the nucleus, tau seems to be predominantly localized to the nucleolus (49, 56, 58).

In adult mice, which express 4R but not 3R tau, isoform-specific tau antibodies revealed that 1N4R tau is enriched in the nuclear-enriched fraction of brain lysates (54). It remains unclear how 1N4R tau gets into the nucleus because none of the CNS tau isoforms carries a (known) nuclear localization signal (NLS) that would enable its transport through nuclear pores into the nucleoplasm. For now we can only speculate about scenarios that would explain the occurrence of tau protein in the nucleus: specific PTMs, e.g., phosphorylation or SUMO-lation, alter the ability of transcription factors to interact with nuclear transport factors and enable their nuclear import (59, 60); a similar PTM-based mechanism could facilitate tau protein transport from the cytosol through the nuclear pore into the nucleoplasm. Another possibility could be nuclear targeting of tau transcripts, which could direct tau mRNA into the nucleus, where local transcription could produce tau protein. In fact, it has been suggested that the majority of nuclear tau may be produced by a less abundant 2-kb transcript that contains the entire tau coding region (61). Transcription of both the 2- and 6-kb tau mRNA starts at the same unique site at the start of exon 1; however, the two transcripts utilize two alternate polyadenylation sites downstream of exon 14 (62).

Both phosphorylated tau and tau dephosphorylated at certain residues have been reported in the nucleus (47, 49, 63). By immunofluorescence imaging and western blot using an antibody specific for the absence of phosphate groups at residues S195/198/199/202 (Tau-1 antibody), the majority of nuclear tau appears to be dephosphorylated, at least at these residues (48, 61). A pronounced accumulation of Tau-1 positive tau in the nucleus was observed upon acute oxidative and heat stress both *in vitro* and *in vivo* (52). Tau colocalizing with the nucleolus also seems to be mostly non-phosphorylated at residues S195/198/199/202 (Tau-1 positive) (56). However, in models of tau-induced neurodegeneration, phosphorylated nuclear tau appears to be associated with neurodegeneration (28, 64–66). In tau overexpressing SH-SY5Y cells—a model manifold used to study nuclear tau—phospho-site-specific tau antibodies revealed nuclear tau phosphorylated at specific sites such as S205, T181, T212, S404, and others (29, 67).

On a general note, the detection of nuclear tau in the mentioned studies relies mainly on the use of antibodies—for example, Tau-1 (non-P S195/198/199/202/) and AT8 (pS202/205)—that may show some unknown cross-reaction with other nuclear proteins. Therefore, more complementary proteomics studies, as that's performed by Ulrich et al. (29), will be needed to clarify which tau isoforms and PTMs occur in the nucleus. Furthermore, the biochemical detection of nuclear tau is usually based on cellular fractionation protocols, in which nuclear-enriched fractions are analyzed for their tau content; from these data, it remains unclear whether tau is present in the nucleoplasm or is associated with the inside or the outside of the nuclear envelope. As of now, it also remains unclear how nuclear tau (PTMs and isoforms) differs between cell types, differentiation state, and host species and which nuclear tau species may be relevant for neuronal function in the human brain.

Intranuclear Tau: Interactions of Nuclear Tau With Intranuclear Components

Tau–DNA Interactions

Different microtubule-associated proteins, such as tau and MAP2, were shown to interact with DNA (68, 69). Tau–DNA

interactions appear to be, to some degree, nucleic acid sequence-specific for single-stranded DNA, with some preference for GC-rich regions, whereas some studies identified no or little sequence specificity for tau binding to double-stranded DNA (70); preferential binding of tau to AG-rich sequences was also reported (55). For both ssDNA and dsDNA, tau binding was reported to be facilitated by the minor DNA groove *via* electrostatic interactions (2, 71, 72), similar to the DNA binding mechanism of histones and other chromatin architectural proteins (73–75), which may suggest a potential chaperone-like function of tau for DNA folding (28, 75).

By nuclear magnetic resonance (NMR) spectroscopy, the tau binding motif for DNA was assigned to the C-terminal half of the proline-rich region and repeat 2 in the repeat domain of human tau (76). Both of these regions in tau are commonly phosphorylated in physiological and pathological conditions (77), which indicates a potential role of phosphorylation (or other PTMs in these regions) for the regulation of tau binding to DNA. Interestingly, the tau-interacting regions in genomic DNA of mouse primary neurons were found to be distributed across different chromosomes and between genic and intergenic regions as shown by chromatin immunoprecipitation with the anti-tau antibody Tau-1 (55). Heat stress, which induces tau phosphorylation at certain epitopes and de-phosphorylation at others, induced an increase in nuclear tau and a global dissociation and redistribution of tau on chromatin (55). Interestingly, hypothermia also induces tau phosphorylation by GSK3β and CDK5 (78) but it is—to our knowledge—not known whether it changes the abundance of tau in the nucleus.

Heat-stress-induced nuclear tau is unphosphorylated at sites T212, T231, T235, S262, S356, S396, and S404 (52), which indicates that the binding of tau to negatively charged DNA could be regulated by phosphorylation, similar to the MT binding of tau. This idea is supported by *in vitro* NMR and surface plasmon resonance (SPR) measurements that show a pronounced reduction of DNA binding ability of phosphorylated compared to unphosphorylated recombinant protein (75, 76). In addition to electrostatic interactions, hydrophobic interactions were found to further stabilize tau–DNA interactions (75, 76).

Suggested Functions of Tau–DNA Interactions

The physiological and the pathological roles of tau binding to DNA are still unclear, and different potential functions have been suggested. For example, the binding of tau to DNA seems to induce a bending and associated conformational changes in the DNA backbone, similar to what is commonly observed for proteins that physically protect DNA from damage (28). Accordingly, primary mouse neurons that are lacking tau show a higher rate of DNA damage in Comet assays, which detects *inter alia* single- and double-stranded DNA breaks (79). This suggests that tau can protect DNA against oxidative and hyperthermic stress, which indicates that tau may function as a protector of genomic integrity under stress conditions (52). In hypothermic mice, which show a transient increase in reactive oxygen species in the brain, the presence of tau also protected against heat shock, suggesting a role of tau in modulating double-strand break DNA repair (53). Recently, tau's involvement in DNA damage response was further defined by Sola et al., who used tau-knockout human neuroblastoma cells (tau-KO SH-SY5Y) to shown that tau-deficient cells are less sensitive to DNA damage-induced apoptosis mediated by p53 modulation (80). A protective role of tau was also suggested on the chromatin level: using immunostainings of fibroblasts from FTD patients carrying the tau P301L mutation, Rossi et al. found chromosome aberrations as well as chromatin and spindle abnormalities and concluded that tau could promote chromosome stability (67, 81). Changes in chromatin and in gene expression in response to tau were also found in other studies (82, 83). For example, the clustering of histone H3 trimethylated at lysine 9 (H3K9me3) and heterochromatin protein 1α (HP1α), markers of heterochromatic DNA is disrupted in tau-deficient mice, indicating that tau may be involved in the epigenetic regulation of gene expression (84). Frost et al. provided a link between mutant tau expression, oxidative stress, and heterochromatin relaxation: upon human mutant tau P301L expression in *Drosophila*, genes that were normally silenced by heterochromatin (such as *Ago3*, the *Drosophila* homolog of human *PIWIL1*) had an increased expression, and neurons showed cell cycle reactivation, a condition that can drive the apoptosis of post-mitotic neuronal cells (82).

A structure-building role of tau in the nucleus was implicated by Sjöberg et al., who reported the binding of tau to pericentromeric DNA in human fibroblasts, lymphoblasts, and HeLa cells and suggested the involvement of tau in nucleolar organization (85). With the nucleolus being the center of ribosomal DNA (rDNA) metabolism and ribosomal complex formation, tau could thus control the rate of ribosome assembly and thereby influence RNA translation (86) or "heterochromatize" (=silence) rRNA genes as observed for other heterochromatin-associated proteins (85). SH-SY5Y cells also showed that tau associates with nucleolar TIP5, a key factor in heterochromatin stability and rDNA transcriptional repression, suggesting a role of tau in rDNA silencing (50).

In summary, intranuclear tau may directly protect DNA integrity, participate in DNA repair mechanisms, be involved in gene regulation, or help to control ribosomal gene translation and assembly.

Intranuclear Tau in Pathology

It has been shown that phosphorylation reduces the nuclear localization of tau (63, 87) and its ability to bind and protect DNA (29, 75, 76, 88), suggesting a potentially harmful loss of nuclear function for hyperphosphorylated tau. The overall absence of tau—and therefore also absence of nuclear tau—in tau-knockout mice has been shown to alter the chromatin arrangement and render neurons more vulnerable to heat stress (53). An increase in cytosolic tau phosphorylation may also be upstream of oxidative stress-induced DNA breakage (63, 82, 89). In any case, nuclear tau alteration capable of disrupting the chromatin organization or inducing DNA damage would dysregulate neuronal gene expression (82), which ultimately could cause neuronal death. However, it is yet unclear to what extent and how intranuclear tau contributes to neurotoxicity and if disease-associated tau mutations contribute to nuclear alterations.

Extranuclear Tau: Interaction of Cytoplasmic Tau With the Nuclear Envelope

In NDDs like AD and tauopathies, a substantial amount of tau is found in the somatodendritic compartment where it can interact with the outside of the nucleus, the outer NE. The transport of RNA and proteins across the NE is regulated by nuclear pores and is essential for many cellular functions. In the following, we introduce the architecture and the function of nuclear pores, and then we will review what is known about interactions between cytosolic tau and the nucleus, which can be of either direct or indirect nature.

Nuclear Pore Complexes and Nucleocytoplasmic Transport

The nucleus is enclosed by the NE, a double lipid bilayer that separates the nuclear interior from the cytoplasm. The outer nuclear membrane is continuously connected to the endoplasmic reticulum membrane system. The inner nuclear membrane is lined with the nuclear lamina, a fibrous meshwork of lamin proteins that provides structural support to the NE (90) and also serves as a scaffold for chromatin attachment (91). The linker of nucleoskeleton and cytoskeleton (LINC) protein complex contributes to nuclear stability and positioning by physically linking the lamin-rich nucleoskeleton to the cytosolic cytoskeleton that comprises *inter alia* actin microfilaments or microtubules (92, 93). To allow for controlled macromolecular trafficking of proteins and RNA between the nuclear interior and the surrounding cytoplasm—a basic process essential for cellular protein homeostasis—the NE is homogenously "perforated" by nuclear pores, which are built by nuclear pore complexes (NPCs) (94, 95). NPCs are among the largest cellular macromolecular assemblies: vertebrate NPCs, for example, have a molecular weight of ∼120 MDa (96). Multiple copies of around 30 different proteins, called nucleoporins (Nups), constitute the building blocks of the NPC, yielding a total of ∼500–1,000 proteins (97). The overall structure of the NPC is conserved across different cell types; however, studies indicate that cells may express unique combinations of NUPs to generate NPCs with specialized functions (98). The center of the nuclear pore is built by a complex cylindrical structure that displays a rotational symmetry of eight subunits surrounding a central tube, through which the nucleoplasm is connected to the cytoplasm and where the exchange of macromolecules between these two cellular compartments takes place (99). From the central pore, largely unstructured, filamentous proteins extend into both the cytoplasmic and the nuclear spaces. On the nuclear side of the pore, eight protein filaments form a basketlike structure by joining into a distal ring (96).

The different Nups are classified regarding their function and location in the NPC (97, 99–101): (i) scaffold or coat Nups determine the structure of the nuclear and the cytoplasmic rings (e.g., Sec13, Seh1, Nup96, Nup75, Nup107, Nup160, Nup133, Nup37, Nup43, and ELYS), (ii) transmembrane Nups or pore membrane proteins (POMs) hold the NPC in position through transmembrane domains that interact with the NE (NDC1, POM210, and POM121), (iii) central channel Nups form the pore of the NPC (Nup205, Nup188, Nup93, Nup155, Nup53, Nup54, Nup58, Nup62, and Nup98), (iv) cytoplasmic ring/filament Nups are projecting into the cytoplasm from the NPC (Rae1, Nu42, Nup88, Nup214, DDX19, Gle1, and RanBP2/Nup358), and (v) nuclear ring/basket Nups are involved in the organization of the NPC cargo transport machinery by facilitating the recognition and the binding of nuclear import and export factors on the nuclear side of the NPC (Nup153, Nup50, Tpr). Of special importance to the NCT of biomolecules through the nuclear pore are the so called FG-Nups, which are central-channel Nups with intrinsically disordered domains rich in phenylalanine-glycine repeats (FG) (102–104). FG-Nups are attached to the nuclear scaffold *via* coiled-coil protein motifs in their non-FG domains, whereby their long FG-domain containing N-terminal parts extends as unstructured polypeptides into the central channel; here they create a hydrogel-like polymer brush that acts as a selectively permeable diffusion barrier for the transport of proteins and other biomolecules (95, 104–106). In their free state *in vitro*, FG-rich Nups spontaneously undergo liquid–liquid phase separation (LLPS) and form hydrogel-like droplets (107). Small nonpolar molecules and ions <40 kDa can passively co-partition into the Nup hydrogel phase and diffuse through the nuclear pore, whereas polar or bigger macromolecules have to be actively transported through the pore in an energy-dependent manner (99, 105, 108).

Intriguingly, multiple proteins that aggregate and form intracellular inclusions in NDDs with detectable NCT impairment are able to also undergo LLPS, for example, the RNA binding proteins FUS (109) and TDP-43 (44, 110), polyQ–Htt (111, 112), and also tau (113–115). One may thus suspect a (mis) functional connection between the liquid protein phase behavior of Nups and these proteinopathic hallmark proteins—e.g., due to co-phase separation, co-aggregation, or NTF loss or gain of function—which in neurodegenerative diseases could then result in NPC dysfunction with neurotoxic consequences.

Active NCT requires interactions between soluble nuclear transport receptors (NTRs) and Nups in the central channel of the NPC. The most common family of NTRs are the karyopherins, also called importins or exportins depending on their transport function into or out of the nucleus (96, 116). Cargo molecules that are supposed to be shuttled into the nucleus or exported from the nucleus are equipped with specific amino acid sequences: a nuclear localization signal (NLS) mediates the import, and a nuclear export signal (NES) mediates the export from the nucleus. Notably, proteins that need to shuttle between the nucleoplasm and the cytoplasm, such as the RNA-binding proteins TDP-43 (117) and FUS (118, 119), can carry both a NLS and a NES. The NCT of NTR-bound cargo molecules further depends on the nucleocytoplasmic gradient of RanGTP and RanGDP, with a high RanGTP concentration inside the nucleus and high cytoplasmic levels of RanGDP (95, 120, 121). If the RanGTP or the RanGDP gradient is destroyed, NCT is not possible (122). In an import scenario, NLS-cargo is bound to cytoplasmic importin-β–either directly or indirectly *via* the adaptor karyopherin importin-α–and is then shuttled through the NPC *via* hydrophobic interactions with FG-Nups (123).

In the nucleus, the NLS-cargo is released when the importin transport receptor interacts with intranuclear RanGTP (124). In an export scenario, the exported NES-cargo is released into the cytoplasm upon GTP hydrolysis of RanGTP by RanGAP1, a GTPase-activating protein located on the cytoplasmic filaments of the NPC (45).

Different NPC models try to explain the molecular mechanism of nucleocytoplasmic transport. The "virtual gating/polymer brush" model suggests that non-interacting FG-Nups extend into the pore and form a polymer brush that functions as an entropic diffusion barrier on both sides of the NPC. While large macromolecules are generally hampered from NPC passage, the binding of transport receptors to the FG-repeats in central pore Nups can facilitate the translocation of their entrained cargo (104). The "selective phase/hydrogel" model anticipates the formation of a hydrogel-like molecular sieve that is formed *via* hydrophobic interactions among FG-repeats (104, 122). While smaller molecules can easily diffuse through the FG-Nup hydrogel meshwork, larger biomolecules cannot penetrate the hydrogel and are thus restrained. NTR–cargo complexes can bind to and dissolve into the FG-Nup meshwork and therefore can be translocated (125).

The regulated bidirectional transport of proteins and RNA in and out of the nucleus is important for many key cellular processes, for example, chromatin assembly, DNA metabolism, RNA synthesis and processing, signal transduction, and ribosome biogenesis. It is therefore obvious that any deregulation and impairment of the NCT can have detrimental consequences for the cell, leading to toxicity and cell death at worst (95). For instance, loss of the nuclear–cytoplasmic Ran-gradient, maintained by RanGAP1, can lead to cell death within minutes (45, 126).

Interestingly, Nups have also been shown to be involved in NCT-independent functions such as microtubule attachment to kinetochores, regulation of genome organization and gene expression, cell differentiation and development, RNA processing, and quality control (45, 96, 100). FG-Nups like Nup62, Nup153, and Nup98 are of special importance for transcription and chromatin organization (127, 128). These findings suggest that even disturbances at the level of the NPC building blocks can have vast cellular consequences.

Tau-Induced Irregularities of the Nuclear Membrane

In AD, ALS, FTD, and HD, pronounced irregularities and invaginations in the normally smooth neuronal NE have been identified by immunohistology and electron microscopy of post-mortem patient brain tissue (38, 39, 129–131).

In the case of tau, nuclear membrane abnormalities and clumping of nuclear pores have been observed in the nuclei of both NFT-neurons and neighboring pre-tangle (37, 132, 133). Alterations in the nuclear architecture were also observed in SH-SY5Y cells overexpressing human tau in the cytosol (134), which induced extensive lobulations in the NE and re-arrangements of the filamentous lamin nucleoskeleton. However, neither degradation of nuclear lamins nor cell death was observed in these cells. Tau-induced lamin dysfunctions were also shown *in vivo* in a *Drosophila* tau FTD-model, where they seemed to occur downstream of aberrant tau phosphorylation and led to neurotoxicity (135). Pathological tau was found to overstabilize F-actin, which led to a disruption of the LINC complex organization and thereby reduction and disorganization of lamin in neurons. As a consequence of the lamin dysfunction, relaxation of heterochromatic DNA was accompanied by subsequent DNA damage, aberrant cell cycle activation, and apoptosis (135). More recently, Frost and colleagues were able to show that the observed FTD-mutant tau-induced NE invagination can also cause a toxic accumulation of mRNA (39). Interestingly, a defective nuclear lamina and NCT impairment—similar to the NE distortions observed in neurons with tau accumulation (38, 82, 135)—occurs also in the premature aging disease Hutchinson-Gilford progeria syndrome (136), suggesting that NE distortions could be a common phenotype in neurodegenerative protein aggregation diseases and aging.

Two more indirect tau–nucleus interactions were recently presented: Autosomal-dominant FTD-tau mutations were shown to cause microtubule-mediated deformation of the nuclear membrane in human induced pluripotent stem cell (iPSC)-derived neurons (131), which resulted in defective NCT, and rod-like cytoplasmic tau aggregates at the nuclear envelope were shown to distort the nuclear membrane in striatal neurons in HD and in pre-tangle neurons in AD, and in mice expressing FTD-mutant tauP310S (37, 137, 138).

Direct Interactions of Tau With the Nuclear Envelope

Evidence for a direct interaction of cytoplasmic tau with NPCs was recently provided by Eftekharzadeh et al. (38). Hippocampal neurons in post-mortem AD brain had a distorted NE and abnormal irregular NPC distribution, and certain FG-Nups accumulated in the cytoplasm of NFT-neurons (**Figure 1**). In tangle-free neurons, phospho-tau accumulated at the nuclear membrane. Using SPR of recombinant proteins and co-immunoprecipitation of tau and Nup98 from human AD brain tissue, a direct interaction of tau with the FG-Nups Nup98 and Nup62 was shown. Interestingly, the C-terminal half of Nup98—one of the most abundant Nups with the highest FG content (107)—was able to trigger tau aggregation *in vitro*, suggesting a possible contribution of soluble cytoplasmic Nup98 to tau tangle formation (38). The C-terminal part of Nup98, which is usually buried in the NPC scaffold, is highly negatively charged and may therefore efficiently induce tau aggregation, similar to other polyanionic macromolecules like heparin and RNA (13–15, 139). In the same study, it was also shown that cytosolic tau can induce neuronal NCT impairments (38). In tau-overexpressing transgenic mice, primary mouse neurons, and human AD brain tissue, the presence of phosphorylated tau in the neuronal soma led to a depletion of nuclear Ran and an impairment of both nucleocytoplasmic import and export of proteins. Notably, NCT and Nup98 defects could be rescued in FTD-tau transgenic mice by reducing soluble transgenic tau, suggesting a new pathogenic mechanism, in which the somatodendritic accumulation of tau enables abnormal interactions of tau with components of the NPC and leads to NCT impairment, which is further accompanied by cytoplasmic aggregation of nucleoporins.

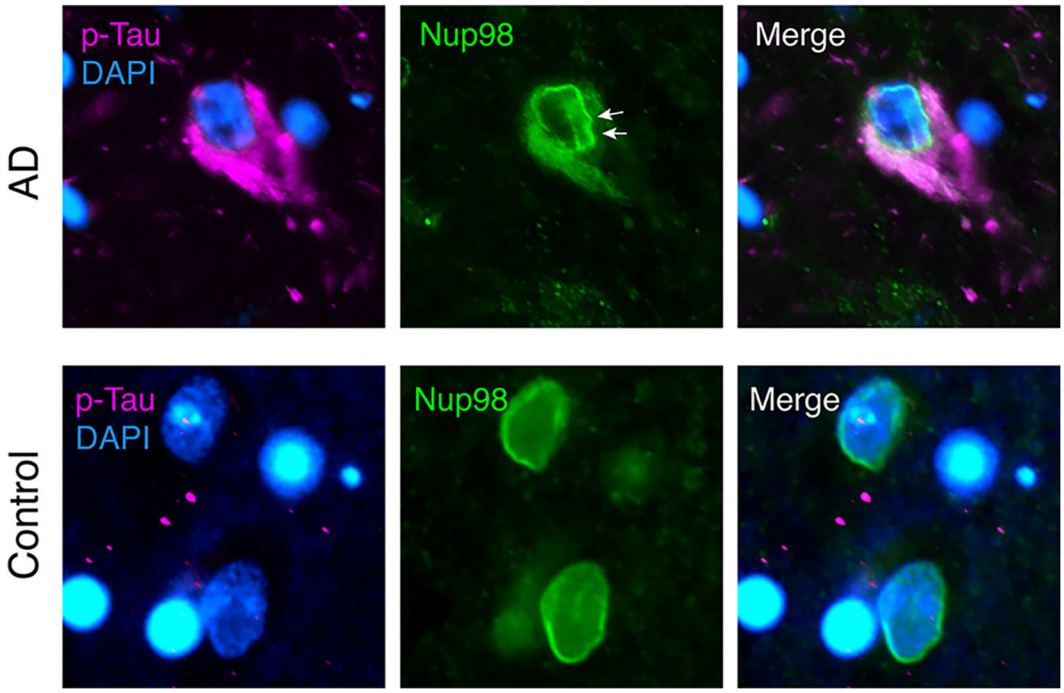


FIGURE 1 | Nuclear envelope distortion and cytoplasmic mislocalization of Nup98 in neurofibrillary tangles (NFTs) of Alzheimer's disease (AD) brain. Human AD (Braak V) and age-matched control brain sections were immunolabeled for phospho-tau (magenta; p-tau mix of anti-phospho-tau antibodies, pS199, pT205, pS262, pT231, and pS409), Nup98 (green), and Dapi (blue). In AD cortex, NFTs filled with phospho-tau show a crinkled/distorted nuclear envelope (white arrows) and Nup98 accumulation in the cytosol. In control nuclei, Nup98 is localized evenly to the nuclear membrane.

Indirect Effects of Tau on the NCT

Despite the direct interaction of tau with Nups, indirect effects of tau on the NCT have also been reported. For example, abnormal cytoplasmic accumulation of NTF2, a RanGDP transporter and key NCT factor, indicated an impaired NCT in the hippocampal neurons of AD brains (133). In another study, importin-α1 localized to "Hirano bodies"—inclusions containing actin and actin-associated proteins—in AD hippocampal neurons, whereas control brains showed a diffuse cytoplasmic localization of importin-α1 (133, 140). Notably, importin-α1 did not co-localize with NFTs or amyloid-β plaques in AD brains and not with Lewy bodies in PD brains.

The disease-associated depletion of Nups from NPCs, as suggested by the cytoplasmic sequestration of Nup98 into NFTs in AD brain (38), could deplete Nup98 from NPCs and lead to NPC disassembly and loss of function. Unspecific clogging of the nuclear pore by tau aggregates could comprise another tau–NPC interaction, leading to NCT impairment.

Another concept for a potential indirect interaction of tau with Nups is based on a study by Toda et al. (141): Nup153 associates with the transcription factor Sox2 to regulate the neural fate of neural progenitor cells (141), whereby Nup153 binding to both the 5′ and the 3′ ends of genes enables a bimodal gene regulation. Other Nups also play a role in transcription regulation (128, 142, 143). Even though the interaction of tau with Nup153 has not been investigated, disease-associated binding of tau to Nups that play a role for transcription could induce tau-mediated gene alterations in neurodegenerative diseases.

NCT IMPAIRMENT AS A GENERAL CONCEPT IN NEURODEGENERATION?

In recent years, the disruption of neuronal NCT has been observed in different neurodegenerative protein aggregation diseases such as HD, ALS, and FTD (97, 144, 145) and recently also as an effect of tau in AD (38). It has been suggested that NCT failure is caused by pathological perinuclear protein aggregation in general [e.g., artificial β-sheets, polyQ–Htt fragments, cytoplasmic fragment of TDP-43 (146)]; however, the molecular and the cellular mechanisms as well as the downstream effects of disease-associated NCT impairment need to be further investigated in order to identify similarities and differences across diseases. Observations associated with NCT failure that are common in different protein aggregation diseases seem to be, for example, (i) the mislocalization of nuclear transport receptors and nucleoporins (45, 147–149), (ii) the mislocalization and the aggregation of RNA-binding proteins (149), and (iii) the loss of chaperone activity exhibited by certain nuclear import receptors (149, 150). In the following, we provide an overview of nucleocytoplasmic trafficking defects in neurodegenerative diseases other than AD.

Nup Mutations Linked to NCT Impairment

Only few neurodegenerative-disease-relevant mutations have been identified within proteins of the NPC/NCT machinery. A missense mutation (Q391P) in the FG-Nup Nup62 was found in autosomal recessive infantile bilateral striatal necrosis, a fatal neurological disorder characterized by bilateral symmetric degeneration of the basal ganglia, the caudate nucleus, and the putamen (151). In ALS, two mutations in the human cytoplasmic ring Nup Gle1 were shown to cause the depletion of Gle1 from the NPC; Gle1 is essential for nuclear mRNA export (152).

NCT in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

Most information about nuclear transport failure in neurodegeneration comes from ALS and FTD research. Accordingly, different recent review articles already cover this topic in detail (97, 122, 144, 145, 153, 154), and we therefore give only a short summary of what is known about NCT impairments in the etiology of ALS/FTD.

ALS and FTD share some clinical, neuropathological, and genetic features and therefore are classified in a common disease spectrum with likely similar neurodegenerative pathways (155). ALS is characterized by a progressive degeneration of motor neurons, which leads to increasing muscle weakness and loss of mobility. FTD, the second most frequent form of dementia, is characterized by frontal and temporal lobe degeneration, which clinically leads to social and behavioral changes (155). A common abnormality in both ALS and FTD is the mislocalization of RNA-binding proteins (RBPs) from the nucleus into the cytoplasmic aggregates in the affected neurons (122). These RBPs include the nuclear protein TAR DNA-binding protein of 43 kDa (TDP-43) and fused in sarcoma (FUS) protein (149, 156, 157); for both proteins, a nuclear loss-of-function and a cytoplasmic gain-of-toxicity are discussed (149). Indications for NPC/NCT disruption in ALS are evident from nuclear membrane irregularities and abnormal NTR distribution in motor neurons (122, 158, 159) and in neurons with cytoplasmic TDP-43 inclusions in post-mortem ALS tissue (160).

The most common genetic cause of ALS/FTD is a repeat expansion of the chromosome 9 open reading frame 72 (C9orf72) (97), which has been linked to NCT impairment on different levels. In yeast and fly models of ALS/FTD C9orf72, different Nups act as suppressors (e.g., Nup107, Nup50, and Nup98) or enhancers (e.g., Nup62 and Gle1) of C9orf72-associated cell toxicity (97, 161–163). Furthermore, RanGAP1 can directly interact with the intronic hexanucleotide (G_4C_2) in the C9orf72 repeat expansion (147, 164), and it accumulates in cytoplasmic punctae in the motor cortex of ALS C9orf72 patients in patient-derived iPSC neurons (147, 165).

Besides *C9orf72*, familial ALS-associated mutations in copper- and zinc-superoxide dismutase (SOD1), in TDP-43, and in FUS have been shown to cause NCT failure. For example, in transgenic mutant SOD1 mice, the subcellular redistribution of importin-β and importin-α from the nucleoplasm into the cytosol has been reported (166). Additionally, misfolding of SOD-1 can expose its normally buried NES-like sequence, which leads to exportin-1-mediated nuclear export of misfolded SOD1 (167). Cytoplasmic accumulation of Nups and RanGAP1 in stress granules was also observed in ALS-SOD1 (42, 149).

In the case of ALS/FTD TDP-43, the pathological cytoplasmic aggregation of TDP-43—an essential nuclear RNA-binding protein and splicing regulator—is associated with mislocalization and/or cytoplasmic aggregation of Nups and nuclear transport factors, with a disruption of the nuclear membrane and NPCs, and, consequently, with the reduction of nuclear protein import and mRNA export (43). By proteomic analysis, components of the NPC/NCT, predominantly FG-Nups (e.g., Nup62, Nup98, and Nup153), scaffold Nups (e.g., Nup35 and Nup93), and nuclear export factors such as Xpo5 and Nxf1 were shown to co-aggregate with pathological cytoplasmic TDP-43. Notably, TDP-43 toxicity and defective NCT function in neurons overexpressing the C-terminal fragment of TDP-43 could be rescued upon treatment with selective nuclear export inhibitors (KPT-276 and KPT-335) (43).

For the nuclear RNA-binding protein FUS, about half of the ALS/FTD mutations affect its NLS sequence, which leads to disease-associated mis-localization, stress granule formation, and aggregation of FUS in the cytoplasm (118, 153). In a *Drosophila* model of human FUS overexpression in motor neurons, neurotoxicity could be prevented by the downregulation of Nup154 (fly ortholog of human Nup155) and exportin-1 (168), supporting a role of the NCT for FUS toxicity in this model. Elsewhere it was suggested that NCT proteins (e.g., exportin-1) modulate FUS toxicity by acting on the mislocalization and the aggregation of FUS itself (122).

In conclusion, comprehensive evidence suggests that NCT dysregulation is a pathogenic driver of neurotoxicity in ALS and neurodegeneration (153).

NCT in Huntington's Disease

Huntington's disease is caused by a CAG-repeat expansion in exon 1 of the huntingtin gene, which leads to a long polyglutamine (polyQ; $n = 35$–$60+$) stretch on the N-terminal end of the Huntingtin protein (Htt) (169, 170). Htt is equipped with an internal NLS and NES sequence and therefore can shuttle between the nucleus and the cytoplasm (171–173). Under disease condition, polyQ–Htt aggregates in the nucleus and the cytosol—mostly in neurons of the striatum and the cortical regions, but also in the hippocampus (169, 174, 175)—and thereby induces neurotoxicity (45, 176).

Within intracellular polyQ–Htt aggregates, FG-repeat Nups of the NPC cytoplasmic filaments (DDX19, RanBP2, and Nup214), the nuclear basket (Nup153), and the central channel (Nup62) have been identified (177). Another interactome study identified RanGAP1, nucleoporin Sec13, and the mRNA export factor Rae1 (ribonucleic acid export 1) as interaction partners of polyQ–Htt (178). Grima et al. confirmed the interaction of Nup62 and RanGap1 with intranuclear polyQ–Htt inclusions in HD transgenic mouse and *Drosophila* models, primary neurons expressing polyQ–Htt, HD patient-derived iPSC neurons, and post-mortem human HD brain regions (45). In fact, multiple NPC proteins were severely mislocalized and aggregated in the cytosol, particularly those from the cytoplasmic ring/filaments

(Nup88 and Gle1) and central channel. In neurons with polyQ–Htt inclusions, both passive and active NCT and the Ran gradient were disrupted. Importantly, treatment with the small molecule nuclear export inhibitor KPT-350 as well as overexpression of RanGAP1 were both able to restore the nucleocytoplasmic Ran gradient (45), rescue cell death, and increase cell viability.

In addition to these molecular effects of polyQ–Htt aggregates on Nups, polyQ–Htt dose- and age-dependent morphological changes of the NE also occur in HD cell models with perinuclear polyQ–Htt accumulation, in transgenic animal models, and in postmortem HD brain (46, 176). Together these findings show that polyQ–Htt-mediated NCT disruptions are a common phenotype in HD (149, 179).

NCT Impairments in Normal Aging

Deficits in NCT have not only been linked to age-related neurodegenerative diseases but also appears to be gradually impaired in normal physiological aging (144). The correct assembly, maintenance, and repair of NPCs, which are crucial for cellular health and integrity (145), ask for intact protein homeostasis, a process that is known to be progressively failing during aging. In dividing cells, NPCs disassemble during mitosis and rearrange afterwards in the newly formed cells (180). Rempel et al. showed that NPC quality control is compromised in aging mitotic cells, which results in decreased NPC function and impaired transcription factor shuttling (181). The maintenance of NPCs in long-lived post-mitotic cells, such as neurons, is provided through the renewal of individual NPC subcomplexes, whereby scaffold Nups remain assembled and installed in the NPC during the entire cellular life span (144, 182). These Nups have one of the highest protein lifetimes of organisms; however, they therefore also contribute to NPC vulnerability in advanced age, when molecular damage has accumulated over time. Indeed a study from D'Angelo et al. showed that a subset of scaffolding Nups is oxidatively damaged in aged cells and that the age-related deterioration of NPCs provokes an increase in nuclear permeability accompanied by leakage of cytoplasmic proteins into the nucleus (182).

NCT Problems Related to Nuclear Import Factors

Nuclear import factors do not only mediate active transport of biomolecules through NPCs but also, in some cases, influence the aggregation of their cargo proteins (183–186). The import factors importin-4, importin-5, importin-7, and importin-β were shown to act as chaperones for exposed basic domains of ribosomal proteins, histones, and other cargos that would otherwise easily aggregate in the polyanionic environment of the cytoplasm (183). A loss of chaperone activity, for example, due to the decrease of import factor RanBP17 with cellular age can lead to NCT impairment, as shown by comparative transcriptomics in fibroblasts and corresponding induced neurons from differently aged donors (187).

Nuclear import factors have also been shown to reverse aberrant liquid–liquid phase separation (LLPS) of proteins and to disaggregate insoluble protein aggregates occurring in neurodegenerative diseases (97, 149, 150). For example, importin-α together with karyopherin-β can disassemble TDP-43 aggregates (150). TNPO1 (= karyopherin-β2) suppresses FUS LLPS and stress granule association (184, 185), whereby disease-linked mutations in the FUS-NLS impair TNPO1 chaperoning and enhance FUS aggregation (185).

THERAPEUTIC APPROACHES FOR NCT FAILURE

Despite major evidence for NCT problems in ALS, FTD, HD, and AD, up to now no therapeutic approach targeting nuclear transport deficits exists for neurodegenerative diseases. Major challenges in developing therapeutic strategies are given not only by the high molecular and structural complexity of the NPC but also by the importance of NCT for virtually all cellular processes: an intact nucleocytoplasmic trafficking of RNA and proteins is essential to change the transcription profile of a cell, for example, as a response of changes in cellular, substrate, or chemical environment; the NCT of biomolecules is both at the end of all signaling cascades and at the beginning of all cellular responses (40, 41, 188). Finding ways to rescue NCT disruption in neurodegenerative proteinopathies thus holds a tempting new opportunity to prevent neuronal death in these diseases but is also a great challenge.

In the recent years, small molecule nuclear export inhibitors were used with some success for therapeutically targeting nucleocytoplasmic export in cancer and viral disease therapies; however, the lack of compounds that inhibit the nuclear transport of specific cargos compromises the development of therapeutic strategies (153, 189). Disease-induced NPC disruptions often cause an imbalance in the nucleocytoplasmic gradient of NTFs, transcription factors, nuclear proteins, and RNA, which in principle can, to some extent, be reverted by either increasing or inhibiting nuclear import or export (97, 150). For example, it has been shown that inhibition of nuclear import rescues the polyQ–Htt toxicity in a yeast model (190), whereas inhibition of nuclear export was neuroprotective in a cell model of ALS (43, 45, 147, 191). Structure-based design of inhibitors that target exportin-1/CRM, the major receptor for the export of proteins out of the nucleus, yielded selective inhibitors of nuclear export (KPT-350, KPT-335, and KPT-276) that also proved successful in preclinical models. However, due to the broad range of molecular cargos shuttled out of the nucleus with the help of exportin-1/CRM1, off-target effects and potential toxicity remain as important issues when targeting this pathway (153, 192). A phase 1 safety trial using the exportin1 inhibitor XPO1 has recently been launched to investigate the safety and beneficial vs. the off-target effects of exportin-1 inhibition in ALS patients (193). This trial will hopefully also clarify whether targeting nucleocytoplasmic export will be sufficient to alleviate pathological neuronal death in the human brain.

In any case, since differences exist in the molecular and the cellular disease mechanisms between neurodegenerative diseases and aging seems to play a role for NPC function as well, physiological and disease-specific NPC/NCT alterations need to be investigated in more detail. For the development

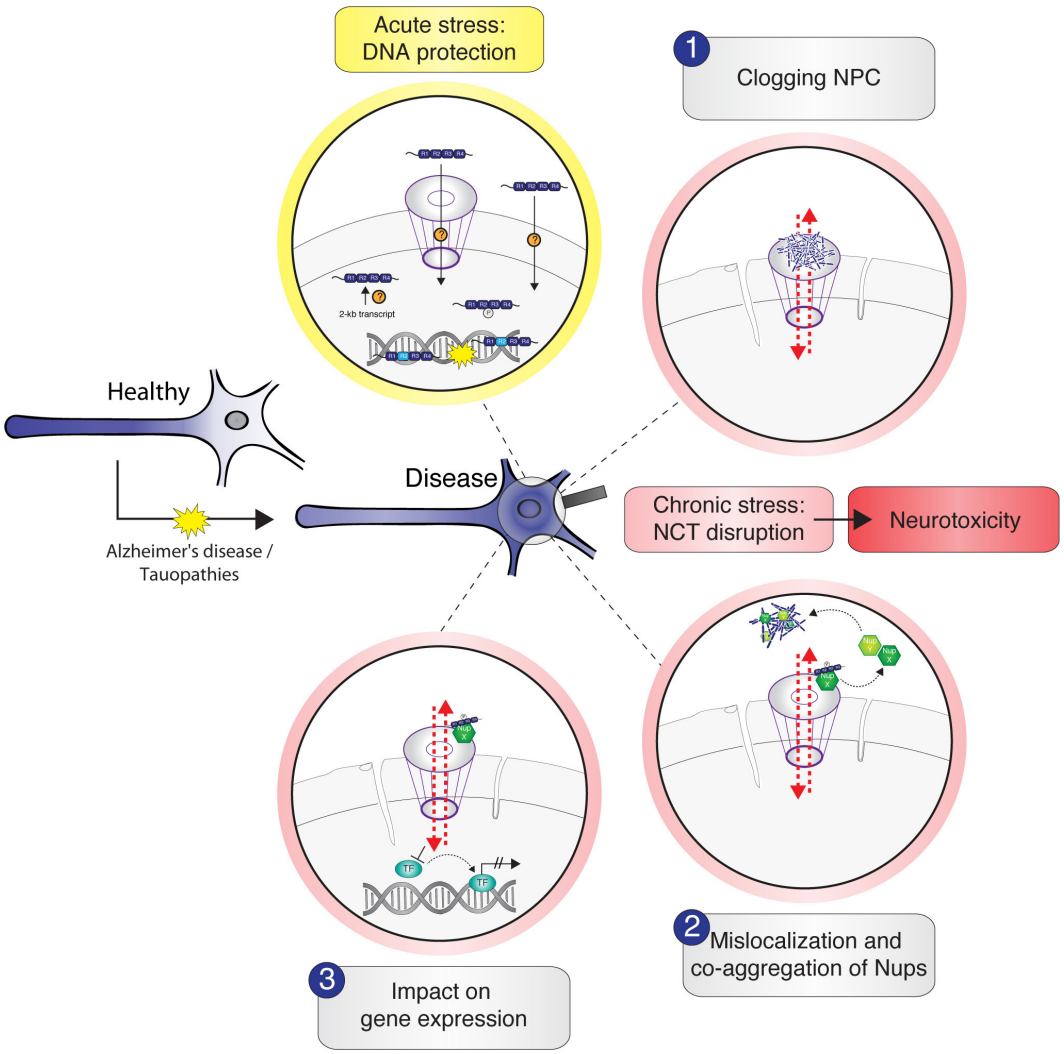


FIGURE 2 | Schematic illustration of potential tau–nucleus interactions leading to nucleocytoplasmic transport (NCT) impairment in tau-related neurodegeneration. Under physiological conditions, cytosolic soluble tau is mainly localized to the neuronal axon to stabilize the microtubules. In stress conditions and in the context of neurodegenerative diseases like Alzheimer's disease and tauopathies, tau mislocalizes from the axon into the somatodendritic compartment where it gets in close proximity to the nucleus. Acute stress, for example, via heat shock, transiently increases the amount of intranuclear tau, either by active transport of tau through the nuclear pore complexes (NPC) or other unknown import mechanisms or by the enhanced expression and/or local translation of nuclear tau transcripts. Nuclear tau binds and stabilizes DNA during the time of insult, undertaking a DNA-protective role. Under persistent stress—as in the context of neurodegenerative diseases—the amount of hyperphosphorylated tau in the soma increases further and leads to different possible scenarios of tau-induced NCT disruption, which are accompanied with nuclear envelope abnormalities (e.g., invaginations) and result in neurotoxicity: (1) soluble and/or aggregated tau binds and thereby clogs the nuclear pore, resulting in cargo transport inhibition; (2) tau interacts with specific Nups of the NPC, leading to NPC disassembly and sequestration of Nups from the NPC into the cytosol, resulting in nuclear pore leakiness and co-aggregation of cytoplasmic Nups with tau; (3) somatodendritic tau interacts with Nups that under physiological conditions would associate with transcription factors to regulate gene expression. These interactions "distract" Nups and thereby indirectly affect gene expression.

of tau-targeted NCT-based therapies, we are still at the very beginning, and systematic analyses of tau–NPC interactions and their downstream effects are needed.

CONCLUSION

Whereas, the presence of tau inside the nucleus has been reported for several years, tau interactions with the NE and their consequences for neuronal NCT were described only recently. NCT impairment and concomitant neurotoxicity in tau-associated NDDs could result from different (hypothetical) scenarios of tau interactions with nuclear pore complexes, with individual Nups, or with NTRs (**Figure 2**). However, to decipher the physiological role of tau in chromatin regulation and the consequences of tau–NPC interactions in pathophysiological conditions, we need to systematically identify tau–NPC, tau–NTR, and tau–chromatin interactions in order to understand the molecular mechanisms and the (dys) functional role of tau's interactions with the nucleus.

Furthermore, we want to acknowledge that the occurrence of NCT problems in protein aggregation diseases (including

tau-related ones) is a rather young observation; however, it yet is a new interesting emerging field in disease neurobiology that offers complementary interpretations to established disease mechanisms associated with neuronal protein aggregation.

AUTHOR CONTRIBUTIONS

LD wrote the first draft of the manuscript. SW and LD edited the manuscript to the final version. All authors contributed to the article and approved the submitted version.

FUNDING

This work has been funded by the German Center for Neurodegenerative Diseases (DZNE) of the Helmholtz Society.

ACKNOWLEDGMENTS

We thank Roderick Lim, Larisa Kapinos-Schneider, and Tomohisa Toda for discussions on tau in the nuclear context.

REFERENCES

1. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci USA*. (1975) 72:1858–62. doi: 10.1073/pnas.72.5.1858
2. Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous central nervous. *J Cell Biol*. (1985) 101:1371–8. doi: 10.1083/jcb.101.4.1371
3. Black MM, Slaughter T, Moshiach S, Obrocka M, Fischer I. Tau is enriched on dynamic microtubules in the distal region of growing axons. *J Neurosci*. (1996) 16:3601–19. doi: 10.1523/JNEUROSCI.16-11-03601.1996
4. Ballatore C, Lee VMY, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci*. (2007) 8:663–72. doi: 10.1038/nrn2194
5. Goedert M, Spillantini MG. Pathogenesis of the tauopathies. *J Mol Neurosci*. (2011) 45:425–31. doi: 10.1007/s12031-011-9593-4
6. Braak H, Braak E. Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol Aging*. (1995) 16:271–8. doi: 10.1016/0197-4580(95)00021-6
7. Hyman BT, Van Hoesen GW, Damasio AR, Barnes CL. Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. *Science*. (1984) 225:1168–70. doi: 10.1126/science.6474172
8. Gómez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol*. (1997) 41:17–24. doi: 10.1002/ana.410410106
9. Goedert M. Tau filaments in neurodegenerative diseases. *FEBS Lett*. (2018) 592:2383–91. doi: 10.1002/1873-3468.13108
10. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature*. (2017) 547:185–90. doi: 10.1038/nature23002
11. Falcon B, Zhang W, Murzin A, Murshudov G, Garringer H, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature*. (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y
12. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature*. (2019) 568:420–3. doi: 10.1038/s41586-019-1026-5
13. Goedert M, Jakes R, Spillantini MG, Hasegawa M, Smith MJ, Crowther RA. Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. *Nature*. (1996) 383:550–3. doi: 10.1038/383550a0
14. Pérez M, Valpuesta JM, Medina M, Montejo De Garcini E, Avila J. Polymerization of τ into filaments in the presence of heparin: the minimal sequence required for τ-τ interaction. *J Neurochem*. (1996) 67:1183–90. doi: 10.1046/j.1471-4159.1996.67031183.x
15. Kampers T, Friedhoff P, Biernat J, Mandelkow EM, Mandelkow E. RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett*. (1996) 399:344–9. doi: 10.1016/S0014-5793(96)01386-5
16. Wilson DM, Binder LI. Free fatty acids stimulate the polymerization of tau and amyloid β peptides: In vitro evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol*. (1997) 150:2181–95.
17. Ward SM, Himmelstein DS, Lancia JK, Binder LI. Tau oligomers and tau toxicity in neurodegenerative disease. *Biochem Soc Trans*. (2012) 40:667–71. doi: 10.1042/BST20120134
18. Cowan CM, Mudher A. Are tau aggregates toxic or protective in tauopathies? *Front Neurol*. (2013) 4:114. doi: 10.3389/fneur.2013.00114
19. Spires-Jones TL, Hyman BT. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. *Neuron*. (2014) 82:756–71. doi: 10.1016/j.neuron.2014.05.004
20. DeVos SL, Corjuc BT, Oakley DH, Nobuhara CK, Bannon RN, Chase A, et al. Synaptic tau seeding precedes tau pathology in human Alzheimer's disease brain. *Front Neurosci*. (2018) 12:267. doi: 10.3389/fnins.2018.00267
21. Lee G, Todd Newman S, Gard DL, Band H, Panchamoorthy G. Tau interacts with src-family non-receptor tyrosine kinases. *J Cell Sci*. (1998) 111:3167–77.
22. Sottejeau Y, Bretteville A, Cantrelle FX, Malmanche N, Demiaute F, Mendes T, et al. Tau phosphorylation regulates the interaction between BIN1's SH3 domain and Tau's proline-rich domain. *Acta Neuropathol Commun*. (2015) 3:58. doi: 10.1186/s40478-015-0237-8
23. Butner KA, Kirschner MW. Tau protein binds to microtubules through a flexible array of distributed weak sites. *J Cell Biol*. (1991) 115:717–30. doi: 10.1083/jcb.115.3.717
24. Brandt R, Lee G. Functional organization of microtubule-associated protein tau. Identification of regions which affect microtubule growth, nucleation, and bundle formation *in vitro*. *J Biol Chem*. (1993) 268:3414–9.
25. Gustke N, Trinczek B, Biernat J, Mandelkow EM, Mandelkow E. Domains of τ protein and interactions with microtubules. *Biochemistry*. (1994) 33:9511–22. doi: 10.1021/bi00198a017
26. Ludin B, Ashbridge K, Fünfschilling U, Matus A. Functional analysis of the MAP2 repeat domain. *J Cell Sci*. (1996) 109:91–9.
27. Von Bergen M, Barghorn S, Biernat J, Mandelkow EM, Mandelkow E. Tau aggregation is driven by a transition from random coil to beta sheet structure. *Biochim Biophys Acta Mol Basis Dis*. (2005) 1739:158–66. doi: 10.1016/j.bbadis.2004.09.010
28. Multhaup G, Huber O, Buée L, Galas MC. Amyloid Precursor Protein (APP) metabolites APP intracellular fragment (AICD), Aβ42, and Tau in nuclear roles. *J Biol Chem*. (2015) 290:23515–22. doi: 10.1074/jbc.R115.677211
29. Ulrich G, Salvadè A, Boersema P, Calì T, Foglieni C, Sola M, et al. Phosphorylation of nuclear Tau is modulated by distinct cellular pathways. *Sci Rep*. (2018) 8:17702. doi: 10.1038/s41598-018-36374-4
30. Pevalova M, Filipcik P, Novak M, Avila J, Iqbal K. Post-translational modifications of tau protein. *Bratisl Lek Listy*. (2006) 107:346–53.
31. Morris M, Knudsen GM, Maeda S, Trinidad JC, Ioanoviciu A, Burlingame AL, et al. Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice. *Nat Neurosci*. (2015) 18:1183–9. doi: 10.1038/nn.4067
32. Mandelkow EM, Biernat J, Drewes G, Gustke N, Trinczek B, Mandelkow E. Tau domains, phosphorylation, and interactions with microtubules.

Neurobiol Aging. (1995) 16:355–62. doi: 10.1016/0197-4580(95)00025-A
33. Maccioni RB, Cambiazo V. Role of microtubule-associated proteins in the control of microtubule assembly. *Physiol Rev*. (1995) 75:835–64. doi: 10.1152/physrev.1995.75.4.835
34. Zempel H, Mandelkow E. Lost after translation: Missorting of Tau protein and consequences for Alzheimer disease. *Trends Neurosci*. (2014) 37:721–32. doi: 10.1016/j.tins.2014.08.004
35. Levine ZA, Larini L, LaPointe NE, Feinstein SC, Shea JE. Regulation and aggregation of intrinsically disordered peptides. *Proc Natl Acad Sci USA*. (2015) 112:2758–63. doi: 10.1073/pnas.1418155112
36. Sotiropoulos I, Galas MC, Silva JM, Skoulakis E, Wegmann S, Maina MB, et al. Atypical, non-standard functions of the microtubule associated Tau protein. *Acta Neuropathol Commun*. (2017) 5:91. doi: 10.1186/s40478-017-0489-6
37. Metuzals J, Robitaille Y, Houghton S, Gauthier S, Leblanc R. Paired helical filaments and the cytoplasmic-nuclear interface in Alzheimer's disease. *J Neurocytol*. (1988) 17:827–33. doi: 10.1007/BF01216709
38. Eftekharzadeh B, Daigle JG, Kapinos LE, Coyne A, Schiantarelli J, Carlomagno Y, et al. Tau protein disrupts nucleocytoplasmic transport in Alzheimer's Disease. *Neuron*. (2018) 99:925–40.e7. doi: 10.1016/j.neuron.2018.07.039
39. Cornelison GL, Levy SA, Jenson T, Frost B. Tau-induced nuclear envelope invagination causes a toxic accumulation of mRNA in *Drosophila*. *Aging Cell*. (2019) 18:e12847. doi: 10.1111/acel.12847
40. Xu L, Massagué J. Nucleocytoplasmic shuttling of signal transducers. *Nat Rev Mol Cell Biol*. (2004) 5:209–19. doi: 10.1038/nrm1331
41. Gama-Carvalho M, Carmo-Fonseca M. The rules and roles of nucleocytoplasmic shuttling proteins. *FEBS Lett*. (2001) 498:157–63. doi: 10.1016/S0014-5793(01)02487-5
42. Zhang K, Daigle JG, Cunningham KM, Coyne AN, Ruan K, Grima JC, et al. Stress granule assembly disrupts nucleocytoplasmic transport. *Cell*. (2018) 173:958–71.e17. doi: 10.1016/j.cell.2018.03.025
43. Chou CC, Zhang Y, Umoh ME, Vaughan SW, Lorenzini I, Liu F, et al. TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. *Nat Neurosci*. (2018) 21:228–39. doi: 10.1038/s41593-017-0047-3
44. Gasset-Rosa F, Lu S, Yu H, Chen C, Melamed Z, Guo L, et al. Cytoplasmic TDP-43 De-mixing independent of stress granules drives inhibition of nuclear import, loss of nuclear TDP-43, and cell death. *Neuron*. (2019) 102:339–57.e7. doi: 10.1016/j.neuron.2019.02.038
45. Grima JC, Daigle JG, Arbez N, Cunningham KC, Zhang K, Ochaba J, et al. Mutant huntingtin disrupts the nuclear pore complex. *Neuron*. (2017) 94:93–107.e6. doi: 10.1016/j.neuron.2017.03.023
46. Gasset-Rosa F, Chillon-Marinas C, Goginashvili A, Atwal RS, Artates JW, Tabet R, et al. Polyglutamine-expanded huntingtin exacerbates age-related disruption of nuclear integrity and nucleocytoplasmic transport. *Neuron*. (2017) 94:48–57.e4. doi: 10.1016/j.neuron.2017.03.027
47. Rady RM, Zinkowski RP, Binder LI. Presence of tau in isolated nuclei from human brain. *Neurobiol Aging*. (1995) 16:479–86. doi: 10.1016/0197-4580(95)00023-8
48. Loomis PA, Howard TH, Castleberry RP, Binder LI. Identification of nuclear T isoforms in human neuroblastoma cells. *Proc Natl Acad Sci USA*. (1990) 87:8422–6. doi: 10.1073/pnas.87.21.8422
49. Greenwood JA, Johnson GVW. Localization and *in situ* phosphorylation state of nuclear tau. *Exp Cell Res*. (1995) 220:332–7. doi: 10.1006/excr.1995.1323
50. Maina MB, Bailey LJ, Wagih S, Biasetti L, Pollack SJ, Quinn JP, et al. The involvement of tau in nucleolar transcription and the stress response. *Acta Neuropathol Commun*. (2018) 6:70. doi: 10.1186/s40478-018-0565-6
51. Lambert MP, Sabo S, Zhang C, Enam SA, Klein WL. Constitutive Alzheimer's-type tau epitopes in a neuritogenic rat CNS cell line. *Neurobiol Aging*. (1995) 16:583–9. doi: 10.1016/0197-4580(95)00042-D
52. Sultan A, Nesslany F, Violet M, Bégard S, Loyens A, Talahari S, et al. Nuclear Tau, a key player in neuronal DNA protection. *J Biol Chem*. (2011) 286:4566–75. doi: 10.1074/jbc.M110.199976
53. Violet M, Delattre L, Tardivel M, Sultan A, Chauderlier A, Caillierez R, et al. A major role for Tau in neuronal DNA and RNA protection in vivo under physiological and hyperthermic conditions. *Front Cell Neurosci*. (2014) 8:84. doi: 10.3389/fncel.2014.00084
54. Liu C, Götz J. Profiling murine tau with 0N, 1N and 2N isoform-specific antibodies in brain and peripheral organs reveals distinct subcellular localization, with the 1N isoform being enriched in the nucleus. *PLoS ONE*. (2013) 8:e84849. doi: 10.1371/journal.pone.0084849
55. Benhelli-Mokrani H, Mansuroglu Z, Chauderlier A, Albaud B, Gentien D, Sommer S, et al. Genome-wide identification of genic and intergenic neuronal DNA regions bound by Tau protein under physiological and stress conditions. *Nucleic Acids Res*. (2018) 46:11405–22. doi: 10.1093/nar/gky929
56. Thurston VC, Zinkowski RP, Binder LI. Tau as a nucleolar protein in human nonneural cells in vitro and in vivo. *Chromosoma*. (1996) 105:20–30. doi: 10.1007/BF02510035
57. Cross D, Tapia L, Garrido J, Maccioni RB. Tau-like proteins associated with centrosomes in cultured cells. *Exp Cell Res*. (1996) 229:378–87. doi: 10.1006/excr.1996.0383
58. Maina MB, Al-Hilaly YK, Serpell LC. Nuclear tau and its potential role in alzheimer's disease. *Biomolecules*. (2016) 6:2–20. doi: 10.3390/biom6010009
59. Ptak C, Wozniak RW. SUMO and nucleocytoplasmic transport. *Adv Exp Med Biol*. (2017) 963:111–26. doi: 10.1007/978-3-319-50044-7_7
60. Turpin P, Ossareh-Nazari B, Dargemont C. Nuclear transport and transcriptional regulation. *FEBS Lett*. (1999) 452:82–6. doi: 10.1016/S0014-5793(99)00533-5
61. Wang Y, Loomis PA, Zinkowski RP, Binder LI. A novel tau transcript in cultured human neuroblastoma cells expressing nuclear tau. *J Cell Biol*. (1993) 121:257–67. doi: 10.1083/jcb.121.2.257
62. Andreadis A. Tau gene alternative splicing: expression patterns, regulation and modulation of function in normal brain and neurodegenerative diseases. *Biochim Biophys Acta Mol Basis Dis*. (2005) 1739:91–103. doi: 10.1016/j.bbadis.2004.08.010
63. Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. *Acta Neuropathol*. (2017) 133:665–704. doi: 10.1007/s00401-017-1707-9
64. Papasozomenos CHS. Nuclear tau immunoreactivity in presenile dementia with motor neuron disease: a case report. *Clin Neuropathol*. (1995) 14:100–4.
65. Álvarez G, Aldudo J, Alonso M, Santana S, Valdivieso F. Herpes simplex virus type 1 induces nuclear accumulation of hyperphosphorylated tau in neuronal cells. *J Neurosci Res*. (2012) 90:1020–9. doi: 10.1002/jnr.23003
66. Lu J, Miao J, Su T, Liu Y, He R. Formaldehyde induces hyperphosphorylation and polymerization of Tau protein both in vitro and in vivo. *Biochim Biophys Acta Gen Subj*. (2013) 1830:4102–16. doi: 10.1016/j.bbagen.2013.04.028
67. Rossi G, Dalprà L, Crosti F, Lissoni S, Sciacca FL, Catania M, et al. A new function of microtubule-associated protein tau: Involvement in chromosome stability. *Cell Cycle*. (2008) 7:1788–94. doi: 10.4161/cc.7.12.6012
68. Wiche G, Corces VG, Avila J. Preferential binding of hog brain microtubule-associated proteins to mouse satellite versus bulk DNA preparations. *Nature*. (1978) 273:403–5. doi: 10.1038/273403a0
69. Villasante A, Corces VG, Manso-Martínez R, Avila J. Binding of microtubule protein to DNA and chromatin: possibility of simultaneous linkage of microtubule to nucleic acid and assembly of the microtubule structure. *Nucleic Acids Res*. (1981) 9:895–908. doi: 10.1093/nar/9.4.895
70. Krylova SM, Musheev M, Nutiu R, Li Y, Lee G, Krylov SN. Tau protein binds single-stranded DNA sequence specifically - the proof obtained *in vitro* with non-equilibrium capillary electrophoresis of equilibrium mixtures. *FEBS Lett*. (2005) 579:1371–5. doi: 10.1016/j.febslet.2005.01.032
71. Goldbaum O, Richter-Landsberg C. Activation of PP2A-like phosphatase and modulation of tau phosphorylation accompany stress-induced apoptosis in cultured oligodendrocytes. *Glia*. (2002) 40:271–82. doi: 10.1002/glia.10119
72. Hua Q, He RQ, Haque N, Qu MH, Del Carmen Alonso A, Grundke-Iqbal I, et al. Microtubule associated protein tau binds to double-stranded but not single-stranded DNA. *Cell Mol Life Sci*. (2003) 60:413–21. doi: 10.1007/s000180300034
73. Bewley CA, Gronenborn AM, Clore GM. Minor groove-binding architectural proteins: Structure, function, and DNA recognition. *Annu Rev Biophys Biomol Struct*. (1998) 27:105–31. doi: 10.1146/annurev.biophys.27.1.105
74. Wei Y, Qu MH, Wang XS, Chen L, Wang DL, Liu Y, et al. Binding to the minor groove of the double-strand, Tau protein prevents DNA damage by peroxidation. *PLoS ONE*. (2008) 3:e2600. doi: 10.1371/journal.pone.0002600

75. Camero S, Benítez MJ, Cuadros R, Hernández F, Avila J, Jiménez JS. Thermodynamics of the interaction between Alzheimer's disease related Tau protein and DNA. *PLoS ONE.* (2014) 9:e104690. doi: 10.1371/journal.pone.0104690
76. Qi H, Cantrelle FX, Benhelli-Mokrani H, Smet-Nocca C, Buée L, Lippens G, et al. Nuclear magnetic resonance spectroscopy characterization of interaction of Tau with DNA and its regulation by phosphorylation. *Biochemistry*. (2015) 54:1525–33. doi: 10.1021/bi5014613
77. Kimura T, Sharma G, Ishiguro K, Hisanaga SI. Phospho-tau bar code: analysis of phosphoisotypes of tau and its application to tauopathy. *Front Neurosci.* (2018) 12:44. doi: 10.3389/fnins.2018.00044
78. Bretteville A, Marcouiller F, Julien C, El Khoury NB, Petry FR, Poitras I, et al. Hypothermia-induced hyperphosphorylation: a new model to study tau kinase inhibitors. *Sci Rep.* (2012) 2:480. doi: 10.1038/srep00480
79. Olive PL, Banath JP, Durand RE. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet" assay. *Radiat Res*. (1990) 122:86–94. doi: 10.2307/3577587
80. Sola M, Magrin C, Pedrioli G, Pinton S, Salvadè A, Papin S, et al. Tau affects P53 function and cell fate during the DNA damage response. *Commun Biol.* (2020) 3:245. doi: 10.1038/s42003-020-0975-4
81. Rossi G, Conconi D, Panzeri E, Redaelli S, Piccoli E, Paoletta L, et al. Mutations in MAPT gene cause chromosome instability and introduce copy number variations widely in the genome. *J Alzheimer's Dis*. (2013) 33:969–82. doi: 10.3233/JAD-2012-121633
82. Frost B, Hemberg M, Lewis J, Feany MB. Tau promotes neurodegeneration through global chromatin relaxation. *Nat Neurosci*. (2014) 17:357–66. doi: 10.1038/nn.3639
83. Klein HU, McCabe C, Gjoneska E, Sullivan SE, Kaskow BJ, Tang A, et al. Epigenome-wide study uncovers large-scale changes in histone acetylation driven by tau pathology in aging and Alzheimer's human brains. *Nat Neurosci*. (2019) 22:37–46. doi: 10.1038/s41593-018-0291-1
84. Mansuroglu Z, Benhelli-Mokrani H, Marcato V, Sultan A, Violet M, Chauderlier A, et al. Loss of Tau protein affects the structure, transcription and repair of neuronal pericentromeric heterochromatin. *Sci Rep*. (2016) 6:33047. doi: 10.1038/srep33047
85. Sjöberg MK, Shestakova E, Mansuroglu Z, Maccioni RB, Bonnefoy E. Tau protein binds to pericentromeric DNA: A putative role for nuclear tau in nucleolar organization. *J Cell Sci*. (2006) 119:2025–34. doi: 10.1242/jcs.02907
86. Koren SA, Hamm MJ, Meier SE, Weiss BE, Nation GK, Chishti EA, et al. Tau drives translational selectivity by interacting with ribosomal proteins. *Acta Neuropathol.* (2019) 137:571–83. doi: 10.1007/s00401-019-01970-9
87. Lefebvre T, Ferreira S, Dupont-Wallois L, Bussière T, Dupire MJ, Delacourte A, et al. Evidence of a balance between phosphorylation and O-GlcNAc glycosylation of Tau proteins - a role in nuclear localization. *Biochim Biophys Acta Gen Subj*. (2003) 1619:167–76. doi: 10.1016/S0304-4165(02)00477-4
88. Lu Y, He HJ, Zhou J, Miao JY, Lu J, He YG, et al. Hyperphosphorylation results in tau dysfunction in DNA folding and protection. *J Alzheimer's Dis*. (2013) 37:551–63. doi: 10.3233/JAD-130602
89. Mondragón-Rodríguez S, Perry G, Zhu X, Moreira PI, Acevedo-Aquino MC, Williams S. Phosphorylation of tau protein as the link between oxidative stress, mitochondrial dysfunction, and connectivity failure: Implications for Alzheimer's disease. *Oxid Med Cell Longev*. (2013) 2013:940603. doi: 10.1155/2013/940603
90. Gruenbaum Y, Margalit A, Goldman RD, Shumaker DK, Wilson KL. The nuclear lamina comes of age. *Nat Rev Mol Cell Biol*. (2005) 6:21–31. doi: 10.1038/nrm1550
91. Aebi U, Cohn J, Buhle L, Gerace L. The nuclear lamina is a meshwork of intermediate-type filaments. *Nature*. (1986) 323:560–4. doi: 10.1038/323560a0
92. Crisp M, Liu Q, Roux K, Rattner JB, Shanahan C, Burke B, et al. Coupling of the nucleus and cytoplasm: role of the LINC complex. *J Cell Biol*. (2006) 172:41–53. doi: 10.1083/jcb.200509124
93. Lombardi ML, Lammerding J. Keeping the LINC: The importance of nucleocytoskeletal coupling in intracellular force transmission and cellular function. *Biochem Soc Trans*. (2011) 39:1729–34. doi: 10.1042/BST20110686
94. Tran EJ, Wente SR. Dynamic nuclear pore complexes: life on the edge. *Cell*. (2006) 125:1041–53. doi: 10.1016/j.cell.2006.05.027
95. Floch AG, Palancade B, Doye V. Fifty years of nuclear pores and nucleocytoplasmic transport studies: Multiple tools revealing complex rules. *Methods Cell Biol*. (2014) 122:1–40. doi: 10.1016/B978-0-12-417160-2.00001-1
96. Lim RYH, Ullman KS, Fahrenkrog B. Biology and biophysics of the nuclear pore complex and its components. *Int Rev Cell Mol Biol*. (2008) 267:299–342. doi: 10.1016/S1937-6448(08)00632-1
97. Moore S, Rabichow BE, Sattler R. The Hitchhiker's guide to nucleocytoplasmic trafficking in neurodegeneration. *Neurochem Res*. (2020) 45:1306–27. doi: 10.1007/s11064-020-02989-1
98. Ori A, Banterle N, Iskar M, Andrés-Pons A, Escher C, Khanh Bui H, et al. Cell type-specific nuclear pores: A case in point for context-dependent stoichiometry of molecular machines. *Mol Syst Biol*. (2013) 9:648. doi: 10.1038/msb.2013.4
99. Wente SR, Rout MP. The nuclear pore complex and nuclear transport. *Cold Spring Harb Perspect Biol*. (2010) 2:a000562. doi: 10.1101/cshperspect.a000562
100. Raices M, D'Angelo MA. Nuclear pore complexes and regulation of gene expression. *Curr Opin Cell Biol*. (2017) 46:26–32. doi: 10.1016/j.ceb.2016.12.006
101. Hinshaw JE, Carragher BO, Milligan RA. Architecture and design of the nuclear pore complex. *Cell*. (1992) 69:1133–41. doi: 10.1016/0092-8674(92)90635-P
102. Radu A, Moore MS, Blobel G. The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. *Cell*. (1995) 81:215–22. doi: 10.1016/0092-8674(95)90331-3
103. Denning DP, Patel SS, Uversky V, Fink AL, Rexach M. Disorder in the nuclear pore complex: The FG repeat regions of nucleoporins are natively unfolded. *Proc Natl Acad Sci USA*. (2003) 100:2450–5. doi: 10.1073/pnas.0437902100
104. Lemke EA. The Multiple Faces of Disordered Nucleoporins. *J Mol Biol*. (2016) 428:2011–24. doi: 10.1016/j.jmb.2016.01.002
105. Frey S, Richter RP, Görlich D. FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties. *Science*. (2006) 314:815–17. doi: 10.1126/science.1132516
106. Dultz E, Zanin E, Wurzenberger C, Braun M, Rabut G, Sironi L, et al. Systematic kinetic analysis of mitotic dis- and reassembly of the nuclear pore in living cells. *J Cell Biol*. (2008) 180:857–65. doi: 10.1083/jcb.200707026
107. Schmidt HB, Görlich D. Nup98 FG domains from diverse species spontaneously phase-separate into particles with nuclear pore-like permselectivity. *Elife*. (2015) 4:1–30. doi: 10.7554/eLife.04251
108. Timney BL, Raveh B, Mironska R, Trivedi JM, Kim SJ, Russel D, et al. Simple rules for passive diffusion through the nuclear pore complex. *J Cell Biol*. (2016) 215:57–76. doi: 10.1083/jcb.201601004
109. Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, et al. A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell*. (2015) 162:1066–77. doi: 10.1016/j.cell.2015.07.047
110. Babinchak WM, Haider R, Dumm BK, Sarkar P, Surewicz K, Choi JK, et al. The role of liquid-liquid phase separation in aggregation of the TDP-43 low-complexity domain. *J Biol Chem*. (2019) 294:6306–17. doi: 10.1074/jbc.RA118.007222
111. Peskett TR, Rau F, O'Driscoll J, Patani R, Lowe AR, Saibil HR. A liquid to solid phase transition underlying pathological huntingtin exon1 aggregation. *Mol Cell*. (2018) 70:588–601.e6. doi: 10.1016/j.molcel.2018.04.007
112. Aktar F, Burudpakdee C, Polanco M, Pei S, Swayne TC, Lipke PN, et al. The huntingtin inclusion is a dynamic phase-separated compartment. *Life Sci Alliance.* (2019) 2:e201900489. doi: 10.26508/lsa.201900489
113. Wegmann S, Eftekharzadeh B, Tepper K, Zoltowska KM, Bennett RE, Dujardin S, et al. Tau protein liquid–liquid phase separation can initiate tau aggregation. *EMBO J*. (2018) 37:e98049. doi: 10.15252/embj.201798049
114. Ambadipudi S, Biernat J, Riedel D, Mandelkow E, Zweckstetter M. Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. *Nat Commun*. (2017) 8:1–13. doi: 10.1038/s41467-017-00480-0
115. Zhang X, Lin Y, Eschmann NA, Zhou H, Rauch JN, Hernandez I, et al. RNA stores tau reversibly in complex coacervates. *PLoS Biol.* (2017) 15:e2002183. doi: 10.1101/111245
116. Stewart M. Molecular mechanism of the nuclear protein import cycle. *Nat Rev Mol Cell Biol*. (2007) 8:195–208. doi: 10.1038/nrm2114

117. Cohen TJ, Lee VMY, Trojanowski JQ. TDP-43 functions and pathogenic mechanisms implicated in TDP-43 proteinopathies. *Trends Mol Med*. (2011) 17:659–67. doi: 10.1016/j.molmed.2011.06.004
118. Dormann D, Rodde R, Edbauer D, Bentmann E, Fischer I, Hruscha A, et al. ALS-associated fused in sarcoma (FUS) mutations disrupt transportin-mediated nuclear import. *EMBO J*. (2010) 29:2841–57. doi: 10.1038/emboj.2010.143
119. Ederle H, Funk C, Abou-Ajram C, Hutten S, Funk EBE, Kehlenbach RH, et al. Nuclear egress of TDP-43 and FUS occurs independently of exportin-1/CRM1. *Sci Rep*. (2018) 8:7084. doi: 10.1038/s41598-018-25007-5
120. Moore MS, Blobel G. A G protein involved in nucleocytoplasmic transport: the role of Ran. *Trends Biochem Sci*. (1994) 19:211–6. doi: 10.1016/0968-0004(94)90024-8
121. Dasso M, Pu RT. Nuclear transport: Run by ran? *Am J Hum Genet*. (1998) 63:311–6. doi: 10.1086/301990
122. Fallini C, Khalil B, Smith CL, Rossoll W. Traffic jam at the nuclear pore: all roads lead to nucleocytoplasmic transport defects in ALS/FTD. *Neurobiol Dis*. (2020) 140:104835. doi: 10.1016/j.nbd.2020.104835
123. Aramburu IV, Lemke EA. Floppy but not sloppy: Interaction mechanism of FG-nucleoporins and nuclear transport receptors. *Semin Cell Dev Biol*. (2017) 68:34–41. doi: 10.1016/j.semcdb.2017.06.026
124. Görlich D, Henklein P, Laskey RA, Hartmann E. A 41 amino acid motif in importin-α confers binding to importin-β and hence transit into the nucleus. *EMBO J*. (1996) 15:1810–7. doi: 10.1002/j.1460-2075.1996.tb00530.x
125. Yang W. "Natively unfolded" nucleoporins in nucleocytoplasmic transport: clustered or evenly distributed? *Nucleus*. (2011) 2:10–6. doi: 10.4161/nucl.13818
126. Hetzer M, Gruss OJ, Mattaj IW. The Ran GTPase as a marker of chromosome position in spindle formation and nuclear envelope assembly. *Nat Cell Biol*. (2002) 4:e177–84. doi: 10.1038/ncb0702-e177
127. Capelson M, Liang Y, Schulte R, Mair W, Wagner U, Hetzer MW. Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. *Cell*. (2010) 140:372–83. doi: 10.1016/j.cell.2009.12.054
128. Kalverda B, Pickersgill H, Shloma VV, Fornerod M. Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. *Cell*. (2010) 140:360–71. doi: 10.1016/j.cell.2010.01.011
129. Sheffield LG, Miskiewicz HB, Tannenbaum LB, Mirra SS. Nuclear pore complex proteins in Alzheimer disease. *J Neuropathol Exp Neurol*. (2006) 65:45–54. doi: 10.1097/01.jnen.0000195939.40410.08
130. Brettschneider J, Del Tredici K, Lee VM-Y, Trojanowski JQ. Spreading of pathology in neurodegenerative diseases: a focus on human studies. *Nat Rev Neurosci*. (2015) 16:109–20. doi: 10.1038/nrn3887
131. Paonessa F, Evans LD, Solanki R, Larrieu D, Wray S, Hardy J, et al. Microtubules deform the nuclear membrane and disrupt nucleocytoplasmic transport in tau-mediated frontotemporal dementia. *Cell Rep*. (2019) 26:582–93.e5. doi: 10.1016/j.celrep.2018.12.085
132. Mirra SS, Wood JG, Pollock NJ, Bakay RA, Binder LI, Ellisman MH. New observations on the fine structure of Alzheimer's disease. *J Neuropathol Exp Neurol*. (1987) 46:377. doi: 10.1097/00005072-198705000-00142
133. Patel VP, Chu CT. Nuclear transport, oxidative stress, and neurodegeneration. *Int J Clin Exp Pathol*. (2011) 4:215–29.
134. Monroy-Ramírez HC, Basurto-Islas G, Mena R, Cisneros B, Binder LI, Avila J, et al. Alterations in the nuclear architecture produced by the overexpression of tau protein in neuroblastoma cells. *J Alzheimer's Dis*. (2013) 36:503–20. doi: 10.3233/JAD-122401
135. Frost B, Bardai FH, Feany MB. Lamin dysfunction mediates neurodegeneration in tauopathies. *Curr Biol*. (2016) 26:129–36. doi: 10.1016/j.cub.2015.11.039
136. Kelley JB, Datta S, Snow CJ, Chatterjee M, Ni L, Spencer A, et al. The defective nuclear lamina in hutchinson-gilford progeria syndrome disrupts the nucleocytoplasmic ran gradient and inhibits nuclear localization of Ubc9. *Mol Cell Biol*. (2011) 31:3378–95. doi: 10.1128/MCB.05087-11
137. Fernández-Nogales M, Cabrera JR, Santos-Galindo M, Hoozemans JJM, Ferrer I, Rozemuller AJM, et al. Huntington's disease is a four-repeat tauopathy with tau nuclear rods. *Nat Med*. (2014) 20:881–5. doi: 10.1038/nm.3617
138. Fernández-Nogales M, Santos-Galindo M, Merchán-Rubira J, Hoozemans JJM, Rábano A, Ferrer I, et al. Tau-positive nuclear indentations in P301S tauopathy mice. *Brain Pathol*. (2017) 27:314–22. doi: 10.1111/bpa.12407
139. Ramachandran G, Udgaonkar JB. Understanding the kinetic roles of the inducer heparin and of rod-like protofibrils during amyloid fibril formation by tau protein. *J Biol Chem*. (2011) 286:38948–59. doi: 10.1074/jbc.M111.271874
140. Lee H-g, Ueda M, Miyamoto Y, Yoneda Y, Perry G, Smith MA, et al. Aberrant localization of importin α1 in hippocampal neurons in Alzheimer disease. *Brain Res*. (2006) 1124:1–4. doi: 10.1016/j.brainres.2006.09.084
141. Toda T, Hsu JY, Linker SB, Hu L, Schafer ST, Mertens J, et al. Nup153 interacts with Sox2 to enable bimodal gene regulation and maintenance of neural progenitor cells. *Cell Stem Cell*. (2017) 21:618–34.e7. doi: 10.1016/j.stem.2017.08.012
142. Vaquerizas JM, Suyama R, Kind J, Miura K, Luscombe NM, Akhtar A. Nuclear pore proteins Nup153 and megator define transcriptionally active regions in the *Drosophila* genome. *PLoS Genet*. (2010) 6:846 doi: 10.1371/journal.pgen.1000846
143. Sarma NJ, Willis K. The new nucleoporin: regulator of transcriptional repression and beyond. *Nucleus*. (2012) 3:508. doi: 10.4161/nucl.22427
144. Kim HJ, Taylor JP. Lost in transportation: nucleocytoplasmic transport defects in ALS and other neurodegenerative diseases. *Neuron*. (2017) 96:285–97. doi: 10.1016/j.neuron.2017.07.029
145. Hutten S, Dormann D. Nucleocytoplasmic transport defects in neurodegeneration — cause or consequence? *Semin Cell Dev Biol*. (2020) 99:151–62. doi: 10.1016/j.semcdb.2019.05.020
146. Woerner AC, Frottin F, Hornburg D, Feng LR, Meissner F, Patra M, et al. Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and RNA. *Science*. (2016) 351:173–6. doi: 10.1126/science.aad2033
147. Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, et al. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature*. (2015) 525:56–61. doi: 10.1038/nature14973
148. Shang J, Yamashita T, Nakano Y, Morihara R, Li X, Feng T, et al. Aberrant distributions of nuclear pore complex proteins in ALS mice and ALS patients. *Neuroscience*. (2017) 350:158–68. doi: 10.1016/j.neuroscience.2017.03.024
149. Fahrenkrog B, Harel A. Perturbations in traffic: aberrant nucleocytoplasmic transport at the heart of neurodegeneration. *Cells*. (2018) 7:232. doi: 10.3390/cells7120232
150. Guo L, Kim HJ, Wang H, Monaghan J, Freyermuth F, Sung JC, et al. Nuclear-import receptors reverse aberrant phase transitions of RNA-binding proteins with prion-like domains. *Cell*. (2018) 173:677–92.e20. doi: 10.1016/j.cell.2018.03.002
151. Basel-Vanagaite L, Muncher L, Straussberg R, Pasmanik-Chor M, Yahav M, Rainshtein L, et al. Mutated nup62 causes autosomal recessive infantile bilateral striatal necrosis. *Ann Neurol*. (2006) 60:214–22. doi: 10.1002/ana.20902
152. Kaneb HM, Folkmann AW, Belzil VV, Jao LE, Leblond CS, Girard SL, et al. Deleterious mutations in the essential mRNA metabolism factor, hGle1, in amyotrophic lateral sclerosis. *Hum Mol Genet*. (2015) 24:1363–73. doi: 10.1093/hmg/ddu545
153. Ferreira PA. The coming-of-age of nucleocytoplasmic transport in motor neuron disease and neurodegeneration. *Cell Mol Life Sci*. (2019) 76:2247–73. doi: 10.1007/s00018-019-03029-0
154. Boeynaems S, Bogaert E, Van Damme P, Van Den Bosch L. Inside out: the role of nucleocytoplasmic transport in ALS and FTLD. *Acta Neuropathol*. (2016) 132:159–73. doi: 10.1007/s00401-016-1586-5
155. Ferrari R, Kapogiannis DD. Huey E. Momeni FTDP, and ALS: a tale of two diseases. *Curr Alzheimer Res*. (2011) 8:273–94. doi: 10.2174/156720511795563700
156. Zhang K, Grima JC, Rothstein JD, Lloyd TE. Nucleocytoplasmic transport in C9orf72-mediated ALS/FTD. *Nucleus*. (2016) 7:132–7. doi: 10.1080/19491034.2016.1172152
157. Ederle H, Dormann D. TDP-43 and FUS en route from the nucleus to the cytoplasm. *FEBS Lett*. (2017) 591:1489–507. doi: 10.1002/1873-3468.12646
158. Kinoshita Y, Ito H, Hirano A, Fujita K, Wate R, Nakamura M, et al. Nuclear contour irregularity and abnormal transporter protein distribution in anterior horn cells in amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol*. (2009) 68:1184–92. doi: 10.1097/NEN.0b013e3181bc3bec

159. Nagara Y, Tateishi T, Yamasaki R, Hayashi S, Kawamura M, Kikuchi H, et al. Impaired cytoplasmic-nuclear transport of hypoxia-inducible factor-1α in amyotrophic lateral sclerosis. *Brain Pathol*. (2013) 23:534–46. doi: 10.1111/bpa.12040
160. Aizawa H, Yamashita T, Kato H, Kimura T, Kwak S. Impaired nucleoporins are present in sporadic amyotrophic lateral sclerosis motor neurons that exhibit mislocalization of the 43-kDa TAR DNA-binding protein. *J Clin Neurol*. (2019) 15:62–7. doi: 10.3988/jcn.2019.15.1.62
161. Freibaum BD, Lu Y, Lopez-Gonzalez R, Kim NC, Almeida S, Lee KH, et al. GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature*. (2015) 525:129–33. doi: 10.1038/nature14974
162. Jovi̇c̈ic A, Mertens J, Boeynaems S, Bogaert E, Chai N, Yamada SB, et al. Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat Neurosci*. (2015) 18:1226–9. doi: 10.1038/nn.4085
163. Boeynaems S, Bogaert E, Michiels E, Gijselinck I, Sieben A, Jovičić A, et al. *Drosophila* screen connects nuclear transport genes to DPR pathology in c9ALS/FTD. *Sci Rep*. (2016) 6:2087. doi: 10.1038/srep20877
164. Donnelly CJ, Zhang PW, Pham JT, Heusler AR, Mistry NA, Vidensky S, et al. RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron*. (2013) 80:415–28. doi: 10.1016/j.neuron.2013.10.015
165. Mihevc SP, Darovic S, Kovanda A, Cesnik AB, Župunski V, Rogelj B. Nuclear trafficking in amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *Brain*. (2017) 140:13–26. doi: 10.1093/brain/aww197
166. Zhang J, Ito H, Wate R, Ohnishi S, Nakano S, Kusaka H. Altered distributions of nucleocytoplasmic transport-related proteins in the spinal cord of a mouse model of amyotrophic lateral sclerosis. *Acta Neuropathol*. (2006) 112:673–80. doi: 10.1007/s00401-006-0130-4
167. Zhong Y, Wang J, Henderson MJ, Yang P, Hagen BM, Siddique T, et al. Nuclear export of misfolded SOD1 mediated by a normally buried NES-like sequence reduces proteotoxicity in the nucleus. *Elife*. (2017) 6:e23759. doi: 10.7554/eLife.23759
168. Steyaert J, Scheveneels W, Vanneste J, Van Damme P, Robberecht W, Callaerts P, et al. FUS-induced neurotoxicity in Drosophila is prevented by downregulating nucleocytoplasmic transport proteins. *Hum Mol Genet*. (2018) 27:4103–16. doi: 10.1093/hmg/ddy303
169. Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR, et al. Huntington disease. *Nat Rev Dis Prim*. (2015) 1:15005. doi: 10.1038/nrdp.2015.5
170. Saudou F, Humbert S. The Biology of Huntingtin. *Neuron*. (2016) 89:910–26. doi: 10.1016/j.neuron.2016.02.003
171. Bessert DA, Gutridge KL, Dunbar JC, Carlock LR. The identification of a functional nuclear localization signal in the Huntington disease protein. *Mol Brain Res*. (1995) 33:165–73. doi: 10.1016/0169-328X(95)00124-B
172. Xia J, Lee DH, Taylor J, Vandelft M, Truant R. Huntingtin contains a highly conserved nuclear export signal. *Hum Mol Genet*. (2003) 12:1393–403. doi: 10.1093/hmg/ddg156
173. Desmond ARS, Xia J, Truant R. Identification of a karyopherin β1/β2 proline-tyrosine nuclear localization signal in huntingtin protein. *J Biol Chem*. (2012) 287:39626–33. doi: 10.1074/jbc.M112.412379
174. Graveland GA, Williams RS, Difiglia M. Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science*. (1985) 227:770–3. doi: 10.1126/science.3155875
175. Reiner A, Dragatsis I, Dietrich P. Genetics and neuropathology of huntington's disease. *Int Rev Neurobiol*. (2011) 98:325–72. doi: 10.1016/B978-0-12-381328-2.00014-6
176. Liu KY, Shyu YC, Barbaro BA, Lin YT, Chern Y, Thompson LM, et al. Disruption of the nuclear membrane by perinuclear inclusions of mutant huntingtin causes cell-cycle re-entry and striatal cell death in mouse and cell models of Huntington's disease. *Hum Mol Genet*. (2015) 24:1602–16. doi: 10.1093/hmg/ddu574
177. Suhr ST, Senut MC, Whitelegge JP, Faull KF, Cuizon DB, Gage FH. Identities of sequestered proteins in aggregates from cells with induced polyglutamine expression. *J Cell Biol*. (2001) 153:283–94. doi: 10.1083/jcb.153.2.283
178. Hosp F, Vossfeldt H, Heinig M, Vasiljevic D, Arumughan A, Wyler E, et al. Quantitative interaction proteomics of neurodegenerative disease proteins. *Cell Rep*. (2015) 11:1134–46. doi: 10.1016/j.celrep.2015.04.030
179. Veldman MB, Yang XW. Huntington's disease: nuclear gatekeepers under attack. *Neuron*. (2017) 94:1–4. doi: 10.1016/j.neuron.2017.03.032
180. Rabut G, Doye V, Ellenberg J. Mapping the dynamic organization of the nuclear pore complex inside single living cells. *Nat Cell Biol*. (2004) 6:1114–21. doi: 10.1038/ncb1184
181. Rempel IL, Crane MM, Thaller DJ, Mishra A, Jansen DPM, Janssens G, et al. Age-dependent deterioration of nuclear pore assembly in mitotic cells decreases transport dynamics. *Elife*. (2019) 8:1–26. doi: 10.7554/eLife.48186
182. D'Angelo MA, Raices M, Panowski SH, Hetzer MW. Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. *Cell*. (2009) 136:284–95. doi: 10.1016/j.cell.2008.11.037
183. Jakel S. Importins fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains. *EMBO J*. (2002) 21:377–86. doi: 10.1093/emboj/21.3.377
184. Yoshizawa T, Ali R, Jiou J, Fung HYJ, Burke KA, Kim SJ, et al. Nuclear import receptor inhibits phase separation of FUS through binding to multiple sites. *Cell*. (2018) 173:693–705.e22. doi: 10.1016/j.cell.2018.03.003
185. Hofweber M, Hutten S, Bourgeois B, Spreitzer E, Niedner-Boblenz A, Schifferer M, et al. Phase separation of FUS is suppressed by its nuclear import receptor and arginine methylation. *Cell*. (2018) 173:706–19.e13. doi: 10.1016/j.cell.2018.03.004
186. Qamar S, Wang GZ, Randle SJ, Ruggeri FS, Varela JA, Lin JQ, et al. FUS phase separation is modulated by a molecular chaperone and methylation of arginine cation-π interactions. *Cell*. (2018) 173:720–34.e15. doi: 10.1016/j.cell.2018.03.056
187. Mertens J, Paquola ACM, Ku M, Hatch E, Böhnke L, Ladjevardi S, et al. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. *Cell Stem Cell*. (2015) 17:705–18. doi: 10.1016/j.stem.2015.09.001
188. Chatel G, Fahrenkrog B. Dynamics and diverse functions of nuclear pore complex proteins. *Nucleus*. (2012) 3:162–71. doi: 10.4161/nucl.19674
189. Kosyna F, Depping R. Controlling the gatekeeper: therapeutic targeting of nuclear transport. *Cells*. (2018) 7:221. doi: 10.3390/cells7110221
190. Zheng J, Yang J, Choe YJ, Hao X, Cao X, Zhao Q, et al. Role of the ribosomal quality control machinery in nucleocytoplasmic translocation of polyQ-expanded huntingtin exon-1. *Biochem Biophys Res Commun*. (2017) 493:708–17. doi: 10.1016/j.bbrc.2017.08.126
191. Archbold HC, Jackson KL, Arora A, Weskamp K, Tank EMH, Li X, et al. TDP43 nuclear export and neurodegeneration in models of amyotrophic lateral sclerosis and frontotemporal dementia. *Sci Rep*. (2018) 8:1–8. doi: 10.1038/s41598-018-22858-w
192. Kirli K, Karaca S, Dehne HJ, Samwer M, Pan KT, Lenz C, et al. A deep proteomics perspective on CRM1-mediated nuclear export and nucleocytoplasmic partitioning. *Elife*. (2015) 4:e11466. doi: 10.7554/eLife.11466
193. Clinicaltrails.gov. *An Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics Study of BIIB067 in Adults With Inherited Amyotrophic Lateral Sclerosis (ALS) - Full Text View* - ClinicalTrials.gov. US National Library *of* Medicine (2015).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

PERSPECTIVE
published: 29 September 2020
doi: 10.3389/fneur.2020.578720

Advances and Challenges in Understanding MicroRNA Function in Tauopathies: A Case Study of miR-132/212

Emmanuelle Boscher[1,2†], *Julia Hernandez-Rapp*[1,2†], *Serena Petry*[1,2], *Remi Keraudren*[1,2], *Sara Rainone*[1,2], *Andréanne Loiselle*[1,2], *Claudia Goupil*[1,2], *Andréanne Turgeon*[1,2], *Isabelle St-Amour*[1,2], *Emmanuel Planel*[1,2] *and Sébastien S. Hébert*[1,2*]

[1] *Axe Neurosciences, Centre de Recherche du CHU de Québec-Université Laval, Québec, QC, Canada,* [2] *Département de Psychiatrie et Neurosciences, Université Laval, Québec, QC, Canada*

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Ismael Santa-Maria Perez, Columbia University, United States
Jose Rodriguez Alvarez, Autonomous University of Barcelona, Spain

***Correspondence:**
Sébastien S. Hébert
sebastien.hebert@crchudequebec.ulaval.ca

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *01 July 2020*
Accepted: *01 September 2020*
Published: *29 September 2020*

Citation:
Boscher E, Hernandez-Rapp J, Petry S, Keraudren R, Rainone S, Loiselle A, Goupil C, Turgeon A, St-Amour I, Planel E and Hébert SS (2020) Advances and Challenges in Understanding MicroRNA Function in Tauopathies: A Case Study of miR-132/212. Front. Neurol. 11:578720. doi: 10.3389/fneur.2020.578720

In the past decade, several groups have reported that microRNAs (miRNAs) can participate in the regulation of tau protein at different levels, including its expression, alternative splicing, phosphorylation, and aggregation. These observations are significant, since the abnormal regulation and deposition of tau is associated with nearly 30 neurodegenerative disorders. Interestingly, miRNA profiles go awry in tauopathies such as Alzheimer's disease, progressive supranuclear palsy, and frontotemporal dementia. Understanding the role and impact of miRNAs on tau biology could therefore provide important insights into disease risk, diagnostics, and perhaps therapeutics. In this Perspective article, we discuss recent advances in miRNA research related to tau. While proof-of-principle studies hold promise, physiological validation remains limited. To help fill this gap, we describe herein a pure tauopathy mouse model deficient for the miR-132/212 cluster. This miRNA family is strongly downregulated in human tauopathies and shown to regulate tau *in vitro* and *in vivo*. No significant differences in survival, motor deficits or body weight were observed in PS19 mice lacking miR-132/212. Age-specific effects were seen on tau expression and phosphorylation but not aggregation. Moreover, various miR-132/212 targets previously implicated in tau modulation were unaffected (GSK-3β, Foxo3a, Mapk1, p300) or, unexpectedly, reduced (Mapk3, Foxo1, p300, Calpain 2) in miR-132/212-deficient PS19 mice. These observations highlight the challenges of miRNA research in living models, and current limitations of transgenic tau mouse models lacking functional miRNA binding sites. Based on these findings, we finally recommend different strategies to better understand the role of miRNAs in tau physiology and pathology.

Keywords: tau, microRNA, PS19, miR-132, tauopathies

INTRODUCTION

Tauopathies comprise a group of ∼30 neurodegenerative disorders characterized by the pathological accumulation of hyperphosphorylated and insoluble tau in neurons and/or glia (1). In humans, the *MAPT* gene encoding tau contains 16 exons, with the first exon as part of the promoter region and last exons comprising the 3′ untranslated region (3′UTR). The tau mRNA

transcript undergoes different steps of regulation including fine-tuning of expression, alternative splicing of exons 2, 3, and 10, and multiple levels of phosphorylation (1). These modifications play a central role in tau function related to the binding and stabilization of microtubules (2). While rare mutations in the *MAPT* gene underlie familial forms of disease (e.g., frontotemporal dementia with parkinsonism-17), the majority of tauopathies are sporadic and of unknown origin. The most prevalent tauopathy is Alzheimer's disease (AD), where tau aggregates into neurofibrillary tangles (NFTs) in conjunction with amyloid-β (Aβ) plaques (3). So far, it remains uncertain which mechanisms surrounding tau biology contribute to brain degeneration and clinical outcomes.

The small (∼21 nts) non-coding microRNAs (miRNAs) play a fundamental role in brain development, function, and survival (4, 5). They function as part of the endogenous RNA-induced silencing complex (RISC) to control protein output. This occurs by binding to mRNA transcripts within the 3′UTR to promote translational repression or mRNA degradation. Interestingly, the brain contains a rich repertoire of miRNAs, some of which go awry in tauopathies. While affected miRNAs have been associated with tau pathology in humans and animal models, the cause-consequence relationship between these factors remains ill defined. Nonetheless, specific miRNAs have emerged as promising diagnostic and therapeutic targets in tauopathies (6–8).

Several methods currently exist to study miRNA:mRNA interaction and biological function (9). These range from bioinformatic predictions, 3′UTR reporter assays, overexpression and inhibition studies, to cross-linking with immunoprecipitation (CLIP). The most common and straightforward approach is introducing a mutation within the miRNA target site (in particular the seed sequence) to inhibit miRNA:mRNA binding and gene expression regulation. This strategy is however quite challenging *in vivo* with only one known report in the mammalian brain (10), unrelated to tau. Most miRNA literature is therefore based on indirect or artificial paradigms that await physiological validation.

In this Perspective article, we provide an overview of advances related to the regulation of tau by miRNAs. As the reader will notice, the literature is promising but lacks consistency and *in vivo* validation. To aid in this effort, we also describe herein PS19 mice deficient for the miR-132/212 cluster. This is the first description of a pure tauopathy mouse model genetically deficient for specific miRNAs. Some paradoxical results obtained in this model prompted us to address certain "barriers" regarding experimental reproducibility and propose guidelines to help move forward this line of research.

PERSPECTIVE ARTICLE

Evidence That tau Is a MicroRNA Target

The human *MAPT* gene produces two 3′UTR isoforms of 256 and 4,163 nucleotides in length (11). The longest isoform is conserved and highly expressed in the brain (frontal cortex) (12). Previous studies have shown that different domains within the tau 3′UTR are important for mRNA structure, stability, and transport (13–15). Since tau is a dose-sensitive gene candidate (16, 17), and that its mis-regulation is associated with disease (18), it seems logical that different regulatory mechanisms have evolved to keep tau expression levels in check.

A simple search of common miRNA target site prediction programs (e.g., targetscan.org) reveals several conserved binding sites within the tau 3′UTR. Consistent with this, a handful of miRNAs have been shown to bind to tau mRNA, including miR-132 (19, 20), miR-34 (11), miR-186 (21), miR-219 (22), miR-362 (23), and miR-766 (23). Most groups have relied on 3′UTR luciferase reporter assays and mutagenesis to confirm gene expression regulation *in vitro*. One report could not confirm the interaction between miR-132 and its corresponding seed region however (11). Whether this is due to technical issues (type of mutagenesis, miRNA titration) or unknown regulatory mechanisms remains to be determined. Taken together, these observations provide strong evidence that tau is a *bone fide* miRNA target that now awaits *in vivo* validation using gene editing technologies. Interestingly, the ratio between tau 3′UTR isoforms seems to differ between healthy and AD brain (12, 24). Whether this results in altered miRNA regulation requires further investigation.

MicroRNA Regulation of tau Pre- and Post-translational Modifications: Unlimited Possibilities?

As stated above, alternative splicing and phosphorylation are key elements of tau regulation and function. Nearly 15 miRNAs have been implicated so far in the *indirect* modulation of tau (8, 25). These "tau modifier" genes are mostly kinases, and include Gsk-3β [miR-132 (26), miR-125b (27), miR-124 (28), miR-219 (29, 30), miR-138 (31)], Cdk5 [miR-125b (32, 33), miR-26b (34), miR-195 (35)], Erk [miR-125b (32)], Itpkb [miR-132 (36)], Fyn [miR-369 (37), miR-106b (38)], and Rock1 [miR-146a (39)]. Tau phosphatases include: Ppp1ca [miR-125b (32)] and Ptpn1 [miR-124 (40)]. Other genes *a priori* unrelated to kinases or phosphatases include p300 [miR-132 (26)], RbFox1 [miR-132 (26)], Nos1 [miR-132 (41)], BNDF [miR-322 (42)], RARα [miR-138 (31)], Cacna1c [miR-137 (43)], Uchl1 [miR-922 (44)], and HspB8 [miR-425 (45)].

The number of miRNA targets involved in tau splicing (exon 10) is more limited. These include Ptbp1 [miR-132 (19)] and yet unidentified genes [miR-124, miR-9, miR-153, miR-137 (19)]. Overall, while some miRNAs (miR-132, miR-125b, miR-124) and target genes (GSK-3β, Cdk5) seem recurrent, no clear pathway or trigger stands out and *in vivo* validation is again largely lacking. To study miRNAs in living organisms, especially in mammals, is particularly challenging since most tau modifier genes contain several miRNA-binding sites themselves (e.g., GSK-3β 3′UTR contains ∼100 putative miRNA sites). Thus, the amount of potential miRNA:mRNA networks surrounding tau biology seem almost limitless, at least in appearance.

Indeed, bioinformatics and *in vitro* paradigms need to be tested and experimentally validated using *in vivo* models

recapitulating the cellular and miRNA-target interaction networks occurring in human physiology and pathology. Furthermore, not all miRNAs modulated in cultured cells are biologically relevant since expressed at low or insufficient levels *in vivo* (as with their target counterparts). Also, not all target genes are sensitive to small changes in mRNA transcript or protein levels (46–48). This said, it is likely that only a limited number of miRNAs and dosage-sensitive genes are involved in the physiological regulation of tau (see also Barrier 2 below). This adds to other potential modes of miRNA action implicating competitive endogenous RNAs (ceRNA) (49) as well as cooperative binding and target site competition (50). An important step will be to identify the functional miRNA:mRNA pairs within the biological networks in brain cells.

A Mouse Model to Study miRNA Deficiency in Pure Tauopathies

Recent RNA deep-sequencing efforts have shown that ∼50–100 miRNAs are expressed at moderate to high levels in the mouse and human brain (51–53). Several of these are enriched in neurons, glia, or other cell types (54). Given the diversity of tau pathologies, selecting a candidate miRNA, and biological model for functional studies is a daunting task. Interestingly, accumulating studies highlight the potential biological importance of specific miRNAs. For example, loss of the miR-132/212 cluster shows a strong correlation with memory decline, NFTs, and Braak (tau pathological) stages in AD (20, 26, 51, 55–59). This cluster is also downregulated in other tauopathies such as frontotemporal dementia (60) and progressive supranuclear palsy (19). Deletion of the miR-132/212 cluster, or inhibition of neuron-specific miR-132, causes an increase in tau phosphorylation or aggregation in 3xTg-AD and APP/PS1 mice (36, 41, 61). Inversely, the brain delivery of miR-132 using viruses reduced tau pathologies in 3xTg-AD (61) and PS19 (26) mice. While promising, different target genes and underlying mechanisms have been proposed, and therefore the mode of action remains unresolved.

To further build on these findings, we investigated the effects of genetically removing the miR-132/212 cluster in PS19 mice, a model of pure tauopathy that overexpresses human tau with a mutation (P301S) that causes FTD in humans (62). This model develops motor deficits, tau deposition, and neuronal loss between 6 and 12 months, with a high mortality rate at late-stage disease.

We observed no significant differences in mouse survival (**Figure 1A**) and motor deficits (**Figure 1B**) following miR-132/212 deletion in PS19 mice (PS19 vs. PS19-KO). A trend for increased body weight was seen in 11–12-month-old PS19-KO females but not males (**Figure 1C**). A small but significant increase in tau expression was seen in pre-symptomatic (3 months) PS19-KO mice with a trend in aged (12 months) mice (**Figure 1D**). In contrast, tau phosphorylation at Ser422 and PHF1 (Ser396/Ser404) epitopes was reduced in aged PS19-KO mice (**Figure 1E**). This was not attributed to changes in body temperature known to influence tau phosphorylation (63) (**Figure 1F**). Non-significant trends were noticed in tau aggregation (sarkosyl-insoluble tau) in PS19-KO mice, owing to ∼20% of KO mice with more aggregates (**Figure 1G** and not shown). In addition, no or minimal effects were seen on different markers of brain integrity, including NeuN (neuron), Snap25 (presynaptic), PSD95 (postsynaptic), GFAP (astrocyte), and Iba1 (microglia) (**Figure 1H**). Taken together, the deletion of the miR-132/212 cluster had no major effects on disease phenotypes tested with divergent effects on tau biology.

We finally investigated a panel of miR-132/212 targets (other than tau) previously associated with tau metabolism or disease, including GSK-3β (26, 61), Mapk3/Erk1 (5, 64), Mapk1/Erk2 (5, 64), p300 (20, 26), Calpain 2 (26), Foxo1a (20), and Foxo3a (20, 26). We observed no significant differences caused by miR-132/212 deficiency on endogenous GSK-3β, Mapk1, and Foxo3a *in vivo* at all ages of study (**Figure 2A**). On the other hand, and unexpectedly, Mapk3, p300, Calpain2, and Foxo1a levels were reduced in PS19-KO mice, albeit at different ages (**Figure 2A**).

Of note, we could confirm the regulatory effects of ectopic miR-132 on all genes in cells (**Figure 2B**). Some cell-type specific effects were noticed, however, including an upregulation of p300 in miR-132-treated HEK293 cells. Representative miRNA qRT-PCR quantification and Western blots of targets are shown in **Figure 2C**. Inversely, inhibition of endogenous miR-132 in cells had only modest and sporadic effects on a subset of target genes (**Figure 2D**). Representative results are shown in **Figure 2E**. Clearly, some discrepancies exist in target gene regulation by miRNAs depending on models and methodological approach. Potential reasons for such paradoxes are discussed below.

Barrier 1: Animal Models

To date, nearly 40 rodent models are available to study different tau species, isoforms and mutations (Alzforum.org) (66). In all cases, the study of human (or humanized) tau is essential to model human pathologies including aggregation and deposition. To our knowledge, only two rodent models contain the complete human tau 3′UTR. These include hTau mice (67) and MAPT knock-in mice (68). Obviously, only these or similar models can validate the direct binding of miRNAs to human tau mRNA. Only a few studies have investigated the effects of miRNA deficiency or overexpression on endogenous murine tau expression with its native 3′UTR (36, 61), but with no direct confirmation using miRNA seed mutagenesis or other techniques. Unfortunately, no group has yet evaluated the contribution of the human tau 3′UTR in disease progression and pathology in mice or other animals.

Of note, most findings linking miRNAs with tau were obtained in mice with human Aβ pathology (3xTg-AD, APP/PS1, 5xFAD) (36, 39, 61, 69). This cannot be underestimated since miR-132/212 deletion promoted tau aggregation in 3xTg-AD (61) but not (robustly) in PS19 mice (**Figure 1G**). Of course, we cannot exclude all other distinct features of each model (e.g., backgrounds, promoters, transgenes, disease onset) including model-specific variations in gene expression networks. The fact that PS19-KO mice display also lower tau phosphorylation

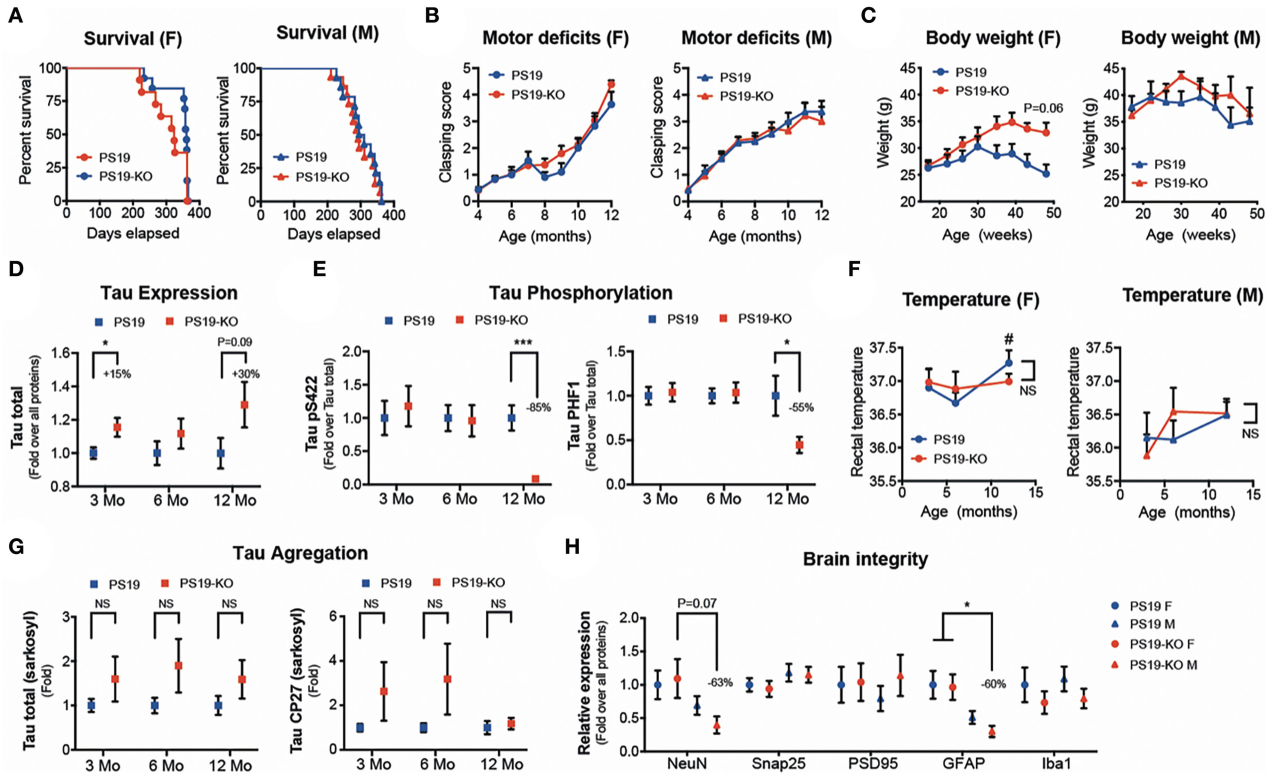


FIGURE 1 | Characterization of PS19 mice lacking the miR-132/212 cluster. **(A)** Kaplan-Meier survival curves, **(B)** clasping scores, and **(C)** body weight of PS19 and PS19-KO mice that are deficient for the miR-132/212 cluster. PS19 mice (JAX No. 008169) were bred with full miR-132/212 KO mice as before (61). Graphs were divided by sex. No significant changes were observed between mouse models. Kaplan-Meier and one-way ANOVA. $N = 6$–16 mice per group. **(D)** Western blot quantifications of cortical total tau expression and phosphorylation (Ser422 and PHF1 epitopes) **(E)** at different ages (3–12 months). $N = 12$–16 mice per group, mixed sex. Unpaired *t*-test, where $^{*}P < 0.05$, $^{***}P < 0.001$. **(F)** Rectal temperature at sacrifice. # denotes significant changes (multiple *t*-tests, $P < 0.05$) between PS19 males and females at 12 months. **(G)** Western blot quantifications of cortical sarkosyl-insoluble tau (Tau total and CP27) at different ages. $N = 9$–16 mice per group, mixed sex. Unpaired *t*-test. **(H)** Western blot quantifications of cortical endogenous NeuN, Snap25, PSD95, GFAP, and Iba1 in 12-month-old PS19 and PS19-KO mice. $N = 9$–10 mice per group, divided by sex. One-way ANOVA with multiple comparison, where $^{*}P < 0.05$. Error bars represent SEM. In **(D-G)**, the groups of mice were analyzed separately per age.

and reduced (instead of increased) expression of some miR-132 targets could be accountable to these and other factors as well, including regulatory feedback loops and the absence of the human tau 3′UTR. Fortunately, recent advances in gene editing technologies *in vivo* (e.g., CRISPR-Cas9) and humanized models (e.g., induced pluripotent stem cells, knock-in mice) can help us to address these issues. The observation that other miRNAs can modulate tau pathology in PS19 mice (40) also opens the door to independent validation studies, that is, taking into consideration the pros and cons of transgenic mice without a human tau 3′UTR.

Barrier 2: Physiological Regulation of tau and Other Targets

Proof-of-principle studies have shown that removing Dicer, the major enzyme responsible for miRNA maturation, induced significant changes in tau metabolism in both neurons (5, 19) and glia (70). However, this approach is largely inadequate to study single miRNAs and targets involved in tau regulation. The function of individual miRNAs is typically (and historically) inferred from overexpression studies in cells or animals, where a single miRNA can regulate tens (up to hundreds) of targets as predicted by bioinformatics tools. However, we know now that most overexpression studies do not reflect physiological context (47, 71–73). Indeed, they can result in the saturation of miRNA maturation products, induce off-target effects, or promote toxicity (74, 75). Lastly, and most importantly, gain-of-function (GOF) tells if a miRNA can exert a specific function, while loss-of-function (LOF) tests whether it is required for that function (72).

Interestingly, recent genetic inactivation studies in *C. elegans* suggest that some miRNAs function mainly through one or a limited number of master genes (76). Proof-of-principle exists in mice (for miR-155) but not yet in neuronal cells (77, 78). If true, most of the genes (modestly) influenced by miRNAs could be biologically "inactive" (47, 48). The results obtained herein *in vivo* (**Figure 2A**), often in theoretical disagreement with

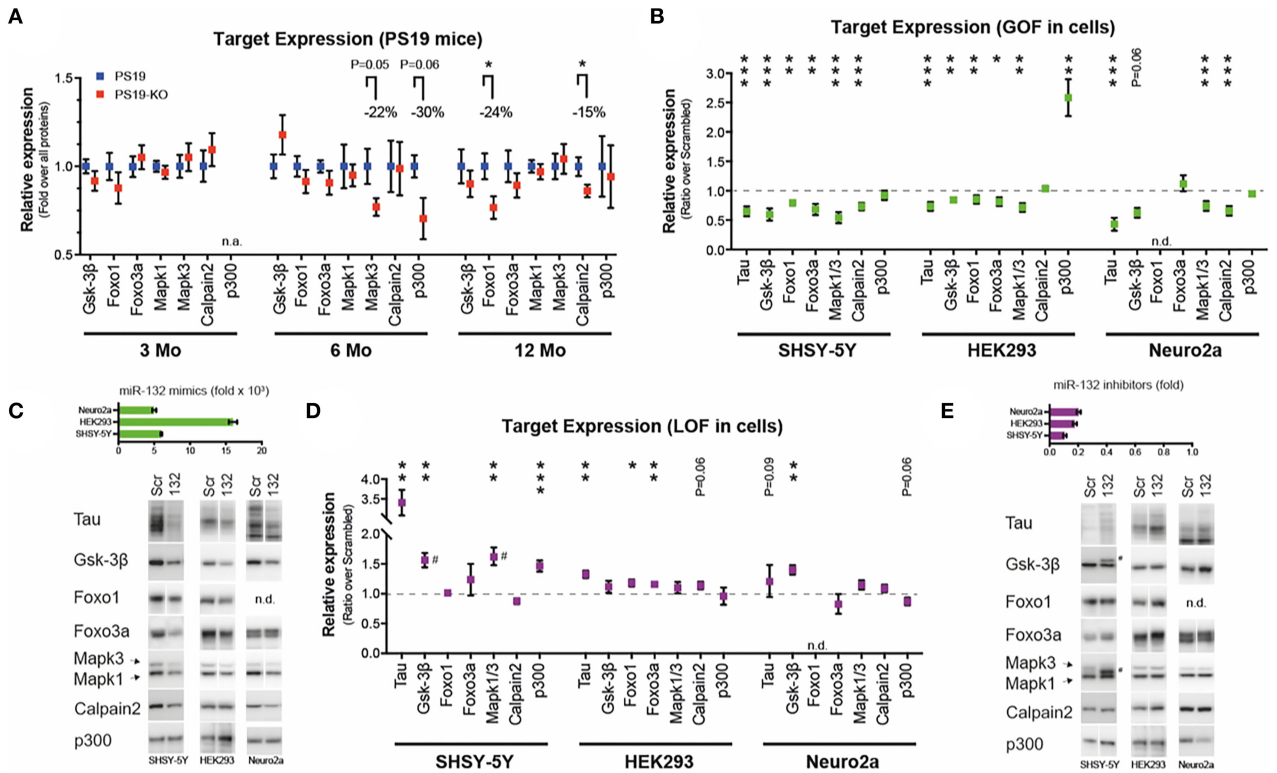


FIGURE 2 | Analysis of miR-132 targets *in vitro* and *in vivo*. **(A)** Western blot quantifications of endogenous miR-132 targets (Gsk-3β, Foxo1, Foxo3a, Mapk1/Erk2, Mapk3/Erk1, Calpain2, p300) in PS19 and PS19-KO mice at different ages in the cortex. All significant trends and changes indicate lower expression in KO mice. $N =$ 6–15 per group, mixed sex. Unpaired *t*-test, where $^{*}P < 0.05$. **(B)** Western blot quantification of endogenous miR-132 targets, including tau, in native human SHSY-5Y, human HEK293, and murine Neuro2a cells. Cells were treated with 50 nM miR-132 mimics or scrambled control for 48 h prior to protein extraction. $N =$ 2–4 experiments performed in triplicate. Results are shown as ratios between miR-132 over scrambled mimics (normalized to 1). Unpaired *t*-test, where $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. **(C)** Upper panel: Ectopic miR-132 levels measured by qRT-PCR in each cell line. Relative to scrambled mimic control. Lower panel. Representative western blot analyses. **(D)** Western blot quantifications of endogenous miR-132 targets. Here, cells were treated with 50 nM miR-132 inhibitors or scrambled control for 48 h. Unpaired *t*-test, where $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. **(E)** Upper panel: Endogenous miR-132 levels measured by qRT-PCR in each cell line following treatment. Relative to scrambled inhibitor control (normalized to 1). Lower panel. Representative western blot analyses. miRNA qRT-PCR were normalized using RNU19 as before (61, 64). Blots were normalized to all proteins using Ponceau or Stain-Free technology. # denotes possible alternate splicing (65) or phosphorylated form of targets. N.A., not available; N.D., not detected; GOF, gain-of-function; LOF, loss-of-function. Error bars represent SEM. In A, the groups of mice were analyzed separately per age. See **Supplementary Methods** for additional details.

the mode of miRNA function, is somewhat in agreement with this hypothesis, more so given the subtle or negligible effects on tau aggregation and mouse phenotypes in PS19-KO mice. Ideally, the selection of dosage-sensitive genes, combined with prior documented effects on tau *in vivo*, would be important for functional validation studies.

A variety of technical and other biological factors need also to be considered in the future, including cell-type specificity, spatiotemporal regulation, statistical power, and potential compensation mechanisms from development or aging. Other tau modifications (e.g., acetylation) (79) could also influence its processing, expression, and analysis using more conventional antibodies. Adapted tools and models are now required to fully understand the regulation of tau by individual miRNAs in its physiological milieu, and at the single cell level.

Barrier 3: Biological State and Context

There is increasing evidence that cell or biological context also influences miRNA function (80). The probability that miRNAs regulate tau differently according to the pathological state of the brain and peripheral system is therefore quite high. Contributing factors include inflammation, oxidative stress, immune response, and co-pathologies if present. Obviously, such systemic effects are difficult to reproduce in single animal models and even more in cells. And, of course, all of these factors influence miRNA expression to some degree. Interestingly, changes in the immune response and neuroinflammation are known to play a role on tau pathology in PS19 mice (62, 81). While some of these markers seem unaffected in PS19-KO mice (**Figure 1H**), a detailed analysis remains to be done. The challenge now is to decipher the individual and combined role of above-mentioned factors on miRNA-mediated tau pathology during disease onset.

Barrier 4: Cause or Consequence

Understanding why, where, and when miRNAs become misregulated in tauopathies is key to elucidating their function and potential use in diagnostics and therapeutics. Interestingly, changes in miRNA levels occur at all stages of disease in tauopathy mouse models. Examples include miR-142, miR-10, miR-146, miR-155, miR-455, and miR-211 in THY-Tau22 mice (82), miR-142 in Tg4510 mice (83), miR-132, miR-146a, miR-22, and miR-455 in hTau mice (84), and miR-132 in PS19 mice (26). These results suggest that human tau (wildtype or mutant) itself can promote miRNA changes in the mammalian brain. The link with human disease remains, at this stage, uncertain given that most mouse models tested so far (THY-Tau22, Tg4510, PS19) overexpress human mutant tau.

The identification of causal mutations or risk factors within miRNA genes or binding sites provides an alternative strategy to elucidate the cause and consequence relationship between tau and miRNAs in humans. So far, there is little evidence that MAPT 3′UTR polymorphisms are associated with AD risk (85). Nevertheless, a role for 3′UTR polymorphisms in other tauopathies cannot be excluded. Interestingly, a study recently identified a polymorphism within the miR-142 promoter that confers risk for AD (86). Given the complexity of MAPT haplotypes and their importance in disease risk (87), one cannot exclude also a role for miRNA-mediated regulation in this context.

Another strategy to determine causality involves a cure or relief of disease symptoms using miRNA therapeutics. MiRNA mimics can rescue in part disease-related phenotypes in AD and tauopathy mice (40, 61, 69). As inferred above, it will be important to define a therapeutic window for the *in vivo* use of miRNA oligonucleotides in humans. Note that a miRNA replacement therapy, with the goal of restoring physiological miRNA levels, could provide an attractive alternative to overexpression *per se*. Taking into consideration all published studies so far, candidate miRNAs for therapeutics include, but are not limited to, miR-132, miR-142, miR-219, miR-455, and miR-146.

CONCLUSION

To date, nearly 30 miRNAs have been implicated in the regulation of tau. The precise role and mode of action of individual miRNAs remains however unsettled. So far, the miR-132/212 cluster stands out for its potential role in regulating tau expression (11, 20, 26, 36, 61), splicing (19), acetylation (26), secretion (26, 88), proteolysis (26, 61), and aggregation (61). This adds to its correlation with disease progression and cognitive impairments in humans and mouse models. However, the choice of living model(s) and hypothesis(es) that need to be addressed are critical. Central elements of tau biology that have not yet been explored is the role of miRNAs in tau function (e.g., microtubule binding, DNA damage, gene expression, cell signaling) and propagation (e.g., spreading and seeding). The importance of miRNAs in familial tauopathies and many other sporadic tauopathies also remain unexplored. Clearly, much more work is needed to fully understand and appreciate the complexity of tau regulation by miRNAs and other non-coding RNAs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Requests should be directed to Sebastien.hebert@crchudequebec.ulaval.ca.

ETHICS STATEMENT

The animal study was reviewed and approved by CHU de Québec Research Center Ethics Committee.

AUTHOR CONTRIBUTIONS

EB, JH-R, SR, SP, AL, CG, AT, IS-A, and RK planned and performed the experiments. EP provided valuable material and helped with experimental planning and writing. SH designed, planned and supervised the experiments, and wrote the article. All authors discussed the results and contributed to the final manuscript.

FUNDING

This study was supported by the Canadian Institutes of Health Research (CIHR) and the Fonds de recherche du Québec Santé (FRQS), including a fellowship to JH-R and AT, and the Huntington's Disease Society of America (HDSA), including a fellowship to SP.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.578720/full#supplementary-material

REFERENCES

1. Sergeant N, Delacourte A, Buee L. Tau protein as a differential biomarker of tauopathies. *Biochim Biophys Acta.* (2005) 1739:179–97. doi: 10.1016/j.bbadis.2004.06.020
2. Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev.* (2000) 33:95–130. doi: 10.1016/S0165-0173(00)00019-9
3. Buee L, Delacourte A. [Tauopathy and alzheimer disease: a full degenerating process]. *Psychol Neuropsychiatr Vieil.* (2006) 4:261–73. doi: 10.1684/pnv.2006.0022
4. Follert P, Cremer H, Beclin C. MicroRNAs in brain development and function: a matter of flexibility and stability. *Front Mol Neurosci.* (2014) 7:5. doi: 10.3389/fnmol.2014.00005
5. Hebert SS, Papadopoulou AS, Smith P, Galas MC, Planel E, Silahtaroglu AN, et al. Genetic ablation of dicer in adult forebrain neurons results in abnormal

tau hyperphosphorylation and neurodegeneration. *Hum Mol Genet.* (2010) 19:3959–69. doi: 10.1093/hmg/ddq311
6. Junn E, Mouradian MM. MicroRNAs in neurodegenerative diseases and their therapeutic potential. *Pharmacol Ther.* (2012) 133:142–50. doi: 10.1016/j.pharmthera.2011.10.002
7. Coughlin D, Irwin DJ. Emerging diagnostic and therapeutic strategies for tauopathies. *Curr Neurol Neurosci Rep.* (2017) 17:72. doi: 10.1007/s11910-017-0779-1
8. Hebert SS, Sergeant N, Buee L. MicroRNAs and the regulation of tau metabolism. *Int J Alzheimer's Dis.* (2012) 2012:406561. doi: 10.1155/2012/406561
9. Thomson DW, Bracken CP, Goodall GJ. Experimental strategies for microRNA target identification. *Nucleic Acids Res.* (2011) 39:6845–53. doi: 10.1093/nar/gkr330
10. Eom TY, Han SB, Kim J, Blundon JA, Wang YD, Yu J, et al. Schizophrenia-related microdeletion causes defective ciliary motility and brain ventricle enlargement via microRNA-dependent mechanisms in mice. *Nat Commun.* (2020) 11:912. doi: 10.1038/s41467-020-14628-y
11. Dickson JR, Kruse C, Montagna DR, Finsen B, Wolfe MS. Alternative polyadenylation and miR-34 family members regulate tau expression. *J Neurochem.* (2013) 127:739–49. doi: 10.1111/jnc.12437
12. Goedert M, Wischik CM, Crowther RA, Walker JE, Klug A. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of alzheimer disease: identification as the microtubule-associated protein tau. *Proc Natl Acad Sci USA.* (1988) 85:4051–5. doi: 10.1073/pnas.85.11.4051
13. Aronov S, Marx R, Ginzburg I. Identification of 3'UTR region implicated in tau mRNA stabilization in neuronal cells. *J Mol Neurosci.* (1999) 12:131–45. doi: 10.1007/BF02736927
14. Aronov S, Aranda G, Behar L, Ginzburg I. Axonal tau mRNA localization coincides with tau protein in living neuronal cells and depends on axonal targeting signal. *J Neurosci.* (2001) 21:6577–87. doi: 10.1523/JNEUROSCI.21-17-06577.2001
15. Chen JL, Moss WN, Spencer A, Zhang P, Childs-Disney JL, Disney MD. The RNA encoding the microtubule-associated protein tau has extensive structure that affects its biology. *PLoS ONE.* (2019) 14:e0219210. doi: 10.1371/journal.pone.0219210
16. Rice AM, McLysaght A. Dosage sensitivity is a major determinant of human copy number variant pathogenicity. *Nat Commun.* (2017) 8:14366. doi: 10.1038/ncomms14366
17. Le Guennec K, Quenez O, Nicolas G, Wallon D, Rousseau S, Richard AC, et al. 17q21.31 duplication causes prominent tau-related dementia with increased MAPT expression. *Mol Psychiatry.* (2017) 22:1119–25. doi: 10.1038/mp.2016.226
18. Caillet-Boudin ML, Buee L, Sergeant N, Lefebvre B. Regulation of human MAPT gene expression. *Mol Neurodegener.* (2015) 10:28. doi: 10.1186/s13024-015-0025-8
19. Smith PY, Delay C, Girard J, Papon MA, Planel E, Sergeant N, et al. MicroRNA-132 loss is associated with tau exon 10 inclusion in progressive supranuclear palsy. *Hum Mol Genet.* (2011) 20:4016–24. doi: 10.1093/hmg/ddr330
20. Lau P, Bossers K, Janky R, Salta E, Frigerio CS, Barbash S, et al. Alteration of the microRNA network during the progression of alzheimer's disease. *EMBO Mol Med.* (2013) 5:1613–34. doi: 10.1002/emmm.201201974
21. Ye J, Zhang Z, Sun L, Fang Y, Xu X, Zhou G. miR-186 regulates chemosensitivity to paclitaxel via targeting MAPT in non-small cell lung cancer (NSCLC). *Mol Biosyst.* (2016) 12:3417–24. doi: 10.1039/C6MB00576D
22. Santa-Maria I, Alaniz ME, Renwick N, Cela C, Fulga TA, Van Vactor D, et al. Dysregulation of microRNA-219 promotes neurodegeneration through post-transcriptional regulation of tau. *J Clin Invest.* (2015) 125:681–6. doi: 10.1172/JCI78421
23. Cai Y, Jia R, Xiong H, Ren Q, Zuo W, Lin T, et al. Integrative gene expression profiling reveals that dysregulated triple microRNAs confer paclitaxel resistance in non-small cell lung cancer via co-targeting MAPT. *Cancer Manag Res.* (2019) 11:7391–404. doi: 10.2147/CMAR.S215427
24. Garcia-Escudero V, Gargini R, Martin-Maestro P, Garcia E, Garcia-Escudero R, Avila J. Tau mRNA 3'UTR-to-CDS ratio is increased in alzheimer disease. *Neurosci Lett.* (2017) 655:101–8. doi: 10.1016/j.neulet.2017.07.007
25. Bazrgar M, Khodabakhsh P, Mohagheghi F, Prudencio M, Ahmadiani A. Brain microRNAs dysregulation: Implication for missplicing and abnormal post-translational modifications of tau protein in alzheimer's disease and related tauopathies. *Pharmacol Res.* (2020) 155:104729. doi: 10.1016/j.phrs.2020.104729
26. El Fatimy R, Li S, Chen Z, Mushannen T, Gongala S, Wei Z, et al. MicroRNA-132 provides neuroprotection for tauopathies via multiple signaling pathways. *Acta Neuropathol.* (2018) 136:537–55. doi: 10.1007/s00401-018-1880-5
27. Zhang L, Dong H, Si Y, Wu N, Cao H, Mei B, et al. miR-125b promotes tau phosphorylation by targeting the neural cell adhesion molecule in neuropathological progression. *Neurobiol Aging.* (2019) 73:41–9. doi: 10.1016/j.neurobiolaging.2018.09.011
28. Kang Q, Xiang Y, Li D, Liang J, Zhang X, Zhou F, et al. MiR-124-3p attenuates hyperphosphorylation of Tau protein-induced apoptosis via caveolin-1-PI3K/Akt/GSK3beta pathway in N2a/APP695swe cells. *Oncotarget.* (2017) 8:24314–26. doi: 10.18632/oncotarget.15149
29. Li J, Chen W, Yi Y, Tong Q. miR-219-5p inhibits tau phosphorylation by targeting TTBK1 and GSK-3beta in alzheimer's disease. *J Cell Biochem.* (2019) 120:9936–46. doi: 10.1002/jcb.28276
30. Arnes MK, Lannes YA, Alaniz J, Cho ME, McCabe JD, Santa-Maria BD, et al. MiR-219 deficiency in alzheimer's disease contributes to neurodegeneration and memory dysfunction through post-transcriptional regulation of tau-kinase network. *bioRxiv*. doi: 10.1101/607176
31. Wang X, Tan L, Lu Y, Peng J, Zhu Y, Zhang Y, et al. MicroRNA-138 promotes tau phosphorylation by targeting retinoic acid receptor alpha. *FEBS Lett.* (2015) 589:726–9. doi: 10.1016/j.febslet.2015.02.001
32. Banzhaf-Strathmann J, Benito E, May S, Arzberger T, Tahirovic S, Kretzschmar H, et al. MicroRNA-125b induces tau hyperphosphorylation and cognitive deficits in alzheimer's disease. *EMBO J.* (2014) 33:1667–80. doi: 10.15252/embj.201387576
33. Ma X, Liu L, Meng J. MicroRNA-125b promotes neurons cell apoptosis and Tau phosphorylation in alzheimer's disease. *Neurosci Lett.* (2017) 661:57–62. doi: 10.1016/j.neulet.2017.09.043
34. Absalon S, Kochanek DM, Raghavan V, Krichevsky AM. MiR-26b, upregulated in alzheimer's disease, activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons. *J Neurosci.* (2013) 33:14645–59. doi: 10.1523/JNEUROSCI.1327-13.2013
35. Sun LH, Ban T, Liu CD, Chen QX, Wang X, Yan ML, et al. Activation of Cdk5/p25 and tau phosphorylation following chronic brain hypoperfusion in rats involves microRNA-195 down-regulation. *J Neurochem.* (2015) 134:1139–51. doi: 10.1111/jnc.13212
36. Salta E, Sierksma A, Vanden Eynden E, De Strooper B. miR-132 loss de-represses ITPKB and aggravates amyloid and TAU pathology in alzheimer's brain. *EMBO Mol Med.* (2016) 8:1005–18. doi: 10.15252/emmm.201606520
37. Yao X, Xian X, Fang M, Fan S, Li W. Loss of miR-369 promotes tau phosphorylation by targeting the fyn and serine/threonine-protein kinase 2 signaling pathways in alzheimer's disease mice. *Front Aging Neurosci.* (2019) 11:365. doi: 10.3389/fnagi.2019.00365
38. Liu W, Zhao J, Lu G. miR-106b inhibits tau phosphorylation at Tyr18 by targeting Fyn in a model of alzheimer's disease. *Biochem Biophys Res Commun.* (2016) 478:852–7. doi: 10.1016/j.bbrc.2016.08.037
39. Wang G, Huang Y, Wang LL, Zhang YF, Xu J, Zhou Y, et al. MicroRNA-146a suppresses ROCK1 allowing hyperphosphorylation of tau in alzheimer's disease. *Sci Rep.* (2016) 6:26697. doi: 10.1038/srep26697
40. Hou TY, Zhou Y, Zhu LS, Wang X, Pang P, Wang DQ, et al. Correcting abnormalities in miR-124/PTPN1 signaling rescues tau pathology in alzheimer's disease. *J Neurochem.* (2020) 154:441–57. doi: 10.1111/jnc.14961
41. Wang Y, Veremeyko T, Wong AH, El Fatimy R, Wei Z, Cai W, et al. Downregulation of miR-132/212 impairs S-nitrosylation balance and induces tau phosphorylation in alzheimer's disease. *Neurobiol Aging.* (2017) 51:156–66. doi: 10.1016/j.neurobiolaging.2016.12.015
42. Zhang J, Liu Z, Pei Y, Yang W, Xie C, Long S. MicroRNA-322 cluster promotes tau phosphorylation via targeting brain-derived neurotrophic factor. *Neurochem Res.* (2018) 43:736–44. doi: 10.1007/s11064-018-2475-1
43. Jiang Y, Xu B, Chen J, Sui Y, Ren L, Li J, et al. Micro-RNA-137 inhibits tau hyperphosphorylation in alzheimer's disease and targets the CACNA1C gene in transgenic mice and human neuroblastoma SH-SY5Y cells. *Med Sci Monit.* (2018) 24:5635–44. doi: 10.12659/MSM.908765

44. Zhao ZB, Wu L, Xiong R, Wang LL, Zhang B, Wang C, et al. MicroRNA-922 promotes tau phosphorylation by downregulating ubiquitin carboxy-terminal hydrolase L1 (UCHL1) expression in the pathogenesis of alzheimer's disease. *Neuroscience.* (2014) 275:232–7. doi: 10.1016/j.neuroscience.2014.06.013
45. Yuan J, Wu Y, Li L, Liu C. MicroRNA-425-5p promotes tau phosphorylation and cell apoptosis in alzheimer's disease by targeting heat shock protein B8. *J Neural Transm.* (2020) 127:339–46. doi: 10.1007/s00702-019-02134-5
46. Bosson AD, Zamudio JR, Sharp PA. Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. *Mol Cell.* (2014) 56:347–59. doi: 10.1016/j.molcel.2014.09.018
47. Natalia Pinzón BL, Martinez L, Sergeeva A, Presumey J, Apparailly F. The number of biologically relevant microRNA targets has been largely overestimated. *Genome Res.* (2017) 26:1–11. doi: 10.1101/gr.205146.116
48. Pinzon N, Li B, Martinez L, Sergeeva A, Presumey J, Apparailly F, et al. microRNA target prediction programs predict many false positives. *Genome Res.* (2017) 27:234–45. doi: 10.1101/gr.205146.116
49. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the rosetta stone of a hidden RNA language? *Cell.* (2011) 146:353–8. doi: 10.1016/j.cell.2011.07.014
50. Denzler R, McGeary SE, Title AC, Agarwal V, Bartel DP, Stoffel M. Impact of MicroRNA levels, target-site complementarity, and cooperativity on competing endogenous RNA-regulated gene expression. *Mol Cell.* (2016) 64:565–79. doi: 10.1016/j.molcel.2016.09.027
51. Hebert SS, Wang WX, Zhu Q, Nelson PT. A study of small RNAs from cerebral neocortex of pathology-verified alzheimer's disease, dementia with lewy bodies, hippocampal sclerosis, frontotemporal lobar dementia, and non-demented human controls. *J Alzheimer's Dis.* (2013) 35:335–48. doi: 10.3233/JAD-122350
52. Babiarz JE, Hsu R, Melton C, Thomas M, Ullian EM, Blelloch R. A role for noncanonical microRNAs in the mammalian brain revealed by phenotypic differences in Dgcr8 versus Dicer1 knockouts and small RNA sequencing. *RNA.* (2011) 17:1489–501. doi: 10.1261/rna.2442211
53. Hebert SS, Nelson PT. Studying microRNAs in the brain: technical lessons learned from the first ten years. *Exp Neurol.* (2011) 235:397–401. doi: 10.1016/j.expneurol.2011.12.004
54. Pomper N, Liu Y, Hoye ML, Dougherty JD, Miller TM. CNS microRNA profiles: a database for cell type enriched microRNA expression across the mouse central nervous system. *Sci Rep.* (2020) 10:4921. doi: 10.1038/s41598-020-61307-5
55. Wong HK, Veremeyko T, Patel N, Lemere CA, Walsh DM, Esau C, et al. De-repression of FOXO3a death axis by microRNA-132 and−212 causes neuronal apoptosis in alzheimer's disease. *Hum Mol Genet.* (2013) 22:3077–92. doi: 10.1093/hmg/ddt164
56. Pichler S, Gu W, Hartl D, Gasparoni G, Leidinger P, Keller A, et al. The miRNome of alzheimer's disease: consistent downregulation of the miR-132/212 cluster. *Neurobiol Aging.* (2017) 50:167.e1–e10. doi: 10.1016/j.neurobiolaging.2016.09.019
57. Annese A, Manzari C, Lionetti C, Picardi E, Horner DS, Chiara M, et al. Whole transcriptome profiling of late-onset alzheimer's disease patients provides insights into the molecular changes involved in the disease. *Sci Rep.* (2018) 8:4282. doi: 10.1038/s41598-018-22701-2
58. Patrick E, Rajagopal S, Wong HA, McCabe C, Xu J, Tang A, et al. Dissecting the role of non-coding RNAs in the accumulation of amyloid and tau neuropathologies in alzheimer's disease. *Mol Neurodegener.* (2017) 12:51. doi: 10.1186/s13024-017-0191-y
59. Zhu QB, Unmehopa U, Bossers K, Hu YT, Verwer R, Balesar R, et al. MicroRNA-132 and early growth response-1 in nucleus basalis of meynert during the course of alzheimer's disease. *Brain.* (2016) 139:908–21. doi: 10.1093/brain/awv383
60. Chen-Plotkin AS, Unger TL, Gallagher MD, Bill E, Kwong LK, Volpicelli-Daley L, et al. TMEM106B, the risk gene for frontotemporal dementia, is regulated by the microRNA-132/212 cluster and affects progranulin pathways. *J Neurosci.* (2012) 32:11213–27. doi: 10.1523/JNEUROSCI.0521-12.2012
61. Smith PY, Hernandez-Rapp J, Jolivette F, Lecours C, Bisht K, Goupil C, et al. miR-132/212 deficiency impairs tau metabolism and promotes pathological aggregation *in vivo*. *Hum Mol Genet.* (2015) 24:6721–36. doi: 10.1093/hmg/ddv377
62. Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* (2007) 53:337–51. doi: 10.1016/j.neuron.2007.01.010
63. El Khoury NB, Gratuze M, Petry F, Papon MA, Julien C, Marcouiller F, et al. Hypothermia mediates age-dependent increase of tau phosphorylation in db/db mice. *Neurobiol Dis.* (2016) 88:55–65. doi: 10.1016/j.nbd.2016.01.005
64. Hernandez-Rapp J, Rainone S, Goupil C, Dorval V, Smith PY, Saint-Pierre M, et al. microRNA-132/212 deficiency enhances Abeta production and senile plaque deposition in alzheimer's disease triple transgenic mice. *Sci Rep.* (2016) 6:30953. doi: 10.1038/srep30953
65. Mukai F, Ishiguro K, Sano Y, Fujita SC. Alternative splicing isoform of tau protein kinase I/glycogen synthase kinase 3beta. *J Neurochem.* (2002) 81:1073–83. doi: 10.1046/j.1471-4159.2002.00918.x
66. Dujardin S, Colin M, Buee L. Invited review: animal models of tauopathies and their implications for research/translation into the clinic. *Neuropathol Appl Neurobiol.* (2015) 41:59–80. doi: 10.1111/nan.12200
67. Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, et al. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem.* (2003) 86:582–90. doi: 10.1046/j.1471-4159.2003.01879.x
68. Saito T, Mihira N, Matsuba Y, Sasaguri H, Hashimoto S, Narasimhan S, et al. Humanization of the entire murine mapt gene provides a murine model of pathological human tau propagation. *J Biol Chem.* (2019) 294:12754–65. doi: 10.1074/jbc.RA119.009487
69. Mai H, Fan W, Wang Y, Cai Y, Li X, Chen F, et al. Intranasal administration of miR-146a agomir rescued the pathological process and cognitive impairment in an AD mouse model. *Mol Ther Nucleic Acids.* (2019) 18:681–95. doi: 10.1016/j.omtn.2019.10.002
70. Kodama L, Guzman E, Etchegaray JI, Li Y, Sayed FA, Zhou L, et al. Microglial microRNAs mediate sex-specific responses to tau pathology. *Nat Neurosci.* (2020) 23:167–71. doi: 10.1038/s41593-019-0560-7
71. Jin HY, Oda H, Chen P, Yang C, Zhou X, Kang SG, et al. Differential sensitivity of target genes to translational repression by miR-17∼92. *PLoS Genet.* (2017) 13:e1006623. doi: 10.1371/journal.pgen.1006623
72. Vidigal JA, Ventura A. The biological functions of miRNAs: lessons from *in vivo* studies. *Trends Cell Biol.* (2015) 25:137–47. doi: 10.1016/j.tcb.2014.11.004
73. Jin J, Kim SN, Liu X, Zhang H, Zhang C, Seo JS, et al. miR-17-92 cluster regulates adult hippocampal neurogenesis, anxiety, and depression. *Cell Rep.* (2016) 16:1653–63. doi: 10.1016/j.celrep.2016.06.101
74. Jin HY, Gonzalez-Martin A, Miletic AV, Lai M, Knight S, Sabouri-Ghomi M, et al. Transfection of microRNA mimics should be used with caution. *Front Genet.* (2015) 6:340. doi: 10.3389/fgene.2015.00340
75. Merhautova J, Demlova R, Slaby O. MicroRNA-based therapy in animal models of selected gastrointestinal cancers. *Front Pharmacol.* (2016) 7:329. doi: 10.3389/fphar.2016.00329
76. Park CY, Choi YS, McManus MT. Analysis of microRNA knockouts in mice. *Hum Mol Genet.* (2010) 19:R169–75. doi: 10.1093/hmg/ddq367
77. Dorsett Y, McBride KM, Jankovic M, Gazumyan A, Thai TH, Robbiani DF, et al. MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc-Igh translocation. *Immunity.* (2008) 28:630–8. doi: 10.1016/j.immuni.2008.04.002
78. Teng G, Hakimpour P, Landgraf P, Rice A, Tuschl T, Casellas R, et al. MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. *Immunity.* (2008) 28:621–9. doi: 10.1016/j.immuni.2008.03.015
79. Gorsky MK, Burnouf S, Sofola-Adesakin O, Dols J, Augustin H, Weigelt CM, et al. Pseudo-acetylation of multiple sites on human Tau proteins alters Tau phosphorylation and microtubule binding, and ameliorates amyloid beta toxicity. *Sci Rep.* (2017) 7:9984. doi: 10.1038/s41598-017-10225-0
80. Erhard F, Haas J, Lieber D, Malterer G, Jaskiewicz L, Zavolan M, et al. Widespread context dependency of microRNA-mediated regulation. *Genome Res.* (2014) 24:906–19. doi: 10.1101/gr.166702.113
81. Leyns CEG, Ulrich JD, Finn MB, Stewart FR, Koscal LJ, Remolina Serrano J, et al. TREM2 deficiency attenuates neuroinflammation and protects against neurodegeneration in a mouse model of tauopathy. *Proc Natl Acad Sci USA.* (2017) 114:11524–9. doi: 10.1073/pnas.1710311114

82. Sierksma A, Lu A, Salta E, Vanden Eynden E, Callaerts-Vegh Z, D'Hooge R, et al. Deregulation of neuronal miRNAs induced by amyloid-beta or TAU pathology. *Mol Neurodegener.* (2018) 13:54. doi: 10.1186/s13024-018-0285-1
83. Sharma S, Khadimallah I, Corya AW, Ali YO, Rao X, Liu Y, et al. Presymptomatic change in microRNAs modulates Tau pathology. *Sci Rep.* (2018) 8:9251. doi: 10.1038/s41598-018-27527-6
84. Lauretti E, Dincer O, Pratico D. Regional and temporal miRNAs expression profile in a transgenic mouse model of tauopathy: implication for its pathogenesis. *Mol Psychiatry.* (2020) doi: 10.1038/s41380-020-0655-2
85. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. *Nat Genet.* (2019) 51:414–30. doi: 10.1038/s41588-019-0358-2
86. Ghanbari M, Munshi ST, Ma B, Lendemeijer B, Bansal S, Adams HH, et al. A functional variant in the miR-142 promoter modulating its expression and conferring risk of alzheimer disease. *Hum Mutat.* (2019) 40:2131–45. doi: 10.1002/humu.23872
87. Santa-Maria I, Haggiagi A, Liu X, Wasserscheid J, Nelson PT, Dewar K, et al. The MAPT H1 haplotype is associated with tangle-predominant dementia. *Acta Neuropathol.* (2012) 124:693–704. doi: 10.1007/s00401-012-1017-1
88. Cha DJ, Mengel D, Mustapic M, Liu W, Selkoe DJ, Kapogiannis D, et al. miR-212 and miR-132 are downregulated in neurally derived plasma exosomes of alzheimer's patients. *Front Neurosci.* (2019) 13:1208. doi: 10.3389/fnins.2019.01208

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 30 September 2020
doi: 10.3389/fneur.2020.579434

Tau Oligomers and Fibrils Exhibit Differential Patterns of Seeding and Association With RNA Binding Proteins

Lulu Jiang[1†], *Jian Zhao*[2†], *Ji-Xin Cheng*[2,3] *and Benjamin Wolozin*[1,3,4,5*]

[1] Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA, United States, [2] Boston University Photonics Center, Boston, MA, United States, [3] Center for Neurophotonics, Boston University School of Medicine, Boston, MA, United States, [4] Department of Neurology, Boston University School of Medicine, Boston, MA, United States, [5] Center for Systems Neuroscience, Boston University School of Medicine, Boston, MA, United States

OPEN ACCESS

Edited by:
Sonia Do Carmo,
McGill University, Canada

Reviewed by:
Anita H. Corbett,
Emory University, United States
Rakez Kayed,
University of Texas Medical Branch at Galveston, United States
Nicholas T. Seyfried,
Emory University, United States

***Correspondence:**
Benjamin Wolozin
bwolozin@bu.edu

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: 02 July 2020
Accepted: 01 September 2020
Published: 30 September 2020

Citation:
Jiang L, Zhao J, Cheng J-X and Wolozin B (2020) Tau Oligomers and Fibrils Exhibit Differential Patterns of Seeding and Association With RNA Binding Proteins. Front. Neurol. 11:579434. doi: 10.3389/fneur.2020.579434

Tau aggregates are pleiotropic and exhibit differences in conformation, structure, and size. These aggregates develop endogenously but are also propagated among neurons in disease. We explored the actions of two distinct types of tau aggregates, tau oligomers (oTau) and tau fibrils (fTau), using a seeding assay in primary neuron cultures expressing human 4R0N tau. We find that oTau and fTau elicit distinct patterns of tau inclusions in the neurons and distinct molecular interactions. The exogenously applied oTau and fTau both clear rapidly from the neurons, but both also seed intracellular inclusions composed of endogenously produced tau. The two types of seeds elicit differential dose–response relationships for seed uptake and the number of resulting intracellular inclusions. Immunocytochemical studies show that co-localization with RNA binding proteins associated with stress granules is much greater for seeds composed of oTau than fTau. Conversely, co-localization with p62/SQSTM1 and thioflavine S is much greater for fTau than oTau. These results suggest that oTau seeds inclusions that modulate the translational stress response and are physiologically active, whereas fTau seeds inclusions that are fibrillar and shunted to the autolysosomal cascade.

Keywords: tau oligomer, neurofibrillary tangle (NFT), image proceeding, RNA translation, stress, stress granules, TIA1, PABP

INTRODUCTION

The microtubule association protein tau is one of the principal components of pathology in Alzheimer's disease and related disorders (ADRD) (1). Tau exists primarily in the axon under basal conditions, with smaller amounts in the dendritic arbor. However, stress induces phosphorylation of tau and localization to the somato-dendritic compartment (2, 3). As the stress persists, the tau begins to accumulate as oligomers and then fibrils, ultimately forming neurofibrillary tangles, which are a pathological hallmark of Alzheimer's disease and other tauopathies.

Our understanding of the patterns and properties of tau aggregates has evolved steadily. *In vitro* studies showed that tau exhibits an intrinsic affinity for RNA, an anionic agent, and a tendency to fibrillize in the presence of anionic agents, including RNA, heparin sulfate, dextran sulfate, and arachidonic acid (4–6). Aggregation of purified tau with anionic agents

leads to an ordered assembly of tau into oligomers and then fibrils, with formation of oligomers thought to be rate limiting (7). However, the physiology of tau is quite different because stress induces phosphorylation of tau at proline directed serines and threonines, which accelerates oligomerization and subsequent fibrillization, and also leads to the somatodendritic accumulation of tau (8, 9).

Studies increasingly reveal remarkable differences in the properties of tau aggregates. The evidence showing that tau conformations differ among diseases is perhaps most evident in the multiple Cryo-EM studies that have been recently published, showing the structural differences among fibrils isolated from brains of subjects with Alzheimer's disease, Pick's disease, corticobasal disease, and chronic traumatic encephalopathy (10–14). Although tau fibrils are the pathological hallmarks of disease, some studies suggest that they are end-stage species that might not be the actual tau aggregate responsible for toxicity and neurodegeneration. Studies in transgenic mouse models of tauopathy demonstrate that neurofibrillary tangles do not induce degeneration on their own, and that neurons lacking neurofibrillary pathology exhibit more functional deficits than neurons containing neurofibrillary pathology (15–17).

Seeding and propagation studies provide a powerful means to compare the biological actions of different conformations of tau aggregates. Studies of tau propagation demonstrate the presence of strains of tau aggregates that differ in their pattern of propagation (18). Direct comparison of tau oligomers and fibrils using an *in vivo* propagation assay demonstrates that both species propagate robustly but that oligomeric tau (oTau) elicits much more neurodegeneration (19). This study also showed a striking association between tau oligomers and RNA binding protein (RBP) pathology (19).

Multiple studies demonstrate associations between tau and elements of RNA metabolism, including RBPs and ribosomal proteins (20–27). Tau binds to ribosomal proteins (23, 24, 28). Tau plays a role in the nucleolus, possibly regulating ribosome biogenesis during stress (29). Tau regulates the formation of stress granules (SGs), which are an important element of the translational stress response, and reducing TIA1, a core SG nucleating RBP, delays progression in a mouse model of tauopathy (20, 21). The link between tau and stress is particularly important because stress induces hyperphosphorylation, oligomerization, and somatodendritic translation of tau, and stress is an inherent element of the disease process. In contrast, tau fibrillization evolves more slowly and is less tightly linked to physiological responses to stress. These observations support a hypothesis that species such as tau oligomers function as part of an integrated stress response and affect neurons in a manner that differs from tau fibrils.

The current study directly compares the actions of oTau and fibrillar tau (fTau) using a seeding assay in primary neuron cultures expressing human 4R0N tau (tau containing 4 repeats in the microtubule binding domain and 0 repeats in the amino-terminal domain). We find oTau and fTau elicit distinct patterns of tau inclusions in the neurons even though the exogenously applied oTau and fTau clear rapidly from the neurons. We also observe that oTau inclusions co-localize strongly with RBPs but exhibit little thioflavine S reactivity, whereas fTau inclusions co-localize strongly with thioflavine S reactivity but weakly with RBPs. These results point to a distinct biological activity of oTau.

MATERIALS AND METHODS

Animals

Use of all animals was approved by the Boston University Institutional and Animal Care and Use Committee. All animals were housed in an IACUC-approved vivarium at Boston University School of Medicine. Breeders of P301S/PS19 mice were obtained from the Jackson Lab (stock no. 008169). Generation F2 mice were bred in-house and aged to 9 months for experiment. Timed pregnant C57BL/6 were purchased from Charles River Laboratories and delivered at E-14, and then the postnatal P0 pups were used for primary hippocampal cultures.

S1p and P3 Fractions Extraction From PS19 Brain Tissue

The frozen hippocampus and cortex tissues (100–250 mg) were weighed and placed in a Beckman polycarbonate thick-wall centrifuge tube (cat no. 362305). A 10 × volume of homogenization buffer was then added to homogenize brain tissue with TBS buffer (50 mM Tris, pH 8.0, 274 mM NaCl, 5 mM KCl) supplemented with protease and phosphatase inhibitor cocktails (Roche, cat no. 05892791001 and cat no. 04906837001), as described previously (20). The homogenate was then ultracentrifuged at 28,000 rpm for 20 min at 4°C. After that, the supernatant was aliquoted to new microfuge tubes as S1 fraction (TBS-soluble). Then the pellet (P1 fraction) was homogenized with buffer B (10 mM Tris, pH 7.4, 800 mM NaCl, 10% sucrose, 1 mM EGTA, 1 mM PMSF), which was ∼5× volume of wet weight of the original tissue. The P1 homogenate was then ultracentrifuged at 22,000 rpm for 20 min at 4 °C. Next, the supernatant (S2 fraction) was aliquoted to a new Beckman polycarbonate thick-wall tube and incubated with 1% Sarkosyl rotating in the bench top thermomixer at 37°C for 1 h. After the incubation, the fraction mix was ultracentrifuged at 55,000 rpm for 1 h at 4°C. Then the sarkosyl-insoluble pellet (P3 fraction) was resuspended with 50 μl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). For the extraction of S1p fraction, the supernatant (S1) fractions were ultracentrifuged a second time at 55,000 rpm at 4°C for 40 min. The TBS-extractable pellet (S1p) fractions were then resuspended in 4 × volume of TE buffer relative to the starting weight of the tissue homogenate.

The molecular weight of tau in these two fractions (S1p and P3) was documented by native polyacrylamide gel electrophoresis as described previously (19). Briefly, the concentration of total tau was measured by immunoblot using 3–12% reducing SDS-PAGE gel by comparison with gradient concentrations of recombinant tau ladders, using the tau-5 antibody (detecting total tau) by immunoblot. All the fractions were then normalized and divided into fractions of 20 μg/ml tau for storage and future use.

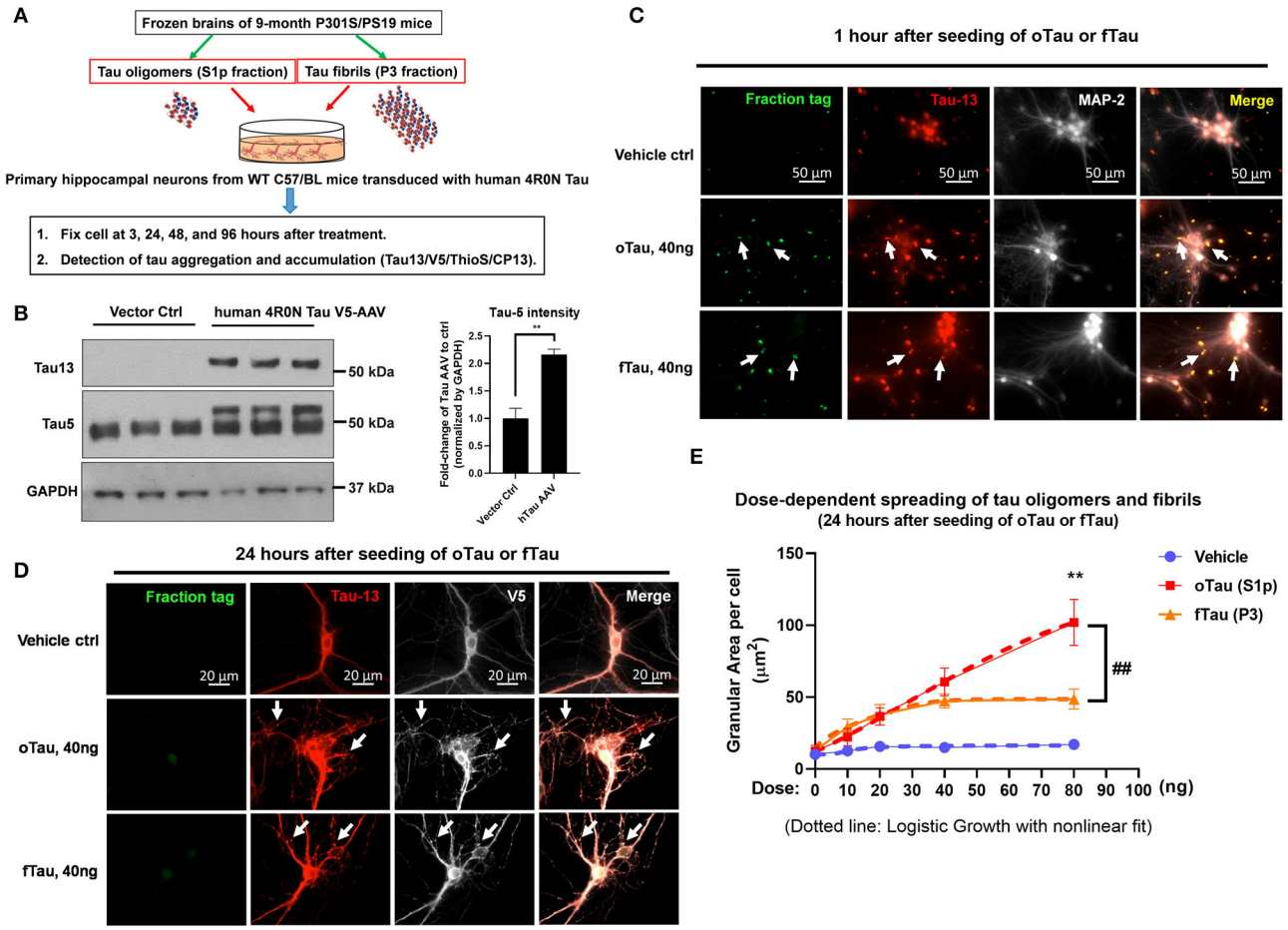


FIGURE 1 | The uptake and self-templating of tau oligomers and fibrils in primary neurons. **(A)** Scheme of experiment design with primary cultures, including the preparation of tau oligomers and fibrils, cell culture plating, transduction, treatment, and harvest in a time course. **(B)** Immunoblot with Tau5 and Tau13 antibody was used to confirm the expression of human tau in transfected C57 neurons. Data are shown as mean ± SEM, data analysis was by two-tailed *t*-test, $^{**}p < 0.01$. **(C)** Detection for the uptake of DyLight-488 conjugated tau oligomers and fibrils by recipient neurons after 1 h of treatment. The neurons were washed three times with PBS before fixed by 4% PFA and subjected to immunofluorescence labeling. Green is the DyLight-488 conjugated tau oligomers and fibrils. Red is the Tau13 antibody labeled human tau, including the fractions added and the overexpressed human tau in the recipient neurons. Bright is the MAP-2 to label neuronal cells. Scale bar 50 μm. **(D)** The self-templating of tau aggregates in the recipient neurons. Detection for the uptake of DyLight-488 conjugated tau oligomers and fibrils by recipient neurons after 24 h of treatment. At 24 h after the treatment of DyLight-488 conjugated tau oligomers and fibrils, the cells were washed with PBS three times followed by fixation with 4% PFA. Then the digestion of DyLight-488 conjugated tau oligomers and fibrils as well as the self-templating of tau aggregates in the recipient neurons were detected by immunofluorescence labeling. Green is the DyLight-488 conjugated tau oligomers and fibrils. Red is the Tau13 antibody labeled human tau, including the fractions added and the overexpressed human tau in the recipient neurons. Bright is the V5-tagged human tau in the recipients. Scale bar 20 μm. **(E)** The quantification for the dose response of tau oligomer and fibril uptake in the recipient neurons. The dosing was arranged at 0, 10, 20, 40, and 80 ng of tau in each tau oligomer or fibril fractions. The dotted line showed the logistic growth of the accumulated tau oligomers and fibrils with non-linear fit. Data are shown as mean ± SEM, data analysis was by two-way ANOVA, multiple comparison test by Fisher's LSD, $^{**}p < 0.01$ in comparison with oTau and fTau dosing groups. ##$p < 0.01$ in comparison with the growth line between oTau and fTau dosing groups.

Primary Hippocampal Culture With P0 Pups

The sterilized 12-mm coverslips were placed into each well of a 24-well-plate and then coated with 1 mg/ml poly-D-lysine for 1 h at room temperature in the culture hood. Then the plates were washed three times with sterile biology-grade water and dried in hood overnight. For the dissection of hippocampus from P0 pups, the pups were anesthetized via hypothermia by wrapping in gauze and placing in aluminum foil pouch on ice. After the brain was isolated from the skull, the meninges need to be completely removed from the brain tissue and unfurl the hippocampus. Then all the hippocampi were transferred into 15-ml conical tubes with 5 ml 0.25% Trypsin–EDTA supplemented with 150 μl DNase. The brain tissue was incubated in a 37 °C water bath for 15 min before being resuspended and triturated in 2 ml plating medium (MEM Gibco no. 11090, 2.5% FBS, 1× penicillin/streptomycin, L-glutamine, 0.6% D-glucose). Then the cells were passed through a 70-μm cell strain before cell count. A total of 60,000 cells/coverslip were plated in 80 μl medium (7.5×10^5 cells/ml) for a 24-well plate. Thirty minutes later, 1 ml of feeding medium (Neurobasal media, 1 × B27

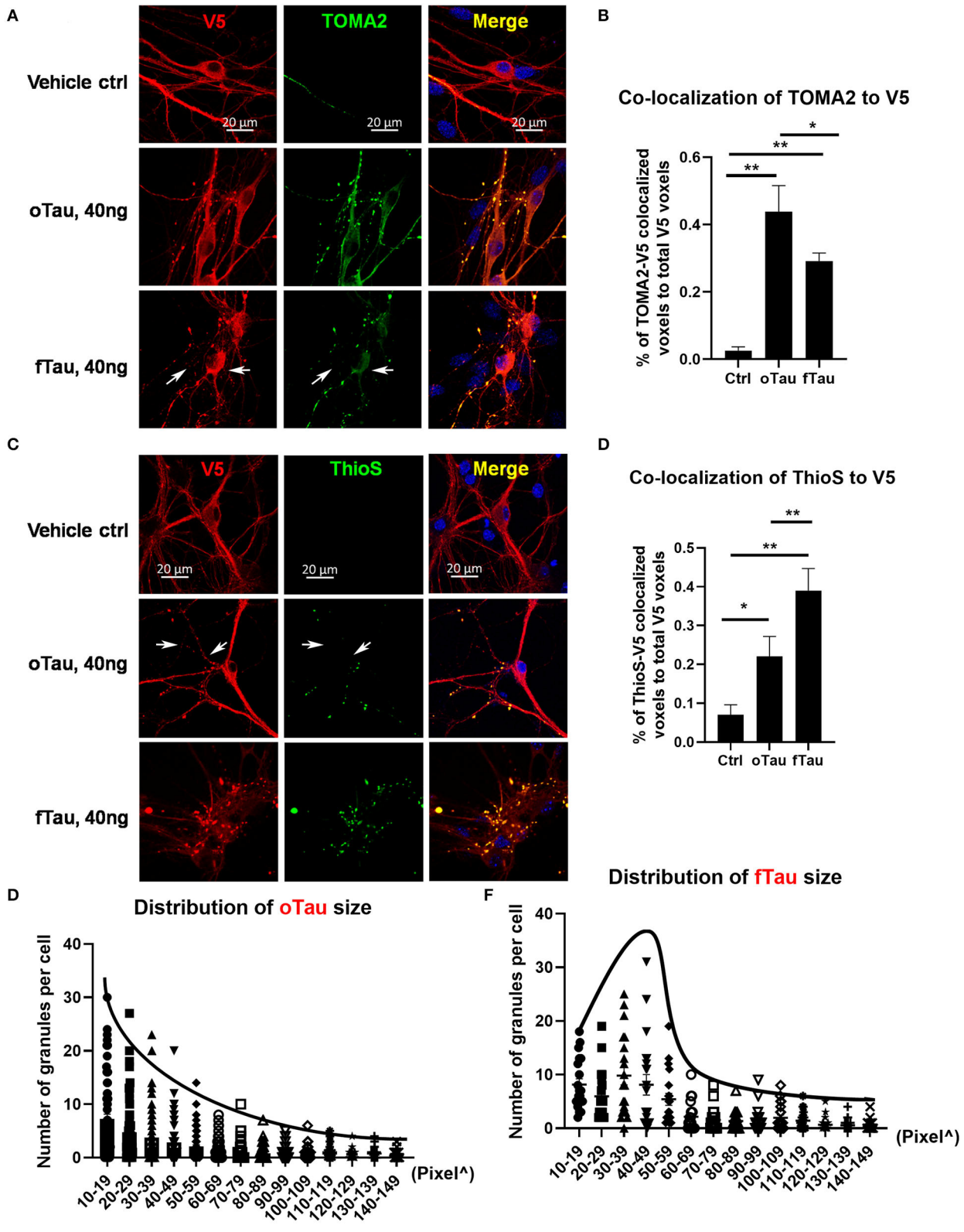


FIGURE 2 | oTau seeds propagate into small tau inclusions while fTau seeds propagate into large tau inclusions. **(A,B)** The hippocampal neurons were treated with oTau (S1p) or fTau (P3) fractions for 24 h. Co-labeling of human tau (V5 tagged, red) in recipient neurons and the oligomeric tau marker TOMA2 (green) showed that

(Continued)

FIGURE 2 | seeding of oTau induced accumulation of oligomeric tau, which is significantly higher than in the fTau group. The white arrows point to V5-tagged inclusions present in the fTau group that showed little or no labeling with TOMA2. Data are shown as mean ± SEM, $N = 20$, data analysis was by one-way ANOVA, multiple comparison test by Fisher's LSD, $^{**}p < 0.01$. Scale bar 20 μm. **(C,D)** The hippocampal neurons were treated with oTau (S1p) or fTau (P3) fractions for 24 h. Co-labeling of human tau (V5 tagged, red) in recipient neurons and the fibril tau marker Thioflavine S (green) showed that seeding of fTau induced accumulation of fibril tau, which is significantly higher than in the oTau group. The white arrows point to V5-tagged inclusions present in the oTau group that showed little or no labeling with Thioflavine S. Data are shown as mean ± SEM, $N = 20$, data analysis was by one-way ANOVA, multiple comparison test by Fisher's LSD, $^{*}p < 0.05$, $^{**}p < 0.01$. Scale bar 20 μm. **(E,F)** Analysis of the size distribution of the propagated tau oligomers and fibrils (V5-positive inclusions).

supplement, 1 × penicillin/streptomycin, 1 × L-glutamine) was added into each well.

Cell Transduction

For cell transduction, at day 2 of cell culture, neurons were transduced with AAV1 vectors of human 4R0N WT tau at MOI 200. The culture medium was replaced ∼1/2 volume of feeding media every 3–4 days for cell maintenance until ready to use for experiment on days 14 to 21.

oTau (S1p) and fTau (P3) Fraction Treatments

S1p and P3 stock solution (20 μg/ml) were diluted in feeding medium for each well in 24-well plates and added into the cells by completely replacing the old medium. Then the cells were fixed by 4% PFA or snap frozen in a time series (3, 24, 48, 96 h) for further analysis.

Immunofluorescence Labeling of Fixed Primary Culture

Cells on a 24-well coverslip were fixed with 0.5 ml 4% paraformaldehyde (PFA)/phosphate buffered saline (PBS) for 15 min followed by washing three times in PBS. The cells were then permeabilized in 0.5 ml PBS/0.1% Triton X-100 (PBST) for 15–30 min. Blocking was done in 0.5 ml of 5% bovine serum albumin (BSA)−5% donkey serum in PBST for 1 h. The primary antibodies were diluted in 5% BSA/PBST and incubated with the cells overnight at 4 °C. On the 2nd day, the neurons were continued to be incubated in secondary antibodies, which were diluted in 5% BSA/PBST, for 2 h at room temperature (RT). All the secondary antibodies were purchased from Thermo Fisher Scientific made in donkey and used for 1:800 dilution in labeling. After secondary antibody, cells were incubated in DAPI diluted 1:10,000 in PBST (5 mg/ml stock solution) for 5 min after first wash. After washing three times with PBS, coverslips were mounted onto glass microscope slides using 8–10 μl Prolong Gold Antifade mounting media (Thermo Fisher Scientific, cat no. P36930) per coverslip. Slides were naturally dried in fume hood (or stored at 4°C until ready to dry in fume hood). The primary antibodies used in this study for immunocytochemistry are as follows: MAP-2 (chicken, AVES, MAP, 1: 250), MAP-2 (rabbit, Millipore, AB5622, 1:1000), V5 (rabbit, Sigma-Aldrich, V8137, 1:1000), CP-13 (mouse, provided by Peter Davies, 1:300), TIA1 (rabbit, Abcam, ab40693, specifically lot no. GR3202325-1, 1:400), PABP (rabbit, Abcam, ab21060, 1:400), EIF3η (rabbit, Santa Cruz, sc-137214, 1:400), p62 (rabbit, Thermo Fisher Scientific, PA5-27247, 1:400), TOMA2 (mouse, provided by Dr. Rakez Kayed, 1:300), and Tau13 (mouse, provided by Dr. Nicholas M. Kanaan from Michigan State University, 1:1000). Images were captured by a Zeiss Axio Observer microscope in **Figures 1**, **3**, **5**. Images were captured by Zeiss LSM700 confocal microscope in **Figures 2**, **4**.

Immunoblot

For p62 detection in primary culture, cell lysates were collected from frozen cultures with RIPA lysis buffer. Then 40 μg of reducing and non-reducing protein samples was separated by gel electrophoresis and transferred to 0.2-μm nitrocellulose membranes using the Bolt SDS-PAGE system (Life Technologies). Membranes were blocked in 5% non-fat dry milk in PBS supplemented with 0.025% Tween-20 (PBST) for 1 h at RT, followed by incubation overnight at 4°C in primary antibody diluted in 5% BSA/PBST. Primary antibodies used were as follows: Tau13 (mouse, provided by Dr. Nicholas M. Kanaan from Michigan State University, 1:5000); Tau 5 (mouse, provided by Dr. Nicholas M. Kanaan from Michigan State University, 1:500); p62 polyclonal antibody (Invitrogen, cat no. PA5-27247); GAPDH polyclonal antibody (Thermo Fisher Scientific, cat no. PA1987). Membranes were then washed three times with PBST and incubated in horseradish peroxidase–conjugated secondary antibodies (Jackson ImmunoResearch) diluted in 1% BSA/PBST at RT for 1 h. After incubation in secondary antibody, membranes were washed three times in PBST and developed using SuperSignal West Pico Chemiluminescent ECL substrate (Thermo Fisher Scientific, cat no. 34080).

Image Analysis

To quantify Tau protein aggregates distribution as well as evaluate the colocalization of the Tau protein and the TIA1 stress granules, we developed a customized MATLAB program to process large amounts of immunofluorescence images automatically (**Supplementary Figure 1**). This algorithm takes the red-fluorescent image that mainly labels the Tau oligomers and fibrils, and has the MATLAB program process the imaging data according to the following procedures: (1) the single-color RGB image is converted to a grayscale image with a bit depth of 8; (2) the noisy background and features are filtered out through the thresholding and the morphological operation; (3) the grayscale image is then converted to a binary image, and the residual filament structures, mostly from the axons, are further removed from this binary image; (4) based on post-processed binary images above, the distribution of Tau protein locations and sizes are calculated, extracted, and exported into a Microsoft Excel file for further statistical analysis. For evaluation of the Tau protein/TIA1 (or PABP, EIF3η) stress granules colocalization, the algorithm processes

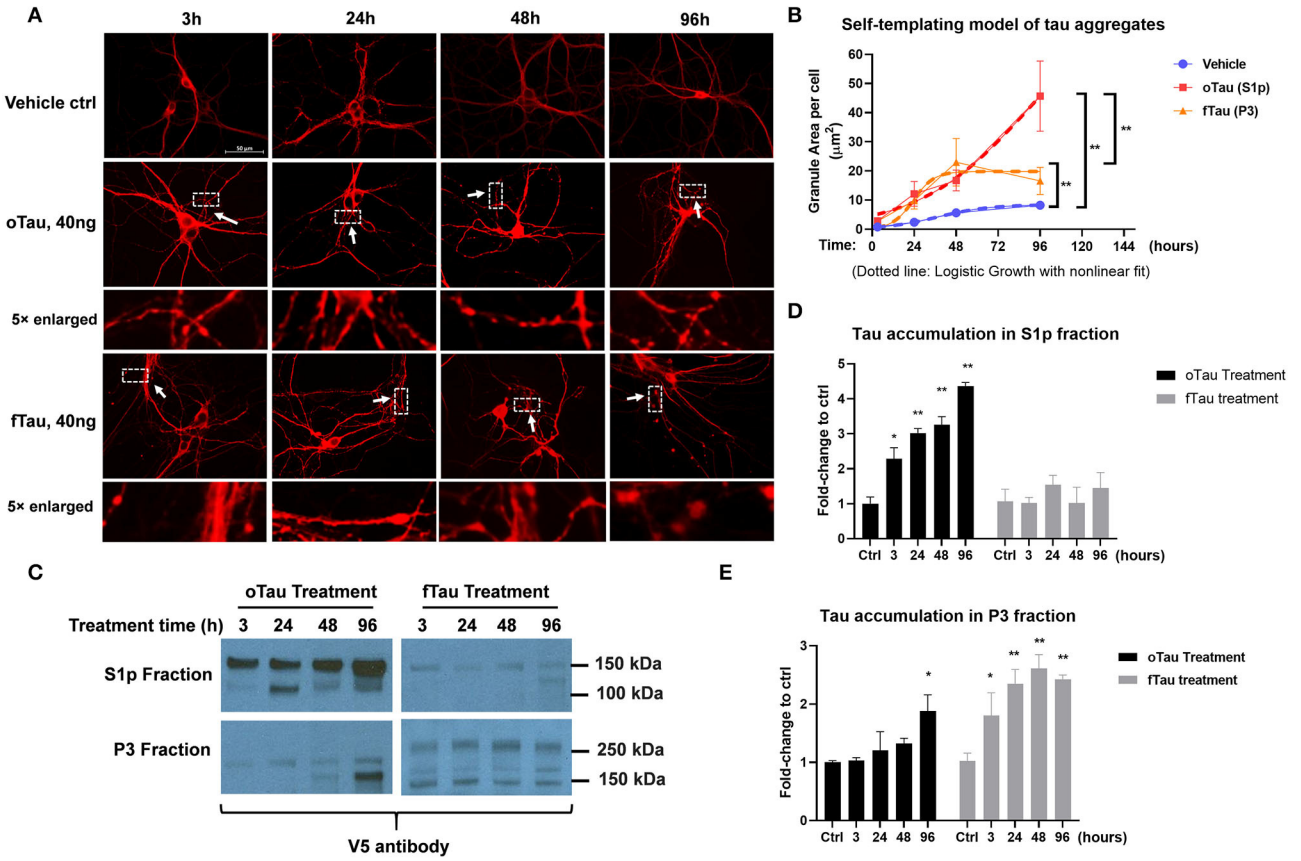


FIGURE 3 | oTau and fTau displayed distinctive propagation cycle in the recipient neurons. **(A)** The representative images of CP13 labeling showed the accumulation of tau inclusions after 3, 24, 48, and 96 h of oTau or fTau seeding. The arrows and boxes highlight regions that are magnified 5-fold immediately below. Scale bar 50 μm. **(B)** Quantification for the accumulation of tau inclusions after seeding of oTau and fTau in a time course of 24, 48, and 96 h after seeding. Dotted lines are the logistic growth with non-linear fit of oTau and fTau in the time course. Data are shown as mean ± SEM, $N = 30$, data analysis was by two-way ANOVA, multiple comparison test by Fisher's LSD, $^{**}p < 0.01$. **(C)** Immunoblots by V5 antibody after fractionation of S1p and P3 from cells treated with the exogenous seeds oTau or fTau. Cell lysate was collected at 3, 24, 48, and 96 h of oTau or fTau seeding. After extraction of S1p and P3 fractions, 10 μg protein was loaded to the gel for V5-positive tau detection. **(D,E)** Immunoblot quantification of V5-positive tau in S1p and P3 fraction of hippocampal cultures overexpressing human 4R0N WT tau, treated with vehicle control, oTau, or fTau on day 14, and harvested at 3, 24, 48, and 96 h after treatment. Data are shown as mean ± SEM, $N = 3$, data analysis was done by two-way ANOVA, multiple comparison test by Fisher's LSD, $^{*}p < 0.05$, $^{**}p < 0.01$ in comparison with vehicle control.

the red-fluorescent images using the same program to obtain a post-processed binary image. The algorithm also converts the green-fluorescent images into another binary map. Then, the algorithm calculates the overlapping between these two binary images, which determines the percentage of colocalization. There are several predefined parameters applied in the MATLAB programs, which are optimized using iterative testing.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 6.00 for Windows with two-sided α of 0.05. All group data are expressed as mean ± SEM. Column means were compared using one-way ANOVA with treatment as the independent variable. Also, group means were compared using two-way ANOVA with factors on genotype and fraction treatments, respectively. When ANOVA showed a significant difference, pairwise comparisons between group means were examined by Tukey, Dunnett, or uncorrected Fisher's LSD multiple comparison test.

RESULTS

Seeding of Exogenous Oligomeric Tau (oTau) and Fibrillar Tau (fTau) Fractions in Recipient Primary Neurons

The experimental is shown in **Figure 1A**. We extracted the S1p (mainly containing oligomeric tau) and P3 fractions (mainly containing fibrillar tau) from PS19 P301S mice brain aged 9 months. The primary hippocampal neurons were plated on a 24-well plate and transduced with 4R0N human Tau AAV1 on day 2. Overexpression of the 4R0N human tau was utilized to create an abundant pool of endogenous tau that might facilitate propagation. On day 14 of cell culture when the neurons are mature, the cells were treated with S1p (oTau) or

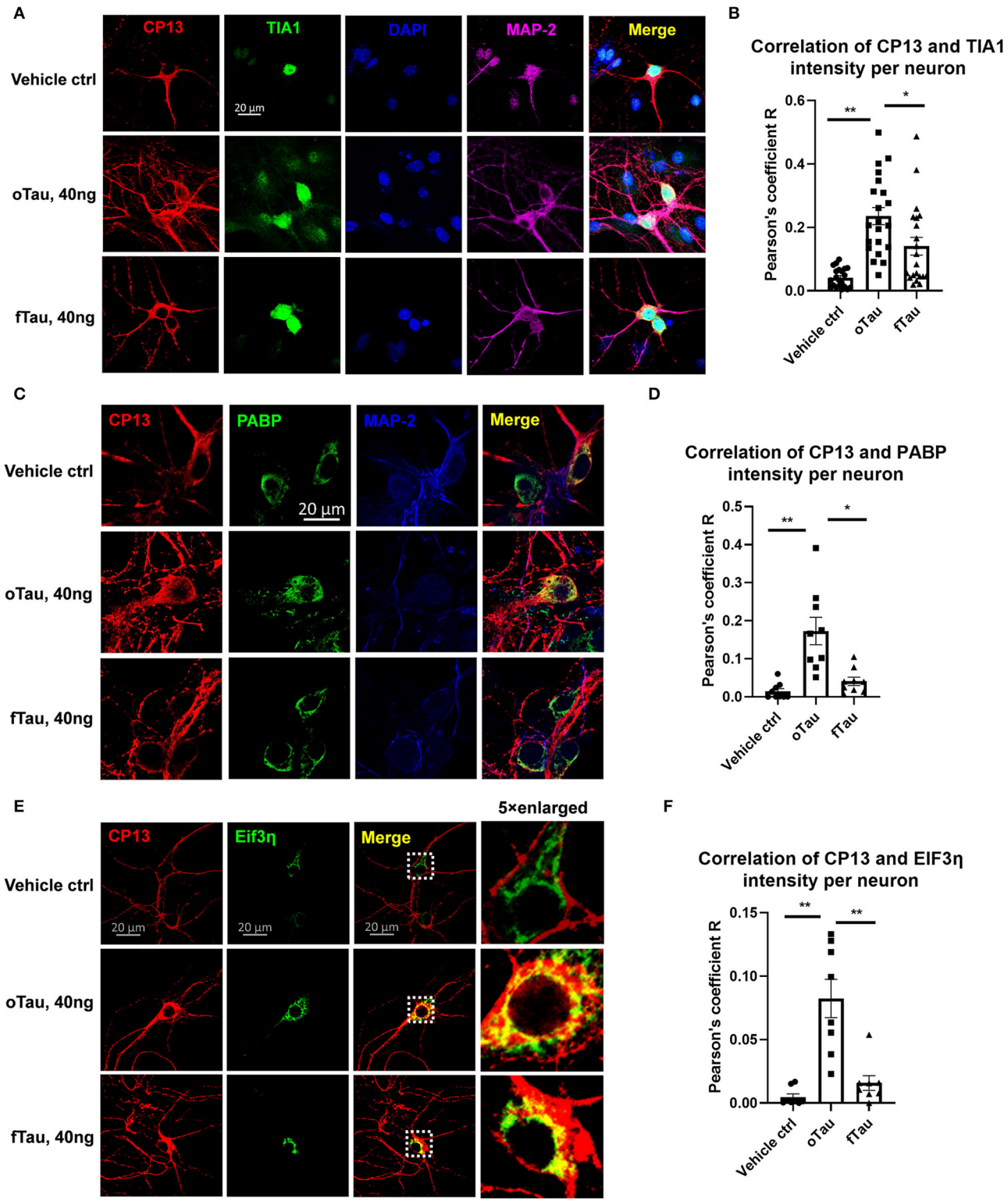


FIGURE 4 | Co-localization of small tau inclusions with stress granules. **(A)** Representative images showing the co-localization of phosphorylated tau inclusions CP13 (red) with TIA1 granules (green) at 3 h after oTau, fTau, or vehicle treatment in hippocampal neurons overexpressing human 4R0N tau. Co-stained markers are MAP-2 (violet) for neuron and DAPI (blue) for nucleus. Scale bar 20 μm. **(B)** Pearson coefficient of correlation between CP13-positive tau with RBP TIA1 is graphed for

(Continued)

FIGURE 4 | individual neurons using ImageJ. Data are shown as mean ± SEM, $N = 20$, data analysis was by one-way ANOVA, multiple comparison test by Fisher's LSD, $^{*}p < 0.05$, $^{**}p < 0.01$ in comparison with vehicle control. **(C)** Representative images showing the co-localization of phosphorylated tau inclusions CP13 (red) with PABP granules (green) at 3 h after oTau, fTau, or vehicle treatment in hippocampal neurons overexpressing human 4R0N tau. Co-stained marker is MAP-2 (blue) for neuron. Scale bar 20 μm. **(D)** Pearson coefficient of correlation between CP13-positive tau with RBP PABP is graphed for individual neurons using ImageJ. Data are shown as mean ± SEM, $N = 10$, data analysis was done by one-way ANOVA, multiple comparison test by Fisher's LSD, $^{*}p < 0.05$, $^{**}p < 0.01$ in comparison with vehicle control. **(E)** Representative images showing the co-localization of phosphorylated tau inclusions CP13 (red) with EIF3η granules (green) at 3 h after oTau, fTau, or vehicle treatment in hippocampal neurons overexpressing human 4R0N tau. The right panel is the 5 × enlarged images to show the detail of co-localization. Scale bar 20 μm. **(F)** Pearson coefficient of correlation between CP13-positive tau with EIF3η is graphed for individual neurons using ImageJ. Data are shown as mean ± SEM, $N = 10$, data analysis was done by one-way ANOVA, multiple comparison test by Fisher's LSD, $^{**}p < 0.01$ in comparison with vehicle control.

P3 (fTau) fractions dosing from 10 to 80 ng, and harvested at the indicated time point for the experiment presented in this article (**Figure 1A**). Immunoblot with Tau5 and Tau13 antibody confirmed the expression of human tau in transfected C57 neurons (**Figure 1B**).

To detect the uptake of tau fractions by recipient neurons, the cells were fixed at 1 h after DyLight-488 conjugated tau oligomers or fibril fractions were introduced into the culture medium. The result showed that both oTau and fTau were effectively taken up by neurons (**Figure 1C**, green); immunocytochemistry with the anti-human tau antibody, Tau13, showed the physical relationship of the exogenous tau to the endogenous human 4R0N tau expressed in these neurons (**Figure 1C**, red). Next, we repeated the experiment but the primary hippocampal neurons were harvested after 24 h to characterize potential propagation of tau fractions in the recipient neurons. Tau was identified in these cultures by labeling with antibody to human tau (tau13) as well as antibody to a V5 tag, which is also present as part of the chimeric human 4R0N tau expressed in these neurons. Fluorescence from the exogenously applied DyLight-488 conjugated tau oligomers and fibrils had largely faded by 24 h after application (**Figure 1D**). However, at 24 h, both the S1p and P3-treated groups showed the appearance of abundant V5 positive tau inclusions, corresponding to endogenously produced V5 tagged human tau (**Figure 1D**). Hippocampal neuron cultures exposed to vehicle exhibited equally strong V5 labeling, but had very smooth labeling without the appearance of inclusions (**Figure 1D**).

We proceeded to test the dose–response relationship comparing the dose of exogenous tau applied (oTau or fTau) and the resulting level of inclusion formation within the neuron at 24 h. The area per cell covered by the granular tau inclusions at 24 h were quantified after treatment with 0, 10, 20, 40, and 80 ng of oTau or fTau (**Figure 1E**). For fTau, formation of intracellular tau granules increased up to the 40 ng dose but not beyond (**Figure 1E**), whereas formation of intracellular tau granules in response to oTau increased linearly at all doses (**Figure 1E**). Application of non-linear curve fitting by logistic growth analysis showed plateauing for the fTau binding curve, which suggests that the seeding of fTau is limited by the interaction with the rate-limiting binding site or receptor-mediated entry pathway. In contrast, seeding by oTau occurs through a mechanism involving either a high abundance binding site or an entry pathway that does not require binding to a specific receptor (**Figure 1E**).

oTau Fractions Seed Small Tau Inclusions While fTau Seed Large Tau Inclusions

We proceeded to compare the distribution of oligomeric, TOMA2-positive tau inclusions with fibrillar, ThioS-positive tau inclusion after exposure to oTau or fTau. The oTau-specific antibody TOMA2 was used to map the distribution of tau oligomers in the recipient neurons at 24 h after tau seeding of oTau or fTau (**Figure 2A**). V5 antibody was used to label the total tau expression and calculate the fraction of tau inclusions that were TOMA2 positive. These results showed that oTau seeded higher fraction TOMA2-positive tau oligomers than fTau (**Figure 2B**). Meanwhile, Thioflavine S and V5 co-labeling was performed to detect the fraction of tau inclusions that exhibited fibrillar tau (**Figure 2C**). These results showed that fTau fraction seeded a higher fraction of fibrillar tau than did oTau (**Figure 2D**).

We also quantified the size distribution of the tau inclusions in the recipient neurons after 24 h of oTau or fTau seeding. We observed that the number of tau inclusions induced by oTau was highest at the smallest diameters and steadily decreased with size (**Figure 2E**). In contrast, inclusion induction by fTau peaked at a larger size, with fewer inclusions at the very small or very large size range (**Figure 2F**).

oTau and fTau Displayed Distinctive Seeding Cycles in the Recipient Neurons

To determine the replication cycle of tau oligomers and fibrils in the recipient neurons, we seeded the neurons with 40 ng of oTau or fTau, which is a dose that produces similar levels of seeding at 24 h. We then harvested cells over a time course of 3, 24, 48, and 96 h after treatment. The hippocampal neurons were stained with CP13 antibody (pSer202 Tau) to identify the tau inclusions (**Figure 3A**). By quantifying the granular area in the individual neurons that were treated with oTau and fTau fractions, we found that the accumulation of fTau-induced inclusions plateaued after 48 h, while the accumulation of oTau-induced inclusions continued to grow past 48 h (**Figure 3B**).

Small oTau Inclusions Co-Localize to Stress Granules in the Recipient Neurons

Our previous studies found that RNA binding protein TIA1 appeared to stabilize oligomeric tau and reduce conversion into larger fibrillar tau tangles (20). Subsequent studies of tau propagation *in vivo* also suggested that oTau selectively co-localizes with RNA binding proteins (RBPs) linked to SGs,

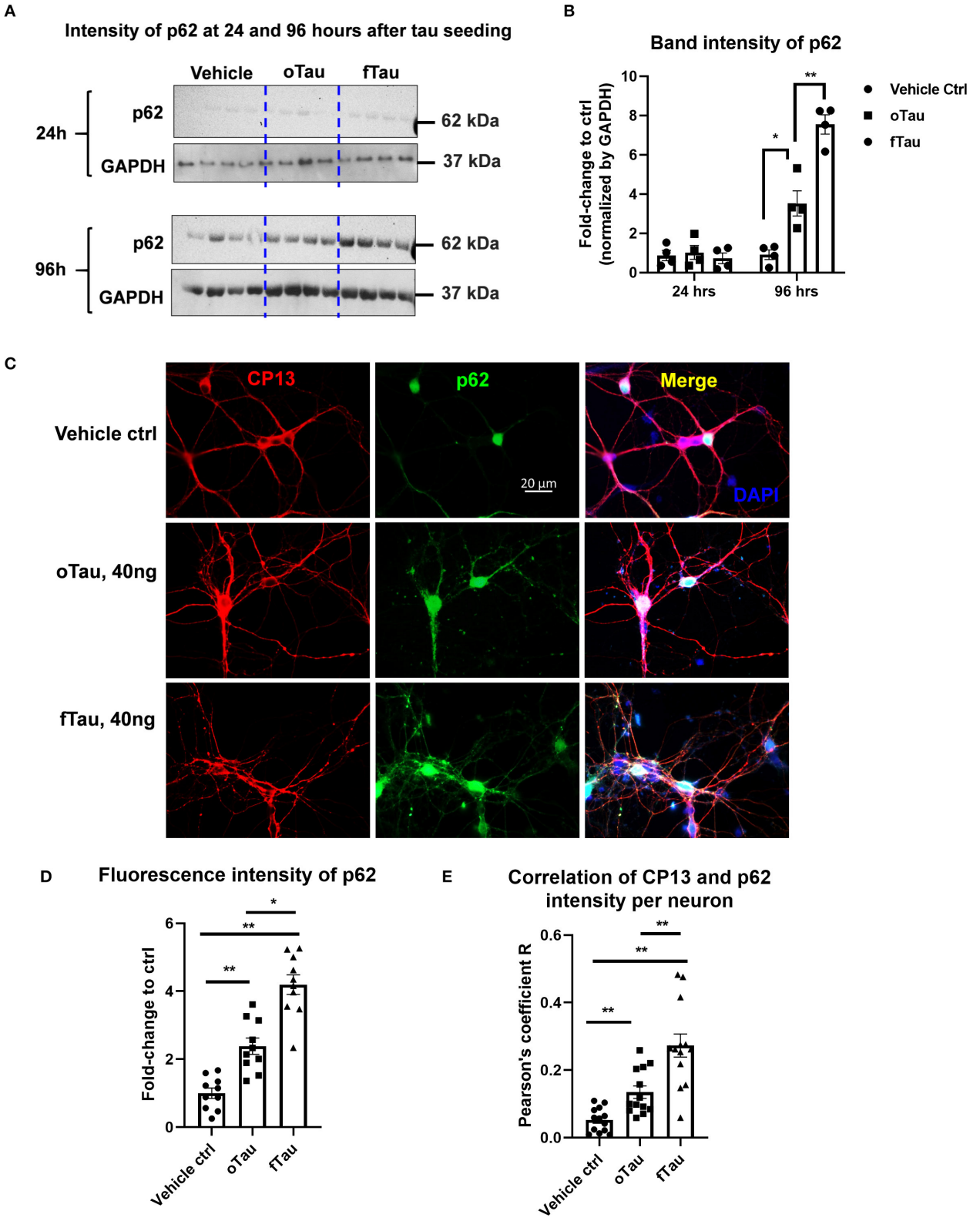


FIGURE 5 | Co-localization of large tau inclusions with p62. **(A)** Immunoblotting detecting p62 expression in neurons after oTau or fTau treatment. Each lane represents an independent biological replicate. The immunoblotting was performed with cell lysate from hippocampal cultures overexpressing human 4R0N WT tau

(Continued)

FIGURE 5 | and treated with vehicle, oTau, or fTau. The cells were harvested at 24 and 96 h after treatment. The p62 antibody was used to identify the expression level of p62 protein. **(B)** Quantification of p62 immunoblot in cell lysate of hippocampal cultures overexpressing human 4R0N WT tau, treated with vehicle control, oTau, or fTau on day 14, and harvested at 24 or 96 h after treatment. Data are shown as mean ± SEM, $N = 4$, data analysis was done by two-way ANOVA, multiple comparison test by Fisher's LSD, $^{*}p < 0.05$, $^{**}p < 0.01$ in comparison with vehicle control. **(C)** Representative images showing the co-localization of phosphorylated tau inclusions CP13 (red) with p62 granules (green) at 96 h after oTau, fTau, or vehicle treatment in hippocampal neurons overexpressing human 4R0N tau. Co-labeling marker is DAPI (blue) to show the cell nucleus. Scale bar 20 μm. **(D)** Quantification of p62 fluorescence intensity in **(C)**, which is the immunofluorescence labeling of hippocampal cultures overexpressing human 4R0N WT tau, treated with vehicle control, oTau, or fTau on day 14, and harvested at 96 h after treatment. Data are shown as mean ± SEM, $N = 10$, data analysis was done by one-way ANOVA, multiple comparison test by Fisher's LSD, $^{*}p < 0.05$, $^{**}p < 0.01$. **(E)** Pearson coefficient of correlation between CP13-positive tau with p62 is graphed for individual neurons using ImageJ. Data are shown as mean ± SEM, $N = 12$, data analysis was done by one-way ANOVA, multiple comparison test by Fisher's LSD, $^{**}p < 0.01$ in comparison with vehicle control.

such as TIA1 (19). We preceded to quantitatively compare the association of oTau and fTau with RBPs and SGs in the hippocampal neuron seeding model. We quantified the co-localization of propagated tau with RBPs, including TIA1, PABP, and EIF3η. These RBPs are the key components of SGs which have been found to play important roles in the progression of neurodegenerative diseases (30). Hippocampal neurons overexpressing human 4R0N tau were seeded with oTau or fTau fractions (40 ng/well), and fixed at 3 h after treatment. The cells were co-labeled with antibodies to phospho-tau (CP13) and each RBP. In each case, the result showed stronger co-localization of cytoplasmic RBPs with inclusions induced by oTau than those induced with fTau (**Figure 4**). The results for TIA1 showed that oTau induced strong TIA1 translocation from the nucleus into neuronal soma and co-localization with oTau inclusions (**Figures 4A,B**); we also note an overall increase in TIA1 fluorescence intensity in neurons exposed to oTau and fTau. Co-labeling of CP13 with PABP also showed stronger co-localization with oTau-induced inclusions than with inclusions induced by fTau (**Figures 4C,D**). Labeling with EIF3η also showed the same trend as with PABP; EIF3η showed stronger co-localization with inclusions induced by oTau in neuronal soma than those induced by fTau (**Figures 4E,F**); we also note an overall increase in EIF3η fluorescence intensity in neurons exposed to oTau and fTau. These results indicate that RNA binding proteins co-localize with the oTau complex.

The Propagated Large Tau Inclusions Co-Localize to p62 Foci in the Recipient Neurons

Previous studies suggested that fibrillar tau aggregates are recognized by p62/SQSTM1, which are then shunted toward the autolysosomal cascade for degradation (31–33). The results presented earlier suggest that fTau induces large, fibrillar tau inclusions, whereas oTau induces smaller, non-fibrillar inclusions (**Figure 3**). Because p62 recognizes fibrillar aggregates, we hypothesized that the fibrillar inclusions stimulated by fTau might also be recognized by p62. To test this hypothesis, we harvested the hippocampal neurons at 24 and 96 h after oTau or fTau treatment. Quantification of p62 expression in total cell lysates by immunoblotting showed that there was significantly more p62 expression at 96 h in the neurons seeded with fTau than those seeded with oTau (**Figures 5A,B**). Next, we examined the degree of co-localization of p62 with tau inclusions at 96 h after seeding with oTau or fTau tau. p62 inclusions were strongly apparent in neurons seeded with either oTau or fTau, and were increased over vehicle-treated neurons (**Figures 5C,D**). Importantly, p62 was significantly more abundant and showed more co-localization with tau inclusions in neurons seeded with fTau than oTau (**Figures 5C,D**). These results support the hypothesis that tau inclusions induced by fTau have more fibrillar structures than those induced by oTau, leading to greater recognition by p62 and potentially promoting increased shunting through the autolysosomal cascade.

DISCUSSION

Protein aggregates show a strong propensity to seed and propagate in cell culture and *in vivo*. Although the morphological changes associated with varying conformers of these aggregates are easily documented, little is known about the biological activities of the varying conformers. In this study, we compared the morphological and biological characteristics of two types of tau aggregates, oTau and fTau. We used tau species generated from 9-month-old P301S tau mice because the oTau generated from these mice is associated with neurodegeneration and previously shown to be toxic (19). We also used primary cultures of hippocampal neurons expressing V5-tagged human tau to increase the rate of seeding and to distinguish between the exogenously applied tau species (that lack a V5 tag) and the endogenously generated tau species that contain a V5 tag. The exogenously applied oTau and fTau were both rapidly transported into the neurons in a dose-dependent manner, but then disappeared within 24 h, consistent with clearance of the exogenous species. In contrast, the resulting inclusions that formed showed labeling by the anti-V5 antibody, demonstrating that these inclusions were predominantly generated from endogenously produced 4R0N tau.

oTau and fTau exhibited striking differences in their uptake and the characteristics of the inclusions subsequently seeded. oTau showed a dose-dependent uptake that plateaued. Such a plateau is typically consistent with receptor binding and saturation of the binding site. Although we did not identify the binding site, a recent study suggests that the lipoprotein receptor LRP1 is an important mediator of tau propagation *in vivo* (34). LRP1 might also mediate uptake of oTau in neuron culture because it is also abundant in primary cultures of hippocampal neurons (35). The uptake of fTau did not exhibit plateauing at the doses used, although we cannot rule out plateauing at higher doses of fTau. The lack of plateauing might reflect uptake through

a non-specific mechanism, such as heparin sulfate proteoglycans, as has been reported previously. The differential uptake suggests different mechanisms of uptake between the two species, which has been noted for other aggregates, such as β-amyloid (36). Once taken up, oTau and fTau also elicited different types of inclusions, with the most abundant inclusions induced by oTau being smaller than those induced by fTau. Previous studies of tau seeding consistently note differences in the morphological patterns of seeding associated with each strain of tau conformer (18, 37).

The biological basis for the differing behaviors of tau conformers is typically poorly understood, but in the current case the differential patterns might be linked to clear differences in the resulting processes associated with each type of aggregate. The inclusions induced by fTau were observed to label with ThioS and anti-p62 antibody. Such labeling indicates the presence of amyloids that are recognized by the cell and targeted by p62 to the autolysosomal system for degradation (38–40). The labeling by both ThioS and anti-p62 antibody supports the hypothesis that exogenous fTau seeds endogenous fTau. In contrast to the strong ThioS labeling, the seeded fTau inclusions exhibit relatively modest association with RBPs and SGs, which suggest that they have proportionately modest activity toward the translational stress response and RNA metabolism.

The behavior of oTau seeding contrasts sharply with that of fTau. Inclusions seeded by oTau showed much stronger association with RBPs associated with SGs, including TIA1, PABP, and EIF3η, than with ThioS and p62. This is consistent with previous work showing the association of tau with SGs in cells, in mice, and in human brain (19, 20, 23–28). Overexpressing tau induces SGs, whereas reducing tau appears to inhibit SG formation (21). Studies of mice with reduced TIA1 showed a surprising reduction in oTau but increase in fTau (20). The current study supports this putative linkage between oTau and SG biology, and is consistent with a role for tau in stimulating the translational stress response (30). It seems likely that tau phosphorylation by proline-directed stress kinases, such as GSK-3β, CDK5, and MARK/Par-1, represents an integrated part of this biological pathway. These kinases are all activated by stress, phosphorylation of tau by these kinases stimulates its oligomerization, and phosphorylated oligomeric tau occurs in concert with the translational stress response (9). Thus, tau phosphorylation, tau oligomerization, and the translational stress response are all linked and integrated.

A major question that remains to be definitively answered is causation. Tau oligomerization could result as an unintended byproduct of stress response or could occur as a biological process designed to drive the stress response. It is noteworthy that oligomerization is a normal common biological process that drives many signaling pathways. For instance, oligomerization is a necessary part in the activation of the proteins p53 and p62 (40–43). However, disease-related accumulation of tau oligomers might stimulate the translational stress response as an indirect response to toxicity induced by tau oligomers. Experiments showing that tau reduction protects against β-amyloid toxicity represent a partial step suggesting a biological role for tau in modulating stress, but more work must be done to rigorously determine whether oligomerization of tau presents a bona fide biological function or an unintended toxic byproduct.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving animals were reviewed and approved by the Boston University Institutional and Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

LJ and JZ performed the experiments and helped write, conceptualized, and edited the article. J-XC conceptualized and edited the article. BW conceptualized, helped write, and edited the article. All authors contributed to the article and approved the submitted version.

FUNDING

We would like to thank the following funding agencies for their support: BW: NIH (AG050471, NS089544, AG056318, AG064932 and AG061706), J-XC: NIH (R35GM136223), and the BrightFocus Foundation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.579434/full#supplementary-material

REFERENCES

1. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci.* (2007) 8:663–72. doi: 10.1038/nrn2194
2. Zempel H, Mandelkow E. Lost after translation: missorting of Tau protein and consequences for Alzheimer disease. *Trends Neurosci.* (2014) 37:721–32. doi: 10.1016/j.tins.2014.08.004
3. Christensen KR, Beach TG, Serrano GE, Kanaan NM. Pathogenic tau modifications occur in axons before the somatodendritic compartment in mossy fiber and Schaffer collateral pathways. *Acta Neuropathol Commun.* (2019) 7:29. doi: 10.1186/s40478-019-0675-9
4. Friedhoff P, Schneider A, Mandelkow EM, Mandelkow E. Rapid assembly of Alzheimer-like paired helical filaments from microtubule-associated protein tau monitored by fluorescence in solution. *Biochemistry.* (1998) 37:10223–30. doi: 10.1021/bi980537d

5. Kampers T, Friedhoff P, Biernat J, Mandelkow EM, Mandelkow E. RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett.* (1996) 399:344–9. doi: 10.1016/S0014-5793(96)01386-5
6. Wilson DM, Binder LI. Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. *In vitro* evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol.* (1997) 150:2181–95.
7. Dobson CM. Protein folding and misfolding. *Nature.* (2003) 426:884–90. doi: 10.1038/nature02261
8. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med.* (2016) 8:595–608. doi: 10.15252/emmm.201606210
9. Stoothoff WH, Johnson GV. Tau phosphorylation: physiological and pathological consequences. *Biochim Biophys Acta.* (2005) 1739:280–97. doi: 10.1016/j.bbadis.2004.06.017
10. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature.* (2017) 547:185–90. doi: 10.1038/nature23002
11. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature.* (2019) 568:420–423. doi: 10.1038/s41586-019-1026-5
12. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature.* (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y
13. Kellogg EH, Hejab NMA, Poepsel S, Downing KH, DiMaio F, Nogales E. Near-atomic model of microtubule-tau interactions. *Science.* (2018) 360:1242–6. doi: 10.1126/science.aat1780
14. Arakhamia T, Lee CE, Carlomagno Y, Duong DM, Kundinger SR, Wang K, et al. Posttranslational modifications mediate the structural diversity of tauopathy strains. *Cell.* (2020) 180:633–44.e12. doi: 10.1016/j.cell.2020.01.027
15. Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, et al. Tau suppression in a neurodegenerative mouse model improves memory function. *Science.* (2005) 309:476–81. doi: 10.1126/science.1113694
16. Crimins JL, Rocher AB, Luebke JI. Electrophysiological changes precede morphological changes to frontal cortical pyramidal neurons in the rTg4510 mouse model of progressive tauopathy. *Acta Neuropathol.* (2012) 124:777–95. doi: 10.1007/s00401-012-1038-9
17. Rocher AB, Crimins JL, Amatrudo JM, Kinson MS, Todd-Brown MA, Lewis J, et al. Structural and functional changes in tau mutant mice neurons are not linked to the presence of NFTs. *Exp Neurol.* (2010) 223:385–93. doi: 10.1016/j.expneurol.2009.07.029
18. Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron.* (2014) 82:1271–88. doi: 10.1016/j.neuron.2014.04.047
19. Jiang L, Ash PEA, Maziuk BF, Ballance HI, Boudeau S, Abdullatif AA, et al. TIA1 regulates the generation and response to toxic tau oligomers. *Acta Neuropathol.* (2019) 137:259–77. doi: 10.1007/s00401-018-1937-5
20. Apicco DJ, Ash PEA, Maziuk B, LeBlang C, Medalla M, Al Abdullatif A, et al. Reducing the RNA binding protein TIA1 protects against tau-mediated neurodegeneration *in vivo*. *Nat Neurosci.* (2018) 21:72–80. doi: 10.1038/s41593-017-0022-z
21. Vanderweyde T, Apicco DJ, Youmans-Kidder K, Ash PE, Cook C, Lummertz da Rocha E, et al. Interaction of tau with the RNA-binding protein TIA1 regulates tau pathophysiology and toxicity. *Cell Rep.* (2016) 15:1–12. doi: 10.1016/j.celrep.2016.04.045
22. Vanderweyde T, Yu H, Varnum M, Liu-Yesucevitz L, Citro A, Ikezu T, et al. Contrasting pathology of stress granule proteins TIA-1 and G3BP in tauopathies. *J Neurosci.* (2012) 32:8270–83. doi: 10.1523/JNEUROSCI.1592-12.2012
23. Koren S, Galvis-Escobar S, Abisambra JF. Tau-mediated dysregulation of RNA: evidence for a common molecular mechanism of toxicity in frontotemporal dementia and other tauopathies. *Neurobiol Dis.* (2020) 141:104939. doi: 10.1016/j.nbd.2020.104939
24. Meier S, Bell M, Lyons DN, Rodriguez-Rivera J, Ingram A, Fontaine SN, et al. Pathological Tau promotes neuronal damage by impairing ribosomal function and decreasing protein synthesis. *J Neurosci.* (2016) 36:1001–7. doi: 10.1523/JNEUROSCI.3029-15.2016
25. Silva JM, Rodrigues S, Sampaio-Marques B, Gomes P, Neves-Carvalho A, Dioli C, et al. Dysregulation of autophagy and stress granule-related proteins in stress-driven Tau pathology. *Cell Death Differ.* (2019) 26:1411–27. doi: 10.1038/s41418-018-0217-1
26. Sengupta U, Montalbano M, McAllen S, Minuesa G, Kharas M, Kayed R. Formation of toxic oligomeric assemblies of rna-binding protein: musashi in alzheimer's disease. *Acta Neuropathol Commun.* (2018) 6:113. doi: 10.1186/s40478-018-0615-0
27. Younas N, Zafar S, Shafiq M, Noor A, Siegert A, Arora AS, et al. SFPQ and tau: critical factors contributing to rapid progression of Alzheimer's disease. *Acta Neuropathol.* (2020) 140:317–39. doi: 10.1007/s00401-020-02178-y
28. Maziuk BF, Apicco DJ, Cruz AL, Jiang L, Ash PEA, da Rocha EL, et al. RNA binding proteins co-localize with small tau inclusions in tauopathy. *Acta Neuropathol Commun.* (2018) 6:71. doi: 10.1186/s40478-018-0574-5
29. Maina MB, Bailey LJ, Wagih S, Biasetti L, Pollack SJ, Quinn JP, et al. The involvement of tau in nucleolar transcription and the stress response. *Acta Neuropathol Commun.* (2018) 6:70. doi: 10.1186/s40478-018-0565-6
30. Wolozin B, Ivanov P. Stress granules and neurodegeneration. *Nat Rev Neurosci.* (2019) 20:649–66. doi: 10.1038/s41583-019-0222-5
31. Moreau K, Fleming A, Imarisio S, Lopez Ramirez A, Mercer JL, Jimenez-Sanchez M, et al. PICALM modulates autophagy activity and tau accumulation. *Nat Commun.* (2014) 5:4998. doi: 10.1038/ncomms5998
32. Zhang Y, Chen X, Zhao Y, Ponnusamy M, Liu Y. The role of ubiquitin proteasomal system and autophagy-lysosome pathway in Alzheimer's disease. *Rev Neurosci.* (2017) 28:861–8. doi: 10.1515/revneuro-2017-0013
33. Fu H, Possenti A, Freer R, Nakano Y, Hernandez Villegas NC, Tang M, et al. A tau homeostasis signature is linked with the cellular and regional vulnerability of excitatory neurons to tau pathology. *Nat Neurosci.* (2019) 22:47–56. doi: 10.1038/s41593-018-0298-7
34. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, et al. LRP1 is a master regulator of tau uptake and spread. *Nature.* (2020) 580:381–5. doi: 10.1038/s41586-020-2156-5
35. Sen A, Alkon DL, Nelson TJ. Apolipoprotein E3 (ApoE3) but not ApoE4 protects against synaptic loss through increased expression of protein kinase C epsilon. *J Biol Chem.* (2012) 287:15947–58. doi: 10.1074/jbc.M111.312710
36. Yu Y, Ye RD. Microglial Abeta receptors in Alzheimer's disease. *Cell Mol Neurobiol.* (2015) 35:71–83. doi: 10.1007/s10571-014-0101-6
37. Jackson SJ, Kerridge C, Cooper J, Cavallini A, Falcon B, Cella CV, et al. Short fibrils constitute the major species of seed-competent tau in the brains of mice transgenic for human P301S Tau. *J Neurosci.* (2016) 36:762–72. doi: 10.1523/JNEUROSCI.3542-15.2016
38. Bjorkoy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol.* (2005) 171:603–14. doi: 10.1083/jcb.200507002
39. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem.* (2007) 282:24131–45. doi: 10.1074/jbc.M702824200
40. Wurzer B, Zaffagnini G, Fracchiolla D, Turco E, Abert C, Romanov J, et al. Oligomerization of p62 allows for selection of ubiquitinated cargo and isolation membrane during selective autophagy. *Elife.* (2015) 4:e08941. doi: 10.7554/eLife.08941.024
41. Fischer NW, Prodeus A, Malkin D, Gariepy J. p53 oligomerization status modulates cell fate decisions between growth, arrest and apoptosis. *Cell Cycle.* (2016) 15:3210–19. doi: 10.1080/15384101.2016.1241917

42. Sun XJ, Wang Z, Wang L, Jiang Y, Kost N, Soong TD, et al. A stable transcription factor complex nucleated by oligomeric AML1-ETO controls leukaemogenesis. *Nature.* (2013) 500:93–7. doi: 10.1038/nature 12287
43. Nguyen TV, Yao S, Wang Y, Rolfe A, Selvaraj A, Darman R, et al. The R882H DNMT3A hot spot mutation stabilizes the formation of large DNMT3A oligomers with low DNA methyltransferase activity. *J Biol Chem.* (2019) 294:16966–77. doi: 10.1074/jbc.RA119.010126

Conflict of Interest: BW is Co-founder and Chief Scientific Officer for Aquinnah Pharmaceuticals Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 19 October 2020
doi: 10.3389/fneur.2020.590059

Much More Than a Cytoskeletal Protein: Physiological and Pathological Functions of the Non-microtubule Binding Region of Tau

Roland Brandt[1,2,3]*, *Nataliya I. Trushina*[1] and *Lidia Bakota*[1]

[1] Department of Neurobiology, University of Osnabrück, Osnabrück, Germany, [2] Center for Cellular Nanoanalytics, University of Osnabrück, Osnabrück, Germany, [3] Institute of Cognitive Science, University of Osnabrück, Osnabrück, Germany

Tau protein (MAPT) is classified as a microtubule-associated protein (MAP) and is believed to regulate the axonal microtubule arrangement. It belongs to the tau/MAP2/MAP4 family of MAPs that have a similar microtubule binding region at their carboxy-terminal half. In tauopathies, such as Alzheimer's disease, tau is distributed more in the somatodendritic compartment, where it aggregates into filamentous structures, the formation of which correlates with cognitive impairments in patients. While microtubules are the dominant interaction partners of tau under physiological conditions, tau has many additional interaction partners that can contribute to its physiological and pathological role. In particular, the amino-terminal non-microtubule binding domain (N-terminal projection region, NTR) of tau interacts with many partners that are involved in membrane organization. The NTR contains intrinsically disordered regions (IDRs) that show a strong evolutionary increase in the disorder and may have been the basis for the development of new, tau-specific interactions. In this review we discuss the functional organization of the tau protein and the special features of the tau non-microtubule binding region also in the connection with the results of Tau KO models. We consider possible physiological and pathological functions of tau's non-microtubule interactions, which could indicate that interactions mediated by tau's NTR and regulated by far-reaching functional interactions of the PRR and the extreme C-terminus of tau contribute to the pathological processes.

Keywords: Alzheimer's disease, membranes, microtubule-associated protein, tau, tauopathy

OPEN ACCESS

Edited by:
Sonia Do Carmo,
McGill University, Canada

Reviewed by:
Peter Jinwoo Chung,
University of Chicago, United States
Akitoshi Takeda,
Osaka City University, Japan

***Correspondence:**
Roland Brandt
robrandt@uni-osnabrueck.de

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *31 July 2020*
Accepted: *16 September 2020*
Published: *19 October 2020*

Citation:
Brandt R, Trushina NI and Bakota L (2020) Much More Than a Cytoskeletal Protein: Physiological and Pathological Functions of the Non-microtubule Binding Region of Tau. Front. Neurol. 11:590059. doi: 10.3389/fneur.2020.590059

INTRODUCTION

Tau is a neuronal microtubule-associated protein (MAP) that is thought to be involved in the regulation of axonal microtubule assembly. In the human genome, tau is encoded by a single gene on chromosome 17q21 (1) and is expressed in several alternatively spliced isoforms. In the human central nervous system (CNS), six different isoforms are present, which differ by the presence or absence of three alternatively spliced exons, exon 2, 3, and 10 (2). The longest isoform in the CNS is encoded by 11 exons and contains 441 amino acids. In the peripheral nervous system (PNS) and the retina (retinal ganglion cells), also longer tau isoforms are present (3).

Tau belongs to a family of structural MAPs that share a conserved carboxy-terminal domain containing the microtubule-binding region (MBR). Besides tau, the family includes the neuronal MAP2 and the non-neuronal MAP4. Bioinformatics analysis indicates that the genes of the two neuronal MAPs, tau and MAP2, are the result of a gene duplication event that occurred at the dawn of the vertebrates (4). Since tau is enriched in the axon while MAP2 is mainly localized to the somatodendritic part of neurons, the N-terminal regions of the two proteins may provide the specific interactions, which are involved in the differential subcellular localization of the two MAPs. During neuronal development, tau localizes early to the axonal compartment, whereas MAP2 becomes restricted to the somatodendritic compartment at a later stage, at least in cultured neurons (5–7).

During Alzheimer's disease (AD) and other tauopathies, tau aggregates in the somatodendritic compartment into neurofibrillary tangles (NFTs) composed of tau proteins with increased stoichiometry of phosphorylation ("hyperphosphorylation") (8). Dysregulation of tau splicing, increased expression of longer tau isoforms containing exon 10, and disease-like tau modifications have been shown to be associated with the development of tauopathies indicating a major role of changes in tau expression and post-translational modifications for disease development (9–11). It is noteworthy that the lack of tau protein only causes subtle changes in the corresponding mouse models and in particular does not have a major influence on the stability of the axonal microtubules. Even acute inactivation of tau in cultured nerve cells does not affect the stability of neuronal microtubules (12). Thus, the data strongly indicates that tau acts not as a stabilizer of microtubules in the axon contrary to the view, which is still quite common in the literature, but instead contributes to regulating microtubule dynamicity (13). On the other hand, the lack of tau reduces pathological changes in various mouse models of AD, stress, excitotoxicity and autism (14–17). This could indicate a "gain of function" mechanism of tau at pathological conditions that is not associated with its role as a microtubule-modulating protein. Bioinformatics analysis showed a minimal interactome of 73 direct binding partners (18), and 175 potential new and known tau interacting proteins were recently identified by MALDI-TOF mass spectrometry (19). Thus, tau appears to be a multifunctional protein with many interaction partners, and pathological changes in its interactome could contribute significantly to disease development in AD and other tauopathies.

Four regions of tau protein can be distinguished on the basis of sequence properties, the N-terminal projection region (NTR), the proline-rich region (PRR), the microtubule-binding region (MBR) and the carboxy-terminal region (CTR) (**Figure 1**). In particular, the NTR protrudes from the microtubule surface when tau is bound to microtubules and could mediate interactions with other cellular components. The NTR is acidic and highly negatively charged at physiological pH, while the PRR and the MBR are both very basic (**Figures 2A,C**). A striking feature that distinguishes the regions is also the level of disorder. Intrinsically disordered regions (IDRs) are characterized by the presence of low sequence complexity and amino acid compositional bias (26, 27). Functionally, IDRs could be relevant because they offer a large interaction area, are known to interact with many binding partners, and are involved in cellular signaling and regulation processes (18). The extent of disorder is highest for the NTR and PRR where disorder-promoting residues except glutamine are overrepresented (**Figure 2B**) (23). This indicates that in particular the non-microtubule binding part of tau has a high degree of binding promiscuity (**Figures 2A,D**). It is noteworthy that the disorder extent of the NTR and PRR shows a strong increase during vertebrate evolution, while the MBR and CTR showed a negative trend (20).

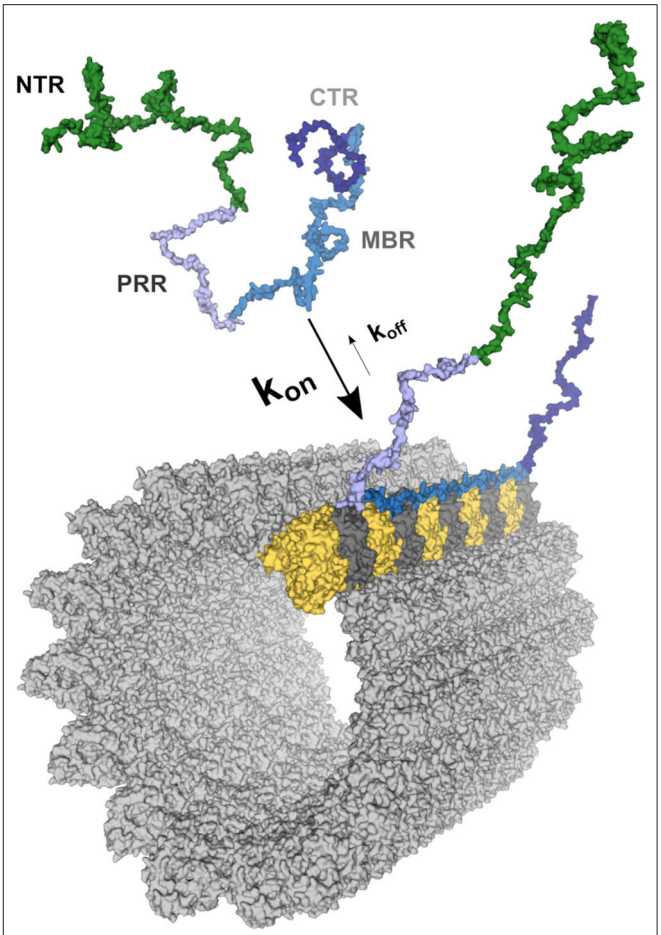


FIGURE 1 | Schematic representation of the tau-microtubule interaction. A free molecule of tau is represented as one of the potential conformations of tau (441 aa long CNS isoform) generated as described previously (20). The different tau regions were mapped onto the model and color-coded as follows: NTR (aa 1–171)—green; PRR (aa 172–243)—light blue; MBR (aa 244–368)—blue; CTR (aa 369–441)—dark blue. The structure of the MBR binding to microtubules is based on PDB:6CVJ and PDB:6CVN structures showing interactions of first two microtubule-binding repeats of tau, R1 and R2, respectively (21). Further repeats, R3 and R4, were based on PDB:6CVN. The rest of tau molecule was artistically rendered based on the free molecule of tau. Binding to a single protofilament of a microtubule segment is depicted. α-tubulin is shown in yellow and β-tubulin in dark gray. All 3D structures are represented as surfaces and were visualized and rendered using PyMOL (www.pymol.org).

The projection domain of tau's neural relative, MAP2, also showed some regions of disorder, but displayed no apparent trend in their change during evolution (28). The organization and the different evolution of the tau sequence therefore suggest that tau is a multifunctional protein in which the N-terminus and the C-terminus have different interactions and contribute to different signaling events that are probably relevant for its physiological and pathological role. In fact, interactions between different tau regions with many different proteins have been found, which showed a certain degree of overlap. However, also several interactions, which are specific for certain regions, particularly with respect to the NTR and the MBR were described (**Figure 3**).

In this article, we want to examine the hypothesis that the different regions of tau have distinct interactions and are involved in various physiological functions and pathological alterations. To this end, we are evaluating data on the functional interactions mediated by the different regions of the tau protein, and are also taking a closer look at studies of tau knockout animals that could provide clues to non-microtubule-related functions of tau. In addition, we discuss the role of possible pathological changes in functional interactions that are mediated by the different tau regions.

FUNCTIONAL INTERACTIONS OF DIFFERENT TAU REGIONS

Tau as a Microtubule Binding Protein: The MBR and CTR

Tau is an abundant protein in neurons of vertebrates where it is enriched in the axonal compartment at physiological conditions. Microtubules are the dominant interaction partner of tau, with the majority of the tau population being bound to cellular microtubules (31, 32). However, the interaction of tau with axonal microtubules is remarkably dynamic (33). Tau rapidly binds and detaches from the microtubule surface in cultured neurons, a feature that has been termed "kiss and hop," with a mean dwell time of single tau molecules in the millisecond range (**Figure 1**) (34).

Tau's microtubule-binding region consists of three or four repeat regions (RRs) dependent on the presence of the alternatively spliced exon 10. Binding to microtubules occurs via small groups of evolutionary conserved residues (35–37). The MBR is highly positively charged at physiological pH due to an overrepresentation of lysine residues (**Figures 2A,B**). On the other hand, microtubules are negatively charged on their surface, which is mostly due to the exposed acidic glutamate-rich C-terminal regions of tubulin (E-hooks) (**Figure 2C**) (38, 39). Thus, electrostatic interactions of the positively charged MBR with the E-hooks is likely to play an important role in the tau microtubule interaction. This is also consistent with a recent near-atomic model of microtubule-tau interactions in which tau follows the ridge on the microtubule surface defined by the H11 and H12 helices of α-tubulin (**Figure 1**) (21). Such an exposed binding site is also consistent with the highly dynamic interaction of tau with microtubules and the short dwell time (34).

In addition to the RRs, a highly conserved pseudorepeat region (PRR) following the RRs on the C-terminal side strongly contributes to microtubule binding *in vitro* and in cells (37, 40). The PRR is also present in the other members of the tau/MAP2/MAP4 family suggesting that it acts as a general modulator of the microtubule interaction in all members of this protein family (40).

Post-translational modifications of tau, in particular phosphorylation, affect tau's binding to microtubules. The phosphorylation of serine 262, which is located in the MBR and forms hydrogen bonds with α-tubulin Glu434, greatly reduces the binding of tau to microtubules (21, 41). However, most phosphorylation sites that affect tau's microtubule interaction are located in two regions that flank the MBR at both sites (20). Most of these sites are present in the PRR and the CTR, which show a clear overrepresentation of serine residues followed by threonine (**Figure 2**). Another post-translational modification of tau with potential functional relevance is acetylation. Lysine residues are typical spots for acetylation and are highly overrepresented in the MBR. Acetylation leads to charge neutralization and has been shown to impair the interaction of tau with microtubules (42). It has been shown that tau itself has acetyltransferase activity and can be acetylated through its own autocatalytic activity (43).

Several binding partners have been identified, whose interactions have been mapped specifically to tau's MBR. In addition to microtubules these include heat shock proteins (44–46), tau itself (47), actin (48–50), and end-binding protein [EB2; (51)] [**Figure 4**; for a complete list with references see Table 1.1. in (20)]. A new addition to the proteins that interacts with tau's MBR is low-density lipoprotein receptor-related protein 1 (LRP1) which may be involved in the endocytosis of tau and its subsequent spread (52). The interaction of tau with LRP1 is mediated by lysine residues in the microtubule-binding repeat region of tau, which mainly contribute to the positive charge of the MBR (**Figure 2B**). Gene ontology (GO)-term grouping shows that the majority of the interacting proteins are associated with microtubule-dependent processes and signaling mechanisms. Few interactions also show associations with cell death mechanisms (**Figure 3**).

Membrane-Related Functions of the Amino-Terminal Binding Domain of Tau

The most obvious peculiarity of the primary sequence of the N-terminal region of tau is a high content of aspartate and glutamate which makes this region highly acidic (**Figures 2A,B**). This is in marked contrast to the PRR and the MBR, which are both positively charged at physiological pH. Tau's NTR shows also the lowest degree of evolutionary conservation compared to the other tau regions (4) except two conserved sequence motifs in the extreme N-terminus, which may be involved in the interaction with annexins [amino acids 6–13 and 27–34; (53)].

GO-term groupings of the proteins, that have been mapped to specifically interact with the NTR reveals an enrichment of proteins implicated in plasma membrane binding and function, and endo-/exocytosis. The interaction of the NTR with a variety of membrane-associated proteins is a unique feature

A

	Full-length	Regions			
		NTR	PRR	MBR	CTR
Molecular weight, kDa	45.85	17.64	7.31	13.18	7.76
Isoelectric point	8.24	4.48	11.44	9.70	7.03
Estimated charge at pH 7	4.80	-16.10	9.90	10.10	0.60
Disorder	0.78	0.94	0.94	0.61	0.54

B

Amino Acids	Full-length	Regions			
		NTR	PRR	MBR	CTR
Proline	4%	1%	**20%**	0%	-2%
Glutamine	-3%	-1%	-7%	-1%	-4%
Serine	2%	-3%	**11%**	2%	**6%**
Glutamate	1%	**6%**	**-2%**	**-2%**	1%
Lysine	4%	1%	**4%**	**9%**	4%
Alanine	1%	6%	-1%	-6%	3%
Glycine	5%	6%	2%	7%	-1%
Aspartate	2%	**5%**	**-3%**	1%	2%
Threonine	3%	5%	**4%**	-2%	**3%**
Arginine	-3%	-3%	**3%**	-5%	-3%
Methionine	-1%	0%	-2%	-1%	-1%
Asparagine	-1%	-3%	-4%	3%	-1%
Valine	0%	-3%	-2%	4%	2%
Histidine	0%	0%	-3%	1%	2%
Leucine	-5%	-7%	-7%	-4%	-2%
Phenylalanine	-3%	-3%	-4%	-3%	-2%
Tyrosine	-2%	-2%	-1%	-2%	-1%
Isoleucine	-1%	-2%	-4%	2%	0%
Tryptophan	-1%	-1%	-1%	-1%	-1%
Cysteine	-2%	-2%	-2%	-1%	-2%

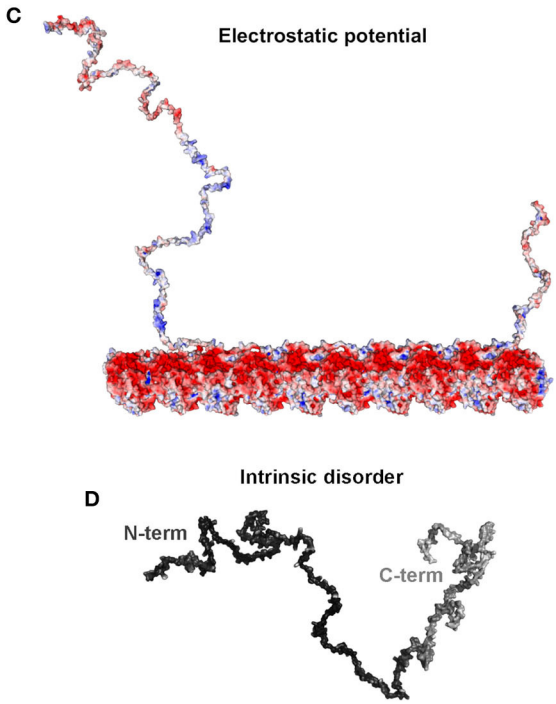


FIGURE 2 | Physicochemical properties of tau and individual tau regions. **(A)** Isoelectric point, charge and disorder of full-length tau and the NTR, PRR, MBR, and CTR. The estimated charge at pH 7 is color-coded from acidic (red) to basic (blue). The extent of disorder is indicated as gray value. **(B)** Differences in frequencies of amino acids in tau and its four regions compared to the frequencies of amino acids encoded by the human genome according to (22). Overrepresented values are indicated in green, underrepresented values in orange color. Amino acids are sorted from disorder-promoting to order-promoting; disorder-promoting amino acids are underlined (23). **(C)** Electrostatic potential of tau and a microtubule protofilament. Electrostatic surface of tau was calculated with APBS Electrostatics Plugin (24). The electrostatic potential is color coded from red (negative) to blue (positive) at physiological pH. **(D)** Extent of intrinsic disorder of tau. Disorder was predicted using IUPred2A long (25). The extent of disorder was mapped onto the tau surface and indicated by white to black values as low or high disorder prediction, respectively.

compared to the other regions of tau. Respective proteins include different annexins [A2, A5, and A6; (53, 54)], a multigene family of Ca^{2+}-regulated membrane binding proteins that are thought to organize the interface between the cytoplasm and the cytoplasmic face of cellular membranes (55). Other membrane associated proteins are synaptic vesicle-associated proteins, such as Synapsin-1 and Synaptotagmin-1 (54), which modulate exocytosis and transmitter release (56). Recently, it has also been shown that tau's NTR interacts with synaptogyrin-3, an integral membrane protein of synaptic vesicles (57). For several interactions of the NTR with membrane components it has been observed that binding is phosphorylation-dependent indicating a potential regulation by signaling mechanisms. Generally, membrane-associated tau appears to be less phosphorylated than the cytosolic fraction (58–60). *In vitro*, also direct interaction of tau with lipids has been reported but it appears that these interactions are mainly mediated by the MBR and not the NTR since it involves short amphipathic helices located in tau's microtubule-binding repeats (61–65).

The GO-term grouping shows that some of the interacting proteins are also associated with signaling mechanisms. This includes, for example, glycogen synthase kinase 3 beta (GSK3β) (66), a proline-directed serine-threonine kinase that is involved in regulating neuronal survival, plasticity and metabolism (67). Tau itself is also a substrate for phosphorylation by GSK3β, which appears to be the main tau kinase *in vivo* (68). Other proteins in this group are several members of the 14-3-3 protein family (54, 69), which are highly expressed in the brain. 14-3-3 proteins are known to modulate the phosphorylation and interactions of many proteins thereby having the potential to modulate a variety of different cellular processes (70). However, it has also been found that some members of the 14-3-3 protein family interact with the PRR and MBR (71), indicating that this interaction may not contribute to the region-specific functions of tau.

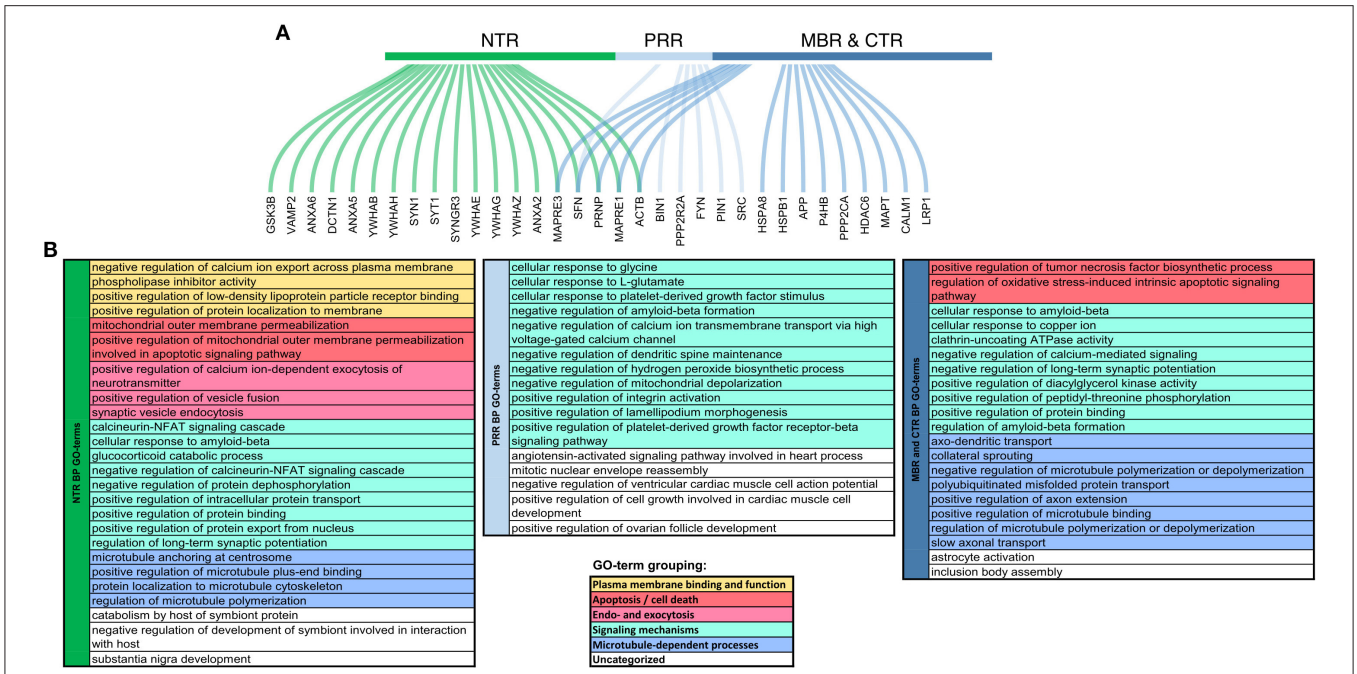


FIGURE 3 | Functional specialization of tau regions with respect to their molecular interactions. **(A)** Genes coding for interaction partners of specific tau regions. Curved lines show to which region of tau the interaction partner was mapped colored by regions, respectively. NTR (1–171), green; PRR (172–243), light blue; MBR and CTR together (244–441), blue. **(B)** Summary representation of GO-terms for the interaction partners, which have been mapped to the different tau regions. GO-term enrichment was performed by ClueGO plugin in Cytoscape (29, 30). Significantly enriched GO-terms ($pV < 0.05$) associated with Biological Processes were identified using a right-sided hypergeometric test with Bonferroni correction. GO-term fusion was used to obtain the more representative terms. GO-terms were grouped and color-coded as presented in the legend below.

The Proline Rich Region as a Module Regulating Signaling

The proline rich region bridges the NTR with the MBR. As the name indicates, the PRR is characterized by a high relative content of proline (20% higher than the average distribution of amino acids in human proteins; **Figure 2B**), which is known for its exceptional conformational rigidity if incorporated in proteins. The PRR is positively charged at physiological pH and has the highest isoelectric point of all tau regions (**Figure 2A**). This is due to a particular high relative content of guanidinium groups (pKa of 12.5) due to the overrepresentation of arginine (**Figure 2B**). The PRR also has the highest relative content of serine making it tau's prime region for phosphorylation, followed by an above-average content of threonine. In fact, the PRR contains 22 predicted phosphorylation sites of which 14 are serine residues (NetPhos 3.1 Server, Technical University of Denmark).

GO-term groupings of the proteins that specifically interact with the PRR show a remarkable enrichment of proteins that are involved in signaling mechanisms. These include kinases, such as the src-family non-receptor tyrosine kinase fyn (72), which have also been shown to phosphorylate tau (73) and the protein phosphatase PP2A/Bα (74). Another interacting protein is the peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (Pin1), which can regulate the tau conformation and function (75, 76).

Phosphorylation within the PRR can affect both the interactions of the NTR and the MBR, suggesting that the PRR can act as a signaling module for the function of both regions. It has been shown that individual phosphorylation events in the PRR, e.g., phosphorylation of Ser-214 by cAMP-dependent protein kinase (PKA) reduces the interaction between tau and microtubules and suppresses *de novo* microtubule polymerization (77, 78). Phosphorylation-mimicking glutamate clusters in the proline-rich region had a similar effect on microtubules and were also sufficient to abolish the tau association with the plasma membrane (79).

LESSONS FROM TAU KNOCKOUT ANIMALS

Analysis of knockout animals can provide important information on the function of a particular protein in a systemic context. Thus, we screened the literature for publications, where tau knockout animals have been characterized. A summary of the studies and key results are shown in **Table 1**. Many of the studies report changes in memory formation and anxiety-related behavior, which could be caused by many deficits, including changes in microtubule-dependent processes, endo- or exocytosis, signaling mechanisms, or plasma-membrane related functions.

Only few studies report effects, which can be directly related to microtubule-dependent processes. In the first study with Tau KO mice, an altered microtubule organization in small-caliber

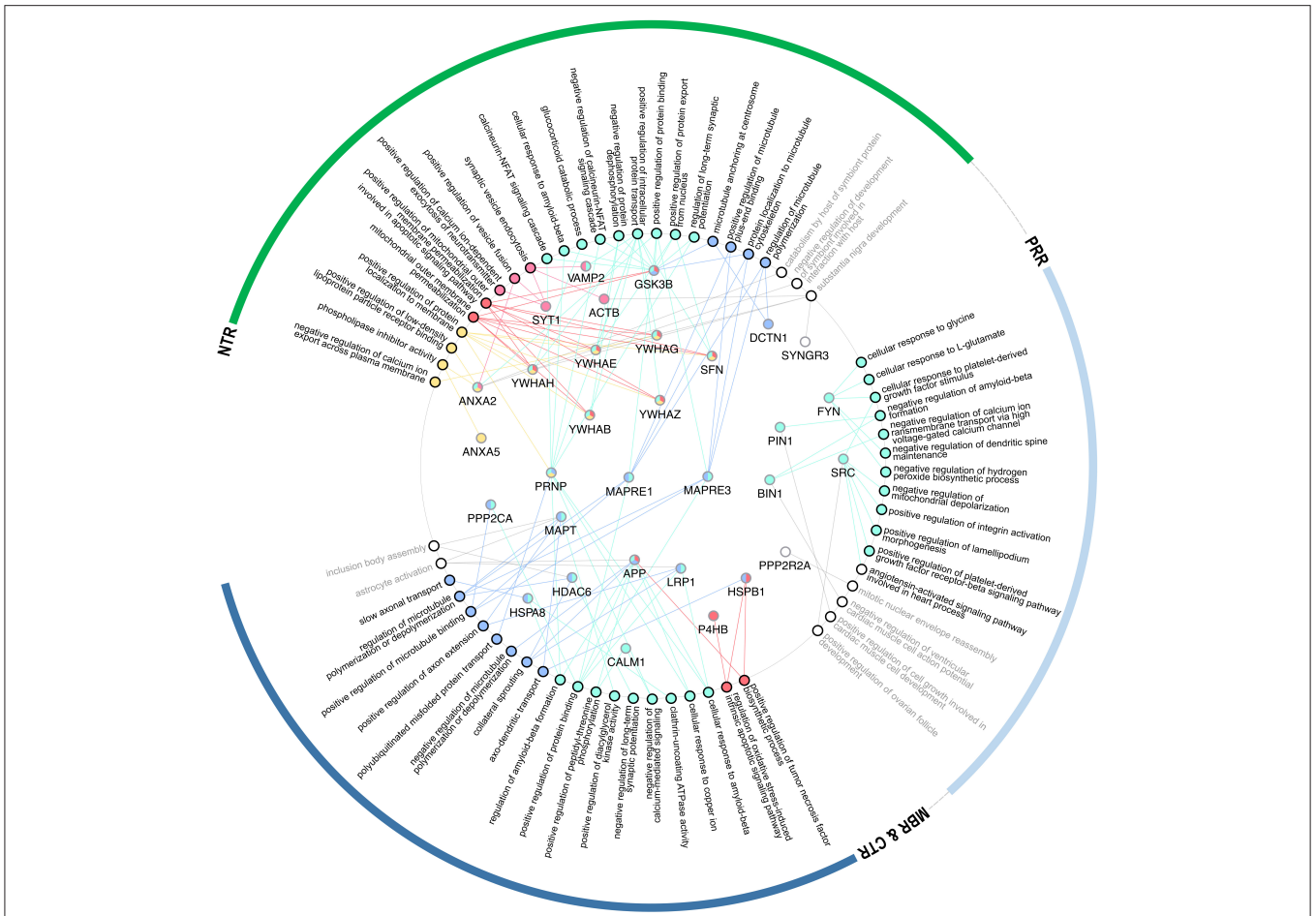


FIGURE 4 | GO-term representation of the individual genes coding for interaction partners that have been mapped to bind to specific regions of tau. GO-terms were color-coded according to the groups as shown in **Figure 3B**.

axons was described (115). Other studies include a report that showed that neurons from Tau KO animals were less affected with respect to transport of Brain-derived neurotrophic factor (BDNF), Beta-secretase 1 (BACE1), or neuropeptide Y (NPY) after treatment with extracellular tau indicating that endogenous tau can be modified by exogenous tau in such a way that transport is impaired (92). In another study, it was observed that Tau KO protects against Aβ-induced loss of microtubules indicating that pathological conditions can cause tau to actively disassemble microtubules (105). It is however noteworthy that none of the studies report obvious destabilization or disassembly of microtubules in Tau KO animals indicating that stabilization of microtubules is not the primary function of tau in neurons.

Several studies report changes, which can be interpreted as being caused by disturbed plasma membrane binding and function. In one study, a decrease in functional extrasynaptic NMDA receptors was observed in Tau KO animals (82) suggesting a participation of tau in anchoring of specific transmembrane proteins of the post-synaptic plasma membrane. In another study, a significant increase in the diameter of synaptic boutons, small swellings that are found at the terminal ends of axons, was observed in Tau KO animals (101). This observation may also be related to a disturbance of plasma membrane organization. Interestingly, one study reports that overexpression of mutant amyloid precursor protein (APP) in Tau KO animals leads to extensive formation of axonal spheroids (109). Spheroids are axonal swellings with discontinuous or absence of myelin sheaths, which may indicate that tau stabilizes the organization of the axonal plasma membrane to support a functional interaction with oligodendrocytes.

Quite some studies report changes in processes, which could be related to disturbances in endo- or exocytosis. In one study hippocampal hyperactivity, which was induced by expression of APP with familial mutations, was found to be attenuated by depletion of tau (90). In another study it was reported that basal synaptic transmission in the hippocampus was decreased in Tau KO mice (101) and one study reports impaired LTD in Tau KO mice (104). In addition, several defects, such as reduced susceptibility to excitotoxic seizures (87), age-dependent hyperactivity and synaptic plasticity defects (88), or facilitation of kainic acid-induced seizures (98) that have been observed in Tau KO animals may be caused by changes in endo- or exocytosis. In fact, also some of the systemic effects, such as changes in

TABLE 1 | Summary and key results of studies using tau knockout animals.

Tau KO animal	Experimental approach	Change in Tau KO animals	References
Scn1aRX/+ and Cntnap2$^{-/-}$ mice × Tau KO mice (Dawson)		Diminished epilepsy, abnormally enlarged brains, and overactivation of the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B).	(17)
Tau KO mouse		Impaired contextual and cued fear memory.	(80)
Tau KO mouse	Fluid percussion injury	Lower anxiety and improved motor function after recovery.	(81)
Tau KO mouse		Decrease in functional extrasynaptic NMDA receptors in the hippocampus.	(82)
Transgenic mouse model of α-synucleinopathy (TgA53T) × Tau KO		Ameliorates cognitive dysfunction and concurrent synaptic deficits of TgA53T mice.	(83)
Tau KO mice		Increased ATP production and improved recognition memory and attentive capacity of juvenile mice.	(84)
Tau KO mice		Olfactory deficit correlated with accumulation of α-synuclein and autophagic impairment.	(85)
Acute Tau KO		Impaired motor coordination and spatial memory.	(86)
Tau KO (tauΔex1)		Reduced susceptibility to excitotoxic seizures.	(87)
Tau KO on B6129PF3/J genetic background		Age-dependent short-term memory deficits, hyperactivity and synaptic plasticity defects.	(88)
Tau KO		Hyperglycemic and glucose intolerance; reduced islet insulin content and elevated proinsulin levels; increased epididymal fat mass and leptin levels; reduced glucose production, and insulin resistance at later ages, leading to complete onset of diabetes.	(89)
Transgenic (J20) mice express human amyloid precursor protein (hAPP) with the Swedish (K670N, M671L) and Indiana (V717F) mutations under the control of the PDGF β-chain promoter × Tau KO mice (Dawson)		Hippocampal hyperactivity, but not mPFC hypoactivity, is attenuated by deletion of tau; tau depletion failed to reverse the memory impairment induced by over-production of APP in MWM; deletion of tau alleviated the hyperlocomotion displayed by APP transgenics.	(90)
Tau KO	Experimental stroke, using a middle cerebral artery occlusion with reperfusion model	Protection from excitotoxic brain damage and neurological deficits.	(16)
Tau KO	Stress-driven suppression of neurogenesis	After exposure to chronic stress no reduction in DG proliferating cells, neuroblasts and newborn neurons.	(91)
Tau KO (Dawson)	Cortical cultures Treatment with extracellular tau	Much less affected transport of BDNF, BACE1 or NPY.	(92)
Tau KO (Dawson)		Heterozygous tau knockout, but not homozygous knockout, induced a selective loss of VTA DA neurons at the early post-natal stage P0, which correlated with a similar reduction in Otx2 expression and increases in prenatal cell death and the unactivated compensation effect of MAP1A in Mapt$^{+/-}$ mice.	(93)
Tau KO (Dawson)	Unilateral, transient middle cerebral artery occlusion (MCAO)	Mice were protected against hemispheric reperfusion injury following MCAO at 3-months of age but not at 12-months.	(94)
Tau KO (Tucker)		Impaired hypothalamic anorexigenic effect of insulin that is associated with energy metabolism alterations.	(95)
Tau KO mice (Dawson)		Increased locomotor activity in 5-months-old animals compared to human wild-type expressing animals.	(96)
Tau KO mice		Tau ablation blocks stress-driven anxious, anhedonic, and passive coping behaviors as well as cognitive impairments; chronic unpredictable stress decreased NA and 5HT levels in WT, but not Tau-KO, animals; stress-driven structural remodeling of hippocampal neurons depends on tau protein.	(15)
Tau KO mice (Dawson)	Primary cultures of hippocampal neurons	Tau is required for normal interactions of RNA binding proteins in brain tissue and tau promotes stress granules, while TIA1 promotes tau misfolding and insolubility.	(97)

(Continued)

TABLE 1 | Continued

Tau KO animal	Experimental approach	Change in Tau KO animals	References
Tau KO mice (Dawson)		Tau facilitates kainic acid (KA)-induced seizures *in vivo*; tau facilitates ROS production in response to excitotoxic insult *in vivo*.	(98)
Tau KO mice	Stereotactic injection of Aβ42 oligomers into the hippocampal dorsal CA1 area bilaterally	Protection against Aβ-induced cognitive impairment, hippocampal neuron loss, and iron accumulation.	(99)
Tau KO mice	Primary cultures of cortical neurons	Protection of mouse primary cortical neurons from loss of mitochondrial membrane potential ($\Delta\Psi m$) caused by low concentrations of Aβ42; absence of tau resulted in significantly greater increases in Ca^{2+}_{cyt} in response to Aβ treatment.	(100)
Tau KO mice		Basal synaptic transmission of mossy fibers measured by input output curves is decreased; bouton diameter increase of ∼45%.	(101)
Tau KO mice on Bl6/129sv and Bl6 backgrounds		Complete tau reduction impairs the performance of mice in accelerated Rotarod test, impairs the performance of mice in Pole test, impairs the performance of mice in Openfield test, alters hindlimb clasping behavior at 12 months of age; motor deficits are related to nigral degeneration.	(102)
Tau KO mice		Treatment with streptozotocin did not lead to impaired hippocampal cognitive behavior in Tau KO mice nor in reduction of PDS-95, synaptophysin and p-CREB.	(103)
Tau KO mice		Impaired LTD but not LTP in Tau KO mice at 7–11 months of age.	(104)
Tau KO mice	Primary neurons	Tau deficiency prevents AβO-induced polyglutamylation, spastin recruitment, and TTLL6 transport into dendrites; tau deficiency does not protect against Aβ association to dendrites and transient spine loss, but protects against loss of MTs, neurofilament invasion, and loss of mitochondria.	(105)
Tau KO mice (Dawson)	Primary cell culture	Tau is required in cultured neurons for ectopic cell cycle re-entry (CCR) induced by Aβ.	(106)
Tau KO mice (Dawson)		Subtle motor deficits at 12–15 months of age connected to mild dopaminergic deficits in Tau KO mice.	(107)
Tau KO mice (Dawson)		Tau-knockout mice develop age-dependent brain atrophy, iron accumulation and substantia nigra neuronal loss, with concomitant cognitive deficits and parkinsonism.	(108)
Tau KO mice (Dawson) and mice expressing APP with the Swedish mutation Tau KO background		Overexpression of mutant APP in tau knockout mice, elicits the extensive formation of axonal spheroids and more severe cognitive deficits.	(109)
Tau KO mice	Primary cultures of cortical neurons	A significantly lower LDH release, with a peak delayed by 24 h, was detected in Tau KO neurons after heat shock.	(110)
Tet/GSK3β mice on Tau KO (Dawson) background		The toxic effect of GSK3 overexpression is milder and slower in the absence of tau.	(111)
Tau KO mice (Dawson)		A deficit in migration of newborn cells in the subgranular zone was observed in Tau KO mice.	(112)
Tau KO mice	Primary cultures of hippocampal neurons	Tau-depleted neurons showed no signs of degeneration in the presence of Aβ.	(113)
Tau KO mice (Dawson)	Primary cultures of hippocampal neurons	Inhibition of neuronal maturation.	(114)
Tau KO mice (Harada)		Altered microtubule organization in small-caliber axons.	(115)

*The following color code was used to assign results to the summary of GO-terms introduced in **Figure 3**: yellow: plasma membrane binding and function; red: apoptosis/cell death; purple: endo- and exocytosis; green: signaling mechanisms; blue: microtubule-dependent processes.*

memory- and anxiety-related behavior (80, 81, 86) could be interpreted as a result of impaired neurotransmitter exocytosis.

Interestingly, also effects on survival of specific cell populations have been reported. This includes a selective loss of dopaminergic neurons in the ventral tegmental area (VTA) and increased prenatal cell death (93), and degeneration of neurons of the Substantia nigra (102) in Tau KO animals. However, opposite effects have also been reported, in which the absence of tau promotes cell survival after toxic attacks. Examples are reports in which Tau KO protects against excitotoxic brain damage (16) or against Aβ-induced loss of hippocampal neurons (99).

Finally, also several reports support a role of tau in modulating signaling mechanisms. In particular it is striking that several papers report impaired glucose metabolism and response to insulin in Tau KO mice (89, 95). In addition, changes in ROS production (98) and Ca^{2+} homeostasis (100) in response to pathological conditions have been reported.

Thus, the results of the analysis of Tau-KO animals support a multifunctional role of Tau, whereby many of the changes observed, in particular with regard to a disturbance in the function and organization of the plasma membrane, to endocytosis and exocytosis, to signaling mechanisms and to the cell death mechanism, are very likely mediated by tau's N-terminal projection domain.

PATHOLOGICAL CHANGES OF FUNCTIONAL INTERACTIONS OF THE DIFFERENT TAU REGIONS

Tau pathology is associated with a decreased binding of tau to axonal microtubules, a redistribution of tau from the axon to the somatodendritic compartment and aggregation of tau protein in NFTs. These events are joined by an increased phosphorylation of tau at selected sites and death of affected neurons. Since it is known that tau's interaction with microtubules is impaired by phosphorylation and familial tauopathy mutations, potential pathological changes of interactions mediated by tau's microtubule binding region were long in the focus of research. However, tau was also found to be present in a membrane-enriched proteome from post-mortem human brain tissue in AD (116) and has been implicated in the formation of toxic complexes with phospholipids (64), suggesting that pathologic changes in tau's membrane interaction mediated by its NTR may also have a central role in the disease process.

Pathological Changes of the Interactions of the Microtubule Binding Region

Phosphorylation of tau at several sites is known to reduce the interaction with microtubules. Most of the regulatory sites are present in the PRR and the CTR, which flank tau's microtubule binding region on both sites. This is also reflected in PHFs that have been isolated from patients with AD, where all of the 10 major phosphorylation sites are located in the PRR and the CTR (20, 117). In addition, Ser262 (which is located in the MBR) has been identified as being phosphorylated in PHFs (118), but appears to be only weakly phosphorylated during the development of AD (119). Progressive phosphorylation during AD has been correlated best for the AT8 site (Ser202/Thr205) (120) but also Ser199 and Thr231, which belong to the major phosphorylated sites in PHFs, show an increase during progression of AD (119). All of these sites are located in the PRR and combined phosphomimicking mutations of five major PHF sites within the PRR (Ser198, Ser199, Ser202, Thr231, and Ser235) suppress de novo microtubule polymerization (79). Thus, a pathologic increase in tau phosphorylation at the PRR appears to impair tau's microtubule related activities. It should however be noted that a phosphomimicking tau construct of 10 major phosphorylation sites that have been identified in PHFs from AD was still able to interact with microtubules, albeit with a reduced dwell time (40). This indicates that phosphorylation-induced changes in tau's microtubule interaction may only partially account for the development of tau pathology, at least with respect to tau's redistribution and toxicity. This is in agreement with the view that tau is not a microtubule stabilizer in the neuron but a multifunctional MAP (121). It should also be noted that the PRR, where many of the sites with increased phosphorylation in AD are located, appears to act as a module that affects not only microtubule-related activities but may regulate also interactions and signaling events, which are mediated by the NTR. This is exemplified by the observation that pseudophosphorylation in the PRR abolished tau's interaction with plasma membrane components (59).

The MBR is also the region that mediates the tau-tau interaction, possibly influencing the formation of tau aggregates. The cores of PHFs and SFs from patients with AD are made of protofilaments comprising residues 306–378 of tau protein (122), which are located within the MBR and CTR. However, the role of phosphorylation in mediating tau aggregation remains unclear. While phosphorylation at different sites parallels tau aggregation in the brain (119), at least *in vitro*, site specific hyperphosphorylation inhibits, rather than promotes, tau fibrillization (123). However, pseudophosphorylated tau at Ser202 and Thr205 showed enhanced aggregation *in vitro* (124) suggesting that some sites have specific effects on filament formation. It is possible that other factors or modifications promote tau aggregation. The ratio of cis/trans isoforms may affect fibrillarization and *in vitro* data suggest that in particular the trans isomer of a tau peptide is prone to aggregate (125). Isomerization is regulated by the protein Pin1, which binds to the PRR of tau. Also, proteolytic degradation of tau may influence filament formation and several studies have provided evidence that tau cleavage by metalloproteinases, caspases, or a lysosomal cysteine proteinase induce tau aggregation (126–129).

Interaction with phospholipids or free fatty acids can also induce the aggregation of full-length tau (62, 130) or a tau fragment comprising the MBR (131). Interestingly, it has also been reported that the NTR can contribute to tau polymerization because a disease-associated R5L mutation in the NTR increases tau polymerization (132). The presence of an N-terminal tau fragment comprising the NTR and part of the PRR was also shown to inhibit tau aggregation, which confirms that the tau N-terminus plays a modulatory role in the aggregation process (133).

Pathological Changes of the Membrane-Related Interactions of Tau

Evidence from cell-free experiments indicated that phospholipids induce changes in the tau conformation over the entire length of the molecule, which affect the phosphorylation of tau and its interaction with microtubules (134). Membranes facilitate tau aggregation *in vitro* (135), and it has been shown that tau binds to membranes via short amphipathic helices located in its microtubule binding repeats (63). In addition, pathological changes in membrane-related interactions of tau, mediated by the NTR that does not bind to microtubules, could play an important role in the redistribution of tau from the axon to the somatodendritic compartment and may also be involved in the induction of toxic effects. Tau's interaction with components of the plasma membrane is affected by mutations,

which have been observed in familial forms of tauopathies, or by phosphorylation at disease-relevant sites. Interestingly, the Frontotemporal Dementia mutation R406W blocks tau's interaction with the membrane in an annexin A2-dependent manner indicating that changes in the C-terminus (mutation at position 406) affect an interaction, which is mediated by the extreme N-terminus (136). A similar observation was made with respect to phosphorylation, where pseudophosphorylation of disease-relevant sites at the C-terminal region blocked tau's membrane interaction (79). It appears surprising that changes in tau's carboxy-terminal region selectively affect a feature that is mediated by the opposite side of the molecule. However, it has been shown previously that tau can adopt a "paperclip" conformation, in which the amino- and carboxy-terminal domains approach each other (137) and MS analysis of the phosphorylation pattern have suggested that the R406W mutation exerts long-range conformational effects on the structure of tau (138). It should also be noted that although pseudophosphorylation in the carboxy terminus of tau abolished tau's interaction with the membrane cortex, tau's ability to promote microtubule assembly remained unchanged (79). This indicates that the interactions of the MBR and the NTR can be influenced differently by disease-associated phosphorylation events in different domains.

Increased phosphorylation as AD progressed was also observed at Tyr18, a site located at the extreme N-terminus of tau (119). Tyr18 is in the first exon of tau, which is also involved in the interaction with annexins, although this particular interaction does not appear to be affected by phosphorylation at this position (53). In the same region, familial tau mutations (R5H and R5L) were also observed in tauopathies (139, 140). In fact, phosphorylation at several tyrosine-residues including those in the N-terminus (Tyr18, Tyr29, and Tyr219) have very recently been shown to abolish tau aggregation and inhibit lipid-binding properties of tau *in vitro* (65).

Besides tau's interaction with plasma membrane components, it has also been shown that tau binds to presynaptic vesicles in AD patient brain. Human tau also interacts with synaptic vesicles in a Drosophila model and tau mutations, which are associated with familial forms of tauopathy, exhibit increased presynaptic localization and lead to reduced synaptic transmission (141). This suggests a presynaptic pathological role of tau, which is associated with a changed disease-associated interaction with synaptic vesicles mediated by the NTR of tau. Also, in this case, the familial mutations were located in the C-terminal part of tau (P301L, V337M, R406W) indicating that changes in the C-terminus can affect interactions, which are mediated by tau's NTR. The pathologic effect on synaptic vesicles may not be mediated via direct interaction with lipids since the same lab has identified the transmembrane vesicle protein synaptogyrin-3 as the binding partner of tau's N-terminus on synaptic vesicles (57).

It is also possible that tau oligomers, which have recently come into the focus as the primary toxic pathological tau species, affect membrane integrity and thereby exert toxic effects. In fact, it has been shown that tau oligomers affect the integrity of artificial phospholipid vesicles and decrease cell viability of a neuronal model (142), however it is unclear, whether membrane-binding properties of the NTR have a role in this process.

CONCLUSIONS

Tau was originally identified as a microtubule-associated protein, but has been shown to have many additional interaction partners and can be considered a multifunctional protein. Indeed, tau knockout studies in mouse models show surprisingly small changes that may be directly related to impaired microtubule stability, impaired microtubule-dependent transport, or changes in microtubule dynamics. Therefore, non-microtubule-related functions mediated by the N-terminal region of tau could play an important role with regard to the functions and malfunctions of tau during tauopathies. There are several indications that are based on the identity of region-specific interaction partners or on the results of tau knockout studies that membrane-related functions of the NTR can disrupt the organization and function of the plasma membrane and other membrane organelles, such as synaptic vesicles. In addition, there are indications of disturbances in signal mechanisms and effects on the survival mechanisms of the cells, which may be mediated by the NTR. It is particularly noteworthy that post-translational modifications or disease-associated mutations in other regions of tau, such as the extreme C-terminus or the PRR, can affect the function of the NTR, suggesting that far-reaching functional interactions contribute to the pathological processes. Gain-in-function mechanisms mediated by the N-terminal region of tau could also explain the obvious paradox that knockout of tau, despite the long-standing (and probably incorrect) dogma of tau as a microtubule-stabilizing protein, has surprisingly few functional consequences in animal models and that the loss of tau can even increase resistance to stress and certain pathological conditions.

The sequence of the N-terminus of tau shows due to the presence of two alternatively spliced exons (exons 2 and 3) in CNS tau isoforms and other exons that are only present in PNS tau (e.g., 4A and 6) considerable variations (3). The PNS-specific exon 4A also shows considerable differences in length between humans, mice and rats. Therefore, it would also be important to gain a better understanding of the role of the N-terminal variants in terms of functional differences mediated by the regions of tau not related to binding to microtubules.

AUTHOR CONTRIBUTIONS

RB, NT, and LB interpreted the data and wrote the article. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by a fellowship of the graduate college EvoCell of the University of Osnabrück (to NT). We acknowledge support by Deutsche Forschungsgemeinschaft (DFG, grant BR1192/14-1 to RB) and Open Access Publishing Fund of Osnabrück University.

REFERENCES

1. Andreadis A, Brown WM, Kosik KS. Structure and novel exons of the human tau gene. *Biochemistry.* (1992) 31:10626–33. doi: 10.1021/bi00158a027
2. 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) 9:4225–30. doi: 10.1002/j.1460-2075.1990.tb07870.x
3. Nunez J, Fischer I. Microtubule-associated proteins (MAPs) in the peripheral nervous system during development and regeneration. *J Mol Neurosci.* (1997) 8:207–22. doi: 10.1007/BF02736834
4. Sundermann F, Fernandez MP, Morgan RO. An evolutionary roadmap to the microtubule-associated protein MAP Tau. *BMC Genomics.* (2016) 17:264. doi: 10.1186/s12864-016-2590-9
5. Dotti CG, Sullivan CA, Banker GA. The establishment of polarity by hippocampal neurons in culture. *J Neurosci.* (1988) 8:1454–68. doi: 10.1523/JNEUROSCI.08-04-01454.1988
6. Kempf M, Clement A, Faissner A, Lee G, Brandt R. Tau binds to the distal axon early in development of polarity in a microtubule- and microfilament-dependent manner. *J Neurosci.* (1996) 16:5583. doi: 10.1523/JNEUROSCI.16-18-05583.1996
7. Kwei SL, Clement A, Faissner A, Brandt R. Differential interactions of MAP2, tau and MAP5 during axogenesis in culture. *Neuroreport.* (1998) 9:1035–40. doi: 10.1097/00001756-199804200-00015
8. Arendt T, Stieler JT, Holzer M. Tau and tauopathies. *Brain Res Bull.* (2016) 126:238–92. doi: 10.1016/j.brainresbull.2016.08.018
9. Goedert M, Crowther RA, Spillantini MG. Tau mutations cause frontotemporal dementias. *Neuron.* (1998) 21:955–8. doi: 10.1016/S0896-6273(00)80615-7
10. Fath T, Eidenmüller J, Brandt R. Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease. *J. Neurosci.* (2002) 22:9733. doi: 10.1523/JNEUROSCI.22-22-09733.2002
11. Schoch KM, Devos SL, Miller RL, Chun SJ, Norrbom M, Wozniak DF, et al. Increased 4R-tau induces pathological changes in a human-tau mouse model. *Neuron.* (2016) 90:941–7. doi: 10.1016/j.neuron.2016.04.042
12. Tint I, Slaughter T, Fischer I, Black MM. Acute inactivation of tau has no effect on dynamics of microtubules in growing axons of cultured sympathetic neurons. *J Neurosci.* (1998) 18:8660–73. doi: 10.1523/JNEUROSCI.18-21-08660.1998
13. Qiang L, Sun X, Austin TO, Muralidharan H, Jean DC, Liu M, et al. Tau does not stabilize axonal microtubules but rather enables them to have long labile domains. *Curr Biol.* (2018) 28:2181–9 e4. doi: 10.1016/j.cub.2018.05.045
14. Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science.* (2007) 316:750–4. doi: 10.1126/science.1141736
15. Lopes S, Vaz-Silva J, Pinto V, Dalla C, Kokras N, Bedenk B, et al. Tau protein is essential for stress-induced brain pathology. *Proc Natl Acad Sci USA.* (2016) 113:E3755. doi: 10.1073/pnas.1600953113
16. Bi M, Gladbach A, Van Eersel J, Ittner A, Przybyla M, Van Hummel A, et al. Tau exacerbates excitotoxic brain damage in an animal model of stroke. *Nat Commun.* (2017) 8:473. doi: 10.1038/s41467-017-00618-0
17. Tai C, Chang CW, Yu GQ, Lopez I, Yu X, Wang X, et al. Tau reduction prevents key features of autism in mouse models. *Neuron.* (2020) 106:421–37 e11. doi: 10.1016/j.neuron.2020.01.038
18. Uversky VN. Intrinsically disordered proteins and their (disordered) proteomes in neurodegenerative disorders. *Front Aging Neurosci.* (2015) 7:18. doi: 10.3389/fnagi.2015.00018
19. Sinsky J, Majerova P, Kovac A, Kotlyar M, Jurisica I, Hanes J. Physiological tau interactome in brain and its link to tauopathies. *J Proteome Res.* (2020) 19:2429–42. doi: 10.1021/acs.jproteome.0c00137
20. Trushina NI, Bakota L, Mulkidjanian AY, Brandt R. The evolution of tau phosphorylation and interactions. *Front Aging Neurosci.* (2019) 11:256. doi: 10.3389/fnagi.2019.00256
21. Kellogg EH, Hejab NMA, Poepsel S, Downing KH, Dimaio F, Nogales E. Near-atomic model of microtubule-tau interactions. *Science.* (2018) 360:1242. doi: 10.1126/science.aat1780
22. Tsuji J, Nydza R, Wolcott E, Mannor E, Moran B, Hesson G, et al. The frequencies of amino acids encoded by genomes that utilize standard and nonstandard genetic codes. *Bios.* (2010) 81:22–31. doi: 10.1893/011.081.0103
23. Uversky VN. Analyzing IDPs in interactomes. *Methods Mol Biol.* (2020) 2141:895–945. doi: 10.1007/978-1-0716-0524-0_46
24. Baker NA, Sept D, Joseph S, Holst MJ, Mccammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc Natl Acad Sci USA.* (2001) 98:10037–41. doi: 10.1073/pnas.181342398
25. Dosztanyi Z, Csizmok V, Tompa P, Simon I. The pairwise energy content estimated from amino acid composition discriminates between folded and intrinsically unstructured proteins. *J Mol Biol.* (2005) 347:827–39. doi: 10.1016/j.jmb.2005.01.071
26. Dyson HJ, Wright PE. Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell Biol.* (2005) 6:197–208. doi: 10.1038/nrm1589
27. Atkins JD, Boateng SY, Sorensen T, Mcguffin LJ. Disorder prediction methods, their applicability to different protein targets and their usefulness for guiding experimental studies. *Int J Mol Sci.* (2015) 16:19040–54. doi: 10.3390/ijms160819040
28. Trushina NI, Mulkidjanian AY, Brandt R. The microtubule skeleton and the evolution of neuronal complexity in vertebrates. *Biol Chem.* (2019) 400:1163–79. doi: 10.1515/hsz-2019-0149
29. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* (2003) 13:2498–504. doi: 10.1101/gr.1239303
30. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics.* (2009) 25:1091–3. doi: 10.1093/bioinformatics/btp101
31. Drubin DG, Kirschner MW. Tau protein function in living cells. *J Cell Biol.* (1986) 103:2739–46. doi: 10.1083/jcb.103.6.2739
32. Weissmann C, Reyher HJ, Gauthier A, Steinhoff HJ, Junge W, Brandt R. Microtubule binding and trapping at the tip of neurites regulate tau motion in living neurons. *Traffic.* (2009) 10:1655–68. doi: 10.1111/j.1600-0854.2009.00977.x
33. Konzack S, Thies E, Marx A, Mandelkow EM, Mandelkow E. Swimming against the tide: mobility of the microtubule-associated protein tau in neurons. *J Neurosci.* (2007) 27:9916–27. doi: 10.1523/JNEUROSCI.0927-07.2007
34. Janning D, Igaev M, Sundermann F, Bruhmann J, Beutel O, Heinisch JJ, et al. Single-molecule tracking of tau reveals fast kiss-and-hop interaction with microtubules in living neurons. *Mol Biol Cell.* (2014) 25:3541–51. doi: 10.1091/mbc.e14-06-1099
35. Butner KA, Kirschner MW. Tau protein binds to microtubules through a flexible array of distributed weak sites. *J Cell Biol.* (1991) 115:717–30. doi: 10.1083/jcb.115.3.717
36. Goode BL, Feinstein SC. Identification of a novel microtubule binding and assembly domain in the developmentally regulated inter-repeat region of tau. *J Cell Biol.* (1994) 124:769–82. doi: 10.1083/jcb.124.5.769
37. Kadavath H, Hofele RV, Biernat J, Kumar S, Tepper K, Urlaub H, et al. Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. *Proc Natl Acad Sci USA.* (2015) 112:7501. doi: 10.1073/pnas.1504081112
38. Nogales E, Whittaker M, Milligan RA, Downing KH. High-resolution model of the microtubule. *Cell.* (1999) 96:79–88. doi: 10.1016/S0092-8674(00)80961-7
39. Okada Y, Hirokawa N. Mechanism of the single-headed processivity: diffusional anchoring between the K-loop of kinesin and the C terminus of tubulin. *Proc Natl Acad Sci USA.* (2000) 97:640–5. doi: 10.1073/pnas.97.2.640
40. Niewidok B, Igaev M, Sundermann F, Janning D, Bakota L, Brandt R. Presence of a carboxy-terminal pseudorepeat and disease-like pseudohyperphosphorylation critically influence tau's interaction with microtubules in axon-like processes. *Mol Biol Cell.* (2016) 27:3537–49. doi: 10.1091/mbc.e16-06-0402
41. Biernat J, Gustke N, Drewes G, Mandelkow EM, Mandelkow E. Phosphorylation of Ser262 strongly reduces binding of tau to microtubules: distinction between PHF-like immunoreactivity and microtubule binding. *Neuron.* (1993) 11:153–63. doi: 10.1016/0896-6273(93)90279-Z

42. Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, et al. The acetylation of tau inhibits its function and promotes pathological tau aggregation. *Nat Commun.* (2011) 2:252. doi: 10.1038/ncomms1255
43. Cohen TJ, Friedmann D, Hwang AW, Marmorstein R, Lee VM. The microtubule-associated tau protein has intrinsic acetyltransferase activity. *Nat Struct Mol Biol.* (2013) 20:756–62. doi: 10.1038/nsmb.2555
44. Sarkar M, Kuret J, Lee G. Two motifs within the tau microtubule-binding domain mediate its association with the hsc70 molecular chaperone. *J Neurosci Res.* (2008) 86:2763–73. doi: 10.1002/jnr.21721
45. Freilich R, Betegon M, Tse E, Mok SA, Julien O, Agard DA, et al. Competing protein-protein interactions regulate binding of Hsp27 to its client protein tau. *Nat Commun.* (2018) 9:4563. doi: 10.1038/s41467-018-07012-4
46. Taylor IR, Ahmad A, Wu T, Nordhues BA, Bhullar A, Gestwicki JE, et al. The disorderly conduct of Hsc70 and its interaction with the Alzheimer's-related tau protein. *J Biol Chem.* (2018) 293:10796–809. doi: 10.1074/jbc.RA118.002234
47. Perez M, Valpuesta JM, Medina M, Montejo De Garcini E, Avila J. Polymerization of tau into filaments in the presence of heparin: the minimal sequence required for tau-tau interaction. *J Neurochem.* (1996) 67:1183–90. doi: 10.1046/j.1471-4159.1996.67031183.x
48. Selden SC, Pollard TD. Interaction of actin filaments with microtubules is mediated by microtubule-associated proteins and regulated by phosphorylation. *Ann N Y Acad Sci.* (1986) 466:803–12. doi: 10.1111/j.1749-6632.1986.tb38464.x
49. Correas I, Padilla R, Avila J. The tubulin-binding sequence of brain microtubule-associated proteins, tau and MAP-2, is also involved in actin binding. *Biochem J.* (1990) 269:61–4. doi: 10.1042/bj2690061
50. Moraga DM, Nunez P, Garrido J, Maccioni RB. A tau fragment containing a repetitive sequence induces bundling of actin filaments. *J Neurochem.* (1993) 61:979–86. doi: 10.1111/j.1471-4159.1993.tb03611.x
51. Ramirez-Rios S, Denarier E, Prezel E, Vinit A, Stoppin-Mellet V, Devred F, et al. Tau antagonizes end-binding protein tracking at microtubule ends through a phosphorylation-dependent mechanism. *Mol Biol Cell.* (2016) 27:2924–34. doi: 10.1091/mbc.E16-01-0029
52. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, et al. LRP1 is a master regulator of tau uptake and spread. *Nature.* (2020) 580:381–5. doi: 10.1038/s41586-020-2156-5
53. Gauthier-Kemper A, Suárez Alonso M, Sündermann F, Niewidok B, Fernandez M-P, Bakota L, et al. Annexins A2 and A6 interact with the extreme N-terminus of tau and thereby contribute to tau's axonal localization. *J Biol Chem.* (2018) 293:8065–76. doi: 10.1074/jbc.RA117.000490
54. Stefanoska K, Volkerling A, Bertz J, Poljak A, Ke YD, Ittner LM, et al. An N-terminal motif unique to primate tau enables differential protein-protein interactions. *J Biol Chem.* (2018) 293:3710–9. doi: 10.1074/jbc.RA118.001784
55. Gerke V, Creutz CE, Moss SE. Annexins: linking Ca^{2+} signalling to membrane dynamics. *Nat Rev Mol Cell Biol.* (2005) 6:449–61. doi: 10.1038/nrm1661
56. Korber C, Kuner T. Molecular machines regulating the release probability of synaptic vesicles at the active zone. *Front Synaptic Neurosci.* (2016) 8:5. doi: 10.3389/fnsyn.2016.00005
57. McInnes J, Wierda K, Snellinx A, Bounti L, Wang YC, Stancu IC, et al. Synaptogyrin-3 mediates presynaptic dysfunction induced by tau. *Neuron.* (2018) 97:823–35 e8. doi: 10.1016/j.neuron.2018.01.022
58. Arrasate M, Perez M, Avila J. Tau dephosphorylation at tau-1 site correlates with its association to cell membrane. *Neurochem Res.* (2000) 25:43–50. doi: 10.1023/A:1007583214722
59. Maas T, Eidenmuller J, Brandt R. Interaction of tau with the neural membrane cortex is regulated by phosphorylation at sites that are modified in paired helical filaments. *J Biol Chem.* (2000) 275:15733–40. doi: 10.1074/jbc.M000389200
60. Pooler AM, Usardi A, Evans CJ, Philpott KL, Noble W, Hanger DP. Dynamic association of tau with neuronal membranes is regulated by phosphorylation. *Neurobiol Aging.* (2012) 33:431 e427–38. doi: 10.1016/j.neurobiolaging.2011.01.005
61. Jones EM, Dubey M, Camp PJ, Vernon BC, Biernat J, Mandelkow E, et al. Interaction of tau protein with model lipid membranes induces tau structural compaction and membrane disruption. *Biochemistry.* (2012) 51:2539–50. doi: 10.1021/bi201857v
62. Kunze G, Barre P, Scheidt HA, Thomas L, Eliezer D, Huster D. Binding of the three-repeat domain of tau to phospholipid membranes induces an aggregated-like state of the protein. *Biochim Biophys Acta.* (2012) 1818:2302–13. doi: 10.1016/j.bbamem.2012.03.019
63. Georgieva ER, Xiao S, Borbat PP, Freed JH, Eliezer D. Tau binds to lipid membrane surfaces via short amphipathic helices located in its microtubule-binding repeats. *Biophys J.* (2014) 107:1441–52. doi: 10.1016/j.bpj.2014.07.046
64. Ait-Bouziad N, Lv G, Mahul-Mellier AL, Xiao S, Zorludemir G, Eliezer D, et al. Discovery and characterization of stable and toxic Tau/phospholipid oligomeric complexes. *Nat Commun.* (2017) 8:1678. doi: 10.1038/s41467-017-01575-4
65. Ait-Bouziad N, Chiki A, Limorenko G, Xiao S, Eliezer D, Lashuel HA. Phosphorylation of the overlooked tyrosine 310 regulates the structure, aggregation, and microtubule- and lipid-binding properties of tau. *J Biol Chem.* (2020) 295:7905–22. doi: 10.1074/jbc.RA119.012517
66. Sun W, Qureshi HY, Cafferty PW, Sobue K, Agarwal-Mawal A, Neufield KD, et al. Glycogen synthase kinase-3beta is complexed with tau protein in brain microtubules. *J Biol Chem.* (2002) 277:11933–40. doi: 10.1074/jbc.M107182200
67. Jaworski T, Banach-Kasper E, Gralec K. GSK-3beta at the intersection of neuronal plasticity and neurodegeneration. *Neural Plast.* (2019) 2019:4209475. doi: 10.1155/2019/4209475
68. Spittaels K, Van Den Haute C, Van Dorpe J, Geerts H, Mercken M, Bruynseels K, et al. Glycogen synthase kinase-3beta phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice. *J Biol Chem.* (2000) 275:41340–9. doi: 10.1074/jbc.M006219200
69. Tugaeva KV, Tsvetkov PO, Sluchanko NN. Bacterial co-expression of human Tau protein with protein kinase A and 14-3-3 for studies of 14-3-3/phospho-Tau interaction. *PLoS ONE.* (2017) 12:e0178933. doi: 10.1371/journal.pone.0178933
70. Cornell B, Toyo-Oka K. 14-3-3 proteins in brain development: neurogenesis, neuronal migration and neuromorphogenesis. *Front Mol Neurosci.* (2017) 10:318. doi: 10.3389/fnmol.2017.00318
71. Joo Y, Schumacher B, Landrieu I, Bartel M, Smet-Nocca C, Jang A, et al. Involvement of 14-3-3 in tubulin instability and impaired axon development is mediated by tau. *FASEB J.* (2015) 29:4133–44. doi: 10.1096/fj.14-265009
72. Lee G, Newman ST, Gard DL, Band H, Panchamoorthy G. Tau interacts with src-family non-receptor tyrosine kinases. *J Cell Sci.* (1998) 111:3167–77.
73. Lee G, Thangavel R, Sharma VM, Litersky JM, Bhaskar K, Fang SM, et al. Phosphorylation of tau by fyn: implications for Alzheimer's disease. *J Neurosci.* (2004) 24:2304–12. doi: 10.1523/JNEUROSCI.4162-03.2004
74. Sontag JM, Nunbhakdi-Craig V, White CL III, Halpain S, Sontag E. The protein phosphatase PP2A/Balpha binds to the microtubule-associated proteins tau and MAP2 at a motif also recognized by the kinase Fyn: implications for tauopathies. *J Biol Chem.* (2012) 287:14984–93. doi: 10.1074/jbc.M111.338681
75. Smet C, Sambo AV, Wieruszeski JM, Leroy A, Landrieu I, Buee L, et al. The peptidyl prolyl cis/trans-isomerase Pin1 recognizes the phospho-Thr212-Pro213 site on tau. *Biochemistry.* (2004) 43:2032–40. doi: 10.1021/bi035479x
76. Albayram O, Herbert MK, Kondo A, Tsai CY, Baxley S, Lian X, et al. Function and regulation of tau conformations in the development and treatment of traumatic brain injury and neurodegeneration. *Cell Biosci.* (2016) 6:59. doi: 10.1186/s13578-016-0124-4
77. Brandt R, Lee G, Teplow DB, Shalloway D, Abdel-Ghany M. Differential effect of phosphorylation and substrate modulation on tau's ability to promote microtubule growth and nucleation. *J Biol Chem.* (1994) 269:11776–82.
78. Sillen A, Barbier P, Landrieu I, Lefebvre S, Wieruszeski JM, Leroy A, et al. NMR investigation of the interaction between the neuronal protein tau and the microtubules. *Biochemistry.* (2007) 46:3055–64. doi: 10.1021/bi061920i
79. Eidenmuller J, Fath T, Maas T, Pool M, Sontag E, Brandt R. Phosphorylation-mimicking glutamate clusters in the proline-rich region are sufficient to simulate the functional deficiencies of hyperphosphorylated tau protein. *Biochem J.* (2001) 357:759–67. doi: 10.1042/bj3570759

80. Goncalves RA, Wijesekara N, Fraser PE, De Felice FG. Behavioral abnormalities in knockout and humanized tau mice. *Front Endocrinol (Lausanne).* (2020) 11:124. doi: 10.3389/fendo.2020.00124
81. Tan XL, Zheng P, Wright DK, Sun M, Brady RD, Liu S, et al. The genetic ablation of tau improves long-term, but not short-term, functional outcomes after experimental traumatic brain injury in mice. *Brain Inj.* (2020) 34:131–9. doi: 10.1080/02699052.2019.1667539
82. Pallas-Bazarra N, Draffin J, Cuadros R, Antonio Esteban J, Avila J. Tau is required for the function of extrasynaptic NMDA receptors. *Sci Rep.* (2019) 9:9116. doi: 10.1038/s41598-019-45547-8
83. Singh B, Covelo A, Martell-Martinez H, Nanclares C, Sherman MA, Okematti E, et al. Tau is required for progressive synaptic and memory deficits in a transgenic mouse model of alpha-synucleinopathy. *Acta Neuropathol.* (2019) 138:551–74. doi: 10.1007/s00401-019-02032-w
84. Jara C, Aranguiz A, Cerpa W, Tapia-Rojas C, Quintanilla RA. Genetic ablation of tau improves mitochondrial function and cognitive abilities in the hippocampus. *Redox Biol.* (2018) 18:279–94. doi: 10.1016/j.redox.2018.07.010
85. Beauchamp LC, Chan J, Hung LW, Padman BS, Vella LJ, Liu XM, et al. Ablation of tau causes an olfactory deficit in a murine model of Parkinson's disease. *Acta Neuropathol Commun.* (2018) 6:57. doi: 10.1186/s40478-018-0560-y
86. Velazquez R, Ferreira E, Tran A, Turner EC, Belfiore R, Branca C, et al. Acute tau knockdown in the hippocampus of adult mice causes learning and memory deficits. *Aging Cell.* (2018) 17:e12775. doi: 10.1111/acel.12775
87. Tan DCS, Yao S, Ittner A, Bertz J, Ke YD, Ittner LM, et al. Generation of a new tau knockout (tauDeltaex1) line using CRISPR/Cas9 genome editing in mice. *J Alzheimers Dis.* (2018) 62:571–8. doi: 10.3233/JAD-171058
88. Biundo F, Del Prete D, Zhang H, Arancio O, D'adamio L. A role for tau in learning, memory and synaptic plasticity. *Sci Rep.* (2018) 8:3184. doi: 10.1038/s41598-018-21596-3
89. Wijesekara N, Goncalves RA, Ahrens R, De Felice FG, Fraser PE. Tau ablation in mice leads to pancreatic beta cell dysfunction and glucose intolerance. *FASEB J.* (2018) 32:3166–73. doi: 10.1096/fj.201701352
90. Yoshikawa M, Soeda Y, Michikawa M, Almeida OFX, Takashima A. Tau depletion in APP transgenic mice attenuates task-related hyperactivation of the hippocampus and differentially influences locomotor activity and spatial memory. *Front Neurosci.* (2018) 12:124. doi: 10.3389/fnins.2018.00124
91. Dioli C, Patricio P, Trindade R, Pinto LG, Silva JM, Morais M, et al. Tau-dependent suppression of adult neurogenesis in the stressed hippocampus. *Mol Psychiatry.* (2017) 22:1110–8. doi: 10.1038/mp.2017.103
92. Swanson E, Breckenridge L, Mcmahon L, Som S, Mcconnell I, Bloom GS. Extracellular tau oligomers induce invasion of endogenous tau into the somatodendritic compartment and axonal transport dysfunction. *J Alzheimers Dis.* (2017) 58:803–20. doi: 10.3233/JAD-170168
93. Zheng M, Jiao L, Tang X, Xiang X, Wan X, Yan Y, et al. Tau haploinsufficiency causes prenatal loss of dopaminergic neurons in the ventral tegmental area and reduction of transcription factor orthodenticle homeobox 2 expression. *FASEB J.* (2017) 31:3349–58. doi: 10.1096/fj.201601303R
94. Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, et al. Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Mol Psychiatry.* (2017) 22:1520–30. doi: 10.1038/mp.2017.171
95. Marciniak E, Leboucher A, Caron E, Ahmed T, Tailleux A, Dumont J, et al. Tau deletion promotes brain insulin resistance. *J Exp Med.* (2017) 214:2257–69. doi: 10.1084/jem.20161731
96. Wobst HJ, Denk F, Oliver PL, Livieratos A, Taylor TN, Knudsen MH, et al. Increased 4R tau expression and behavioural changes in a novel MAPT-N296H genomic mouse model of tauopathy. *Sci Rep.* (2017) 7:43198. doi: 10.1038/srep43198
97. Vanderweyde T, Apicco DJ, Youmans-Kidder K, Ash PEA, Cook C, Lummertz Da Rocha E, et al. Interaction of tau with the RNA-binding protein TIA1 regulates tau pathophysiology and toxicity. *Cell Rep.* (2016) 15:1455–66. doi: 10.1016/j.celrep.2016.04.045
98. Pallo SP, Dimaio J, Cook A, Nilsson B, Johnson GVW. Mechanisms of tau and abeta-induced excitotoxicity. *Brain Res.* (2016) 1634:119–31. doi: 10.1016/j.brainres.2015.12.048
99. Li X, Lei P, Tuo Q, Ayton S, Li QX, Moon S, et al. Enduring elevations of hippocampal amyloid precursor protein and iron are features of beta-amyloid toxicity and are mediated by tau. *Neurotherapeutics.* (2015) 12:862–73. doi: 10.1007/s13311-015-0378-2
100. Pallo SP, Johnson GV. Tau facilitates Abeta-induced loss of mitochondrial membrane potential independent of cytosolic calcium fluxes in mouse cortical neurons. *Neurosci Lett.* (2015) 597:32–7. doi: 10.1016/j.neulet.2015.04.021
101. Decker JM, Kruger L, Sydow A, Zhao S, Frotscher M, Mandelkow E, et al. Pro-aggregant tau impairs mossy fiber plasticity due to structural changes and Ca(++) dysregulation. *Acta Neuropathol Commun.* (2015) 3:23. doi: 10.1186/s40478-015-0193-3
102. Lei P, Ayton S, Moon S, Zhang Q, Volitakis I, Finkelstein DI, et al. Motor and cognitive deficits in aged tau knockout mice in two background strains. *Mol Neurodegener.* (2014) 9:29. doi: 10.1186/1750-1326-9-29
103. Abbondante S, Baglietto-Vargas D, Rodriguez-Ortiz CJ, Estrada-Hernandez T, Medeiros R, Laferla FM. Genetic ablation of tau mitigates cognitive impairment induced by type 1 diabetes. *Am J Pathol.* (2014) 184:819–26. doi: 10.1016/j.ajpath.2013.11.021
104. Kimura T, Whitcomb DJ, Jo J, Regan P, Piers T, Heo S, et al. Microtubule-associated protein tau is essential for long-term depression in the hippocampus. *Philos Trans R Soc Lond B Biol Sci.* (2014) 369:20130144. doi: 10.1098/rstb.2013.0144
105. Zempel H, Luedtke J, Kumar Y, Biernat J, Dawson H, Mandelkow E, et al. Amyloid-beta oligomers induce synaptic damage via tau-dependent microtubule severing by TTLL6 and spastin. *EMBO J.* (2013) 32:2920–37. doi: 10.1038/emboj.2013.207
106. Seward ME, Swanson E, Norambuena A, Reimann A, Cochran JN, Li R, et al. Amyloid-beta signals through tau to drive ectopic neuronal cell cycle re-entry in Alzheimer's disease. *J Cell Sci.* (2013) 126:1278–86. doi: 10.1242/jcs.1125880
107. Morris M, Hamto P, Adame A, Devidze N, Masliah E, Mucke L. Age-appropriate cognition and subtle dopamine-independent motor deficits in aged tau knockout mice. *Neurobiol Aging.* (2013) 34:1523–9. doi: 10.1016/j.neurobiolaging.2012.12.003
108. Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK, et al. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nat Med.* (2012) 18:291–5. doi: 10.1038/nm.2613
109. Dawson HN, Cantillana V, Jansen M, Wang H, Vitek MP, Wilcock DM, et al. Loss of tau elicits axonal degeneration in a mouse model of Alzheimer's disease. *Neuroscience.* (2010) 169:516–31. doi: 10.1016/j.neuroscience.2010.04.037
110. Miao Y, Chen J, Zhang Q, Sun A. Deletion of tau attenuates heat shock-induced injury in cultured cortical neurons. *J Neurosci Res.* (2010) 88:102–10. doi: 10.1002/jnr.22188
111. Gomez De Barreda E, Perez M, Gomez Ramos P, De Cristobal J, Martin-Maestro P, Moran A, et al. Tau-knockout mice show reduced GSK3-induced hippocampal degeneration and learning deficits. *Neurobiol Dis.* (2010) 37:622–9. doi: 10.1016/j.nbd.2009.11.017
112. Fuster-Matanzo A, De Barreda EG, Dawson HN, Vitek MP, Avila J, Hernandez F. Function of tau protein in adult newborn neurons. *FEBS Lett.* (2009) 583:3063–8. doi: 10.1016/j.febslet.2009.08.017
113. Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to beta-amyloid-induced neurotoxicity. *Proc Natl Acad Sci USA.* (2002) 99:6364–9. doi: 10.1073/pnas.092136199
114. Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci.* (2001) 114:1179–87. Available online at: https://jcs.biologists.org/content/114/6/1179.article-info
115. Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, et al. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature.* (1994) 369:488–91. doi: 10.1038/369488a0
116. Donovan LE, Higginbotham L, Dammer EB, Gearing M, Rees HD, Xia Q, et al. Analysis of a membrane-enriched proteome from postmortem human brain tissue in Alzheimer's disease. *Proteomics Clin Appl.* (2012) 6:201–11. doi: 10.1002/prca.201100068
117. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida H, Titani K, et al. Proline-directed and non-proline-directed phosphorylation of PHF-tau. *J Biol Chem.* (1995) 270:823–9. doi: 10.1074/jbc.270.2.823

118. Hanger DP, Byers HL, Wray S, Leung KY, Saxton MJ, Seereeram A, et al. Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis. *J Biol Chem.* (2007) 282:23645–54. doi: 10.1074/jbc.M703269200
119. Neddens J, Temmel M, Flunkert S, Kerschbaumer B, Hoeller C, Loeffler T, et al. Phosphorylation of different tau sites during progression of Alzheimer's disease. *Acta Neuropathol Commun.* (2018) 6:52. doi: 10.1186/s40478-018-0557-6
120. Zhou XW, Li X, Bjorkdahl C, Sjogren MJ, Alafuzoff I, Soininen H, et al. Assessments of the accumulation severities of amyloid beta-protein and hyperphosphorylated tau in the medial temporal cortex of control and Alzheimer's brains. *Neurobiol Dis.* (2006) 22:657–68. doi: 10.1016/j.nbd.2006.01.006
121. Baas PW, Qiang L. Tau: it's not what you think. *Trends Cell Biol.* (2019) 29:452–61. doi: 10.1016/j.tcb.2019.02.007
122. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature.* (2017) 547:185–90. doi: 10.1038/nature23002
123. Haj-Yahya M, Gopinath P, Rajasekhar K, Mirbaha H, Diamond MI, Lashuel HA. Site-specific hyperphosphorylation inhibits, rather than promotes, tau fibrillization, seeding capacity, and its microtubule binding. *Angew Chem Int Ed.* (2020) 59:4059–67. doi: 10.1002/anie.201913001
124. Rankin CA, Sun Q, Gamblin TC. Pseudo-phosphorylation of tau at Ser202 and Thr205 affects tau filament formation. *Brain Res Mol Brain Res.* (2005) 138:84–93. doi: 10.1016/j.molbrainres.2005.04.012
125. Ikura T, Tochio N, Kawasaki R, Matsuzaki M, Narita A, Kikumoto M, et al. The trans isomer of tau peptide is prone to aggregate, and the WW domain of Pin1 drastically decreases its aggregation. *FEBS Lett.* (2018) 592:3082–91. doi: 10.1002/1873-3468.13218
126. Yin H, Kuret J. C-terminal truncation modulates both nucleation and extension phases of tau fibrillization. *FEBS Lett.* (2006) 580:211–5. doi: 10.1016/j.febslet.2005.11.077
127. Nubling G, Levin J, Bader B, Israel L, Botzel K, Lorenzl S, et al. Limited cleavage of tau with matrix-metalloproteinase MMP-9, but not MMP-3, enhances tau oligomer formation. *Exp Neurol.* (2012) 237:470–6. doi: 10.1016/j.expneurol.2012.07.018
128. Zhang Z, Song M, Liu X, Kang SS, Kwon IS, Duong DM, et al. Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease. *Nat Med.* (2014) 20:1254–62. doi: 10.1038/nm.3700
129. Means JC, Gerdes BC, Kaja S, Sumien N, Payne AJ, Stark DA, et al. Caspase-3-dependent proteolytic cleavage of tau causes neurofibrillary tangles and results in cognitive impairment during normal aging. *Neurochem Res.* (2016) 41:2278–88. doi: 10.1007/s11064-016-1942-9
130. Wilson DM, Binder LI. Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. *In vitro* evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol.* (1997) 150:2181–95.
131. Barracchia CG, Tira R, Parolini F, Munari F, Bubacco L, Spyroulias GA, et al. Unsaturated fatty acid-induced conformational transitions and aggregation of the repeat domain of tau. *Molecules.* (2020) 25:2716. doi: 10.3390/molecules25112716
132. Gamblin TC, Berry RW, Binder LI. Tau polymerization: role of the amino terminus. *Biochemistry.* (2003) 42:2252–7. doi: 10.1021/bi0272510
133. Horowitz PM, Lapointe N, Guillozet-Bongaarts AL, Berry RW, Binder LI. N-terminal fragments of tau inhibit full-length tau polymerization *in vitro*. *Biochemistry.* (2006) 45:12859–66. doi: 10.1021/bi061325g
134. Shea TB. Phospholipids alter tau conformation, phosphorylation, proteolysis, and association with microtubules: implication for tau function under normal and degenerative conditions. *J Neurosci Res.* (1997) 50:114–22. doi: 10.1002/(SICI)1097-4547(19971001)50:1<114::AID-JNR12>3.0.CO;2-B
135. Elbaum-Garfinkle S, Ramlall T, Rhoades E. The role of the lipid bilayer in tau aggregation. *Biophys J.* (2010) 98:2722–30. doi: 10.1016/j.bpj.2010.03.013
136. Gauthier-Kemper A, Weissmann C, Golovyashkina N, Sebo-Lemke Z, Drewes G, Gerke V, et al. The frontotemporal dementia mutation R406W blocks tau's interaction with the membrane in an annexin A2-dependent manner. *J Cell Biol.* (2011) 192:647–61. doi: 10.1083/jcb.201007161
137. Jeganathan S, Von Bergen M, Brutlach H, Steinhoff HJ, Mandelkow E. Global hairpin folding of tau in solution. *Biochemistry.* (2006) 45:2283–93. doi: 10.1021/bi0521543
138. Connell JW, Gibb GM, Betts JC, Blackstock WP, Gallo J, Lovestone S, et al. Effects of FTDP-17 mutations on the *in vitro* phosphorylation of tau by glycogen synthase kinase 3beta identified by mass spectrometry demonstrate certain mutations exert long-range conformational changes. *FEBS Lett.* (2001) 493:40–4. doi: 10.1016/S0014-5793(01)02267-0
139. Hayashi S, Toyoshima Y, Hasegawa M, Umeda Y, Wakabayashi K, Tokiguchi S, et al. Late-onset frontotemporal dementia with a novel exon 1 (Arg5His) tau gene mutation. *Ann Neurol.* (2002) 51:525–30. doi: 10.1002/ana.10163
140. Poorkaj P, Muma NA, Zhukareva V, Cochran EJ, Shannon KM, Hurtig H, et al. An R5L tau mutation in a subject with a progressive supranuclear palsy phenotype. *Ann Neurol.* (2002) 52:511–6. doi: 10.1002/ana.10340
141. Zhou L, McInnes J, Wierda K, Holt M, Herrmann AG, Jackson RJ, et al. Tau association with synaptic vesicles causes presynaptic dysfunction. *Nat Commun.* (2017) 8:15295. doi: 10.1038/ncomms15295
142. Flach K, Hilbrich I, Schiffmann A, Gartner U, Kruger M, Leonhardt M, et al. Tau oligomers impair artificial membrane integrity and cellular viability. *J Biol Chem.* (2012) 287:43223–33. doi: 10.1074/jbc.M112.396176

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 12 November 2020
doi: 10.3389/fneur.2020.590199

Cellular Biology of Tau Diversity and Pathogenic Conformers

*Sang-Gyun Kang[1], Ghazaleh Eskandari-Sedighi[2], Lenka Hromadkova[3], Jiri G. Safar[3] and David Westaway[1,2]**

[1] Center for Prions and Protein Folding Diseases, University of Alberta, Edmonton, AB, Canada, [2] Department of Biochemistry, University of Alberta, Edmonton, AB, Canada, [3] Department of Neurology and Pathology, Case Western Reserve University, Cleveland, OH, United States

Tau accumulation is a prominent feature in a variety of neurodegenerative disorders and remarkable effort has been expended working out the biochemistry and cell biology of this cytoplasmic protein. Tau's wayward properties may derive from germline mutations in the case of frontotemporal lobar degeneration (FTLD-MAPT) but may also be prompted by less understood cues—perhaps environmental or from molecular damage as a consequence of chronological aging—in the case of idiopathic tauopathies. Tau properties are undoubtedly affected by its covalent structure and in this respect tau protein is not only subject to changes in length produced by alternative splicing and endoproteolysis, but different types of posttranslational modifications that affect different amino acid residues. Another layer of complexity concerns alternate conformations—"conformers"—of the same covalent structures; *in vivo* conformers can encompass soluble oligomeric species, ramified fibrillar structures evident by light and electron microscopy and other forms of the protein that have undergone liquid-liquid phase separation to make demixed liquid droplets. Biological concepts based upon conformers have been charted previously for templated replication mechanisms for prion proteins built of the PrP polypeptide; these are now providing useful explanations to feature tau pathobiology, including how this protein accumulates within cells and how it can exhibit predictable patterns of spread across different neuroanatomical regions of an affected brain. In sum, the documented, intrinsic heterogeneity of tau forms and conformers now begins to speak to a fundamental basis for diversity in clinical presentation of tauopathy sub-types. In terms of interventions, emphasis upon subclinical events may be worthwhile, noting that irrevocable cell loss and ramified protein assemblies feature at end-stage tauopathy, whereas earlier events may offer better opportunities for diverting pathogenic processes. Nonetheless, the complexity of tau sub-types, which may be present even within intermediate disease stages, likely mitigates against one-size-fits-all therapeutic strategies and may require a suite of interventions. We consider the extent to which animal models of tauopathy can be reasonably enrolled in the campaign to produce such interventions and to slow the otherwise inexorable march of disease progression.

Keywords: cell-to-cell transmission, liquid-liquid phase separation, molecular heterogeneity, tauopathy, transgenic mice, ubiquitin-proteasome system

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Yumiko Motoi, Juntendo University, Japan
Simon Dujardin, Massachusetts General Hospital and Harvard Medical School, United States

***Correspondence:**
David Westaway
david.westaway@ualberta.ca

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *31 July 2020*
Accepted: *05 October 2020*
Published: *12 November 2020*

Citation:
Kang S-G, Eskandari-Sedighi G, Hromadkova L, Safar JG and Westaway D (2020) Cellular Biology of Tau Diversity and Pathogenic Conformers. Front. Neurol. 11:590199. doi: 10.3389/fneur.2020.590199

INTRODUCTION

Propelled by documentation of tau accumulation in a variety of neurodegenerative disorders and a causal role in some instances, as defined by *MAPT* mutation kindreds, the past two decades of work has seen both remarkable efforts applied to this errant cytoplasmic protein and new insights into its biology and pathobiology. Some strides in understanding have been helped by the availability of corresponding rodent models, but advances in this period have also arisen from the emergence of new, generalized biological techniques such as inducible pluripotent stem cells, three-dimensional cell cultures, optogenetics, gene editing, and cryo electron microscopy (cryo-EM)—to name but a few. In the conceptual realm, prion replication mechanisms of templated protein misfolding derived from study of the prion protein (PrP) have been instrumental in considering how tau disease events are perpetuated inside cells and also how they might spread in an infection-like manner between cells. Similarly, the new understanding that there are forms of protein folding and assembly unrecognized by earlier textbook concepts of secondary, tertiary and quaternary structure is also gaining influence. Thus, the concept of liquid-liquid phase separation (LLPS) of proteins arising from curiosity-driven insights into the why's and wherefore's of low complexity domains in proteins has been extended to encompass DNA-binding protein 43 (TDP-43), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) and fused in sarcoma (FUS) in neurodegenerative diseases and now tau itself. Here we recap the remarkable diversity of covalent and conformational variants of tau in tauopathies and some parallels in diverse cell biological effects, these including transit within cells of the same lineage type and across cell lineages. We present an emphasis upon subclinical events, noting that irrevocable cell loss and ramified protein assemblies feature at end-stage tauopathy, whereas earlier malleable events may offer better targets for diverting disease processes. We also discuss uses and limitations of animal models of tauopathy to produce interventions and the trend toward use of low-expresser rodent transgenic models as slower, yet potentially more accurate, embodiments of disease pathogenesis.

In terms of the disease landscape to be considered here, there are no <27 tauopathies described to date. Some examples include frontotemporal lobar degeneration associated with *MAPT* mutations (FTLD-MAPT), Pick's disease (PiD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), globular glial tauopathy (GGT) and argyrophilic grain disease (AGD) (1, 2), with considerations of effects in different cell lineages included in Section cell lineages harboring abnormal forms of tau. All tauopathies share the common feature of tau aggregation and deposition in the brain. They are also categorized into two subgroups: primary and secondary. Primary tauopathies are the diseases in which tau aggregation plays a prominent role in disease pathogenesis. In secondary tauopathies, the disease is fueled by defects of other proteins or by tissue trauma which then lead (by means that are sometimes debated) to changes in the repertoire of tau molecular species. Alzheimer's disease (AD) and the inherited prion disease Gerstmann–Sträussler–Scheinker syndrome (GSS) may be classified as secondary tauopathies (3). Arguably the most well-studied tau accumulations are paired helical filaments (PHFs), the principal constituent of the neurofibrillary tangles (NFTs) in AD patients. These filaments consist of two structurally distinct parts: an external "fuzzy coat" comprised of N- and C-terminal areas of the protein that can be removed by treatment with the broad-spectrum protease pronase and then a distinct pronase-resistant region, closer to the center of the protein, containing the tandem microtubule-binding repeats (4); the domain structure of tau will be considered in more detail in Section tau physiology, spliced forms and posttranslational covalent variations.

FTLD-MAPT [see (5)], as per its name, revolves around *MAPT* mutations and is a neuropathological correlate of frontotemporal dementia (FTD). With respect to the goal of explaining tau diversity in chemical and cell biological terms, as FTLD-MAPT is a primary tauopathy caused by germline mutations, it seems potentially easier to understand the pathogenic process than in cases of idiopathic (sporadic) forms of AD and FTD that lack such mutations. In short, it is perhaps an advantageous model for delineating steps in disease progression. Even so, FTLD-MAPT pathogenesis is not automatically straightforward, noting that cases harboring the same mutation can have a diversity of clinical phenotypes (6–8) including different neuropathological findings. Perhaps surprisingly given a transcriptional profile defining tau mRNA mainly in neurons, unusual forms of tau protein can be found in astrocytes and oligodendrocytes (see below). This is generally the case for FTLD-MAPT with different types of tau mutations and the situation holds for the specific case of the P301L mutation, a case which we have studied in detail using biopsy material from an Iberian P301L kindred with a founder effect mutation (9, 10). Due to the position of the P301L mutation in exon 10 encoding microtubule binding repeat 2, it only affects the 4R form of tau (**Figure 1A**); in this respect, it is notable that P301L pathologies in astrocytes and oligodendrocytes resemble other 4R-tauopathies such as CBD (11). In short, a recurring observation for the FTLD-MAPT pathogenesis is phenotypic heterogeneity. To begin to grapple with this diversity, we will first consider some cardinal features of tau biochemistry and cell biology.

TAU PHYSIOLOGY, SPLICED FORMS, AND POSTTRANSLATIONAL COVALENT VARIATIONS

Tau protein is a microtubule associated protein (MAP), encoded by a single gene, *MAPT*, located on chromosome 17q21 of the human genome and consisting of a total of 16 exons (12) (**Figure 1A**). Tau mRNAs are mainly expressed in neurons and exhibit a developmental change in the ratios of spliced forms (13). Tau mRNAs and proteins have also been detected in oligodendrocytes and astrocytes, but often to a lesser extent (14–20). This observation presents an interesting twist when considering the accumulation of aberrant and potentially pathogenic tau protein in glial cell populations (as considered further below). Primary transcripts of *MAPT* undergo alternative

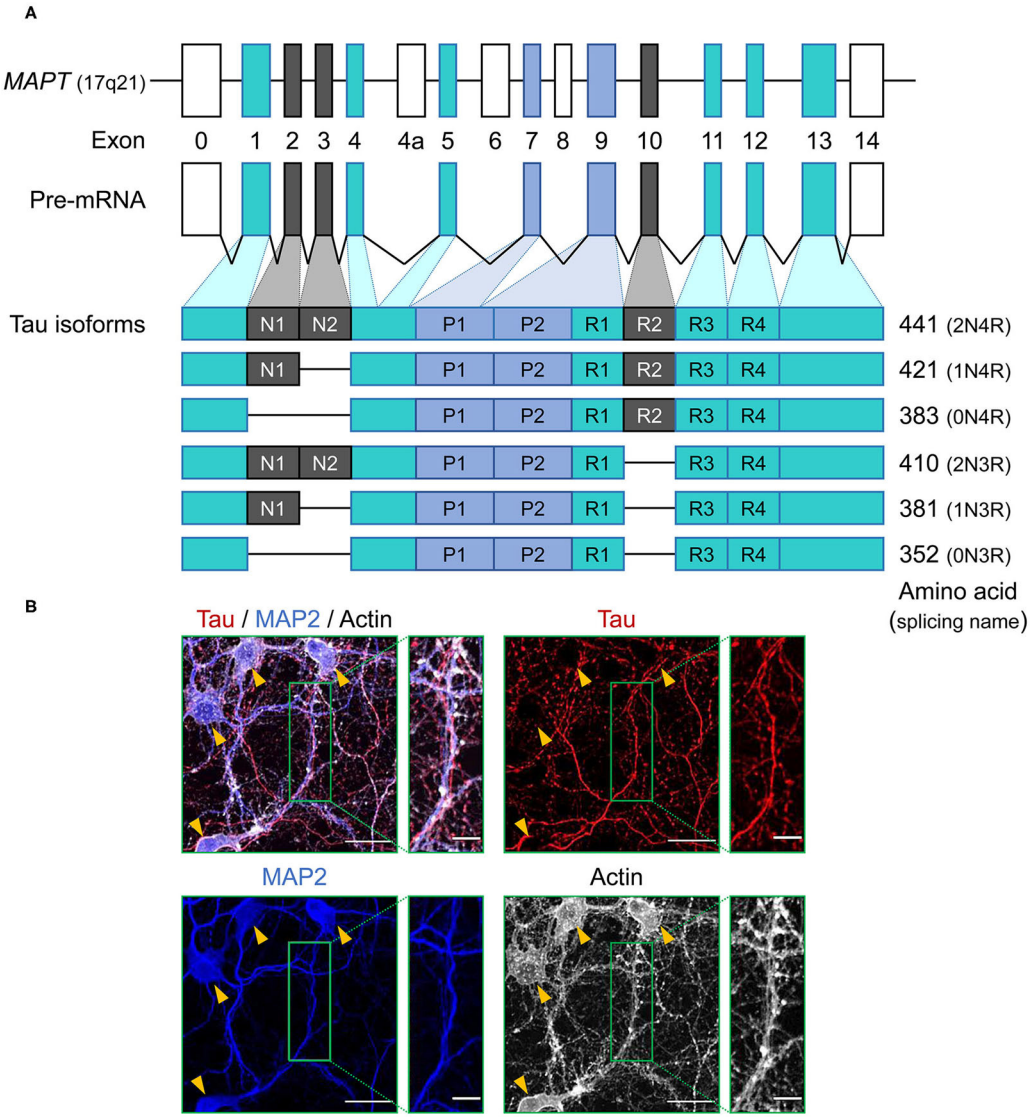


FIGURE 1 | Schematic representation of *MAPT* and the splice isoforms of tau in the human brain. **(A)** Human *MAPT* contains 16 exons. Exons in turquoise boxes (exons 1, 4, 5, 7, 9, 11, 12, 13) are constitutive, while the others are subject to alternative splicing. Exons 0 and 1 encode the 5′ untranslated sequences, and exon 14 is part of the 3′ untranslated region. Exon 4a, 6, and 8 are transcribed only in peripheral tissue, and alternative splicing of exon 2, 3, and 10 generates the six isoforms of tau. Tau isoforms translated from mRNAs that include exon 10, which encodes an additional microtubule-binding motif, are commonly referred to as four-repeat (4R) tau isoforms, whereas isoforms that exclude exon 10 are referred to as three-repeat (3R) tau isoforms. **(B)** Immunocytochemistry of tau (red) in primary hippocampal neurons at 21 days *in vitro* culture using anti-tau monoclonal antibody (RTM47 detecting 2-44 amino acid) with microtubule-associated protein 2 (MAP2) counter stains in blue and actin stain with TRITC-labeled phalloidin in gray. Yellow arrowheads indicate neuronal soma. Scale bars, 30 μm and 10 μm in the boxed images. Image: L. Hromadkova.

splicing events which, upon translation, yield six protein isoforms in the adult human brain. These spliced mRNA forms differ from each other by the presence or absence of exons 2, 3, and 10. Tau mRNAs that include exon 10, which encodes an additional microtubule-binding repeat (repeat 2), are commonly referred to as four-repeat (4R) tau spliced forms while mRNAs that exclude exon 10 are referred to as three-repeat (3R) tau spliced forms (**Figure 1A**). The distribution of spliced isoforms is inter-species variable, affected by brain development stage and varies in both temporal and spatial patterns on cellular and brain regional levels (21–25). *MAPT* knock-out mice develop normally without displaying any overt histological abnormalities, possibly due to tau function being rescued by other MAPs, but morphological phenotypes include a minor decrease in microtubule-stability of small caliber axons and some effects on axonogenesis (26, 27). Translation of these mRNAs yield a protein product distributed predominantly in the neuronal axons, but also found in various cellular locations.

Seen in broad overview, tau is a soluble hydrophilic protein described primarily as an essential factor for microtubule assembly (28). The more acidic N-terminal region is mostly involved in interactions with numerous tau binding partners (even affecting its association with cell and nuclear membranes, etc.) (29) while the positively charged C-terminal region encompasses three or four imperfect repeat domains and plays a crucial role in tau interactions with microtubule proteins (although this region can also be involved in interactions with other proteins) (30). Superimposed on this sketched ground-plan, alternative splicing of exons 2, 3 to make mRNAs encoding 0N, 1N, and 2N proteins isoforms can affect the natively disordered N-terminal region in respect to its binding properties with tau-interacting partners and, thus, even the cellular distribution of the protein (23). The N-terminal can itself be perceived as having sub-regions including an acidic region subject to alternative splices and, most notably, a proline-rich domain that can accept many phosphorylation events. The C-terminal region is home to tandem microtubule-binding repeats and subject to the 3R vs. 4R mRNA splicing already noted. These microtubule-binding repeats are followed by a C-terminal extension. Tau lacks any putative signal peptides, transmembrane helices, lipidation or glycolipidation sites that might integrate it into cell membranes and, while it is reported to have short amphipathic helices that might allow it to interact with membrane lipids (31), it is typically considered to be a "cytoplasmic" protein, albeit one that can end up in different cytoplasmic niches or compartments that abut the cytoplasm.

Despite an incomplete understanding of the functional implications of tau distribution among various cellular compartments, tau may be inferred to be multifunctional (32). The most well-studied function is the ability to regulate microtubule assembly and axonal transport of vesicles and organelles (33, 34). Unlike MAP2 which is another major species of MAPs found in the neuronal bodies and dendrites, tau is abundant in the axon (35) (**Figure 1B**). Tau localization to the other types of microtubule architectures such as growth cones (36, 37) and mitotic spindles (38) are indicative of its dynamic nature and functional repertoire extending beyond axonal microtubule polymerization to encompass developing or regenerating neurites and cell division processes. In neurons, tau has been identified in the synapses (39, 40) and might be involved in the regulation of morphological plasticity of dendrites (41, 42). Provocatively, tau can be released during neuronal activity, an effect which is inferred to involve presynaptic events (43, 44). Tau also binds to and protects neuronal DNA under stress conditions (e.g., oxidative and mild heat stresses) (45, 46) and participates in RNA metabolism through direct association with RNA-binding proteins (47, 48).

Descriptions of biochemistry and cell biology need to consider tau species as they behave physiologically vs. unequivocal disease-associated forms of tau (and there are also versions that might fall into to a middle ground). These issues are apparent from the association of different diseases with 3R- and 4R-tau and continue when one turns to another form of covalent variation, namely post-translational modification (PTM). Tau PTMs are striking and important and we have attempted to touch upon physiological and pathological versions of these. PTM's are described in overview in **Figure 2A** and are inventoried as follows:

Phosphorylation

Tau phosphorylation is arguably one of the most well-known and abundant PTMs targeting this protein. With 85 potential phosphorylation residues in the longest human isoform (441 amino acids) (45 Ser, 35 Thr, and 5 Tyr residues) (56), this protein is a notable target for several kinases and phosphatases. In consequence, tau's phosphorylation state represents the sum total of dynamic processes (57) and, in turn, regulates different capabilities of tau such as its interaction with the microtubule network and assembly, modulation of cell polarity, axonogenesis, and subcellular localization. Under-phosphorylated tau with phosphorylation of ∼1–3 residues is an efficient microtubule network stabilizer whereas hyperphosphorylated tau can have less interaction with microtubule proteins and hence can be more prone to misfolding and consequent aggregation (58). Noting the caveat that some phosphorylation sites are believed to have protective effects and inhibit tau from aggregation and formation of toxic species (59), a broad perspective is that imbalances between tau kinase and phosphatase activities may trigger the non-physiological tau phosphorylation with all the consequences leading to neurodegeneration (60, 61).

A higher amount of phosphorylation in AD brains vs. control brains is extensively documented; normal brain tau has 2–3 mole of phosphate per mole of protein, but AD brains contain tau with an ∼3-fold greater stoichiometry (62). So far, 45 phosphorylation sites were detected in insoluble aggregates of tau extracted from AD brain, herein referred to as PHF-tau, several of them being strictly AD-specific and some being shared with tau preparations isolated from control brains (57, 63). Moreover, some clusters of phosphorylation (e.g., Ser210-Thr217, Thr231-Ser238) are involved in a hierarchy of events, meaning that phosphorylation occurs sequentially with initial phosphorylation sites priming subsequent phosphorylation events on nearby residues (64, 65). The phosphorylation sites in PHF-tau are predominantly located in the proline-rich domain and the regions flanking the microtubule-binding domain (63, 65–68), and are involved in alterations in tau microtubule binding dynamics and interactions with other reactive partners. Four phosphorylated residues occurring specifically in PHF-tau have been identified in the microtubule-binding domain region (Ser258, Ser262, Ser289, and Ser356) and were shown to have an impact on microtubule binding capacity (69, 70). Some of the proposed mechanisms of toxicity for hyperphosphorylated tau species include: mis-sorting from axons to the somato-dendritic compartment, disruption of intracellular proteostasis network, interference with nuclear-cytoplasmic transport and dysregulation of physiological functions by altering the repertoire of protein interactors (71–74).

Proteolysis

As a result of proteolytic processing, generated fragments could behave differently compared to the full-length protein regarding

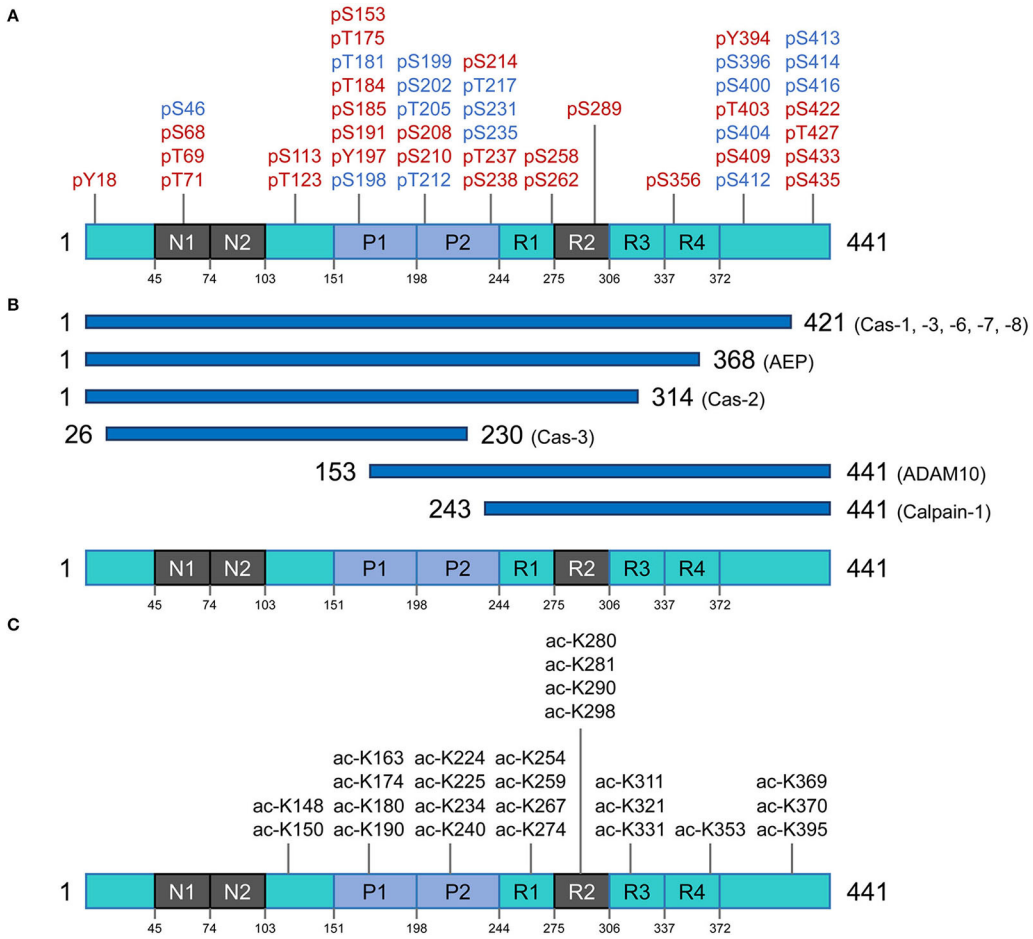


FIGURE 2 | Post translational modifications of the tau protein. **(A)** Phosphorylation residues. Phosphorylated residues found in pathological conditions are represented in red, while phosphorylated residues in blue are observed in both normal and diseased conditions. **(B)** Proteolysis of tau. Potential pathological signatures of tau fragments and the proteases in charge of the cleavage are presented. Further details are described in **Table 1**. **(C)** Acetylation residues. Acetylated Lys residues in pathological conditions are represented on the longest isoform of human tau (2N4R). This specific modification mainly targets the residues in the core region (Pro-rich domain and repeat domains). Cas, caspase; AEP, asparagine endopeptidase; ADAM10, disintegrin and metalloproteinase domain-containing protein 10.

(i) conformation, (ii) solubility, (iii) stability and half-life, (iv) cell localization, and (v) interacting molecules. Under normal circumstances, when tau is no longer needed, it can be efficiently targeted for cytoplasmic degradation pathways such as the ubiquitin-proteasome system (UPS) and autophagy-lysosome system as an attempt to maintain proteostasis (75). However, in disease conditions, tau can become the target of several endoproteases and produce fragments of different sizes that can be found within intracellular tau inclusions and can also be detected in cerebrospinal fluid (CSF), interstitial fluid (ISF) and plasma of patients with different tauopathies. Proteolytic fragments of tau have been shown to be secreted in a variety of cell systems and animal models [reviewed in (76)]. Generation of some of these fragments has been correlated with accumulation of pathologic tau entities and disease progression in several animal models, as well as in post-mortem tissue extracted from tauopathy patients. Moreover, the process of fragmentation is likely superimposed on the phenomenon of tau spreading (below) to generate species with more or less spreading capability. **Figure 2B** and **Table 1** summarize some of these well-characterized cleavage events, the cognate protease and potential relevance to disease conditions. The list of identified tau fragments is longer though, entailing several orphan fragments with corresponding proteases yet to be identified [reviewed in (77)].

Several members of the caspase family of proteases have been identified to cleave tau (caspases-1,−3,−6,−7, and−8) at residue 421 (49). This truncated fragment has the propensity to assemble into aggregates faster than the full-length protein and can be detected in fibrillar pathologies of the brain of AD patients (49). The 34 kDa fragment generated by caspase-2, known as ΔTau314, is another well-studied caspase-cleaved tau fragment (51, 78). This C-terminally truncated fragment of tau mis-localizes to dendritic spines and causes cognitive

TABLE 1 | Proteolysis events and fragmentation of tau.

Cleavage site	Identified fragment	Protease	Impacts of cleavage on disease pathogenesis	References
D421-S422	1–421 (Tau-C)	Cas-1, −3, −6, −7, and −8	Faster aggregation rate compared to full-length tau. Found associated with NFTs in AD brains and increased in AD, FTD-tau, and PSP compared to control brain samples.	(49, 50)
D314-L315, D421-S422 (*in vitro*)	1–314 (tau314)	Cas-2	Lower propensity to aggregate compared to tau441. Impaired synaptic transmission and drives hippocampal neuronal loss.	(51)
D25-Q26, R230-T231	26–230 (20–22 kDa)	Cas-3	Exacerbating mitochondrial dysfunction. Caused NMDAR-mediated cell death in rat CGCs.	(52)
N368-K369	1–368	Asparagine endopeptidase	Reduced ability to induce microtubule polymerization, triggered apoptosis. The C-terminal fragment has increased propensity to aggregate into PHFs compared to tau441.	(53)
A152-T153	153–441 (Tau-A)	ADAM10	Found in serum from patients with AD and inversely correlates with cognitive test scores. Physical interaction with tau is unclear.	(54)
R242-L243	243–441 (Tau-CTF24)	Calpain-1	Accelerates intracellular propagation of tau and has reduced capacity for promoting microtubule assembly compared to tau441.	(55)

The summary recaps tau fragments, proteases responsible for their generation and the impact on disease pathogenesis in animal models and human kindred.
Cas, caspase; CGCs, cerebellar granule cells; NMDAR, N-methyl-D-aspartate receptor (also known as the NMDA receptor); PHFs, paired helical filaments; ADAM10, disintegrin and metalloproteinase domain-containing protein 10, a membrane-tethered protease.

dysfunction in an animal model of tauopathy (51). A recent report indicates elevated levels of this fragment in cognitively impaired human kindreds (78). A 35 kDa N-terminally truncated tau fragment entailing the microtubule-binding repeat domains was reported to be present in post-mortem brains of patients diagnosed with tauopathies in which 4R isoforms predominate (79). Minimal expression of this fragment in mice (Tau35) led to tau neuropathology, deficits in cognitive and motor function, muscle degeneration and impaired proteostasis (51, 80). Since truncation of tau could facilitate subsequent conformational changes and enhance aggregation, modulating this particular PTM in different tauopathies could offer a new approach to therapeutic intervention (80, 81).

Acetylation

The very first report on tau acetylation was from a study on synthetic peptides spanning amino acids 160–182 and 264–287 of the full-length (2N4R) tau, to generate acetylated-tau antibodies. As a result of this study, Sirtuin 1 (SIRT1) was identified as a deacetylase targeting tau (82). Partially akin to phosphorylation, acetylation has a regulatory role on tau-microtubule interactions (83). There are over 20 Lys residues that can be targeted for acetylation, and acetylation of some appears to be of particular pathological significance (**Figure 2C**) (82, 84, 85). Tau protein can also undergo autoacetylation, by the help of catalytic Cys residues in the microtubule binding region (86). By neutralizing the repulsion of positively-charged Lys residues, acetylation tends to make parallel stacking of β-strands more favorable and hence promote tau aggregation (84, 87, 88). Physiological investigations have revealed that acetylation of tau also affects degradation of the protein (by inhibiting ubiquitination of Lys residues) and hence slows the rate of protein turnover; this is associated with attenuation of tau microtubule binding and promotion of aggregation, especially into soluble oligomers (89, 90).

O-glycosylation

O-glycosylation (or O-GlcNAcylation) is a dynamic process that involves the addition of the β-d-N-acetylglucosamine (GlcNAc) molecule to Ser or Thr residues of the target protein via O-linkage. The two enzymes responsible for regulation of this PTM are O-GlcNAc transferase and O-GlcNAcase (91). It has been shown that O-GlcNAcylation can negatively regulate tau phosphorylation in a site-specific manner *in vitro* and *in vivo* (in cell models) (92). The balance between tau hyperphosphorylation and O-glycosylation could also impact the protein's cellular localization (93). Moreover, tau tangles isolated from AD patients are hyperphosphorylated and hypo-O-glycosylated (92). However, it is still not fully clear whether decreased O-GlcNAcylation of tau has any causative effect on hyperphosphorylation or is simply a secondary effect (94). In fact, increasing tau O-GlcNAcylation via inhibition of O-GlcNAcase in JNPL3 tauopathy mouse model [mutant tau P301L under the mouse *PRNP* promoter (95)] hindered tau aggregation and decreased neuronal cell loss by impairing tau's ability to oligomerize and without affecting tau phosphorylation (96).

Other PTM's

Beyond phosphorylation, proteolysis, acetylation and O-glycosylation, multiple Lys residues occurring in tau molecule (44 residues in human full-length tau variant 2N4R) may be modified by other PTMs (ubiquitination, sumoylation, and methylation), which can then play a role in tau assembly and toxicity via participation in electrostatic and hydrophobic interactions (87, 97). N-glycosylation, prolyl-isomerization, nitration, polyamination, and oxidation are yet other PTMs discussed in relation to the tau-mediated pathogenesis of AD.

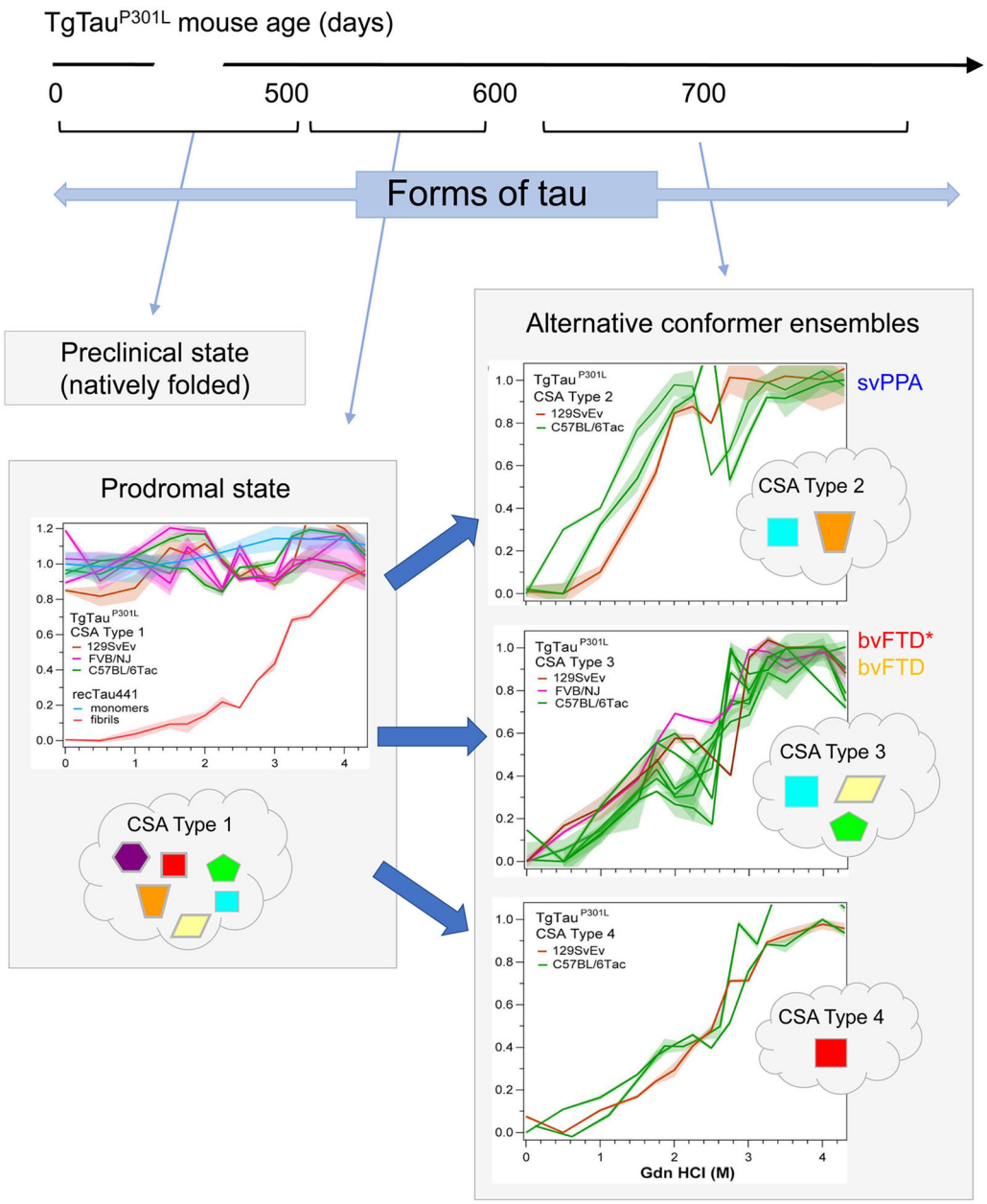


FIGURE 3 | Temporal evolution of conformer ensembles in the pathogenesis of a primary tauopathy. Conformers of protease-sensitive detergent-insoluble tau in TgTau^{P301L} mice are represented by different geometric shapes [modified from (107)]. Different coexisting combinations (i.e., ensembles) of conformers corresponding to different CSA profiles are shown within the cloud outlines while the corresponding CSA traces of the samples are shown above these, with the y-axis representing F_{app} values and the x-axis representing increasing Gdn HCl concentrations up to 4.5 M. Distinct CSA Types 2, 3, and 4 are present in the TgTau^{P301L} mice; each curve represents dissociation and unfolding in one individual. F_{app} values are plotted as mean ± SEM (shades) for each denaturant concentration and assayed in triplicate. Curve analysis was performed with non-linear regression and significance determined with generalized Wilcoxon test. Average ages (days ± *SD*) for CSA Types 1, 2, 3, and 4 were 535 ± 32, 649 ± 56, 629 ± 57, and 682 ± 82 days, respectively and for the types of fibrillar assemblies associated with the CSA profiles, see (107). CSA Types 2–4 are seen in mice with statistically indistinguishable average ages and hence likely represent alternative pathways of ensemble evolution (blue arrows). The closest equivalent human disease profiles to mouse CSA Types 2 and 3 are presented to the right of the CSA plots in the boxes with solid outlines; the initial clinical diagnoses assigned to these FTLD-MAPT-P301L cases are shown (svPPA, semantic variant of primary progressive aphasia; bvFTD, behavioral variant of FTD; bvFTD*, a bvFTD sub-variety). CSA indicates conformational stability assay; F_{app}, indicates values of apparent fractional change.

Even though each tau PTM is formed by a distinct mechanism utilizing different enzymes, cofactors and chemical groups, their net effect is to impact the protein's function, its cellular localization, and turnover (98, 99).

In terms of taking these concepts further, a particular case in point relates to accumulating evidence that sites of tau cleavage may be specific to individual or subgroups of tauopathies, it then being likely that tau fragmentation events may impact the evolution of collections (ensembles) of co-existing alternative tau conformational species (51). This consideration opens a window on the more general issue of non-covalent forms of variation in tau that may impact physiological and pathophysiological output measures.

ASSEMBLY STATES AND CONFORMATIONS OF TAU

Prion Effects, Conformers, and Templated Misfolding

Although the prion concept was formulated to refer to a specific group of proteinopathies caused by misfolding of the cellular prion protein (PrP^C), other proteins were subsequently discovered to undergo a similar process. Unlike PrP^C which is generated in the secretory pathway, these other proteins such as URE2 and Sup35 in yeast, are located in the cytoplasm. Today, the prion paradigm - according to which a fundamental cause of specific disorders is the misfolding and seeded aggregation of specific proteins—is a useful unifying principle to explore the different facets of pathogenesis of many age-related neurodegenerative diseases. In prion diseases, the processes of protein replication (accumulation of misfolded copies), toxicity and infectivity can be uncoupled in different experimental paradigms, removing the constraint for extending insights from prion disease to non-transmissible neurologic syndromes. Tauopathies came to be considered in this broader context following pioneering works starting in 2009 (100–102). Proteins with this behavior [i.e., tau, alpha-synuclein (α-syn)] have been referred to as "prion-like" or "prions" (103–105). This nomenclature has also been applied to amyloid beta (Abeta) (106), this AD pathogenesis-associated peptide deriving from sequential endoproteolysis of a type I transmembrane protein and secreted into the extracellular space.

As per the covalently heterogeneous forms of tau listed above, accumulating evidence supports presence of multiple conformationally distinct conformers (strains) of tau (107–111). Acknowledging the existence of widely-used conformation-specific tau antibodies (112) and noting heterogeneity in PrP structure in prion strains (113), a conceptual approach deriving from prion disease is to consider (i) alternative folding outcomes as key determinants of heterogeneity in clinical presentation of tauopathies and (ii) propagation of tau conformers by templating as a mechanism underpinning the spread of disease-associated forms. In our investigations, conformation-dependent immunoassays (CDIs) and conformational stability assays (CSAs) were utilized to appraise abnormally-folded tau. In this procedure tau is first exposed to the protein denaturant guanidine hydrochloride (Gdn HCl) and then exposed to europium-labeled antibody against epitopes that are hidden under native conditions in the absence of Gdn HCl (107, 113). Signal intensities in the absence and presence of Gdn HCl give ratiometric values for how an epitope is hidden in a misfolded molecule (CDI) and stepwise addition of Gdn HCl in a conformational stability assay (CSA) yields a characteristic profile for gradual chemical unfolding—differences in stability profiles have been described for prions and Abeta, providing evidence of strains with distinct conformations (113–115). Most importantly, CDI ratios and CSA unfolding conformational signatures are independent of the concentrations of the misfolded species and the procedure does not involve pre-purification or *in vitro* amplification steps that can alter the *in vivo* conformational repertoire and biological properties of strain isolates (116, 117). For PrP, CSAs differentiate strains regardless of PTMs such as glycosylation and glycolipidation (117–120). For tau we selected a monitoring antibody for epitope occlusion located in the R3/R4 boundary of microtubule binding repeats, an area less decorated by PTMs (121, 122) but also considered to be conformationally remodeled in tau strains (123). The tau CDI assay performed against recombinant full-length human tau (tau441) that was deliberately misfolded into fibrils demonstrated a broad linear range for these assays. Using human FTLD-MAPT-P301L brain material derived from frontal cortex and mouse P301L brain materials we found related, complex unfolding patterns indicative of multiple co-existing conformers (107), leading to a conclusion that the collection (ensemble) of tau conformers seen at disease endpoint evolves from a precursor population, a complex mixture of early misfolded forms (**Figure 3**).

Oligomers

While descriptions of cryo-EM data on hallmark of tau fibrillar assemblies present at end-stage are listed below, these assemblies are not necessarily the neurotoxic entities leading to disease and instead oligomers may fulfill this role (as considered in section toxic effects of abnormal tau). Also, soluble, non-fibrillar, oligomers are posited to be responsible for the spread of pathology throughout the brain (124); active seeding capacity may correlate poorly with fibrillar deposits seen by light microscopy and high molecular weight soluble forms of tau derived from size exclusion chromatography may be most adept in *in vitro* seeding reactions (108–110, 125, 126). A rare species of high molecular weight, soluble, phosphorylated tau oligomers present in brain of transgenic tau mice, as well as AD patient cortices are believed to be the endogenous form of tau involved in propagation (127). In accordance with this observation, tau seeding strongly correlates with the amount of oligomeric and phosphorylated tau in post-mortem brains of AD patients, strongly suggesting that oligomeric hyperphosphorylated tau species act as seeds (128). Interestingly, these soluble assemblies demonstrate substantial patient-to-patient heterogeneity, perhaps because they include a larger variety of PTMs in comparison to large, non-soluble fibrils. In turn, these heterogeneities could relate to differences in clinical measures such as rate of clinical decline amongst AD patients (107, 128).

For the foregoing discussions of oligomers, there is a caveat concerning a range of definitions, terminology, and methodologies used for these tau assemblies: dimers (disulfide bond-dependent or -independent), multimers (trimer, tetramer, etc.), granular aggregates or small filamentous protofibrils [defined based on observations made in EM or atomic force microscopy (AFM)] have all been considered within this umbrella term.

Liquid-Liquid Phase Separation (LLPS) of Tau

Disruptions of membraneless organelles (MLOs) can induce neurodegenerative processes (129–133). MLOs, unlike canonical membrane-bound cellular organelles such as secretory vesicles, the endoplasmic reticulum and mitochondria, do not have an enclosing membrane yet compartmentalize like oil droplets in water (134). Intrinsically disordered proteins containing low complexity domains and RNA molecules can bind to each other and form liquid droplets, a phenomenon known as LLPS that has been known to regulate reversible dynamics of MLOs in cell milieu (134–136).

Alterations in the biophysical properties of MLOs became evident in the context of amyotrophic lateral sclerosis (ALS)/FTD. Pathogenic mutations in TDP-43, hnRNPA1 and FUS perturb disassembly of MLOs (e.g., stress granules) and predispose to aggregate into amyloid-like fibrils (130–133). Similarly, toxic dipeptide repeat proteins produced from hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (C9ORF72) bind to low sequence complexity domains in RNA-binding proteins; these binding events subsequently interfere with physiological functions carried out by multiple MLOs and in this way contribute to pathogenesis (129). More recently, several lines of evidence suggest that intrinsically disordered structure, inhomogeneous charge distribution, hyperphosphorylation, and/or aggregation-prone mutations allow tau to undergo LLPS under conditions of molecular crowding (137–143). While the *in vivo* parameters and co-factors involved in the LLPS of tau are not fully understood, sustained conditions can coerce droplets to more solid-like forms; for example, conversion to irreversible hydrogels and amyloid-like fibrils in the presence of multivalent polymers (e.g., RNAs) or pathogenic mutations (137, 142). Presumably, the liquid-solid phase transitions would, in turn, trigger regulated cell death starting within the preclinical stage of tauopathies in a similar way to ALS/FTD (129, 130). The molecular mechanism underlying LLPS of tau remains challenging to assess *in vivo*, due to the metastable and reversible property of liquid condensates. Nonetheless, these findings indicate that the demixed state of tau droplets can act as a possible toxic intermediator which occurs in a transitional state between internalization and intracellular tau propagation.

Tau Structures Deduced by Cryo-EM

Recent examples of a variety of atomic-level resolution structures for tau fibrils obtained by cryo-EM examination of brain material (87, 123, 144–146) represent milestones in the field as they provide molecular coordinates for designed ligands and capture in still-life variations in what some might term tau strains. Knowledge at the structural level of tau fibrils before the cryo-EM era was insufficient; although solid-state nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) were able to assign strands to certain peptides in synthetic fibers, no atomic model was available (147). Cryo-EM studies of tau fibrils obtained from brain of human patients with distinct tauopathies [AD, PiD and chronic traumatic encephalopathy (CTE)] have revealed that each tauopathy has characteristic filament folds, which are conserved among individuals with the same disease, yet different from structures obtained from *in vitro* aggregation of recombinant tau (148–150). The first report on cryo-EM structure of pathological tau (with 3.4-3.5 Å resolution) is based on atomic models of PHFs and straight filaments (SFs) obtained from an individual AD patient. This structure shows that the core of both tau filaments is made of identical protofilaments (residues Val306-Phe378) which adopt a combined cross-β/β-helix structure, and the two types of filaments are ultrastructural polymorphs with differences in their inter-protofilament packing (145). The ultrastructure of tau filaments obtained from PiD and CTE came along next (with resolution of 3.2 Å and 2.3 Å, respectively) (144, 146). While the filament core in PiD (a 3R tauopathy) consists of residues Lys254–Phe378 of 3R tau, the filaments in CTE entail residues Lys274–Arg379 of 3R and Ser305–Arg379 of 4R tau isoforms (144, 146). Nonetheless, this current repertoire of folds is superficially narrower than for other types of analyses. Besides technical considerations relating to sampling, there may be intrinsic reasons for this disparity. It could be that soluble tau oligomers exist in multiple conformations, but only a subset of these conformations is represented by the structures present in long-lived fibrils. Alternatively, despite a few common ultrastructures, PTM patterns could add another level of conformational diversity (107, 128). As an example, ubiquitination of tau within the fibril forming core region (Lys369–Glu380) can mediate fibril diversity (87).

CELL LINEAGES HARBORING ABNORMAL FORMS OF TAU

Analyses of cell-free systems or purified protein from autopsy material cannot encompass dynamic relationships applying to genesis and turnover of tau conformers in living cells, nor to the important situation in the sub-clinical phase of disease where therapeutic interventions might best be applied before irrevocable neuronal loss. In prion disease, strains made of different conformers of the pathogenic infectious prion protein (PrP^{Sc}) are often considered to have differing abilities to infect cells; this effect has been studied by using endpoint-titrated samples obtained by serial dilution (i.e., biologically cloned) to infect susceptible animals, which are then in turn scored for different neuropathological patterns of protein accumulation (151). In a seemingly parallel set of observations to protein structural assays, different tauopathies are known to be associated with different cell populations. Thus, (i) 3R tauopathies include PiD with 3R tau in neuronal cytoplasmic inclusions called

Pick Bodies (11); (ii) 4R tauopathies such as CBD include glial cells of the cortex and white matter as well as neuronal accumulation; similarly, in the case of PSP, there are tau tangles in glia and neurons (152). Both CBD and PSP may also include oligodendroglial tau inclusions (11); (iii) 3R+4R tauopathies also exist and are most commonly represented by AD, with tau in neuronal cell bodies as NFTs and within dystrophic neurites lying nearby mature plaques. Noting these points and, because it is known that experimentally-tractable FTLD-MAPT tauopathy manifests in different cell populations sharing the same *MAPT* genotype (108, 153, 154), it is likely that aspects of the cell biology of tau remain to be discovered. It has been reported that synthetic tau-preformed fibrils and pathological tau derived from brains of AD patient are capable of causing tau aggregation in both cultured cells and wild type mice (155, 156), and that the cell-to-cell spread pattern of the seed-competent tau conformers in the central nervous system (CNS) was determined by synaptic connectivity (e.g., afferent and efferent connections) rather than spatial proximity (156–159). Moreover, similar to prion strains, tau conformers derived from distinct tauopathies including AD, PSP, and CBD recapitulated their phenotype characteristics of tau pathology; time-, dose-, and injection site-dependent patterns of spreading and cell type-specific aggregation (159, 160) [also reviewed in (161)]. In prion diseases, a popular idea is that different prion strains perpetuated by experimental inoculation prefer to infect different cells (a concept commonly called tropism) (151) but primary tauopathies derive from germline mutations and contributions of exogenous infection to this process may not exist or may be secondary events. Nonetheless, for malformed tau emerging spontaneously within the CNS, uptake by different cell lineages could play an active role in selective propagation of tau strains, this arising as a consequence of fundamental differences in endogenous processes that distinguish neurons, oligodendrocytes and astrocytes. This latter concept might begin to explain why conformer mixtures can often be encountered within the same brain (107).

Tissue Tropism of Tau Conformers (Glial Tauopathies)

Tau expression is predominantly present in neurons, with lower expression levels or signals below assay threshold applying to oligodendrocytes and astrocytes (160, 162). In the secondary tauopathy AD, tau aggregates are only found in neurons as NFTs and neuropil threads, which are composed of both 3R and 4R tau (163). On the other hand, abundant glial tau deposits are found along with neuronal pathology in primary tauopathies and in other subtypes of FTD/FTLD including PiD, CBD, PSP, GGT, and AGD (1). The majority of glial tau pathologies are observed in oligodendrocytes and astrocytes and, in some instances, tau inclusions are also found in microglia (164, 165). Interestingly, in a neuronal tau knockdown mouse model ($TauKDn^{cre;fl/fl}$), oligodendrocytic tauopathy spread through adjacent brain regions, whereas astrocytic inclusions remained confined to the injection site (160). The various deposition morphologies of glial tau (1, 166) (described below) may have functional correlates and could be drivers underlying the diverse manifestations of neurodegenerative tauopathies.

Ramified inclusions are astrocytic tau fibrils found in PiD. Thick processes and eccentric nuclei are accompanied with ramified inclusions (167, 168).

Tufted tau inclusions are densely packed fibrils found in the proximal processes surrounding astrocytic nuclei and are the pathological signature of PSP. Morphologically, star-like tufts of dense fibers emanate from the cell body (169, 170).

Coiled bodies are intracytoplasmic tau inclusions surrounding the nucleus of oligodendrocytes that form coil-like or comma-like inclusions. They are also common in many FTLD-subtypes such as PSP, CBD, AGD, and FTLD-MAPT-P301L (169, 171, 172).

Astrocytic plaques are hallmarks of CBD and take the forms of densely tau-immunoreactive stubby dilatations in distal processes of astrocytes (173). The inclusions are comprised of twisted and straight tubules with diameters of 15–20 nm (174).

Argyrophilic threads are tau-positive thread-like structures in the processes of astrocytes and oligodendrocytes and are prominent in CBD (11, 175).

Thorn-shaped inclusions are juxtanuclear assemblies with tau-immunoreactivity and extension into the proximal processes of astrocytic endfeet at the pial surface and around blood vessels (173). These appear as argyrophilic masses with flame or thorn-like shapes in both PSP and aging-related tau astrogliopathy (ARTAG) (173, 176).

Globular oligodendrocytic and astrocytic inclusions comprise insoluble globules and granular tau deposits emanating from the cell body that are unique characteristics of GGT (11, 177, 178).

Pick-bodies are neuronal tau inclusions found in PiD and are round in shape but to a lesser extent, ***Pick body-like inclusions*** are also evident in both astrocytes and oligodendroglia in PiD (167, 168).

Different manifestations of astroglial tau are recently reviewed and summarized by Kovacs (166), along with a consideration of potential precursor forms. These data point to a non-trivial role for astroglial tau in pathogenesis of diverse diseases. On the other hand, there seems to be a molecular conundrum regarding the origins of the tau conformers fueling these diverse glial tau pathologies, noting that glial expression of tau mRNA in human tissue is much lower than in neurons and (16, 18) that tau transgenic mice using the *PRNP* promoter (generally considered to drive pan-neuronal expression) nonetheless accumulate hyperphosphorylated and argyrophilic tau in astrocytes (154, 179).

Glial cells constitute roughly half of the cells of the human CNS (180). In healthy conditions, they considerably influence nervous system development, from neuronal birth, migration, axon specification, and growth through circuit assembly and synaptogenesis (181), while in CNS injury, they are responsible for phagocytosis and elimination of microbes, dead cells, and protein aggregates, as well as other particulate and soluble antigens that may endanger the CNS (182, 183). The glial pathologies could be contributed by a cell-to-cell transfer initiated by exocytosis, budding from plasma membrane and synaptic secretion of cellular and pathogenic tau to

the extracellular space, these mechanisms having long been considered as common mechanisms for disease progression in most neurodegenerative diseases (184). A simple extrapolation is that glial tau inclusions are derived from a neuronal source by the active cellular process of efferocytosis; this is a defense mechanism during the resolution of pathological events that involves engulfment and clearance of dead and dying cells by the professional phagocyte (e.g., microglia) and non-professional phagocytes (e.g., oligodendrocytes, astrocytes, neuronal progenitor cells) in the CNS (185). This simple idea starts to address the conundrum presented by most tau expression deriving from neurons, but is not a comprehensive explanation; thus the syndrome called ARTAG (166, 173) has astrocytic tau without neuronal tau accumulation and oligodendroglial tau can be detected in young to middle-aged TgTau^{P301L} mice in the apparent absence of neuronal tau inclusions, which may not appear until many months later (108).

SPREAD OF TAU AT THE CELLULAR AND TISSUE LEVEL

In early stages of most protein misfolding diseases, the pathological changes, including aggregated protein accumulation and neurological dysfunctions are restricted to confined regions of the nervous system. However, as the disease progresses such alterations spread throughout the CNS, suggesting the presence of a cell biological spreading mechanisms for misfolded protein species (186), with these not necessarily being synonymous with conformational templating mechanisms, some of which may take place inside cells. These general thoughts have become embodied in the specific idea that neuropathological staging of tauopathies originally mapped with phospho-specific antibodies and conformation-dependent antibodies (187–190) reflects the sequential spread of misfolded tau species, following patterns of neuroanatomical connectivity rather than simple physical or spatial proximity (191–193).

Generalized Transfer Processes Between Homologous Cells

Intracellular depositions of abnormally folded proteins act as dangerous molecular signals (DAMPs, damage associated molecular patterns) causing stress conditions and provoking diverse responses which can address burdens such as accumulation of misfolded tau, α-syn, Abeta, TDP-43, and PrP^{Sc} by upregulating proteolysis and/or secretion pathways (184). Depending on the secretion pathways (e.g., membrane fusion, ectosomes and exosomes), secreted tau can be found as a free protein and/or within vesicles. However, secretion is not the end of the story and nor is it necessarily a good outcome for the tissue; once bound to the plasma membrane of neighboring cells, tau conformers may yet be internalized by endocytosis, pinocytosis or phagocytosis (regardless of the type of the adjacent cell) (**Figure 4**) (184, 194). To complete this process, extracellular tau conformers may be required to interact with phosphatidylinositol 4,5 phosphate (PI(4,5)P2), cholesterol, sphingolipids and/or heparan sulfate proteoglycans located at the extracellular leaflet of the plasma membrane (195–197). Secreted tau in a vesicular form (e.g., ectosomes and exosomes) can fuse to the plasma membrane or get endocytosed by recipient cells. Exosomes are released on the exocytosis of multivesicular bodies following inward budding of the outer endosomal membrane. Ectosomes are formed by outward budding of the plasma membrane and can deliver larger cargos (194, 198). Heparan sulfate proteoglycan-mediated macropinocytosis is another type of endocytosis that is the preferred entry for tau monomers and oligomers (197, 199). Pathogenic tau conformers can also travel directly between cells via tunneling nanotubes, these being actin-rich membranous protrusions that allow for intercellular transport of various cargos not only between neurons but also astrocytes (200). However, it remains unclear how the internalized tau conformers in recipient cells escape from endosomal (or lysosomal) processing and in turn encounter endogenous substrates for templated fibrillization (161).

Heterologous Transfers

In terms of different lineage origins for donor and recipient cells, there is an emerging stream of literature from seeding paradigms illustrating different pairwise combinations. Indeed, one might imagine six permutations of *heterologous* one-way transfer of abnormal tau between neurons, astrocytes and oligodendrocytes. While the issue of *MAPT* gene expression crops up again here, i.e., the ability of all three lineages in the human brain or transgenic mouse models to express different spliced tau mRNAs (given native tau substrate is required for propagation by templated misfolding), some permutations are already established. In one experimental configuration, the source of malformed tau can derive from a clinical syndrome with multiple affected lineages, hence a heterogeneous tau source, which then allows for the detection of responses in different recipient lineages. Using source material from tau transgenic mice with extensive pathology or from human disease tissue, induced tau pathologies after seeding into indicator mice are not restricted to neurons but also include astrocytic and oligodendrocytic inclusions. Seeding experiments using stereotaxic injections into different neuroanatomical areas (for example, into the corpus callosum, to examine oligodendrocyte responses) allow insight into lineage tropism effects, the contribution of neuroanatomical pathways and trans-synaptic spread and comparisons with staging schemes derived solely from examination of human brain material (100, 158, 159, 169, 187, 188, 201–203).

Tissue Level Effects; Role of the Glymphatic System

Emerging evidence suggests the existence of a mechanism underlying solute clearance from the brain's extracellular space, this being termed the glymphatic pathway. Unlike traditional degradation processes including autophagy and UPS, this pathway conveys protein aggregates from the parenchyma to the CSF as a highly organized fluid transport and clearance system (204–206). This pathway facilitates the flow of CSF

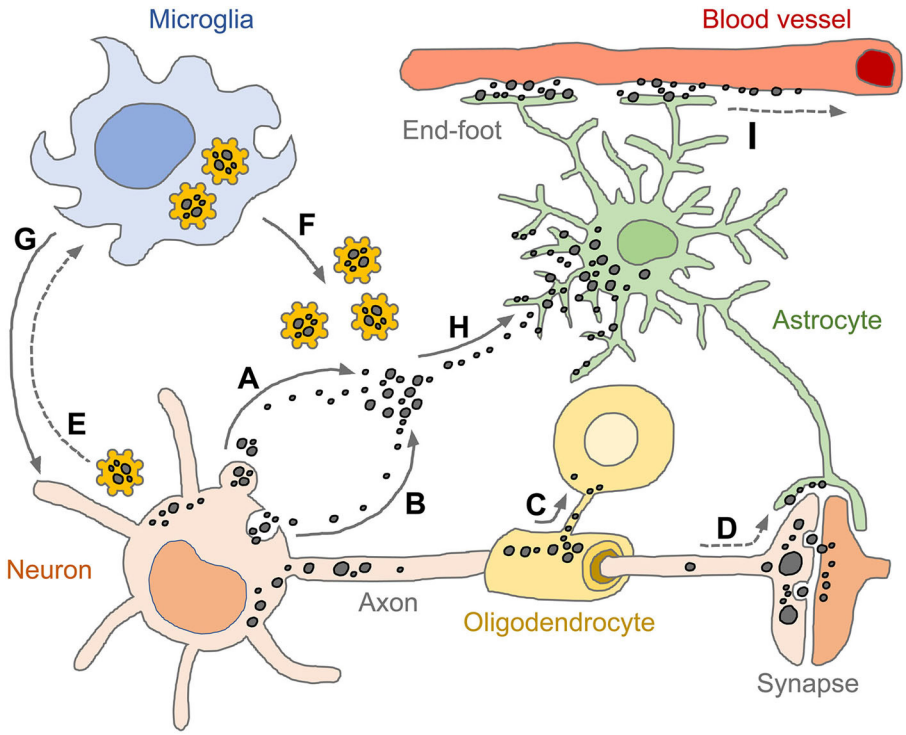


FIGURE 4 | Spread of pathogenic tau conformers and glial tauopathy. Under disease conditions, tau is dissociated from axonal microtubule structure, aggregated (or condensed) and relocalized in somato-dendritic compartments. The pathogenic tau conformers enhance cytoplasmic proteostasis pathways and promote secretion machinery, such as ectosomes **(A)** and exosomes **(B)**, which aim to get rid of the cellular burdens. Oligodendrocytes transmit tau pathology through their own processes near the formation of the myelin sheath **(C)**. Tau conformers also spread to neighboring neurons and astrocytes *via* post-synaptic clefts and astrocytic processes, respectively **(D)**. Intracellular tau aggregates are sensed by pattern recognition receptors (e.g., NLRP3) and primed microglia **(E)**. The reactive microglia amplify innate immune responses by releasing pro-inflammatory effector molecules (e.g., tau-NLRP3-inflammasomes, cogwheel shapes in yellow) **(F)** and subsequently induce pyroptosis which is an inflammatory form of regulated cell death **(G)**. Extracellular tau conformers transferred to astrocytes through both ingestion and phagocytosis **(H)**, appearing as diverse tau pathologies. Tau immunoreactivities are also found in perivascular astrocyte end-feet, indicating that astrocytic tau inclusions could be drained through glymphatic system which is a fluid-clearance pathway in the brain tissue **(I)**. Tau conformers are shown as black dots in various sizes. Solid line arrows indicate experimentally proven pathways (published), while dotted line arrows indicate pathways as yet unidentified.

to arterial perivascular space and subsequently into the brain interstitium which contains pathogenic tau conformers released from neurons and glia. The flow then migrates toward the venous perivascular spaces, clearing solutes from the neuropil into meningeal and cervical lymphatic drainage vessels. The astrocytic aquaporin-4 (AQP4) water channels localized in astrocytic end feet play an important role in CSF-ISF exchanges in both periarterial and perivenous spaces (207). Animals lacking AQP4 gene expression exhibit a ∼70% reduction in interstitial solute clearance compared to wild-type control mice (205). Depletion of AQP4 also exacerbated neuropathology by increasing levels of phosphorylated tau and reactive gliosis in a mouse model of traumatic brain injury (TBI) (204). Pharmacological inhibition of AQP4 using TGN-020 (N-1,3,4-thiadiazol-2-yl-3-pyridinecarboxamide) impaired glymphatic CSF-ISF exchange and tau protein clearance in rTg4510 tau transgenic mouse model (208). Given that thorn-shaped tau inclusions at astrocytic end-feet are hallmarks in both PSP and ARTAG (166, 173), these data may suggest an intriguing connection (or competition) between pathologic spread of toxic tau conformers vs. inactivation of proteinaceous pathological tau seeds (**Figure 4**) (184, 207). Further studies are needed to substantiate the dual and opposing roles of glial cells in tauopathies, being both beneficial and detrimental.

TOXIC EFFECTS OF ABNORMAL TAU

Although results obtained from numerous studies indicate that misfolded protein aggregates are toxic to neurons *in vitro* and *in vivo*, the molecular mechanism(s) through which they induce their toxicity is not always well-established. This is partially due to the heterogeneity of aggregated and misfolded proteins species. Since misfolded proteins can co-exist in several distinct forms with different features and characteristics, they might each induce neurotoxicity in their own idiosyncratic ways. These general considerations about neurodegenerative disease very much apply to tau, a protein with impressive diversity of covalent forms and conformers and an ability to assemble into supramolecular structures in neuronal, astrocytic and oligodendroglial lineages.

It is known that the neuronal loss in AD patients exceed the number of NFTs (89), and neurons containing NFTs are functionally intact *in vivo* (209, 210). Moreover, some studies in animal models have shown that overexpression of tau can lead to cell death and synaptic dysfunction in the absence of tau filaments (211). In fact, reducing tau overexpression in mutant tau transgenic mice (rTg4510) decreases neuronal cell loss despite progressive formation of tau tangles (212). At the same time, the onset of clinical symptoms in AD and PSP brains correlate with elevated levels of multimeric, soluble assemblies, known as tau oligomers. Empirically, one way to test for toxicity is by direct injection of purified material and here it is noted that injection of oligomers into the brain of wild-type mice, rather than monomers or fibrils cause cognitive, synaptic, and mitochondrial abnormalities (126, 213). Collectively, these points suggest that formation of tau tangles (or tangle-like structures) is not essential for neuronal loss and that tau-induced neurotoxicity is in fact dependent upon the formation of non-filamentous, aggregate intermediates known as tau oligomers (89, 214).

Inflammation and Gliosis

Since brain tissue is immune-privileged with the restricted access of immune cells through blood-brain barrier, resident microglia, monocyte and astrocytes are the major effector cells of the innate immune defense against microbial infection, brain injury and neurodegenerative disorders (215). Neuroinflammation in various proteinopathies, where protein aggregates are causing cell damage, is induced by CNS-resident and/or potentially blood-derived innate immune cells. On the other hand, adaptive immune cells such as B and T lymphocytes drive the pathological processes (216) in microbial infections and autoimmune disease (e.g., encephalitides and multiple sclerosis, MS).

The components of the innate immune system have their own inherent protective and defensive functions against various danger signals (DAMPs) as well as pathogens (pathogen-associated molecular patterns, PAMPs), while excessive or non-resolving immune responses have the opposite effect and may damage the host (217, 218). Pathogenic protein conformers in various neurodegenerative diseases have been reported to activate chronic neuroinflammation through pattern recognition receptors which are important sensors of innate immunity found in most CNS cells. For example, oligomeric forms of Abeta and α-syn induce NF-κB-dependent pro-inflammatory gene expression by binding to cell surface receptors such as receptor for advanced glycation end products (RAGE), toll-like receptor (TLR)-2 and TLR-4 (219–222). Extracellular soluble forms of Abeta are internalized into microglia by binding to a protein, triggering receptor expressed on myeloid cells 2 (TREM2), which is capable of promoting phagocytic activity via regulation of C/EBPα and CD36 expression (223). It is known that aggregation-prone proteins including Abeta, α-syn, TDP-43, and superoxide dismutase 1 (SOD1) along with other DAMPs, such as ATP and lysophosphatidylcholine can activate intracellular inflammasomes following interleukin 1 beta (IL-1β) release (224–229).

Concerning tauopathies, there is increasing evidence that inflammasome-mediated gliosis and innate immune responses are recurrent features (230, 231). One may speculate that pathogenic tau conformers taken up into glial cells could act as endogenous DAMPs and be recognized by cytoplasmic pattern recognition receptors such as inflammasomes (232, 233), molecular assemblies which are expressed and activated in different types of CNS-resident cells (231, 234). There is a critical role for the inflammasome-mediated innate immune responses in tau pathogenesis, given that exogenously and non-exogenously seeded tau could activate inflammasomes (232, 233). Upon activation, inflammasome components referred to as PYD and CARD form protein filaments. These polymerization steps are conserved signaling cascades in innate immunity and inflammation (233, 235) and are somewhat "prion-like" as assembly of the ASC specks can transfer to neighboring cells (**Figure 4**) (236, 237). In transgenic mice expressing human MAPT-P301S tau ($MAPT^{P301S}$PS19), the ablation of senescent astrocytes and microglia prevents gliosis, deposition of tangle-like structures, degeneration of cortical and hippocampal neurons, indicating the role of dysregulated glial cells that could initiate and exacerbate tau pathology (238).

Disruption of Cellular/Axonal Transport

Growing evidence suggests that defective neuronal and axonal transport due to early axonal dysfunction could play a contributory role in several neurodegenerative diseases. Standing somewhat in contrast to the lack of deficits in tau knock-out mice (26, 27, 239, 240), there are reports to this effect for tauopathies at their early disease stages (214, 241, 242). In fact, several studies have demonstrated that the most common tauopathies are characterized by several features that point to a significant role for axonal dysfunction that may originate from deficits in fast axonal transport (243–246). One report has proposed that tau oligomers disrupt microtubule stability and trafficking, thus affecting organelle distribution, and inducing toxicity (247). Oligomers can also cause dramatic displacement of endogenous axonal tau into the somato-dendritic compartments, and, in turn dysregulation of microtubule-based fast axonal transport (248).

Disruption of Nuclear Cytoplasmic Transport

Declines in the structural integrity of nuclear pore complex (NPC) and the efficiency of nuclear-cytoplasmic transport (NCT) have been reported in neurodegenerative disorders including FTD, ALS, Huntington's disease and tauopathies (71, 74, 249–254). The constant flow of protein and RNA species is critical for transcriptional regulation, signal transduction, cell growth, and cell cycle (255, 256); these molecular transportation events occur through the NPCs, which are one of the largest embedded macromolecular assemblies of the nuclear envelope and form a channel by fusing the outer and inner nuclear envelope leaflets (256–258). NCT through these pores is mediated by around 30 different nucleoporins (NUPs), which are protein building blocks of NPCs and have remarkably long-lifespans (259).

Nuclear localization of tau species and their interaction with DNA have suggested a protective role in genome surveillance for normal cells. Conversely, in disease conditions such as AD, an alteration of these functions might enhance genomic

vulnerability and neurodegeneration (32). More recently, impairment of NCT has been reported in transgenic mice expressing P301L tau and in AD brains, wherein mislocalization of NUPs is observed with aggregated tau. Concomitant decreases in the levels of NUPs, especially NUPs rich in phenylalanine-glycine repeats (e.g., NUP-98), suggest deterioration of NPC function (71). Others have shown that pathogenic mutations in *MAPT* caused mislocalization of tau into the somato-dendritic compartment and deformation of nuclear membrane as appraised by lamin B staining of nuclear lamina, consequently interfering with NCT (74). P301S and P301L mutant forms of tau may induce mitotic spindle defects during cell division and produce aneuploid cells prone to apoptosis, with these inferences being supported by analyses of brain cell suspensions derived from corresponding transgenic mice (260). Since the aneuploidy-mediated regulated cell death requires cell division, this type of pathogenic event may be more relevant to glial tau pathologies.

DISCUSSION

An emerging area of consensus is the remarkable level of diversity of tau, with implications for the lab, for the clinic and for pharmaceutical companies. In the human context—as briefly inventoried here—there are different tauopathies, but as noted above, heterogeneity can also be evident within a given disease entity having the exact same *MAPT* protein coding sequence, as noted above and recently illustrated for FTLD-MAPT-P301L cases (107). An analogous effect is now documented for AD with wild-type human tau isoforms (128). This effect/challenge being accepted, therapeutic approaches using small molecule compounds might nonetheless need to pass the checkpoint of validation in animal models. Perhaps surprisingly, close inspection of mouse models of tauopathy can reveal heterogeneity too.

One general way to explain heterogeneity in biological systems is via the action of modifier genes. Allelic forms of the apolipoprotein E (ApoE) gene are potent modifiers for both genetic and sporadic forms of AD, but in the context of FTLD, citations for their impact are sparse. Also, for use of animal models of tauopathy, there are no high frequency polymorphisms in mice equivalent to the human ApoE e2, e3, and e4 forms affecting residues 112 and 158. While a mouse variant in residue 163 has been described that may originate from the DBA/2 background (261), in our own studies phenotypic heterogeneity was observed in $TgTau^{P301L}$ mice inbred to three backgrounds other than DBA/2 (108). For these transgenic mice, we considered whether a somatic mutation of the MAPT-P301L transgene might offer an explanation for heterogeneity in the CNS phenotypes but a PCR assay for genome rearrangements (262) failed to yield evidence for re-integrated transgene copies in brain genomic DNA—this assay had a detection limit for altered transgenes 1,300x below the level of an endogenous single-copy gene (107). We concluded that variations in the nuclear genome are unlikely causes of disease heterogeneity. While another type of genome, the microbiome, might ultimately have a bearing upon phenotypic heterogeneity, its association with FTD and FTLD has been less explored than in the context of Parkinson's disease (PD) and accumulation of α-syn (263, 264). One might then conclude that heterogeneity of tau species observed in the lab recapitulates an intrinsic biological effect and not a distortion arising in the course of animal modeling.

A widespread assumption when using models has been that animals of the same age and genotype are phenotypically identical; indeed, one might not embark upon testing a therapy in a model if not subscribing to this unwritten assumption. However, while this view may have originated from an earlier era with extensive use of over-expresser transgenic mouse lines with a compressed timescale for pathogenesis and hence lower husbandry costs, it may be inadequate and need reconsideration (265, 266). Instead, deviations from homogeneity in slow pathogenesis models might be telling us that processes are nuanced enough in these animals to capture the very same biological mechanisms that are driving heterogeneity in human tauopathies. In terms of the molecular mechanisms driving heterogeneity, there is no shortage of possibilities. As inventoried in the section on tau physiology, spliced forms and posttranslational covalent variations, there is a thicket of PTMs for tau (phosphorylation, acetylation, O-glycosylation, ubiquitination, etc.), quite beside the protein having six different primary structures due to alternative RNA splicing. Additional layers of complexity might be imparted as tau transits between cell lineages and neuroanatomical areas, across synapses, across areas of the extracellular matrix with different surveilling cells (Sections cell lineages harboring abnormal forms of tau, spread of tau at the cellular and tissue level, and toxic effects of abnormal tau), all or any of which might impose different spectra of PTM enzymes and proteostatic environments. Nonetheless, as tauopathies (a) can be devastating and are a considerable burden on the healthcare system and (b) can occur in the context of comorbidities, means must be sought to stratify these variations to deal with the most important entities. In practical terms, the complex landscape of tau biology can be approached by placing a focus on a foreground species, e.g., ones that are thought to be particularly toxic. Thus, although heterogeneity in the here and now is an "inconvenient truth," embracing this effect, defining its origins and then adjusting approaches may pave the way for more sophisticated testing and more realistic interventions.

AUTHOR CONTRIBUTIONS

S-GK: conceptualization, validation, investigation, visualization, writing—original draft, writing—review, and editing. GE-S: investigation, visualization, writing—original draft, writing—review, and editing. LH: data curation, investigation, writing—review, and editing. JS: conceptualization, supervision, funding acquisition, writing—review, and editing. DW: conceptualization, supervision, funding acquisition, validation, project administration, writing—original draft, writing—review, and editing. All authors contributed to the article and approved the submitted version.

FUNDING

Work in the Westaway lab was funded by the Canadian Institutes of Health Research (CIHR PS148962 and GER163048) and by Alberta Innovates Biosolutions (ABIBS AEP 201600021 and 20160023). Instrumentation was supported by the Canada Foundation for Innovation (NIF21633) and by the Alberta Synergies in Alzheimer's and Related Disorders (SynAD) program, which was funded by the Alzheimer Society of Alberta and Northwest Territories through the Hope for Tomorrow program and the University Hospital Foundation. DW was supported through a Canada Research Chair (Tier 1). Work in the Safar lab was supported by grants from Alberta Innovates Biosolutions (FP00209618) and from NIH (1RF1AG058267 and 1RF1AG061797).

ACKNOWLEDGMENTS

We are indebted to the Neurological Tissue Bank of the Biobank-Hospital Clinic-IDIBAPS, Barcelona, Spain, and Teresa Ximelis for sample and data procurement and to all brain donors and their families for generous brain donation for research; special thanks go to Drs. Laura Molina-Porcel and Ellen Gelpi.

REFERENCES

1. Gotz J, Halliday G, Nisbet RM. Molecular pathogenesis of the tauopathies. *Annu Rev Pathol.* (2019) 14:239–61. doi: 10.1146/annurev-pathmechdis-012418-012936
2. Ling H. Untangling the tauopathies: current concepts of tau pathology and neurodegeneration. *Parkinsonism Related Disord.* (2018) 46:S34–8. doi: 10.1016/j.parkreldis.2017.07.031
3. Irwin DJ. Tauopathies as clinicopathological entities. *Parkinsonism Related Disord.* (2016) 22:S29–33. doi: 10.1016/j.parkreldis.2015.09.020
4. Goedert M, Spillantini MG. Pathogenesis of the tauopathies. *J Mol Neurosci.* (2011) 45:425. doi: 10.1007/s12031-011-9593-4
5. Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology.* (1998) 51:1546–54. doi: 10.1212/WNL.51.6.1546
6. Mirra SS, Murrell JR, Gearing M, Spillantini MG, Goedert M, Crowther RA, et al. Tau pathology in a family with dementia and a P301L mutation in tau. *J Neuropathol Exp Neurol.* (1999) 58:335–45. doi: 10.1097/00005072-199904000-00004
7. Tacik P, Sanchez-Contreras M, DeTure M, Murray ME, Rademakers R, Ross OA, et al. Clinicopathologic heterogeneity in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) due to microtubule-associated protein tau (MAPT) p.P301L mutation, including a patient with globular glial tauopathy. *Neuropathol Appl Neurobiol.* (2017) 43:200–14. doi: 10.1111/nan.12367
8. van Swieten JC, Stevens M, Rosso SM, Rizzu P, Joosse M, de Koning I, et al. Phenotypic variation in hereditary frontotemporal dementia with tau mutations. *Ann Neurol.* (1999) 46:617–26. doi: 10.1002/1531-8249(199910)46:4<617::AID-ANA10>3.0.CO;2-I
9. Borrego-Ecija S, Morgado J, Palencia-Madrid L, Grau-Rivera O, Rene R, Hernandez I, et al. Frontotemporal dementia caused by the P301L mutation in the MAPT gene: clinicopathological features of 13 cases from the same geographical origin in Barcelona, Spain. *Dement Geriatr Cogn Disord.* (2017) 44:213–21. doi: 10.1159/000480077
10. Palencia-Madrid L, Sanchez-Valle R, Fernandez de Retana I, Borrego S, Grau-Rivera O, Rene R, et al. A unique common ancestor introduced P301L mutation in MAPT gene in frontotemporal dementia patients from Barcelona (Baix Llobregat, Spain). *Neurobiol Aging.* (2019) 84:236 e239–15. doi: 10.1016/j.neurobiolaging.2019.08.015
11. Kovacs GG. Invited review: neuropathology of tauopathies: principles and practice. *Neuropathol Appl Neurobiol.* (2015) 41:3–23. doi: 10.1111/nan.12208
12. Neve RL, Harris P, Kosik KS, Kurnit DM, Donlon TA. Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2. *Brain Res.* (1986) 387:271–80. doi: 10.1016/0169-328X(86)90033-1
13. McMillan P, Korvatska E, Poorkaj P, Evstafjeva Z, Robinson L, Greenup L, et al. Tau isoform regulation is region- and cell-specific in mouse brain. *J Comp Neurol.* (2008) 511:788–803. doi: 10.1002/cne.21867
14. Andreadis A. Tau gene alternative splicing: expression patterns, regulation and modulation of function in normal brain and neurodegenerative diseases. *Biochim Biophys Acta.* (2005) 1739:91–103. doi: 10.1016/j.bbadis.2004.08.010
15. Couchie D, Fages C, Bridoux AM, Rolland B, Tardy M, Nunez J. Microtubule-associated proteins and *in vitro* astrocyte differentiation. *J Cell Biol.* (1985) 101:2095–103. doi: 10.1083/jcb.101.6.2095
16. Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO J.* (1989) 8:393–9. doi: 10.1002/j.1460-2075.1989.tb03390.x
17. Klein C, Kramer EM, Cardine AM, Schraven B, Brandt R, Trotter J. Process outgrowth of oligodendrocytes is promoted by interaction of fyn kinase with the cytoskeletal protein tau. *J Neurosci.* (2002) 22:698–707. doi: 10.1523/JNEUROSCI.22-03-00698.2002
18. Kosik KS, Orecchio LD, Bakalis S, Neve RL. Developmentally regulated expression of specific tau sequences. *Neuron.* (1989) 2:1389–97. doi: 10.1016/0896-6273(89)90077-9
19. LoPresti P, Szuchet S, Papasozomenos SC, Zinkowski RP, Binder LI. Functional implications for the microtubule-associated protein tau: localization in oligodendrocytes. *Proc Natl Acad Sci USA.* (1995) 92:10369–73. doi: 10.1073/pnas.92.22.10369
20. Muller R, Heinrich M, Heck S, Blohm D, Richter-Landsberg C. Expression of microtubule-associated proteins MAP2 and tau in cultured rat brain oligodendrocytes. *Cell Tissue Res.* (1997) 288:239–49. doi: 10.1007/s004410050809
21. 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) 9:4225–30. doi: 10.1002/j.1460-2075.1990.tb07870.x
22. Hanes J, Zilka N, Bartkova M, Caletkova M, Dobrota D, Novak M. Rat tau proteome consists of six tau isoforms: implication for animal models of human tauopathies. *J Neurochem.* (2009) 108:1167–76. doi: 10.1111/j.1471-4159.2009.05869.x
23. Liu C, Gotz J. Profiling murine tau with 0N, 1N and 2N isoform-specific antibodies in brain and peripheral organs reveals distinct subcellular localization, with the 1N isoform being enriched in the nucleus. *PLoS ONE.* (2013) 8:e84849. doi: 10.1371/journal.pone.0084849
24. Takuma H, Arawaka S, Mori H. Isoforms changes of tau protein during development in various species. *Brain Res Dev Brain Res.* (2003) 142:121–7. doi: 10.1016/S0165-3806(03)00056-7
25. Zempel H, Dennissen FJA, Kumar Y, Luedtke J, Biernat J, Mandelkow EM, et al. Axodendritic sorting and pathological missorting of Tau are isoform-specific and determined by axon initial segment architecture. *J Biol Chem.* (2017) 292:12192–207. doi: 10.1074/jbc.M117.784702
26. Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, et al. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature.* (1994) 369:488. doi: 10.1038/369488a0
27. Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci.* (2001) 114:1179–87. Available online at: https://jcs.biologists.org/content/114/6/1179.long

28. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci USA*. (1975) 72:1858–62. doi: 10.1073/pnas.72.5.1858
29. Liu C, Song X, Nisbet R, Gotz J. Co-immunoprecipitation with tau isoform-specific antibodies reveals distinct protein interactions and highlights a putative role for 2N tau in disease. *J Biol Chem.* (2016) 291:8173–88. doi: 10.1074/jbc.M115.641902
30. Buée L, Bussière T, Buée-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Rev.* (2000) 33:95–130. doi: 10.1016/S0165-0173(00)00019-9
31. Georgieva ER, Xiao S, Borbat PP, Freed JH, Eliezer D. Tau binds to lipid membrane surfaces via short amphipathic helices located in its microtubule-binding repeats. *Biophys J.* (2014) 107:1441–52. doi: 10.1016/j.bpj.2014.07.046
32. Bukar Maina M, Al-Hilaly YK, Serpell LC. Nuclear tau and its potential role in Alzheimer's disease. *Biomolecules.* (2016) 6:9. doi: 10.3390/biom6010009
33. Dixit R, Ross JL, Goldman YE, Holzbaur EL. Differential regulation of dynein and kinesin motor proteins by tau. *Science.* (2008) 319:1086–9. doi: 10.1126/science.1152993
34. Trinczek B, Ebneth A, Mandelkow EM, Mandelkow E. Tau regulates the attachment/detachment but not the speed of motors in microtubule-dependent transport of single vesicles and organelles. *J Cell Sci.* (1999) 112:2355–67.
35. Kanai Y, Hirokawa N. Sorting mechanisms of tau and MAP2 in neurons: suppressed axonal transit of MAP2 and locally regulated microtubule binding. *Neuron.* (1995) 14:421–32. doi: 10.1016/0896-6273(95)90298-8
36. Black MM, Slaughter T, Moshiach S, Obrocka M, Fischer I. Tau is enriched on dynamic microtubules in the distal region of growing axons. *J Neurosci.* (1996) 16:3601–19. doi: 10.1523/JNEUROSCI.16-11-03601.1996
37. DiTella M, Feiguin F, Morfini G, Caceres A. Microfilament-associated growth cone component depends upon Tau for its intracellular localization. *Cell Motil Cytoskeleton.* (1994) 29:117–30. doi: 10.1002/cm.970290204
38. Preuss U, Doring F, Illenberger S, Mandelkow EM. Cell cycle-dependent phosphorylation and microtubule binding of tau protein stably transfected into Chinese hamster ovary cells. *Mol Biol Cell.* (1995) 6:1397–410. doi: 10.1091/mbc.6.10.1397
39. Regan P, Whitcomb DJ, Cho K. Physiological and pathophysiological implications of synaptic tau. *Neuroscientist.* (2017) 23:137–51. doi: 10.1177/1073858416633439
40. Sahara N, Murayama M, Higuchi M, Suhara T, Takashima A. Biochemical distribution of tau protein in synaptosomal fraction of transgenic mice expressing human P301L tau. *Front Neurol.* (2014) 5:26. doi: 10.3389/fneur.2014.00026
41. Chen Q, Zhou Z, Zhang L, Wang Y, Zhang YW, Zhong M, et al. Tau protein is involved in morphological plasticity in hippocampal neurons in response to BDNF. *Neurochem Int.* (2012) 60:233–42. doi: 10.1016/j.neuint.2011.12.013
42. Pallas-Bazarra N, Jurado-Arjona J, Navarrete M, Esteban JA, Hernandez F, Avila J, et al. Novel function of Tau in regulating the effects of external stimuli on adult hippocampal neurogenesis. *EMBO J.* (2016) 35:1417–36. doi: 10.15252/embj.201593518
43. Pooler AM, Phillips EC, Lau DH, Noble W, Hanger DP. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep.* (2013) 14:389–94. doi: 10.1038/embor.2013.15
44. Yamada K, Holth JK, Liao F, Stewart FR, Mahan TE, Jiang H, et al. Neuronal activity regulates extracellular tau *in vivo*. *J Exp Med.* (2014) 211:387–93. doi: 10.1084/jem.20131685
45. Padmaraju V, Indi SS, Rao KS. New evidences on Tau-DNA interactions and relevance to neurodegeneration. *Neurochem Int.* (2010) 57:51–7. doi: 10.1016/j.neuint.2010.04.013
46. Sultan A, Nesslany F, Violet M, Begard S, Loyens A, Talahari S, et al. Nuclear tau, a key player in neuronal DNA protection. *J Biol Chem.* (2011) 286:4566–75. doi: 10.1074/jbc.M110.199976
47. Apicco DJ, Ash PEA, Maziuk B, LeBlang C, Medalla M, Al Abdullatif A, et al. Reducing the RNA binding protein TIA1 protects against tau-mediated neurodegeneration *in vivo*. *Nat Neurosci.* (2018) 21:72–80. doi: 10.1038/s41593-017-0022-z
48. Maziuk BF, Apicco DJ, Cruz AL, Jiang L, Ash PEA, da Rocha EL, et al. RNA binding proteins co-localize with small tau inclusions in tauopathy. *Acta Neuropathol Commun.* (2018) 6:71. doi: 10.1186/s40478-018-0574-5
49. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, et al. Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci.* (2003) 100:10032–7. doi: 10.1073/pnas.1630428100
50. Zhao Y, Tseng I-C, Heyser CJ, Rockenstein E, Mante M, Adame A, et al. Appoptosin-mediated caspase cleavage of tau contributes to progressive supranuclear palsy pathogenesis. *Neuron.* (2015) 87:963–75. doi: 10.1016/j.neuron.2015.08.020
51. Zhao X, Kotilinek LA, Smith B, Hlynialuk C, Zahs K, Ramsden M, et al. Caspase-2 cleavage of tau reversibly impairs memory. *Nat Med.* (2016) 22:1268–76. doi: 10.1038/nm.4199
52. Amadoro G, Ciotti MT, Costanzi M, Cestari V, Calissano P, Canu N. NMDA receptor mediates tau-induced neurotoxicity by calpain and ERK/MAPK activation. *Proc Natl Acad Sci.* (2006) 103:2892–7. doi: 10.1073/pnas.0511065103
53. Zhang Z, Song M, Liu X, Kang SS, Kwon I-S, Duong DM, et al. Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease. *Nat Med.* (2014) 20:1254. doi: 10.1038/nm.3700
54. Henriksen K, Wang Y, Sørensen MG, Barascuk N, Suhy J, Pedersen JT, et al. An enzyme-generated fragment of tau measured in serum shows an inverse correlation to cognitive function. *PLoS ONE.* (2013) 8:64990. doi: 10.1371/journal.pone.0064990
55. Matsumoto S-E, Motoi Y, Ishiguro K, Tabira T, Kametani F, Hasegawa M, et al. The twenty-four KDa C-terminal tau fragment increases with aging in tauopathy mice: implications of prion-like properties. *Human Mol Gen.* (2015) 24:6403–16. doi: 10.1093/hmg/ddv351
56. Martin L, Latypova X, Terro F. Post-translational modifications of tau protein: implications for Alzheimer's disease. *Neurochem Int.* (2011) 58:458–71. doi: 10.1016/j.neuint.2010.12.023
57. Martin BA, Allen PA. Where do we stand on the relationship between tau biomarkers and mild cognitive impairment? *Quant Imaging Med Surg.* (2013) 3:189–91. doi: 10.3978/j.issn.2223-4292.2013.07.02
58. Tenreiro S, Eckermann K, Outeiro TF. Protein phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci.* (2014) 7:42. doi: 10.3389/fnmol.2014.00042
59. Ittner A, Chua SW, Bertz J, Volkerling A, van der Hoven J, Gladbach A, et al. Site-specific phosphorylation of tau inhibits amyloid-β toxicity in Alzheimer's mice. *Science.* (2016) 354:904–8. doi: 10.1126/science.aah6205
60. Billingsley ML, Kincaid RL. Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. *Biochem J.* (1997) 323:577–91. doi: 10.1042/bj3230577
61. Gong CX, Liu F, Grundke-Iqbal I, Iqbal K. Impaired brain glucose metabolism leads to Alzheimer neurofibrillary degeneration through a decrease in tau O-GlcNAcylation. *J Alzheimers Dis.* (2006) 9:1–12. doi: 10.3233/JAD-2006-9101
62. Kopke E, Tung YC, Shaikh S, Alonso AC, 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) 268:24374–84.
63. Noble W, Hanger DP, Miller CC, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. *Front Neurol.* (2013) 4:83. doi: 10.3389/fneur.2013.00083
64. Goedert M, Jakes R, Crowther RA, Cohen P, Vanmechelen E, Vandermeeren M, et al. Epitope mapping of monoclonal antibodies to the paired helical filaments of Alzheimer's disease: identification of phosphorylation sites in tau protein. *Biochem J.* (1994) 301:871–7. doi: 10.1042/bj3010871
65. Hanger DP, Byers HL, Wray S, Leung KY, Saxton MJ, Seereeram A, et al. Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis. *J Biol Chem.* (2007) 282:23645–54. doi: 10.1074/jbc.M703269200
66. Hanger DP, Betts JC, Loviny TL, Blackstock WP, Anderton BH. New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using

nanoelectrospray mass spectrometry. *J Neurochem.* (1998) 71:2465–76. doi: 10.1046/j.1471-4159.1998.71062465.x
67. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida H, Watanabe A, et al. Hyperphosphorylation of tau in PHF. *Neurobiol Aging.* (1995) 16:365–71. doi: 10.1016/0197-4580(95)00027-C
68. Vega IE, Cui L, Propst JA, Hutton ML, Lee G, Yen SH. Increase in tau tyrosine phosphorylation correlates with the formation of tau aggregates. *Brain Res Mol Brain Res.* (2005) 138:135–44. doi: 10.1016/j.molbrainres.2005.04.015
69. Ando K, Maruko-Otake A, Ohtake Y, Hayashishita M, Sekiya M, Iijima KM. Stabilization of microtubule-unbound tau via tau phosphorylation at Ser262/356 by Par-1/MARK contributes to augmentation of AD-related phosphorylation and Abeta42-induced tau toxicity. *PLoS Genet.* (2016) 12:e1005917. doi: 10.1371/journal.pgen.1005917
70. Biernat J, Gustke N, Drewes G, Mandelkow EM, Mandelkow E. Phosphorylation of Ser262 strongly reduces binding of tau to microtubules: distinction between PHF-like immunoreactivity and microtubule binding. *Neuron.* (1993) 11:153–63. doi: 10.1016/0896-6273(93)90279-Z
71. Eftekharzadeh B, Daigle JG, Kapinos LE, Coyne A, Schiantarelli J, Carlomagno Y, et al. Tau protein disrupts nucleocytoplasmic transport in Alzheimer's disease. *Neuron.* (2018) 99:925–40 e927. doi: 10.1016/j.neuron.2018.07.039
72. Gilley J, Ando K, Seereeram A, Rodríguez-Martín T, Pooler AM, Sturdee L, et al. Mislocalization of neuronal tau in the absence of tangle pathology in phosphomutant tau knockin mice. *Neurobiol Aging.* (2016) 39:1–18. doi: 10.1016/j.neurobiolaging.2015.11.028
73. Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med.* (2009) 15:112–9. doi: 10.1016/j.molmed.2009.01.003
74. Paonessa F, Evans LD, Solanki R, Larrieu D, Wray S, Hardy J, et al. Microtubules deform the nuclear membrane and disrupt nucleocytoplasmic transport in tau-mediated frontotemporal dementia. *Cell Rep.* (2019) 26:582–93 e585. doi: 10.1016/j.celrep.2018.12.085
75. Klaips CL, Jayaraj GG, Hartl FU. Pathways of cellular proteostasis in aging and disease. *J Cell Biol.* (2018) 217:51–63. doi: 10.1083/jcb.201709072
76. Mudher A, Colin M, Dujardin S, Medina M, Dewachter I, Alavi Naini SM, et al. What is the evidence that tau pathology spreads through prion-like propagation? *Acta Neuropathol Commun.* (2017) 5:99. doi: 10.1186/s40478-017-0488-7
77. Quinn JP, Corbett NJ, Kellett KA, Hooper NM. Tau proteolysis in the pathogenesis of tauopathies: neurotoxic fragments and novel biomarkers. *J Alzheimer's Dis.* (2018) 63:13–33. doi: 10.3233/JAD-170959
78. Liu P, Smith BR, Montonye ML, Kemper LJ, Leinonen-Wright K, Nelson KM, et al. A soluble truncated tau species related to cognitive dysfunction is elevated in the brain of cognitively impaired human individuals. *Sci Rep.* (2020) 10:1–18. doi: 10.1038/s41598-020-60777-x
79. Wray S, Saxton M, Anderton BH, Hanger DP. Direct analysis of tau from PSP brain identifies new phosphorylation sites and a major fragment of N-terminally cleaved tau containing four microtubule-binding repeats. *J Neurochem.* (2008) 105:2343–52. doi: 10.1111/j.1471-4159.2008.05321.x
80. Bondulich MK, Guo T, Meehan C, Manion J, Rodriguez Martin T, Mitchell JC, et al. Tauopathy induced by low level expression of a human brain-derived tau fragment in mice is rescued by phenylbutyrate. *Brain.* (2016) 139:2290–306. doi: 10.1093/brain/aww137
81. Hanger DP, Wray S. *Tau Cleavage and Tau Aggregation in Neurodegenerative Disease*. London: Portland Press Ltd. (2010). doi: 10.1042/BST0381016
82. Min SW, Cho SH, Zhou Y, Schroeder S, Haroutunian V, Seeley WW, et al. Acetylation of tau inhibits its degradation and contributes to tauopathy. *Neuron.* (2010) 67:953–66. doi: 10.1016/j.neuron.2010.08.044
83. Cook C, Stankowski JN, Carlomagno Y, Stetler C, Petrucelli L. Acetylation: a new key to unlock tau's role in neurodegeneration. *Alzheimers Res Ther.* (2014) 6:29. doi: 10.1186/alzrt259
84. Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, et al. The acetylation of tau inhibits its function and promotes pathological tau aggregation. *Nat Commun.* (2011) 2:252. doi: 10.1038/ncomms1255
85. Tracy T, Claiborn KC, Gan L. Regulation of tau homeostasis and toxicity by acetylation. *Adv Exp Med Biol.* (2019) 1184:47–55. doi: 10.1007/978-981-32-9358-8_4
86. Cohen TJ, Friedmann D, Hwang AW, Marmorstein R, Lee VM. The microtubule-associated tau protein has intrinsic acetyltransferase activity. *Nat Struct Mol Biol.* (2013) 20:756–62. doi: 10.1038/nsmb.2555
87. Arakhamia T, Lee CE, Carlomagno Y, Duong DM, Kundinger SR, Wang K, et al. Posttranslational modifications mediate the structural diversity of tauopathy strains. *Cell.* (2020) 180:633–44 e612. doi: 10.1016/j.cell.2020.01.027
88. Trzeciakiewicz H, Tseng JH, Wander CM, Madden V, Tripathy A, Yuan CX, et al. A dual pathogenic mechanism links tau acetylation to sporadic tauopathy. *Sci Rep.* (2017) 7:44102. doi: 10.1038/srep44102
89. Ihara Y. PHF and PHF-like fibrils–cause or consequence? *Neurobiol Aging.* (2001) 22:123–6. doi: 10.1016/S0197-4580(00)00200-1
90. Tracy T, Claiborn KC, Gan L. Regulation of tau homeostasis and toxicity by acetylation. In: *Tau Biology*. Springer. (2019). p. 47–55.
91. Van den Steen P, Rudd PM, Dwek RA, Opdenakker G. Concepts and principles of O-linked glycosylation. *Crit Rev Biochem Mol Biol.* (1998) 33:151–208. doi: 10.1080/10409239891204198
92. Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, Gong C-X. O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. *Proc Natl Acad Sci.* (2004) 101:10804–9. doi: 10.1073/pnas.0400348101
93. Lefebvre T, Ferreira S, Dupont-Wallois L, Bussiere T, Dupire M-J, Delacourte A, et al. Evidence of a balance between phosphorylation and O-GlcNAc glycosylation of Tau proteins—a role in nuclear localization. *Biochim Biophys Acta.* (2003) 1619:167–76. doi: 10.1016/S0304-4165(02)00477-4
94. Wani WY, Chatham JC, Darley-Usmar V, McMahon LL, Zhang J. O-GlcNAcylation and neurodegeneration. *Brain Res Bulletin.* (2017) 133:80–7. doi: 10.1016/j.brainresbull.2016.08.002
95. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, et al. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet.* (2000) 25:402–5. doi: 10.1038/78078
96. Yuzwa SA, Shan X, Macauley MS, Clark T, Skorobogatko Y, Vosseller K, et al. Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. *Nat Chem Biol.* (2012) 8:393–9. doi: 10.1038/nchembio.797
97. Kontaxi C, Piccardo P, Gill AC. Lysine-directed post-translational modifications of tau protein in Alzheimer's disease and related tauopathies. *Front Mol Biosci.* (2017) 4:56. doi: 10.3389/fmolb.2017.00056
98. Gong CX, Liu F, Grundke-Iqbal I, Iqbal K. Post-translational modifications of tau protein in Alzheimer's disease. *J Neural Transm.* (2005) 112:813–38. doi: 10.1007/s00702-004-0221-0
99. Yang XJ, Seto E. Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol Cell.* (2008) 31:449–61. doi: 10.1016/j.molcel.2008.07.002
100. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol.* (2009) 11:909–13. doi: 10.1038/ncb1901
101. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem.* (2009) 284:12845–52. doi: 10.1074/jbc.M808759200
102. Walker LC, Jucker M. Neurodegenerative diseases: expanding the prion concept. *Annu Rev Neurosci.* (2015) 38:87–103. doi: 10.1146/annurev-neuro-071714-033828
103. Holmes BB, Diamond MI. Prion-like properties of Tau protein: the importance of extracellular Tau as a therapeutic target. *J Biol Chem.* (2014) 289:19855–61. doi: 10.1074/jbc.R114.549295
104. Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, Berry DB, et al. Evidence for alpha-synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proc Natl Acad Sci USA.* (2015) 112:E5308–17. doi: 10.1073/pnas.1514475112
105. Woerman AL, Aoyagi A, Patel S, Kazmi SA, Lobach I, Grinberg LT, et al. Tau prions from Alzheimer's disease and chronic traumatic encephalopathy patients propagate in cultured cells. *Proc Natl Acad Sci USA.* (2016) 113:E8187–96. doi: 10.1073/pnas.1616344113
106. Watts JC, Prusiner SB. Beta-amyloid prions and the pathobiology of Alzheimer's disease. *Cold Spring Harb Perspect Med.* (2018) 8:a023507. doi: 10.1101/cshperspect.a023507

107. Daude N, Kim C, Kang SG, Eskandari-Sedighi G, Haldiman T, Yang J, et al. Diverse, evolving conformer populations drive distinct phenotypes in frontotemporal lobar degeneration caused by the same MAPT-P301L mutation. *Acta Neuropathol.* (2020) 139:1045–70. doi: 10.1007/s00401-020-02148-4
108. Eskandari-Sedighi G, Daude N, Gapeshina H, Sanders DW, Kamali-Jamil R, Yang J, et al. The CNS in inbred transgenic models of 4-repeat Tauopathy develops consistent tau seeding capacity yet focal and diverse patterns of protein deposition. *Mol Neurodegener.* (2017) 12:72. doi: 10.1186/s13024-017-0215-7
109. Kaufman SK, Sanders DW, Thomas TL, Ruchinskas AJ, Vaquer-Alicea J, Sharma AM, et al. Tau prion strains dictate patterns of cell pathology, progression rate, and regional vulnerability *in vivo*. *Neuron.* (2016) 92:796–812. doi: 10.1016/j.neuron.2016.09.055
110. Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron.* (2014) 82:1271–88. doi: 10.1016/j.neuron.2014.04.047
111. Sharma AM, Thomas TL, Woodard DR, Kashmer OM, Diamond MI. Tau monomer encodes strains. *Elife.* (2018) 7. doi: 10.7554/eLife.37813.023
112. Weaver CL, Espinoza M, Kress Y, Davies P. Conformational change as one of the earliest alterations of tau in Alzheimer's disease. *Neurobiol Aging.* (2000) 21:719–27. doi: 10.1016/S0197-4580(00)00157-3
113. Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, et al. Eight prion strains have PrP(Sc) molecules with different conformations. *Nat Med.* (1998) 4:1157–65. doi: 10.1038/2654
114. Cohen ML, Kim C, Haldiman T, ElHag M, Mehndiratta P, Pichet T, et al. Rapidly progressive Alzheimer's disease features distinct structures of amyloid-beta. *Brain.* (2015) 138:1009–22. doi: 10.1093/brain/awv006
115. Safar J, Roller PP, Gajdusek DC, Gibbs CJ Jr. Thermal stability and conformational transitions of scrapie amyloid (prion) protein correlate with infectivity. *Protein Sci.* (1993) 2:2206–16. doi: 10.1002/pro.5560021220
116. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med.* (2011) 17:175–8. doi: 10.1038/nm.2294
117. Kim C, Xiao X, Chen S, Haldiman T, Smirnovas V, Kofskey D, et al. Artificial strain of human prions created *in vitro*. *Nat Commun.* (2018) 9:2166. doi: 10.1038/s41467-018-04584-z
118. Haldiman T, Kim C, Cohen Y, Chen W, Blevins J, Qing L, et al. Co-existence of distinct prion types enables conformational evolution of human PrPSc by competitive selection. *J Biol Chem.* (2013) 288:29846–61. doi: 10.1074/jbc.M113.500108
119. Kim C, Haldiman T, Surewicz K, Cohen Y, Chen W, Blevins J, et al. Small protease sensitive oligomers of PrPSc in distinct human prions determine conversion rate of PrP(C). *PLoS Pathog.* (2012) 8:e1002835. doi: 10.1371/journal.ppat.1002835
120. Safar JG, Xiao X, Kabir ME, Chen S, Kim C, Haldiman T, et al. Structural determinants of phenotypic diversity and replication rate of human prions. *PLoS Pathog.* (2015) 11:e1004832. doi: 10.1371/journal.ppat.1004832
121. Buee L, Troquier L, Burnouf S, Belarbi K, Van der Jeugd A, Ahmed T, et al. From tau phosphorylation to tau aggregation: what about neuronal death? *Biochem Soc Trans.* (2010) 38:967–72. doi: 10.1042/BST0380967
122. Stoothoff WH, Johnson GV. Tau phosphorylation: physiological and pathological consequences. *Biochim Biophys Acta.* (2005) 1739:280–97. doi: 10.1016/j.bbadis.2004.06.017
123. Zhang W, Tarutani A, Newell KL, Murzin AG, Matsubara T, Falcon B, et al. Novel tau filament fold in corticobasal degeneration. *Nature.* (2020) 580:283–7. doi: 10.1038/s41586-020-2043-0
124. Gomez-Ramos A, Diaz-Hernandez M, Rubio A, Miras-Portugal M, Avila J. Extracellular tau promotes intracellular calcium increase through M1 and M3 muscarinic receptors in neuronal cells. *Mol Cell Neurosci.* (2008) 37:673–81. doi: 10.1016/j.mcn.2007.12.010
125. Kaufman SK, Del Tredici K, Thomas TL, Braak H, Diamond MI. Tau seeding activity begins in the transentorhinal/entorhinal regions and anticipates phospho-tau pathology in Alzheimer's disease and PART. *Acta Neuropathol.* (2018) 136:57–67. doi: 10.1007/s00401-018-1855-6
126. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Sarmiento J, Troncoso J, Jackson GR, et al. Identification of oligomers at early stages of tau aggregation in Alzheimer's disease. *FASEB J.* (2012) 26:1946–59. doi: 10.1096/fj.11-199851
127. Takeda S, Wegmann S, Cho H, DeVos SL, Commins C, Roe AD, et al. Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain. *Nat Commun.* (2015) 6:8490. doi: 10.1038/ncomms9490
128. Dujardin S, Commins C, Lathuiliere A, Beerepoot P, Fernandes AR, Kamath TV, et al. Tau molecular diversity contributes to clinical heterogeneity in Alzheimer's disease. *Nat Med.* (2020) 26:1256–63. doi: 10.1038/s41591-020-0938-9
129. Lee KH, Zhang P, Kim HJ, Mitrea DM, Sarkar M, Freibaum BD, et al. C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. *Cell.* (2016) 167:774–88 e717. doi: 10.1016/j.cell.2016.10.002
130. Mann JR, Gleixner AM, Mauna JC, Gomes E, DeChellis-Marks MR, Needham PG, et al. RNA binding antagonizes neurotoxic phase transitions of TDP-43. *Neuron.* (2019) 102:321–38 e328. doi: 10.1016/j.neuron.2019.01.048
131. Murakami T, Qamar S, Lin JQ, Schierle GS, Rees E, Miyashita A, et al. ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron.* (2015) 88:678–90. doi: 10.1016/j.neuron.2015.10.030
132. Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, et al. A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell.* (2015) 162:1066–77. doi: 10.1016/j.cell.2015.07.047
133. Kim HJ, Kim NC, Wang YD, Scarborough EA, Moore J, Diaz Z, et al. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature.* (2013) 495:467–73. doi: 10.1038/nature11922
134. Shin Y, Brangwynne CP. Liquid phase condensation in cell physiology and disease. *Science.* (2017) 357:aaf4382. doi: 10.1126/science.aaf4382
135. Alberti S, Gladfelter A, Mittag T. Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. *Cell.* (2019) 176:419–34. doi: 10.1016/j.cell.2018.12.035
136. Ryan VH, Fawzi NL. Physiological, pathological, and targetable membraneless organelles in neurons. *Trends Neurosci.* (2019) 42:693–708. doi: 10.1016/j.tins.2019.08.005
137. Ambadipudi S, Biernat J, Riedel D, Mandelkow E, Zweckstetter M. Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. *Nat Commun.* (2017) 8:275. doi: 10.1038/s41467-017-00480-0
138. Boyko S, Qi X, Chen TH, Surewicz K, Surewicz WK. Liquid-liquid phase separation of tau protein: the crucial role of electrostatic interactions. *J Biol Chem.* (2019) 294:11054–9. doi: 10.1074/jbc.AC119.009198
139. Kanaan NM, Hamel C, Grabinski T, Combs B. Liquid-liquid phase separation induces pathogenic tau conformations *in vitro*. *Nat Commun.* (2020) 11:2809. doi: 10.1038/s41467-020-16580-3
140. Singh V, Xu L, Boyko S, Surewicz K, Surewicz WK. Zinc promotes liquid-liquid phase separation of tau protein. *J Biol Chem.* (2020) 295:5850–6. doi: 10.1074/jbc.AC120.013166
141. Vega IE, Umstead A, Kanaan NM. EFhd2 affects tau liquid-liquid phase separation. *Front Neurosci.* (2019) 13:845. doi: 10.3389/fnins.2019.00845
142. Wegmann S, Eftekharzadeh B, Tepper K, Zoltowska KM, Bennett RE, Dujardin S, et al. Tau protein liquid-liquid phase separation can initiate tau aggregation. *EMBO J.* (2018) 37:98049. doi: 10.15252/embj.201798049
143. Zhang X, Lin Y, Eschmann NA, Zhou H, Rauch JN, Hernandez I, et al. RNA stores tau reversibly in complex coacervates. *PLoS Biol.* (2017) 15:e2002183. doi: 10.1371/journal.pbio.2002183
144. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature.* (2019) 568:420–3. doi: 10.1038/s41586-019-1026-5
145. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature.* (2017) 547:185–90. doi: 10.1038/nature23002
146. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature.* (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y

147. Lippens G, Gigant B. Elucidating Tau function and dysfunction in the era of cryo-EM. *J Biol Chem.* (2019) 294:9316–25. doi: 10.1074/jbc.REV119.008031
148. Goedert M. Tau filaments in neurodegenerative diseases. *FEBS Lett.* (2018) 592:2383–91. doi: 10.1002/1873-3468.13108
149. Zhang W, Falcon B, Murzin AG, Fan J, Crowther RA, Goedert M, et al. Heparin-induced tau filaments are polymorphic and differ from those in Alzheimer's and Pick's diseases. *Elife.* (2019) 8:40. doi: 10.7554/eLife.43584.040
150. Falcon B, Zhang W, Schweighauser M, Murzin AG, Vidal R, Garringer HJ, et al. Tau filaments from multiple cases of sporadic and inherited Alzheimer's disease adopt a common fold. *Acta Neuropathol.* (2018) 136:699–708. doi: 10.1007/s00401-018-1914-z
151. Bartz JC. Prion strain diversity. *Cold Spring Harb Perspect Med.* (2016) 6:a024349. doi: 10.1101/cshperspect.a024349
152. Mirra SS, Hyman BT. Greenfield's Neuropathology In: *Ageing and Dementia. 7th Edn Vol. 2.* Graham DI, Lantos PL, editors. London UK: Arnold. (2002). p. 226–35.
153. Lin WL, Lewis J, Yen SH, Hutton M, Dickson DW. Filamentous tau in oligodendrocytes and astrocytes of transgenic mice expressing the human tau isoform with the P301L mutation. *Am J Pathol.* (2003) 162:213–8. doi: 10.1016/S0002-9440(10)63812-6
154. Murakami T, Paitel E, Kawarabayashi T, Ikeda M, Chishti MA, Janus C, et al. Cortical neuronal and glial pathology in TgTauP301L transgenic mice: neuronal degeneration, memory disturbance, and phenotypic variation. *Am J Pathol.* (2006) 169:1365–75. doi: 10.2353/ajpath.2006.051250
155. Guo JL, Lee VM. Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. *J Biol Chem.* (2011) 286:15317–31. doi: 10.1074/jbc.M110.209296
156. Guo JL, Narasimhan S, Changolkar L, He Z, Stieber A, Zhang B, et al. Unique pathological tau conformers from Alzheimer's brains transmit tau pathology in non-transgenic mice. *J Exp Med.* (2016) 213:2635–54. doi: 10.1084/jem.20160833
157. Ahmed Z, Cooper J, Murray TK, Garn K, McNaughton E, Clarke H, et al. A novel *in vivo* model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. *Acta Neuropathol.* (2014) 127:667–83. doi: 10.1007/s00401-014-1254-6
158. Iba M, McBride JD, Guo JL, Zhang B, Trojanowski JQ, Lee VM. Tau pathology spread in PS19 tau transgenic mice following locus coeruleus (LC) injections of synthetic tau fibrils is determined by the LC's afferent and efferent connections. *Acta Neuropathol.* (2015) 130:349–62. doi: 10.1007/s00401-015-1458-4
159. Narasimhan S, Guo JL, Changolkar L, Stieber A, McBride JD, Silva LV, et al. Pathological tau strains from human brains recapitulate the diversity of tauopathies in non-transgenic mouse brain. *J Neurosci.* (2017) 37:11406–23. doi: 10.1523/JNEUROSCI.1230-17.2017
160. Narasimhan S, Changolkar L, Riddle DM, Kats A, Stieber A, Weitzman SA, et al. Human tau pathology transmits glial tau aggregates in the absence of neuronal tau. *J Exp Med.* (2020) 217:783. doi: 10.1084/jem.20190783
161. Gibbons GS, Lee VMY, Trojanowski JQ. Mechanisms of cell-to-cell transmission of pathological tau: a review. *JAMA Neurol.* (2019) 76:101–8. doi: 10.1001/jamaneurol.2018.2505
162. Seiberlich V, Bauer NG, Schwarz L, Ffrench-Constant C, Goldbaum O, Richter-Landsberg C. Downregulation of the microtubule associated protein tau impairs process outgrowth and myelin basic protein mRNA transport in oligodendrocytes. *Glia.* (2015) 63:1621–35. doi: 10.1002/glia.22832
163. Braak H, Braak E. Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol Aging.* (1995) 16:271–78. doi: 10.1016/0197-4580(95)00021-6
164. Komori T. Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. *Brain Pathol.* (1999) 9:663–79. doi: 10.1111/j.1750-3639.1999.tb00549.x
165. Odawara T, Iseki E, Kosaka K, Akiyama H, Ikeda K, Yamamoto T. Investigation of tau-2 positive microglia-like cells in the subcortical nuclei of human neurodegenerative disorders. *Neurosci Lett.* (1995) 192:145–8. doi: 10.1016/0304-3940(95)11595-N
166. Kovacs GG. Astroglia and tau: new perspectives. *Front Aging Neurosci.* (2020) 12:96. doi: 10.3389/fnagi.2020.00096
167. Ferrer I, Lopez-Gonzalez I, Carmona M, Arregui L, Dalfo E, Torrejon-Escribano B, et al. Glial and neuronal tau pathology in tauopathies: characterization of disease-specific phenotypes and tau pathology progression. *J Neuropathol Exp Neurol.* (2014) 73:81–97. doi: 10.1097/NEN.0000000000000030
168. Yamazaki M, Nakano I, Imazu O, Kaieda R, Terashi A. Astrocytic straight tubules in the brain of a patient with Pick's disease. *Acta Neuropathol.* (1994) 88:587–91. doi: 10.1007/BF00296498
169. Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. *Proc Natl Acad Sci USA.* (2013) 110:9535–40. doi: 10.1073/pnas.1301175110
170. Yamada T, McGeer PL, McGeer EG. Appearance of paired nucleated, Tau-positive glia in patients with progressive supranuclear palsy brain tissue. *Neurosci Lett.* (1992) 135:99–102. doi: 10.1016/0304-3940(92)90145-W
171. Nishimura T, Ikeda K, Akiyama H, Kondo H, Kato M, Li F, et al. Immunohistochemical investigation of tau-positive structures in the cerebral cortex of patients with progressive supranuclear palsy. *Neurosci Lett.* (1995) 201:123–6. doi: 10.1016/0304-3940(95)12151-X
172. Tolnay M, Spillantini MG, Goedert M, Ulrich J, Langui D, Probst A. Argyrophilic grain disease: widespread hyperphosphorylation of tau protein in limbic neurons. *Acta Neuropathol.* (1997) 93:477–84. doi: 10.1007/s004010050642
173. Kovacs GG, Ferrer I, Grinberg LT, Alafuzoff I, Attems J, Budka H, et al. Aging-related tau astrogliopathy (ARTAG): harmonized evaluation strategy. *Acta Neuropathol.* (2016) 131:87–102. doi: 10.1007/s00401-015-1509-x
174. Yoshida M. Astrocytic inclusions in progressive supranuclear palsy and corticobasal degeneration. *Neuropathology.* (2014) 34:555–70. doi: 10.1111/neup.12143
175. Dickson DW. Neuropathologic differentiation of progressive supranuclear palsy and corticobasal degeneration. *J Neurol.* (1999) 246(Suppl.2):II6–15. doi: 10.1007/BF03161076
176. Nishimura M, Namba Y, Ikeda K, Oda M. Glial fibrillary tangles with straight tubules in the brains of patients with progressive supranuclear palsy. *Neurosci Lett.* (1992) 143:35–8. doi: 10.1016/0304-3940(92)90227-X
177. Bigio EH, Lipton AM, Yen SH, Hutton ML, Baker M, Nacharaju P, et al. Frontal lobe dementia with novel tauopathy: sporadic multiple system tauopathy with dementia. *J Neuropathol Exp Neurol.* (2001) 60:328–41. doi: 10.1093/jnen/60.4.328
178. Burrell JR, Forrest S, Bak TH, Hodges JR, Halliday GM, Kril JJ. Expanding the phenotypic associations of globular glial tau subtypes. *Alzheimers Dement.* (2016) 4:6–13. doi: 10.1016/j.dadm.2016.03.006
179. Ikeda M, Shoji M, Kawarai T, Kawarabayashi T, Matsubara E, Murakami T, et al. Accumulation of filamentous tau in the cerebral cortex of human tau R406W transgenic mice. *Am J Pathol.* (2005) 166:521–31. doi: 10.1016/S0002-9440(10)62274-2
180. von Bartheld CS, Bahney J, Herculano-Houzel S. The search for true numbers of neurons and glial cells in the human brain: a review of 150 years of cell counting. *J Comp Neurol.* (2016) 524:3865–95. doi: 10.1002/cne.24040
181. Allen NJ, Lyons DA. Glia as architects of central nervous system formation and function. *Science.* (2018) 362:181–5. doi: 10.1126/science.aat0473
182. Colonna M, Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol.* (2017) 35:441–68. doi: 10.1146/annurev-immunol-051116-052358
183. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature.* (2017) 541:481–7. doi: 10.1038/nature21029
184. Brunello CA, Merezhko M, Uronen RL, Huttunen HJ. Mechanisms of secretion and spreading of pathological tau protein. *Cell Mol Life Sci.* (2020) 77:1721–44. doi: 10.1007/s00018-019-03349-1
185. Boada-Romero E, Martinez J, Heckmann BL, Green DR. The clearance of dead cells by efferocytosis. *Nat Rev Mol Cell Biol.* (2020) 21:398–414. doi: 10.1038/s41580-020-0232-1
186. Polymenidou M, Cleveland DW. Prion-like spread of protein aggregates in neurodegeneration. *J Exp Med.* (2012) 209:889–93. doi: 10.1084/jem.20120741
187. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* (1991) 82:239–59. doi: 10.1007/BF00308809

188. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol.* (2011) 70:960–9. doi: 10.1097/NEN.0b013e318232a379
189. Jicha GA, Bowser R, Kazam IG, Davies P. Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. *J Neurosci Res.* (1997) 48:128–32. doi: 10.1002/(SICI)1097-4547(19970415)48:2<128::AID-JNR5>3.0.CO;2-E
190. Jicha GA, Lane E, Vincent I, Otvos LJr, Hoffmann R, Davies P. A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease. *J Neurochem.* (1997) 69:2087–95. doi: 10.1046/j.1471-4159.1997.69052087.x
191. Calafate S, Buist A, Miskiewicz K, Vijayan V, Daneels G, de Strooper B, et al. Synaptic contacts enhance cell-to-cell tau pathology propagation. *Cell Rep.* (2015) 11:1176–83. doi: 10.1016/j.celrep.2015.04.043
192. de Calignon A, Polydoro M, Suarez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron.* (2012) 73:685–97. doi: 10.1016/j.neuron.2011.11.033
193. Liu L, Drouet V, Wu JW, Witter MP, Small SA, Clelland C, et al. Trans-synaptic spread of tau pathology *in vivo*. *PLoS ONE.* (2012) 7:e31302. doi: 10.1371/journal.pone.0031302
194. Cocucci E, Meldolesi J. Ectosomes and exosomes: shedding the confusion between extracellular vesicles. *Trends Cell Biol.* (2015) 25:364–72. doi: 10.1016/j.tcb.2015.01.004
195. Ait-Bouziad N, Lv G, Mahul-Mellier AL, Xiao S, Zorludemir G, Eliezer D, et al. Discovery and characterization of stable and toxic Tau/phospholipid oligomeric complexes. *Nat Commun.* (2017) 8:1678. doi: 10.1038/s41467-017-01575-4
196. Katsinelos T, Zeitler M, Dimou E, Karakatsani A, Muller HM, Nachman E, et al. Unconventional secretion mediates the trans-cellular spreading of tau. *Cell Rep.* (2018) 23:2039–55. doi: 10.1016/j.celrep.2018.04.056
197. Mirbaha H, Holmes BB, Sanders DW, Bieschke J, Diamond MI. Tau trimers are the minimal propagation unit spontaneously internalized to seed intracellular aggregation. *J Biol Chem.* (2015) 290:14893–903. doi: 10.1074/jbc.M115.652693
198. Dujardin S, Begard S, Caillierez R, Lachaud C, Delattre L, Carrier S, et al. Ectosomes: a new mechanism for non-exosomal secretion of tau protein. *PLoS ONE.* (2014) 9:e100760. doi: 10.1371/journal.pone.0100760
199. Rauch JN, Chen JJ, Sorum AW, Miller GM, Sharf T, See SK, et al. Tau internalization is regulated by 6-O sulfation on heparan sulfate proteoglycans (HSPGs). *Sci Rep.* (2018) 8:6382. doi: 10.1038/s41598-018-24904-z
200. Abounit S, Wu JW, Duff K, Victoria GS, Zurzolo C. Tunneling nanotubes: a possible highway in the spreading of tau and other prion-like proteins in neurodegenerative diseases. *Prion.* (2016) 10:344–51. doi: 10.1080/19336896.2016.1223003
201. Boluda S, Iba M, Zhang B, Raible KM, Lee VM, Trojanowski JQ. Differential induction and spread of tau pathology in young PS19 tau transgenic mice following intracerebral injections of pathological tau from Alzheimer's disease or corticobasal degeneration brains. *Acta Neuropathol.* (2015) 129:221–37. doi: 10.1007/s00401-014-1373-0
202. Ferrer I, Aguilo Garcia M, Carmona M, Andres-Benito P, Torrejon-Escribano B, Garcia-Esparcia P, et al. Involvement of oligodendrocytes in tau seeding and spreading in tauopathies. *Front Aging Neurosci.* (2019) 11:112. doi: 10.3389/fnagi.2019.00112
203. Ferrer I, Andres-Benito P, Zelaya MV, Aguirre MEE, Carmona M, Ausin K, et al. Familial globular glial tauopathy linked to MAPT mutations: molecular neuropathology and seeding capacity of a prototypical mixed neuronal and glial tauopathy. *Acta Neuropathol.* (2020) 139:735–71. doi: 10.1007/s00401-019-02122-9
204. Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, Yang L, et al. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *J Neurosci.* (2014) 34:16180–93. doi: 10.1523/JNEUROSCI.3020-14.2014
205. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. *Sci Transl Med.* (2012) 4:147ra111. doi: 10.1126/scitranslmed.3003748
206. Xu Z, Xiao N, Chen Y, Huang H, Marshall C, Gao J, et al. Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain Abeta accumulation and memory deficits. *Mol Neurodegener.* (2015) 10:58. doi: 10.1186/s13024-015-0056-1
207. Rasmussen MK, Mestre H, Nedergaard M. The glymphatic pathway in neurological disorders. *Lancet Neurol.* (2018) 17:1016–24. doi: 10.1016/S1474-4422(18)30318-1
208. Harrison IF, Ismail O, Machhada A, Colgan N, Ohene Y, Nahavandi P, et al. Impaired glymphatic function and clearance of tau in an Alzheimer's disease model. *Brain.* (2020) 143:2576–93. doi: 10.1093/brain/awaa179
209. Kuchibhotla KV, Wegmann S, Kopeikina KJ, Hawkes J, Rudinskiy N, Andermann ML, et al. Neurofibrillary tangle-bearing neurons are functionally integrated in cortical circuits *in vivo*. *Proc Natl Acad Sci.* (2014) 111:510–4. doi: 10.1073/pnas.1318807111
210. Gómez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol Off J Am Neurol Assoc Child Neurol Soc.* (1997) 41:17–24. doi: 10.1002/ana.410410106
211. Wittmann CW, Wszolek MF, Shulman JM, Salvaterra PM, Lewis J, Hutton M, et al. Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles. *Science.* (2001) 293:711–4. doi: 10.1126/science.1062382
212. Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, et al. Tau suppression in a neurodegenerative mouse model improves memory function. *Science.* (2005) 309:476–81. doi: 10.1126/science.1113694
213. Castillo-Carranza DL, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G, et al. Passive immunization with Tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J Neurosci.* (2014) 34:4260–72. doi: 10.1523/JNEUROSCI.3192-13.2014
214. Shafiei SS, Guerrero-Muñoz MJ, Castillo-Carranza DL. Tau oligomers: cytotoxicity, propagation, and mitochondrial damage. *Front Aging Neurosci.* (2017) 9:83. doi: 10.3389/fnagi.2017.00083
215. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nat Rev Immunol.* (2014) 14:463–77. doi: 10.1038/nri3705
216. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci.* (2015) 16:358–72. doi: 10.1038/nrn3880
217. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* (2015) 14:388–405. doi: 10.1016/S1474-4422(15)70016-5
218. Labzin LI, Heneka MT, Latz E. Innate immunity and neurodegeneration. *Annu Rev Med.* (2018) 69:437–49. doi: 10.1146/annurev-med-050715-104343
219. Deane R, Singh I, Sagare AP, Bell RD, Ross NT, LaRue B, et al. A multimodal RAGE-specific inhibitor reduces amyloid beta-mediated brain disorder in a mouse model of Alzheimer disease. *J Clin Invest.* (2012) 122:1377–92. doi: 10.1172/JCI58642
220. Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, et al. Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. *Glia.* (2013) 61:349–60. doi: 10.1002/glia.22437
221. Kim C, Ho DH, Suk JE, You S, Michael S, Kang J, et al. Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. *Nat Commun.* (2013) 4:1562. doi: 10.1038/ncomms2534
222. Liu S, Liu Y, Hao W, Wolf L, Kiliaan AJ, Penke B, et al. TLR2 is a primary receptor for Alzheimer's amyloid beta peptide to trigger neuroinflammatory activation. *J Immunol.* (2012) 188:1098–107. doi: 10.4049/jimmunol.1101121
223. Kim SM, Mun BR, Lee SJ, Joh Y, Lee HY, Ji KY, et al. TREM2 promotes Abeta phagocytosis by upregulating C/EBPalpha-dependent CD36 expression in microglia. *Sci Rep.* (2017) 7:11118. doi: 10.1038/s41598-017-11634-x
224. Denes A, Coutts G, Lenart N, Cruickshank SM, Pelegrin P, Skinner J, et al. AIM2 and NLRC4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3. *Proc Natl Acad Sci USA.* (2015) 112:4050–5. doi: 10.1073/pnas.1419090112

225. Gustot A, Gallea JI, Sarroukh R, Celej MS, Ruysschaert JM, Raussens V. Amyloid fibrils are the molecular trigger of inflammation in Parkinson's disease. *Biochem J.* (2015) 471:323–33. doi: 10.1042/BJ20150617
226. Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat Immunol.* (2008) 9:857–65. doi: 10.1038/ni.1636
227. Kaushal V, Dye R, Pakavathkumar P, Foveau B, Flores J, Hyman B, et al. Neuronal NLRP1 inflammasome activation of Caspase-1 coordinately regulates inflammatory interleukin-1-beta production and axonal degeneration-associated Caspase-6 activation. *Cell Death Differ.* (2015) 22:1676–86. doi: 10.1038/cdd.2015.16
228. Meissner F, Molawi K, Zychlinsky A. Mutant superoxide dismutase 1-induced IL-1beta accelerates ALS pathogenesis. *Proc Natl Acad Sci USA.* (2010) 107:13046–50. doi: 10.1073/pnas.1002396107
229. Zhao W, Beers DR, Bell S, Wang J, Wen S, Baloh RH, et al. TDP-43 activates microglia through NF-kappaB and NLRP3 inflammasome. *Exp Neurol.* (2015) 273:24–35. doi: 10.1016/j.expneurol.2015.07.019
230. Shi Y, Manis M, Long J, Wang K, Sullivan PM, Remolina Serrano J, et al. Microglia drive APOE-dependent neurodegeneration in a tauopathy mouse model. *J Exp Med.* (2019) 216:2546–61. doi: 10.1084/jem.20190980
231. Voet S, Srinivasan S, Lamkanfi M, van Loo G. Inflammasomes in neuroinflammatory and neurodegenerative diseases. *EMBO Mol Med.* (2019) 11:10248. doi: 10.15252/emmm.201810248
232. Ising C, Venegas C, Zhang S, Scheiblich H, Schmidt SV, Vieira-Saecker A, et al. NLRP3 inflammasome activation drives tau pathology. *Nature.* (2019) 575:669–73. doi: 10.1038/s41586-019-1769-z
233. Stancu IC, Cremers N, Vanrusselt H, Couturier J, Vanoosthuyse A, Kessels S, et al. Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology *in vivo*. *Acta Neuropathol.* (2019) 137:599–617. doi: 10.1007/s00401-018-01957-y
234. Mamik MK, Power C. Inflammasomes in neurological diseases: emerging pathogenic and therapeutic concepts. *Brain.* (2017) 140:2273–85. doi: 10.1093/brain/awx133
235. Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D, et al. Microglia-derived ASC specks cross-seed amyloid-beta in Alzheimer's disease. *Nature.* (2017) 552:355–61. doi: 10.1038/nature25158
236. Baroja-Mazo A, Martin-Sanchez F, Gomez AI, Martinez CM, Amores-Iniesta J, Compan V, et al. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat Immunol.* (2014) 15:738–48. doi: 10.1038/ni.2919
237. Franklin BS, Bossaller L, De Nardo D, Ratter JM, Stutz A, Engels G, et al. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat Immunol.* (2014) 15:727–37. doi: 10.1038/ni.2913
238. Bussian TJ, Aziz A, Meyer CF, Swenson BL, van Deursen JM, Baker DJ. Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. *Nature.* (2018) 562:578–82. doi: 10.1038/s41586-018-0543-y
239. Fujio K, Sato M, Uemura T, Sato T, Sato-Harada R, Harada A. 14-3-3 proteins and protein phosphatases are not reduced in tau-deficient mice. *Neuroreport.* (2007) 18:1049–52. doi: 10.1097/WNR.0b013e32818b2a0b
240. Tucker KL, Meyer M, Barde YA. Neurotrophins are required for nerve growth during development. *Nat Neurosci.* (2001) 4:29–37. doi: 10.1038/82868
241. Combs B, Mueller RL, Morfini G, Brady ST, Kanaan NM. Tau and axonal transport misregulation in tauopathies. *Adv Exp Med Biol.* (2019) 1184:81–95. doi: 10.1007/978-981-32-9358-8_7
242. Cowan CM, Mudher A. Are tau aggregates toxic or protective in tauopathies? *Front Neurol.* (2013) 4:114. doi: 10.3389/fneur.2013.00114
243. Ikeda K, Akiyama H, Haga C, Kondo H, Arima K, Oda T. Argyrophilic thread-like structure in corticobasal degeneration and supranuclear palsy. *Neurosci Lett.* (1994) 174:157–9. doi: 10.1016/0304-3940(94)90010-8
244. McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, et al. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol.* (2009) 68:709–35. doi: 10.1097/NEN.0b013e3181a9d503
245. Probst A, Tolnay M, Langui D, Goedert M, Spillantini MG. Pick's disease: hyperphosphorylated tau protein segregates to the somatoaxonal compartment. *Acta Neuropathol.* (1996) 92:588–96. doi: 10.1007/s004010050565
246. Zhang Y, Walter R, Ng P, Luong PN, Dutt S, Heuer H, et al. Progression of microstructural degeneration in progressive supranuclear palsy and corticobasal syndrome: a longitudinal diffusion tensor imaging study. *PLoS ONE.* (2016) 11:e0157218. doi: 10.1371/journal.pone.0157218
247. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener.* (2011) 6:39. doi: 10.1186/1750-1326-6-39
248. Swanson E, Breckenridge L, McMahon L, Som S, McConnell I, Bloom GS. Extracellular tau oligomers induce invasion of endogenous tau into the somatodendritic compartment and axonal transport dysfunction. *J Alzheimers Dis.* (2017) 58:803–20. doi: 10.3233/JAD-170168
249. Grima JC, Daigle JG, Arbez N, Cunningham KC, Zhang K, Ochaba J, et al. Mutant huntingtin disrupts the nuclear pore complex. *Neuron.* (2017) 94:93–107 e106. doi: 10.1016/j.neuron.2017.03.023
250. Jiang P, Gan M, Yen SH, Moussaud S, McLean PJ, Dickson DW. Proaggregant nuclear factor(s) trigger rapid formation of alpha-synuclein aggregates in apoptotic neurons. *Acta Neuropathol.* (2016) 132:77–91. doi: 10.1007/s00401-016-1542-4
251. Jovicic A, Mertens J, Boeynaems S, Bogaert E, Chai N, Yamada SB, et al. Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat Neurosci.* (2015) 18:1226–9. doi: 10.1038/nn.4085
252. Woerner AC, Frottin F, Hornburg D, Feng LR, Meissner F, Patra M, et al. Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and RNA. *Science.* (2016) 351:173–6. doi: 10.1126/science.aad2033
253. Zhang K, Daigle JG, Cunningham KM, Coyne AN, Ruan K, Grima JC, et al. Stress granule assembly disrupts nucleocytoplasmic transport. *Cell.* (2018) 173:958–71 e917. doi: 10.1016/j.cell.2018.03.025
254. Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, et al. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature.* (2015) 525:56–61. doi: 10.1038/nature14973
255. Beck M, Hurt E. The nuclear pore complex: understanding its function through structural insight. *Nat Rev Mol Cell Biol.* (2017) 18:73–89. doi: 10.1038/nrm.2016.147
256. Timney BL, Raveh B, Mironska R, Trivedi JM, Kim SJ, Russel D, et al. Simple rules for passive diffusion through the nuclear pore complex. *J Cell Biol.* (2016) 215:57–76. doi: 10.1083/jcb.201601004
257. Clarke PR, Zhang C. Spatial and temporal coordination of mitosis by Ran GTPase. *Nat Rev Mol Cell Biol.* (2008) 9:464–77. doi: 10.1038/nrm2410
258. Guttinger S, Laurell E, Kutay U. Orchestrating nuclear envelope disassembly and reassembly during mitosis. *Nat Rev Mol Cell Biol.* (2009) 10:178–91. doi: 10.1038/nrm2641
259. Toyama BH, Savas JN, Park SK, Harris MS, Ingolia NT, Yates JR 3rd, Hetzer MW. Identification of long-lived proteins reveals exceptional stability of essential cellular structures. *Cell.* (2013) 154:971–82. doi: 10.1016/j.cell.2013.07.037
260. Caneus J, Granic A, Rademakers R, Dickson DW, Coughlan CM, Chial HJ, et al. Mitotic defects lead to neuronal aneuploidy and apoptosis in frontotemporal lobar degeneration caused by MAPT mutations. *Mol Biol Cell.* (2018) 29:575–86. doi: 10.1091/mbc.E17-01-0031
261. Neuner SM, Heuer SE, Huentelman MJ, O'Connell KMS, Kaczorowski CC. Harnessing genetic complexity to enhance translatability of Alzheimer's disease mouse models: a path toward precision medicine. *Neuron.* (2019) 101:399–411 e395. doi: 10.1016/j.neuron.2018.11.040
262. Lee MH, Siddoway B, Kaeser GE, Segota I, Rivera R, Romanow WJ, et al. Somatic APP gene recombination in Alzheimer's disease and normal neurons. *Nature.* (2018) 563:639–45. doi: 10.1038/s41586-018-0718-6
263. Breen DP, Halliday GM, Lang AE. Gut-brain axis and the spread of alpha-synuclein pathology: vagal highway or dead end? *Mov Disord.* (2019) 34:307–16. doi: 10.1002/mds.27556

264. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell.* (2016) 167:1469–80 e1412. doi: 10.1016/j.cell.2016.11.018
265. Saito T, Matsuba Y, Mihira N, Takano J, Nilsson P, Itohara S, et al. Single App knock-in mouse models of Alzheimer's disease. *Nat Neurosci.* (2014) 17:661–3. doi: 10.1038/nn.3697
266. Saito T, Mihira N, Matsuba Y, Sasaguri H, Hashimoto S, Narasimhan S, et al. Humanization of the entire murine Mapt gene provides a murine model of pathological human tau propagation. *J Biol Chem.* (2019) 294:12754–65. doi: 10.1074/jbc.RA119.009487

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 12 November 2020
doi: 10.3389/fneur.2020.590754

Tau Filament Self-Assembly and Structure: Tau as a Therapeutic Target

Sebastian S. Oakley[1], *Mahmoud B. Maina*[1,2], *Karen E. Marshall*[1], *Youssra K. Al-Hilaly*[1,3], *Charlie R. Harrington*[4,5], *Claude M. Wischik*[4,5] *and Louise C. Serpell*[1]*

[1] Sussex Neuroscience, School of Life Sciences, University of Sussex, Brighton, United Kingdom, [2] College of Medical Sciences, Yobe State University, Damaturu, Nigeria, [3] Chemistry Department, College of Science, Mustansiriyah University, Baghdad, Iraq, [4] School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, United Kingdom, [5] TauRx Therapeutics Ltd., Aberdeen, United Kingdom

Tau plays an important pathological role in a group of neurodegenerative diseases called tauopathies, including Alzheimer's disease, Pick's disease, chronic traumatic encephalopathy and corticobasal degeneration. In each disease, tau self-assembles abnormally to form filaments that deposit in the brain. Tau is a natively unfolded protein that can adopt distinct structures in different pathological disorders. Cryo-electron microscopy has recently provided a series of structures for the core of the filaments purified from brain tissue from patients with different tauopathies and revealed that they share a common core region, while differing in their specific conformation. This structurally resolvable part of the core is contained within a proteolytically stable core region from the repeat domain initially isolated from AD tau filaments. Tau has recently become an important target for therapy. Recent work has suggested that the prevention of tau self-assembly may be effective in slowing the progression of Alzheimer's disease and other tauopathies. Here we review the work that explores the importance of tau filament structures and tau self-assembly mechanisms, as well as examining model systems that permit the exploration of the mode of action of potential inhibitors.

Keywords: tau, tauopathies, Alzheimer's disease, tau aggregation inhibitors, *in vitro* models, self-assembly, filaments

OPEN ACCESS

Edited by:
Sonia Do Carmo,
McGill University, Canada

Reviewed by:
Edeildo Silva-Júnior,
Federal University of Alagoas, Brazil
Antonio Giuliano Zippo,
Italian National Research Council, Italy

***Correspondence:**
Louise C. Serpell
l.c.serpell@sussex.ac.uk

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *02 August 2020*
Accepted: *04 September 2020*
Published: *12 November 2020*

Citation:
Oakley SS, Maina MB, Marshall KE, Al-Hilaly YK, Harrington CR, Wischik CM and Serpell LC (2020) Tau Filament Self-Assembly and Structure: Tau as a Therapeutic Target. Front. Neurol. 11:590754. doi: 10.3389/fneur.2020.590754

INTRODUCTION

Newly synthesized and unfolded proteins must undergo a carefully controlled folding process to produce their specific biologically active conformation, known as the native state. This is a specific 3D structure required for correct protein function and is encoded in the amino acid sequence (1). This folding process can be thought of as following an energy landscape whereby the unfolded polypeptide folds to form the native state, the most thermodynamically stable configuration for the polypeptide (2). The unfolded proteins at the top of the landscape are initially driven by hydrophobic collapse to form secondary structure and further intramolecular contacts, such as covalent bonds and hydrogen bonds, help the proteins reach their native state (3, 4). Alternatively, proteins can form misfolded intermediates, which can go on to generate a pathological conformation leading to the formation of amyloid fibrils. However, multiple diseases are associated with stable aggregates that have formed as a result of the assembly of misfolded proteins and dysfunctions in the protective mechanisms, such as the molecular chaperones (1, 2, 5).

Contrary to the native state, where hydrophobic residues are protected, misfolded proteins have exposed hydrophobic residues. This facilitates the formation of hydrophobic interactions between misfolded proteins and drives the self-assembly of proteins into protein amyloid (6). Amyloid fibrils are composed of assembled peptides formed of β-sheets with a typical cross-β structure, which is common for all amyloid, regardless of the amino acid sequence and native structure of the precursor protein (7). The conversion of soluble, monomeric protein into amyloid fibrils includes the production of partially folded intermediates, which have become a focus for understanding pathogenesis of disease in recent years (8–11). These include dimers, trimers, tetramers, oligomers, and protofilaments (12–15). Mature amyloid fibrils are made of several individual protofilaments (16). The accumulation of amyloid fibrils, and their related intermediates, are strongly associated with cellular dysfunction and are common pathological hallmarks for neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease and Huntington's disease.

Each neurodegenerative disease has its own disease-specific protein that forms disease-specific amyloid fibrils. Nevertheless, there is a group of neurodegenerative diseases, known as tauopathies, that are characterized by the presence of amyloid fibrils consisting entirely of self-assembled tau protein. Filaments that accumulate in the various tauopathies differ in the specific fibril structure as a result of tau aggregation, but this highlights an opportunity where an understanding of tau self-assembly in one tauopathy, may benefit our research in investigating another tauopathy.

TAU PROTEIN IN DISEASE

Physiological Expression and Function

Human tau protein is encoded by the *MAPT* gene located on chromosome 17 and is expressed in the central nervous system as a family of six isoforms. These isoforms are the product of alternative mRNA splicing of transcripts from *MAPT*, resulting in proteins of varying amino acid lengths from 352 to 441. They differ by the presence of zero, one or two N-terminal inserts encoded by exons 2 and 3 (0N, 1N, 2N), and the inclusion or absence of the second 31 amino acid microtubule-binding repeat (R2) in the C-terminal half (encoded by exon 10). Inclusion of exon 10 results in the production of three tau isoforms each having four repeats (4R) and the absence of exon 10 produces three isoforms having only three repeats (3R) (**Figure 1**) (23, 24).

Tau is a family of microtubule-associated proteins responsible for promoting and stabilizing the microtubule network (25, 26). These functions are facilitated by the microtubule-binding domain in the C-terminal domain, with 4R isoforms shown to be better at promoting microtubule assembly and binding to microtubules than the 3R isoforms (27). Microtubules are essential for a range of cellular processes for the maintenance of neuronal morphology, such as intracellular transport of organelles, cell division and signal transduction (28). It is important to note that the different isoforms may be differentially localized depending on the developmental stages, tissues, cell lines, brain regions and intracellular compartments (29–34). For instance, in the murine brain, 1N tau isoforms are overexpressed in the pituitary gland, compared to the cortex or hippocampus and under-expressed in the olfactory bulb. The 2N isoforms are enriched in the cerebellum compared to any other brain region, but are under-expressed in the olfactory bulb; the 0N isoforms are the predominantly expressed isoforms in the olfactory bulb and cortex (34). Intracellularly, in the murine brain, 1N isoforms predominate in the nuclear fraction and 0N isoforms predominate in the cell body/axons (34). In human immortalized cells, like SH-SY5Y neuroblastoma cells, tau may localize to the nucleus or cytoplasm depending on whether the cells are differentiated or not (35). Moreover, it has been shown that the 3R isoform of tau is preferentially localized to the nucleolus (29).

Tau has been observed to bind nucleic acids, suggesting the involvement of tau in chromatin remodeling. This interaction with DNA indicates that tau proteins may play a role in protection against reactive oxygen species (36, 37), ribosome stability and miRNA activity (38). It has also been shown that tau plays a role in nucleolar transcription and participates in the nucleolar stress response (39, 40). These roles seem to be important in the pathogenesis of tauopathies. For example, tau pathology has been shown to impair nucleocytoplasmic transport in AD and frontotemporal dementia (41, 42), induce nuclear envelope invagination in AD (43, 44), induce neurodegeneration through global chromatin relaxation (45), and transposable element dysregulation in AD (46). These studies emphasize the importance of a nuclear role for tau in tauopathies.

Tauopathies

Tauopathies are clinically, biochemically and morphologically heterogenous neurodegenerative diseases characterized by the deposition of abnormal aggregates of tau in the brain (47). In each tauopathy, tau self-assembles via the repeat domains to form a disease-specific conformation, which associates to form disease-specific amyloid fibrils. It is widely recognized that the accumulated tau pathology seen in tauopathies is directly associated with cognitive decline and clinical dementia in patients suffering with these diseases (48–56). The importance of tau in neurodegeneration was emphasized when it was discovered that dominantly inherited mutations in *MAPT* cause frontotemporal dementia with parkinsonism-linked to chromosome 17 (FTDP-17) (57–59). Unlike AD, FTDP-17 is not characterized by the presence of amyloid β (Aβ) peptide plaques

Abbreviations: AD, Alzheimer's disease; 4R, four-repeats; 3R, three-repeats; FTDP-17, frontotemporal dementia with parkinsonism-linked to chromosome 17; Aβ, amyloid beta; FTD, frontotemporal dementia; PiD, Pick's disease; CTE, chronic traumatic encephalopathy; CBD, corticobasal degeneration; PART, primary age-related tauopathy; PSP, progressive supranuclear palsy; AGD, argyrophilic grain disease; cryo-EM, cryo-electron microscopy; NFT, neurofibrillary tangle; PHF, paired helical filament; SF, straight filament; mAb, monoclonal antibody; bvFTD, behavioral-variant frontotemporal dementia; NPF, narrow Pick filament; WPF, wide Pick filament; PTM, post-translational modification; MTBR, microtubule-binding region; TAI, tau aggregation inhibitor; MT, methylthioninium; LMT, leuco-methylthioninium; MTC, methylthioninium chloride; LMTM, leuco-methylthioninium bis(hydromethanesulfonate).

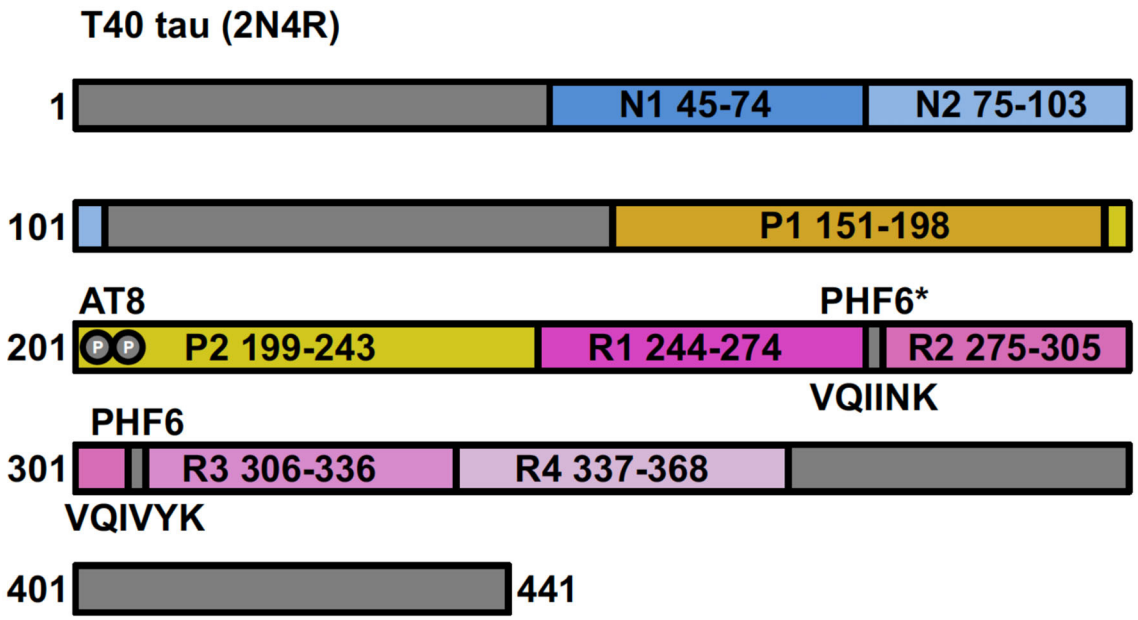


FIGURE 1 | Full-length tau (isoform 2N4R) containing two N-terminal inserts (N1 and N2), two proline-rich regions (P1 and P2) and four microtubule-binding repeats (R1–R4). Antibody AT8 recognizes an epitope dependent on phosphorylation at Ser201 and Thr205. The 6 residue PHF (306–311) and PHF6* (17–22) fragments are from repeat regions R3 and R2, respectively.

but patients exhibit cognitive decline. Pathologically aggregated tau is therefore sufficient, in the absence of Aβ to result in neurodegeneration and dementia in the absence of Aβ (60).

Tauopathies include AD, frontotemporal dementia (FTD: which includes Pick's disease (PiD) as a sub-type), chronic traumatic encephalopathy (CTE), corticobasal degeneration (CBD), primary age-related tauopathy (PART), progressive supranuclear palsy (PSP), and argyrophilic grain disease (AGD). **Table 1** outlines key features of the tau aggregates associated with different tauopathies. They can be classified into primary or secondary depending on whether tau aggregates represent the sole molecular lesion, or if the disease is associated with other pathological features, such as Aβ plaques in AD (73). Tauopathies can be further distinguished by the tau isoforms present in their filaments, with AD and CTE comprising all six isoforms (3R and 4R), CBD, PSP, and AGD only comprising only 4R isoforms, and PiD only containing 3R isoforms (67).

Characteristics of Tau Aggregates

For each tauopathy, revealing the unique molecular signatures for the disease-specific fibrils is a vital step toward understanding key assembly mechanisms and interactions. Recent cryo-electron microscopy (cryo-EM) studies have revealed the atomic structure of the tau fibrils associated with AD, PiD, CTE, and CBD. In this section we explore the similarities and differences between the molecular structures of these aggregates (**Figure 2**).

Alzheimer's Disease (AD)

AD is the leading cause of dementia and is characterized by the presence of extracellular amyloid plaques, composed of Aβ peptide, and intracellular neurofibrillary tangles (NFTs), composed of tau (74–76). The accumulation of these pathological hallmarks causes progressive neurodegeneration in the neocortex, hippocampus and cingulate gyrus, leading to cognitive decline (77).

NFTs are composed of two types of fibrils: paired helical filaments (PHFs) and straight filaments (SFs) (49, 78–80). In AD, PHFs predominate (90%), whereas filaments in PiD are predominately SFs (95%). Initial proteolytic cleavage and antibody labeling studies established that PHFs isolated from AD brain tissue comprise of a core that is resistant to a broad-spectrum exo-protease (Pronase), which is covered with a Pronase-sensitive "fuzzy coat" (74, 81). Pronase treatment removes the "fuzzy coat" leaving the intact PHF-core, which reacts with monoclonal antibody 423 (mAb 423) that recognizes tau truncated at E391 (82). These experiments confirmed that tau is the major structural component of the PHF core (83–85). Negative-stain electron microscopy further revealed that the core comprises of a double helical stack of C-shaped subunits, with the N- and C-terminal regions projecting away from the core in a disordered manner to form the "fuzzy coat" (86).

In a recent cryo-EM study, Fitzpatrick and colleagues solved the structure of the shared common structural core of PHFs and SFs, consisting of residues V306—F378, indicating the inclusion of R3 and R4 repeats plus an additional 10 amino acids at the C-terminus (62). This allows for the incorporation of all six tau isoforms. Labeling with an R2-specific epitope antibody (anti-4R, raised against V275-C291 of R2) was used to suggest the absence of R2 from the core, although R2 sequences were identified in core PHF characterized biochemically (84).

TABLE 1 | List of tauopathies and details of their associated tau pathology.

Tauopathy	Primary or secondary tauopathy	Tau pathology	Filaments	Filament core structure (Figure 2)	Tau isoforms in filaments
Alzheimer's Disease (AD) (62, 63)	Secondary (with amyloid-beta plaques)	Neurofibrillary tangles (NFTs)	Paired helical filaments (PHFs): longitudinal spacing between crossovers of 65–85 nm and a width of 7 nm at the narrowest parts and 15 nm at the widest section Straight filaments (SFs): 10 nm wide with crossovers distances ranging from 70–90 nm	Alzheimer fold: Identified by Fitzpatrick and colleagues, consisting of residues 306-378 of 3R and 4R tau (all R3 and R4 repeats, and 10 amino acids after R4). Eightβ-strands adopting a C-shaped architecture	3R/4R
Pick's Disease (PiD)/Frontal temporal dementia (FTD) (64)	Primary	Pick bodies	Narrow Pick filaments (NPFs): helical twist with a crossover distance of ∼100 nm and a width of 5–15 nm Wide Pick filaments (WPFs): helical twist with a crossover distance of ∼100 nm and a width of 5–30 nm	Pick fold: Identified by Falcon and colleagues, consisting of residues 254–378 of 3R tau (C-terminal part of R1 repeat, all R3 and 4R repeats, and 10 amino acids after 4R). Nine β-strands pack together in a hairpin-like fashion	3R
Chronic traumatic encephalopathy (CTE) (65)	Primary	NFTs and glial tangles (GTs)	CTE type I filaments: Predominant helical filaments widths of 20–25 nm and crossover spacing of 65–80 nm. CTE type II filaments: pronounced helical twists with widths of 15–30 nm. Paired helical filaments (PHFs)	CTE fold: Identified by Falcon and colleagues, consisting of residues 274–379 for 3R tau, 305–379 for 4R tau. Eight β-strands adopting a C-shaped architecture in a more open configuration than the Alzheimer fold, consisting of a hydrophobic cavity	3R/4R
Corticobasal degeneration (CBD) (66)	Primary	NFTs, coiled bodies, argyrophilic threads and astrocytic plaques	Type I/narrow filaments: Consist of a single protofilament with a helical twist with a crossover distance ∼100 nm, width of 8 nm (min) and 13 nm (max) Type II/wide filaments: Consist of a pair of identical protofilament of type I, with a helical twist with crossover distance of ∼140 nm and a width of 8 nm (min) and 26 nm (max)	CBD Fold: Identified by Zhang and colleagues, consisting of residues 274–380 of 4R tau (last residue of R1 repeat, all R2, R3, and R4 repeats, and 12 amino acids after R4 repeat). Eleven β-strands form a four-layered structure containing a hydrophilic cavity	4R
Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (67)	Primary	Massive neuronal and glial tau deposits	Tau protofilament is an 11–12 nm diameter tubule	Not yet resolved	4R, 3R, or 3R/4R
Progressive supranuclear palsy (PSP) (68)	Primary	Tau-positive NFTs, neuropil threads and tau deposits in astrocytes (tufted astrocytes and oligodendroglial coiled bodies)	13–14 nm diameter straight tubules, two pair along longitudinal axes, show periodic narrowing at 220–400 nm and diameters of 22–24 nm (max) and 11–12 nm (min)	Not yet resolved	4R
Primary age-related tauopathy (PART) (69)	Primary	NFTs	Tau fibrils display a typical PHF morphology	Not yet resolved	3R/4R
Argyrophilic grain disease (AGD) (70–72)	Primary	Argyrophilic grains, oligodendrocytic coiled bodies and pre-neurofibrillary tangles	9–18 nm diameter straight filaments or 25 nm diameter tubules	Not yet resolved	4R

The core structure is an in-register parallel β-sheet formed by a β-bend and a β-helix motif linked by β-strands, which forms the previously identified C-shape (termed Alzheimer's fold) (**Figure 2**). In addition, these findings have confirmed the presence of the hexapeptide, ^{306}VQIVYK^{311} (PHF6) in the core of AD filaments, located in the N-terminal part of the cross-β structure and that packs through a heterotypic, non-staggered interface with the opposing residues ^{373}THKLTF^{378} (**Figure 2**). PHF6 has been considered essential for tau self-assembly (87, 88). PHFs and SFs are formed from two identical protofilaments

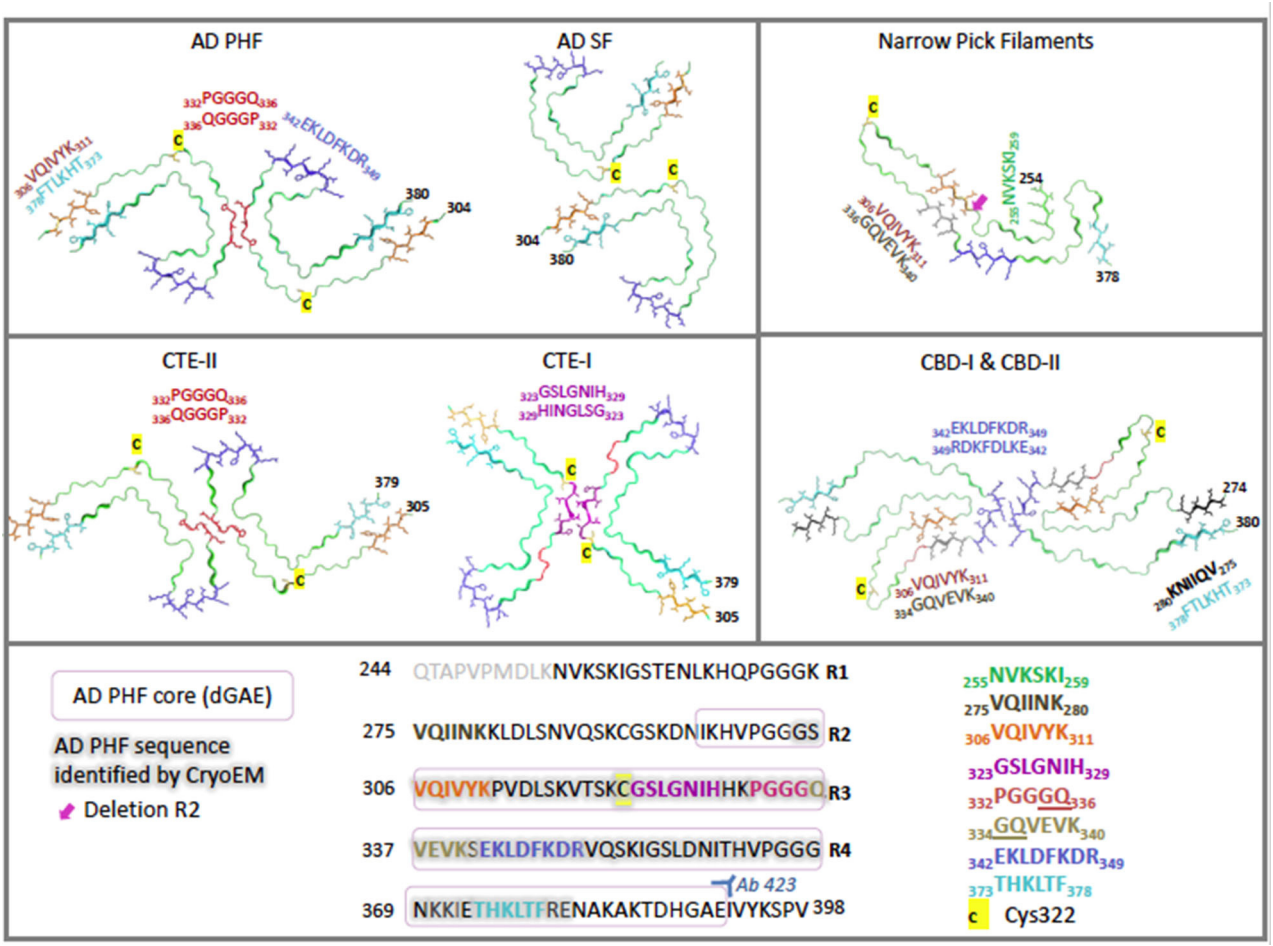


FIGURE 2 | Cryo-electron microscopy structures of *ex-vivo* filaments from AD (6hre, 6hrf), PiD (6gx5), CTE (6nwq, 6nwp), and CBD (6tjx) depicting a single layer slice through the filament with a view down the filament axis using cartoon ribbons in Pymol (61). Key regions are highlighted in color as stick representations. Structures have been arranged to highlight comparisons of key region positions. The primary sequence is shown below, highlighted in the same colors for each region. Cys322 is highlighted in yellow as a stick representation. Pink box signifies AD PHF-core dGAE sequence 297–391. Gray outline signifies region resolved by CryoEM for AD PHF. Blue 342–349 is found at the β-helical bend in AD PHF and SF as well as CTE-I and -II, but is found at the dimer interface for CBD-I and -II and at a kink in PiD. Red 332–336 is found at the dimer interface for AD PHF and for CTE-II interface, while purple 323–329 is found at the dimer interface for CTE-I. Orange 306–311 (PHF6) interdigitates with cyan 373–378 across the sheets between the N- and C- terminal ends of the core in AD PHF, AD SF and CTE-I and II. However, in PiD and CBD-I and II, 306–311 interdigitates with 336–340. In the recently solved CBD structures, black 275–280 (PHF6*) interdigitates with cyan 373–378. Green is found at the N-terminus of PiD. Pink arrow points to the absence of R2 in PiD narrow filament structure. AD: (62, 63) PiD (64); CTE: (65); CBD: (66).

that differ in the way they are packed, displaying ultrastructural polymorphism (62); PHF protofilaments are paired base-to-base, whereas SF protofilaments are paired back-to-base (**Figure 2**).

Pick's Disease (PiD)

PiD, an uncommon subtype of FTD, is a neurodegenerative disorder characterized by the presence of tau-positive filaments, contained in lesions originally described as Pick's bodies found throughout the limbic and neocortical regions. PiD presents clinically with a combination of aphasia and behavioral abnormalities. The most prevalent FTD syndrome is behavioral-variant FTD (bvFTD) and is characterized initially by personality changes and impairment in judgement in the absence of memory impairment. The broader class of frontotemporal lobar degeneration disorders includes primary progressive aphasia, corticobasal syndrome, and a Parkinsonian syndrome (89).

Cryo-EM distinguished narrow and wide Pick filaments (NPFs and WPFs, respectively), with NPFs being the primary filament observed (64). NFPs are composed of a single protofilament with a Pick tau filament fold (termed the Pick fold) (**Figure 2**). Whereas, the WPFs are seen to be two NPF protofilaments interacting at their distal tips. As in AD, these filaments are composed of a Pronase-resistant core surrounded by a "fuzzy coat" of disordered N- and C-terminal projections, which are removed after Pronase treatment. The Pick fold encompasses the core region, which consists of residues K254—F378 of 3R tau, including the C-terminal part of R1, all of R3 and R4, and 10 amino acids after R4. This confirmed the

selective incorporation of 3R tau only in Pick body filaments. The core structure consists of 9 β-strands arranged into four cross-β packing stacks and connected by turns and arcs, which creates an elongated structure that is distinct from the C-shaped sub-structure of protofilaments seen in AD (**Figure 2**) (64).

Chronic Traumatic Encephalopathy (CTE)

CTE is a neurodegenerative tauopathy associated with repeated head trauma, identified in former participants of contact sports (boxing, American football, and soccer), ex-military personnel, and victims of severe physical abuse (90–93). CTE is characterized by an abundance of hyperphosphorylated tau protein within NFTs and glial tangles, with tau aggregates forming in cortical layers II and III (94–97).

Falcon and colleagues found two novel filament structures in CTE brain tissue (termed CTE type I and CTE type II filaments), which are ultrastructural polymorphs that share protofilaments, but with a different protofilament interface (**Figure 2**). In addition to these, they found a small fraction of filaments that were identical to AD PHFs (65). With the use of antibody labeling, it has been shown that all six tau isoforms in a hyperphosphorylated state are incorporated into the protofilaments similar to AD (76). The core of the protofilaments consists of residues K274-R379 of R3 tau and S305-R379 of R4 tau to form a C-shaped conformation, termed the CTE fold, which resembles the Alzheimer's fold (**Figure 2**). However, due to a different β-strand packing in the β-helix motif and a hydrophobic cavity, absent in AD filaments, CTE protofilaments have a more open C-shape conformation than seen in AD (see **Figure 2**). The hydrophobic cavity contains an additional density that is not connected to the density of the tau molecules, suggesting it is not covalently linked and does not appear to be a post-translational modification (PTM) (65). The authors suggest candidate molecules might be non-polar sterols and sterol derivatives, or fatty acids. The hydrophobic cavity containing the additional density has highlighted the structural differences seen in tauopathy filaments, as well as identifying a potentially important step in the pathogenesis of CTE. With the presence of tau inclusions around blood vessels in CTE, the authors suggest the additional density represents cofactors that may be involved in the initiation of tau aggregation in CTE, which may enter the brain after head trauma or other mechanisms (65).

Corticobasal Degeneration (CBD)

CBD is a neurodegenerative tauopathy characterized by neuronal and glial deposits of hyperphosphorylated tau throughout the somatosensory, premotor, supplementary motor cortices, basal ganglia and brainstem (98). Patients present with motor and cognitive decline, including corticobasal syndrome, Richardson syndrome, and Alzheimer's-like dementia (99).

Zhang and colleagues have revealed two unique filaments that comprise the neuronal and glial deposits of CBD, termed type I (narrow) and type II (wide) CBD filaments (66). Type I CBD filaments are composed of a single protofilament with a helical twist that adopts a previously unknown four-layered fold, while type II CBD filaments consist of a pair of identical protofilaments of type I, exhibiting helical twists and related by mirror symmetry. Type I and II filaments contain a common protofilament, consisting of a core structure composed of residues K274-E380, which covers the last residue of R1, all of R2-R4, and 12 amino acids after R4. The core consists of 11 β-strands that are connected by turns and arcs to form a previously unknown four-layered structure, termed the CBD fold (**Figure 2**). Like the CTE fold, the CBD cryo-EM map contains additional density that the authors suggest may be composed of non-proteinaceous, polyanionic molecules. Unlike CTE, the additional density in CBD is contained in a hydrophilic cavity. The authors have suggested that these molecules may enter the brain from the periphery and aid the formation of the CBD fold and filaments, as in the hypothesis of Falcon and colleagues for CTE filament assembly (65, 66).

Identifying the different conformations of tau folds in these different diseases has been a pivotal step in our understanding of tau aggregates and the important interactions involved in tau self-assembly. These structures have demonstrated the ability of tau to form distinct molecular conformations that could account for the structural diversity of tau aggregates within the different tauopathies. The structural differences seen in tau filaments from AD and CTE indicate that tau isoform composition is not the sole determinant of conformation, and that the distinct tau structures observed are the result of precise disease-specific processes (62, 65, 100).

AD is the most prominent tauopathy, thought to account for around 60–70% cases of dementia worldwide and is expected to affect 100 million patients by 2050 (101). The remainder of this review will focus on tau assembly observed in AD and the use of *in vitro* models of tau aggregation. However, it is important to consider the potential significance and relevance of tau self-assembly findings in AD research for other tauopathies.

Tau Self-Assembly in AD

Full-length recombinant tau shows very little intrinsic tendency to aggregate *in vitro*, due to its hydrophilic nature and absence of PTMs that may promote aggregation (102, 103). Our understanding of tau aggregation has come mostly from *in vitro* studies using tau models, in which exogenous additives are required to initiate tau aggregation, or fragments of tau that can spontaneously aggregate. Additives include polyanionic factors, such as heparin or RNA (104–106), and arachidonic acid (107). Early studies demonstrated that pathological tau-tau binding through the repeat domain instigates the formation of the proteolytically stable structure of the PHF core (108, 109). This process exhibits a "prion-like" characteristic, whereby aggregation of tau seems to be auto-catalytic and self-propagating (110). Tau protein undergoes repeated binding and proteolytic digestion cycles, leading to the accumulation of a PHF-core tau fragment at the expense of native tau. The accumulating fragment lacks the N-terminus and terminates at the C-terminus at E391 (108) (**Figure 3**). Key regions that drive filament formation are thought to be the hexapeptides ^{306}VQIVYK^{311} (PHF6) and ^{275}VQIINK^{280} (PHF6*), (highlighted in **Figure 1**) (87, 88). PHF6 has been suggested as essential for tau self-assembly (87, 111) and cryo-EM has revealed its presence in the core region, where it packs through a heterotypic, non-staggered interface with

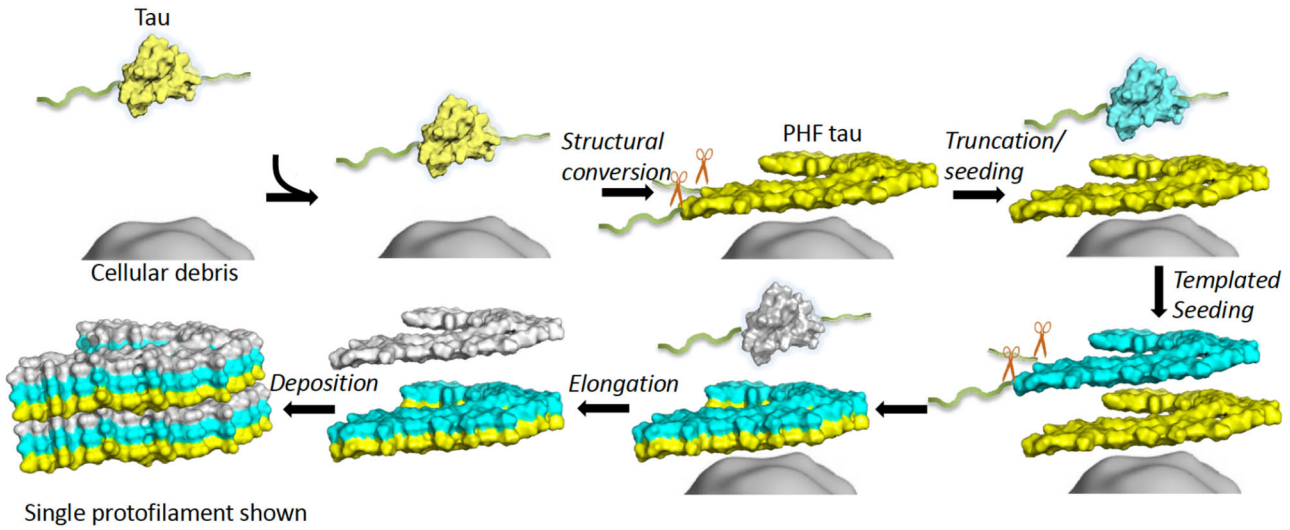


FIGURE 3 | *In vitro* tau aggregation by autocatalytic propagation. Native tau misfolding (yellow) is triggered by unknown cellular components (gray surface) initiating a structural conversion. This misfolded tau (yellow) acts as a seed that binds full-length, native tau (cyan) and converts the repeat domain into a proteolytically stable conformational replicate (cyan). This process repeats (white), leading to formation of elongated fibrils (6 layers shown). Excess regions of tau outside the core (fuzzy coat) are removed prior to or during conversion or after assembly (exact details unknown). The structure of a single protofilament is shown for simplicity, although dimerization is an important step for AD PHF formation. Single chain layers are shown in yellow, cyan and white. The morphological detail is created using a single layer and chain of 6hre.pdb as a template and Pymol was used to prepare a surface representation (61).

^{373}THKLTF^{378} (62) (**Figure 2**). However, it has been suggested that PHF6* is a more powerful driver of 4R tau aggregation and a superior target for inhibiting assembly of 4R tau (112).

It is not known what initiates the self-assembly of tau protein *in vivo*. Lipofuscin deposits have been suggested as a substrate that might capture tau and induce a stable conformational change in the protein. Lipofuscin is composed of undigested products of mitochondrial turnover as a result of a dysfunctional endosomal-lysosomal pathway (109, 113, 114). Alternatively, tau on its own, either as oligomers or short fibrils, can initiate or seed the recruitment of monomers in tau self-assembly (115, 116). This process is discussed later in this review.

The events or factors that initiate the process of autocatalytic aggregation of tau protein remain unclear. No differences in the primary amino acid sequence have been identified in the tau fragment extracted from PHF preparations, which indicates that any pathological processing of tau associated with PHF assembly must be induced by conformational or post-translational changes in the protein (75, 117, 118).

Tau exists as a natively unfolded protein and is subject to many PTMs, such as phosphorylation and truncation, which affect its biological function. Nonetheless, aberrant PTMs may be associated with preventing tau function by inhibiting tau-tubulin binding, while promoting pathological tau self-assembly (119–122). Understanding these PTMs is extremely important when studying the key initiation mechanism of tau self-assembly and are therefore important when considering reliable tau models.

Phosphorylation

Tau is a phosphoprotein and exists in a dynamic equilibrium between a microtubule-bound and -free state, controlled by the extent of its phosphorylation. Tau contains up to 85 potential phosphorylation sites, with the main kinases responsible for tau phosphorylation identified as GSK3β, CDK2, CDK5, CaMKII, and PKA. The major phosphatases, that dephosphorylate tau include PP2A, PP2B, PP2C, with PP2A considered the main phosphatase (123–125).

PHFs from AD brains were shown to contain all six isoforms in a hyperphosphorylated state (84) and PHF-tau contains 4–5-fold more phosphorylated residues than tau from healthy control brains, taken by some to indicate an association between hyperphosphorylation of tau with tau assembly in AD (126, 127). In conjunction with this, higher levels of extracellular hyperphosphorylated tau are measured in patients with AD (128). Hyperphosphorylation of tau may drive some disease-relevant features such as a lower binding affinity for microtubules; reduced biological activity; mis-localization within the cell; increased ability to aggregate into PHFs; and to contribute to neuronal death (49, 129–138). It has been argued that tau phosphorylation could be the trigger of its self-assembly, with an increase in tau phosphorylation observed prior to the formation of tau aggregates (139–143). On the other hand, biochemical studies of tau accumulation in the course of disease do not suggest the existence of a soluble pool of free phosphorylated tau prior to aggregation (144), and indeed the appearance of soluble forms of hyperphosphorylated tau becomes measurable in the cortex only at advanced stages of pathology (56). Other studies have suggested that it is not the overall phosphorylation state of tau that may trigger aggregation, but the phosphorylation of specific sites. Combined phosphorylation of S202/T205/S208 sites in the AT8 epitope (shown in **Figure 1**), with the lack of phosphorylation at S262,

contributes to filament formation (145). In contrast, Zhou and colleagues observed a dramatic increase in the phosphorylation sites, including S262, in association with the formation of high molecular weight oligomers of tau in AD brains (146). In addition, Tepper and colleagues suggested that phosphorylation of tau in cells promotes oligomer formation but not filaments (147). Alternatively, phosphorylation may be secondary to formation of oligomers and consequent mis-sorting of tau (110).

The involvement of tau phosphorylation in tau assembly is supported by the observation that there is a 50% reduction in the prominent phosphatase, PP2A, observed in AD brains (124). Dephosphorylation of PHFs isolated from AD brains results in their dissociation and disaggregation (148), as well as restoring normal tau function to promote assembly of microtubules and abolishing its pathological self-assembly (131, 149). These studies support an association between hyperphosphorylated tau and its self-assembly into PHFs. However, other groups have shown conflicting results. Initial studies of the PHF core revealed that, after Pronase treatment, PHFs were not recognized by phosphorylation-dependent antibodies (74, 81). Other studies have shown that only a minor proportion of PHF-tau is either full-length or hyperphosphorylated (<5%) (150) and tau species isolated from the proteolytically stable PHF-core are not phosphorylated (151). This suggests that the sites of tau phosphorylated in the PHFs are located only in the "fuzzy coat" and are not structural components of the PHF-core. This has been further supported by Arkhamia and colleagues, who showed that phosphorylation occurs largely in the "fuzzy coat" region of CBD and AD filaments using mass spectrometry (152). Furthermore, the structural core revealed by cryo-EM comprises regions downstream of key phosphorylation sites such as the AT8 epitope (153–155) (**Figure 2**). Additional studies have questioned the role of phosphorylated tau in the mechanism of tau self-assembly. Studies have implied that hyperphosphorylation of tau inhibits tau-tau binding (109, 156), inhibits its aggregation (157), and is not sufficient for filament propagation in cellular models (158, 159).

Truncation

Tau is sensitive to proteolytic digestion that is facilitated by a group of proteases. Tau is a substrate for a variety of proteases, such as caspase (160), calpain (161), cathepsin (162), and thrombin (163). As previously stated, initial studies identified truncated tau as the structural component of the Pronase-resistant PHF core (74, 81). Truncation of tau protein can act as a driver of tau self-assembly in the process of autocatalytic propagation by removal of occluding N- and C-terminal domains of the molecule (**Figure 3**) (108, 110). As a natively unfolded protein, tau has little tendency to aggregate in physiological conditions (103). However, there is evidence that tau can change its conformation into a "paperclip" loop, by folding its N- and C-terminal regions back onto the microtubule-binding repeats, thought to inhibit or protect against pathological aggregation (164). Tau fragments truncated at both N- and C-termini that contain the microtubule-binding repeats are more prone to form aggregates, probably because the protective "paperclip" loop has been disrupted (165). It has been suggested that truncated tau can facilitate the assembly of full-length tau for fibril formation and is the driving force behind NFT formation (47, 160, 166, 167). The self-assembly of truncated tau observed with the *in vitro* model dGAE form fibrils that structurally resemble PHFs in AD without inducers (168, 169); dGAE is one of the PHF-core tau species isolated from AD brain tissue (74, 84).

PHF-core tau isolated from Pronase-treated PHF preparations from AD brain comprises a number of different fragments encompassing residues 297–391 and 264–359 from the repeats R2–R4 and repeats (R1–R3) of the 4-repeat isoform, and residues 266–391 of the 3-repeat isoform (74, 82, 83). The 296-390 fragment binds full-length 4-repeat tau with high affinity (156) and generates the species terminating at E391 *in vitro* (108). Tau C-terminally truncated at E391 is found in early amorphous deposits in AD brain tissues and the epitope becomes occluded as filaments form (170). Tau truncated at E391 was identified in PHFs isolated from AD brains after-Pronase treatment using mAb 423, which recognizes the C-terminal E391 in PHFs, but not normal adult tau proteins (85, 171). The E391 truncation and truncations at homologous positions in other isoforms, therefore represent the proteolytically stable footprint of the high affinity core-tau aggregation domain (82).

Tau truncated at D421 is produced by caspase activity, which is elevated in AD brains (172, 173). It has been shown that Aβ (1–16, 23–48) peptide exposure can trigger caspase cleavage of tau to produce the N-terminal tau 1–421 fragment, which assembles more readily into filaments than full-length tau. This study suggests a possible link between Aβ, induction of tau PTMs and the initiation of tau self-assembly into filaments (160). In addition, studies have indicated that caspase-mediated truncation of tau at D421 may precede hyperphosphorylation and aggregate formation in AD, PiD and PSP, which may be an important step in understanding tau self-assembly for tauopathies (174–177). Studies have also demonstrated that tau truncated at D421 may become an effector of apoptosis, further linking pathological tau to neurodegeneration (178, 179).

On the other hand, an *in vivo* study using the P301S transgenic mouse model demonstrated that hyperphosphorylation precedes the truncation of tau at D421 and that truncation occurs after filament formation (180). This suggests that this particular truncation is not necessary for filament formation. Further studies indicate that phosphorylation and N-terminal truncation, but not C-terminal truncation, are more significant in the formation of oligomeric tau species (146). This further demonstrates the complexity of tau self-assembly and highlights the gaps in our understanding of the initial steps in the formation of aggregates. Identifying an *in vitro* model that can accurately replicate the pathological processing of tau in AD brains will greatly assist in studies on tau self-assembly, as well as other important aspects of tau pathology, such as its propagation throughout the brain.

Trans-cellular Propagation of Tau and Seeding

If tau pathology were restricted to affected neurons, then the damage induced by tau would be self-limiting. As discussed

earlier, propagation of tau is thought to be through a prion-like mechanism, which utilizes an initial seed to recruit normal monomeric tau and is able to facilitate autocatalytic amplification (110). The initial seed could be undigested cellular components, such as lipofuscin deposits (109), or it could be tau in an oligomeric state. Recent studies have supported the proposal that tau itself can undergo active cell-to-cell (trans-cellular) transfer and initiate the propagation of tau aggregates in previously unaffected neurons, whereby it seeds further tau aggregation (181, 182). Understanding the mechanism whereby pathological tau can spread throughout the brain is a major focus for dementia research and offers the potential of being an important therapeutic target for attenuating the progression of tauopathies.

Initial studies observed the formation of intracellular tau aggregates after tau was applied extracellularly, demonstrating the ability of tau to seed assembly in adjacent cells *in vitro* (183) and *in vivo* (184). It was further shown that intracellular tau aggregates are released and taken-up by co-cultured cells, whereby the internalized aggregates induced assembly of intracellular tau and promoted fibrillization, demonstrating prion-like propagation of tau (115, 181, 182). Additional studies have shown that the trans-cellular propagation of tau is facilitated by synaptic connections between neurons (trans-synaptic propagation) that seems to be stimulated by depolarization during neuronal activity, which can accelerate the spread of tauopathy (185–191). The spread of tau through trans-synaptic propagation via specifically connected neurons may explain the stereotypical staging of tau pathology seen in AD (192). However, the mechanisms that facilitate tau intercellular propagation through secretion and uptake are not fully understood. Several studies indicate that tau secretion is enabled via vesicles known as exosomes (193–196) and ectosomes (188). Microglial cells may also promote tau propagation through exosome-dependent mechanisms (197). In contrast to these findings, a study indicated the secretion of tau is an exosome-independent process, requiring heat shock cognate 70, chaperone DnaJ and synaptosomal associated protein 23 (198). The secreted pathological tau is required to undergo cellular uptake to gain entry into neighboring cells, which has been linked to multiple cellular uptake mechanisms. These include receptor-mediated endocytosis (199), dynamin-driven endocytosis (187), and actin-dependent proteoglycan-mediated micropinocytosis (200).

In addition to the mechanisms that govern intercellular propagation, it is becoming increasingly important to consider the species of tau that is responsible for initiating seeding. Proteolytically stable oligomers have been the mostly widely documented species responsible for intercellular self-propagating tau and have the greatest seeding activity for initiating the self-assembly cascade of tau in unaffected brain regions (115, 183, 184, 187, 201). Recent work has shown that the dGAE fragment in soluble form can be internalized into neuronal cells and can recruit endogenous tau (202). There is also some evidence for fibril-induced tau propagation (203, 204). Nonetheless, oligomeric tau is still thought to be the key species that gives tau pathology its "prion-like" characteristic.

Recognizing the self-propagating nature of tau and the seeding abilities of different tau species in tauopathies has opened up new possibilities for therapeutic targets and interventions for the treatment of tauopathies. The anti-tau antibody infusions and small molecules to disrupt the propagating mechanism of tauopathies are attractive therapeutic strategies. These therapeutic targets will be discussed later in the review.

Although NFTs are considered histopathological hallmarks of AD, there is increasing evidence that the cytotoxicity associated with tauopathies may not be as a result of these larger aggregates. In addition to trans-cellular propagation, oligomers are associated with the molecular dysfunctions that underlie neurodegeneration and the cognitive decline seen in patients. *In vivo* tau-overexpression studies have demonstrated neuronal loss, behavioral abnormalities, and synaptic dysfunction occurring in the absence of NFTs (205–212). These studies indicate a pathological role for pre-tangle tau species in the dysfunction of neurons. This has been further supported by evidence that injection of tau oligomers into wild-type mice induces memory impairment, synaptic loss, and mitochondrial dysfunction, that are not seen with monomer or fibril injections (213). In addition, following a decrease in oligomeric tau levels, improved cognitive function suggests the potential of anti-tau antibody infusions as a therapeutic approach (214). In response to these findings, it has been proposed that mature tau fibrils could be a protective response to absorb the more toxic oligomeric species, eventually inducing their clearance via protease activity or autophagy (215, 216). However, the oligomers formed through the repeat domain are proteolytically stable and hence resist proteolytic and autophagic activity, and are therefore able to propagate between neurons and induce cytotoxicity. Tau-induced neurodegeneration may be due to an increase in oligomers rather than due to the accumulation of PHF in NFTs. This concept has been proposed for other neurodegenerative diseases, but still remains to be validated with respect to tau and AD (217).

Investigating the molecular mechanisms underlying protein self-assembly has been an important focus for further understanding of neurodegeneration and identifying therapeutic targets. Generating a reliable *in vitro* model that can accurately replicate the protein self-assembly and associated cytotoxicity that occurs in tauopathies will further our understanding of the underlying principles of aggregation and the potential for therapeutic targets. However, it presents a huge challenge to create an *in vitro* model that will effectively replicate pathological processing of tau that occurs *in vivo*, whilst also modeling other aspects of interest, such as the production of representative oligomeric species.

Models of Tau Aggregation

Brain-derived materials are inherently variable, and variations are difficult to control. Hence, there is a need for an *in vitro* system using synthetic tau that can recapitulate the pathological self-assembly of tau observed in the brains of tauopathy patients. Due to its hydrophilic nature, recombinant tau shows very little tendency to aggregate, making it a difficult protein for study of its aggregation and cytotoxicity mechanisms (102). Thus, multiple tau models have been developed that exhibit a higher tendency to aggregate and enable effective *in vitro* research. Here we discuss some of these models, summarized in **Figure 4**.

Full-Length Tau (T40, 2N4R)

The largest tau isoform, also known as full-length tau or T40 (441 residues), incorporates both N-terminal inserts and four microtubule-binding repeats (2N4R tau: **Figure 4**). The full-length tau *in vitro* model will be referred to as T40 for this section of the review. This isoform has been used extensively to study the role of tau in disease. Tagging T40 with diverse fluorescent proteins (GFP, YFP, and CFP) has been a useful tool to visualize tau aggregation *in vitro* and further our knowledge of tau assembly (153, 218). Frost and colleagues used fluorescently tagged endogenously expressed T40 in their studies to demonstrate tau propagation and seeding, as summarized earlier. They showed that tau-YFP does not readily aggregate within the cell but, when they introduced tau as a pre-assembled aggregate into the cell, it induced fibrilization of the tau-YFP (183). Groups have been able to develop cell-based models that can monitor and quantify tau assembly by utilizing fluorescence resonance energy transfer and biomolecular fluorescence complementation technology using T40 (154). Using these techniques, groups were able to achieve spatial and temporal resolution of tau aggregation in living cells following hyperphosphorylation (219), and demonstrating the role of GSK3β and caspase cleavage in intermolecular association of tau (220, 221).

Many investigators have used T40, either as a monomer or having induced filament assembly by the addition of cofactors, such as heparin (104, 105, 155), RNA (106), or fatty acids (107). T40 filaments resemble those found in AD brains. These cofactors enhance tau aggregation by inducing a conformational change from mostly random coil to β-sheet structure in the microtubule-binding domain containing the hexapeptide motifs thought to be necessary for assembly (87, 88, 222, 223). Heparin-induced filaments have become an important tool used to study pathological tau aggregation, such as investigating the effects of *MAPT* mutations on tau assembly (224, 225) and investigating potential aggregation inhibitors (226–228). They have provided a platform to characterize the progressive fibrilization of tau, through monomeric aggregation into intermediate species and ultimately into filaments (229). In addition, heparin-induced aggregates of T40 were used to support the role of PHF6* hexapeptide (^{275}VQIINK^{280}) as a potent driver of aggregation and seeding for full-length tau, suggesting that it is a superior target for inhibition when compared to the PHF6 hexapeptide (112). Crespo and colleagues have used heparin-induced filaments to produce an assay, which they claim to be highly reproducible and closely mimicking *in vivo* tau misfolding and aggregation (230). They suggest that the assay will enable mechanistic studies on tau pathogenesis and can be utilized for evaluating candidate drugs during screening processes.

These studies illustrate the importance of full-length tau as a tool in understanding of both physiological and pathological tau. However, T40 may not be a reliable model for AD research. Evidence shows that heparin-induced tau has reduced seeding activity compared to tau from the brains of mice expressing human P301S tau (231). Additional studies have shown structural differences in tau filaments seeded using tau from AD brains and recombinant tau assembled with heparin (159). Recently, Fichou and colleagues reported that heparin-induced tau filaments do not reproduce the main structural features seen in filaments from AD brains, such as the C-shape Alzheimer's fold and the anti-parallel β-sheet structure between 303–347 and 349–378 (100). Moreover, cryo-EM revealed that heparin-induced filaments of 4R (full-length) tau are polymorphic, adopting at least four different conformations, with 3R tau adopting a conformation of its own (232). All of these conformations are structurally distinct, consisting of an ordered core structure, which is very different to those characterized using cryo-EM of filaments from AD, PiD, CTE, and CBD. The studies also revealed that these filaments lack essential features of AD PHFs, such as the interface between PHF6 and its opposing residues 373–378. These findings indicate that heparin-induced filaments of T40 tau are both structurally heterogeneous and distinct from those found in the disease brain, challenging their reliability for use in a tau self-assembly model or as an assay for screening potential inhibitors. As noted above, heparin-induced filaments from full-length tau were used as a basis for inferring

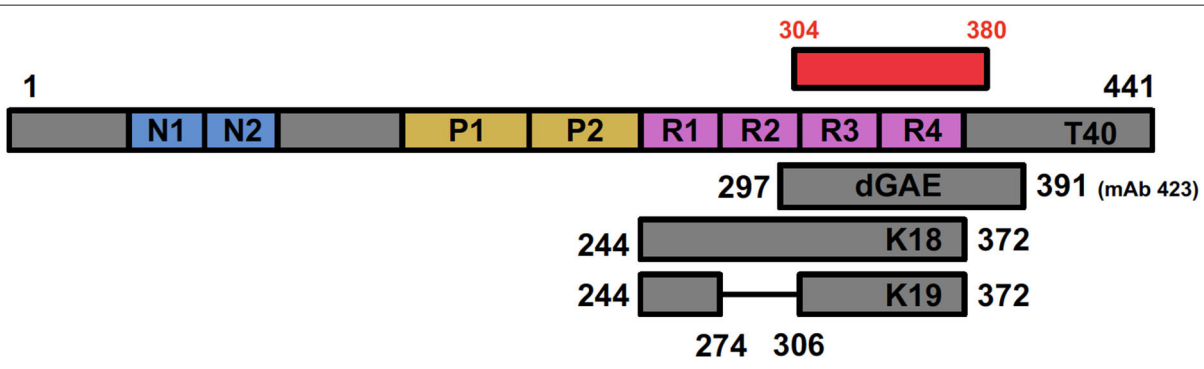


FIGURE 4 | Positioning of tau fragments used as models for studying tau biology showing full-length T40 in relation to the AD cryo-EM derived structure (shown in red). dGAE covers I297—E391, incorporating a C-terminal portion of R2, R3, and R4 (shown in pink). The C-terminal E391, recognized by mAb 423, is absent in dGA, a fragment ending in A390. K18 (Q244—E372) covers all four repeat domains; K19 covers the same region but lacks residues 275–305 (R2) encoded by exon 10 in 4R isoform.

the importance of PHF6* in assembly (112). Recent cryo-EM structures of the heparin-induced filaments indicate that the PHF6* region is located in the cryo-EM core of T40 filaments forming a contiguous β-strand, whereas it not found within the core of AD filaments. It is however visible within the core of the recent cryo-EM structure for CBD filaments at the far N-terminal end (residues 274–280), so it may yet be resolved in more detailed PHF structures (**Figure 2**). This illustrates a potential disadvantage of the heparin-induced T40 model for investigating tau self-assembly. This model produces filament structures that are distinct from those so far characterized from tauopathies and may have a different mechanism of self-assembly and highlighting factors that are not applicable for tauopathies.

Nevertheless, it is important to consider what we can learn from these heparin-induced filaments. Even though there are structural differences between such filaments and tauopathy filaments, they do exhibit similarities. They are composed of tau molecules that form cross-β structures through parallel stacking of β-strands along the helical axis. The β-strands contain short loop regions and the filament structures share a common pattern of β-strand formation (232). Studying the formation of these filaments may shed light on key assembly processes, or they could be more relevant for other tauopathy filaments that have not been solved yet, such as PSP. In addition, cofactor-induced aggregates spontaneously disassemble into monomers when the cofactor is removed (233). The authors of these studies have proposed that unknown cofactors may play a role in tau self-assembly in tauopathies and these could provide a potential therapeutic target.

Overall, T40 has been used extensively to model tau assembly. It has been extremely useful in uncovering the role of tau, both physiologically and in pathology. The use of heparin to induce aggregation of tau into filaments that may have some resemblance to disease-like filaments has made it an attractive model when investigating tau self-assembly. Given the recent evidence of structural dissimilarities from native filaments, T40 heparin-induced filaments should be used with caution in structure-based studies for drug design and antibody development, where structural accuracy is essential. This does not preclude the use of T40 in assembly assays, but rather reveals that the unique structures of heparin-induced T40 filaments differ from the pathologic filaments. The use of full-length tau in the generation of filaments should still be open to further investigation, using a range of biophysical techniques to determine the mechanism of initiation of aggregation. Future research may unveil a novel mechanism for induction of aggregation of T40 which may make it a more suitable candidate for the investigation of tau self-assembly.

Repeat Domain Models (K18/K19)

Since the microtubule-binding repeats domain were shown to compose the core region of PHFs and facilitate the pathological tau-tau binding (74), it has been common practice to use tau fragments that only comprise of the microtubule-binding domain. Some groups simply refer to them as the microtubule-binding region (MTBR) and specify whether they use the 3R or 4R isoforms. Others use two model peptides, termed K18 and K19. K18 represents all four-repeat domain and contains residues 244–372, while K19 is similar to K18 but lacks the second repeat (missing residues 275–305) absent from 3R tau isoforms (**Figure 4**) (234, 235). As stated previously, tau fragments including MTBR and truncated at both N- and C-termini are more prone to form aggregates than full-length tau (165) and aggregation can be further stimulated by including mutations or adding cofactors, such as heparin (102, 236). They produce filaments that resemble AD PHFs in terms of fiber morphology and β-sheet content (87, 88, 227), which provided the basis for using MTBR in tau assembly studies.

As mentioned previously, the Diamond group have used full-length tau in their study on the propagation and seeding of tau (183). The full-length tau did not aggregate spontaneously in cells, so they utilized a MTBR fragment similar to K18, corresponding to residues 243–375. They were able to prepare *in vitro* aggregates of the MTBR fragment with arachidonic acid, which readily entered cells and induced aggregation of intracellular T40-YFP. This demonstrated that T40 does not readily aggregate in cells, but internalized MTBR aggregates were able to induce fibrilization of the intracellular T40-YFP. These studies also used the expression of mutant forms of the MTBR models to further our understanding of tau propagation. Multiple K18 mutants, labeled with YFP or CFP, were used to demonstrate that the secretion of fibrils into the extracellular space leads to the internalization of these fibrils by other cells and the aggregation of the intracellular tau, supporting the concept of trans-cellular propagation of tau aggregates (181). The authors advocate the use of the K18 construct on the grounds that it reliably forms fibrils in cultured cells and aggregation potential can be modulated with the introduction of specific mutations. This includes the combined P301L and V337M mutant (termed LM), enabling efficient formation of intracellular aggregates and providing an effective model of tau propagation. In addition, the expression of the aggregation-competent MTBD fused to YFP was utilized to confirm the prion-like nature of tau (182). Similar to T40, filaments can be prepared using K18 and K19 with the addition of cofactors. The Mandelkow group have used the heparin-induced aggregation of K19 extensively as a model in investigating inhibitors of tau polymerization. They have utilized the K19 model to screen a library of 200,000 compounds in the search for potential inhibitors of tau aggregation, and therefore potential therapeutic agents. They were able to identify rhodamines, phenylthiazolyl-hydrazides, N-phenylamines, anthraquinones and benzothiazoles (237–239) and aminothienopyridazines as a novel class of tau assembly inhibitors (240, 241). They also utilized the K18 and K19 fragments to identify the hexapeptide motifs considered to be essential for tau aggregation, PHF6 and PHF6* (87, 88).

Although these studies have proved informative in tau self-assembly studies, it is now possible to compare filaments composed of K18 and K19, with genuine filaments isolated from AD brains. The cryo-EM of PHFs has shown that the N-terminal cross-β structure is formed by the PHF6 hexapeptide, which is essential for assembly of tau (87, 88) (**Figure 2**). PHF6 packs against its opposing residues 373–378, which are not present in the K18 and K19 fragment because they terminate at E372

(62, 234). Thus, filaments composed of K18 and K19 fragments cannot be said to reproduce the Alzheimer's fold resolved in PHFs and SFs derived from AD brains. In addition, since K18 and K19 terminate at E372, these fragments lack the C-terminus after the MTBR, that has been shown to contribute to the core region of filaments seen in AD, PiD, CTE, and CBD (in AD this extends to residues 378, in PiD to 378, in CTE to 379, and in CBD to 380), which casts further doubt on the use of such filaments as models for assembly assays. As stated previously, the use of heparin to induce K18 and K19 filaments must be used with caution, as it has been shown that heparin-induced filaments are highly heterogenous and differ from those seen in tauopathies (232).

dGAE Model

dGAE is a 95 amino acid truncated form of tau protein corresponding to residues 297–391 encompassing part of R2, and R3 and R4 from full-length 2N4R tau (**Figures 2**, **4**). The fragment corresponds to one of the species isolated from the proteolytically stable AD PHF core preparations (74, 82, 84). The terminology of dGAE refers to the three C-terminal residues, glycine (G), alanine (A), and terminating with glutamate (E), E391; the initial letter corresponds to the N-terminal residue which for "d" refers to I297 (82). Tau truncated at E391 was identified as being part of the Pronase-resistant core during initial proteolytic and antibody studies, which is recognized by mAb 423 (**Figure 2**). PHFs isolated from AD brain without Pronase are also recognized by mAb 423, but require a higher concentration of antibody (82). mAb 423 immunoreactivity can also be revealed in intracellular tangles following treatment with formic acid in dystrophic neurites and in granulovacuolar degeneration in AD brain sections (170, 242, 243) supporting the view that C-terminal truncation at E391 is not a Pronase artifact. Rather, digestion by endogenous proteases may be one of the events leading to PHF formation and depends on a particular configuration of tau found in partially assembled precursors, and also in fully assembled PHFs. The dGAE peptide and other core peptides were proposed as representing an integral part of the stable core region in PHFs (82, 108, 170, 244, 245) and has now been confirmed as encompassing slightly shorter PHF ordered core region (304–380) resolved using cryo-EM (62) (**Figure 2**).

dGAE was chosen for further study because it is also similar to repeats R1/R3/R4 from the 3-repeat isoform apart from 3 residues near the N-terminus (L/I, Q/V, K/S in the 3-repeat and 4-repeat isoforms, respectively) and is therefore likely to be representative of the behavior of both isoforms (**Figure 2**). Studies of the assembly properties of the dGAE peptide has revealed that it has the ability to self-assemble spontaneously in physiological conditions *in vitro*, without the need for added cofactors, such as heparin. Self-assembled dGAE filaments have been shown to share characteristics of amyloid fibrils, displaying β-rich structure and giving a cross-β X-ray fiber diffraction pattern (168), as well as closely resembling PHFs in AD brains (169). Transmission electron microscopy revealed no significant variations between dGAE filaments and no significant variations between dGAE filaments and AD filaments. Atomic force microscopy also demonstrated that dGAE forms filaments that share macromolecular characteristics with PHFs from AD brains. These results suggest that dGAE functions as a consistent and reliable model for understanding pathological tau self-assembly and for screening inhibitors and understanding their mode of action.

Disulphide crosslinking between dimers was proposed to be an essential part of tau assembly and propagation (102, 246–248). Disulphide cross-linkages are formed between cysteine residues, located on tau at C291 and C322 (numbering for 2N4R tau), in which C322 exists in all tau isoforms, whereas C291 is only found in 4R tau isoforms. The assembly of filaments from dGAE was found to be enhanced in conditions unfavorable for disulphide crosslinking formation and with a variant lacking the cysteine residue all together, dGAE-C322A (168). The authors concluded that assembly of dGAE is independent of disulphide crosslinking and suggested that the formation of disulphide bonds competes with crucial conformations required for filament formation. This is at odds with previous suggestions that disulphide-linked dimer formation is an important step in filament formation, but is consistent with the structure of *ex vivo* PHFs by cryo-EM (62). This shows that the C322 residue is buried between sheets and would be inaccessible for disulphide crosslinking (62, 168) (**Figure 2**). This evidence suggests that dGAE provides a more accurate representation of pathological tau in AD, in comparison to the models used in previous studies (1N3R, 1N4R, and K18), which require exogenous heparin for assembly. In addition, Al-Hilaly and colleagues identified two monomeric isoforms of dGAE, one with a gel mobility of 10 kDa and the second with a mobility of 12 kD. The same doublet was seen in the proteins extracted from the core region of PHFs isolated from AD brain tissue, which first lead to the identification of truncated tau as a structural component of the PHF cores (74, 168).

The dGAE fragment has also been utilized for the investigation of tau aggregation inhibitors (TAI). dGAE, and a fragment similar to dGAE but lacking the E391 residue (dGA), were used in the study which identified the methylthioninium (MT) moiety as the first TAI (108). Since then, other groups have attempted to understand the mechanisms of MT action by using heparin-induced filaments composed of the MTBR fragments, K18 and K19 (249, 250). These studies hypothesized that the TAI activity of MT requires cysteine oxidation, which was consistent with the previous proposals stating that tau assembly was dependent on disulphide crosslinking. However, a recent study using the dGAE fragment has shown that MT acts optimally as a TAI in its reduced form as leuco-MT (LMT) rather than the oxidized form at MT^{+} (251). The evidence that LMT is the active form of MT required to inhibit pathological aggregation of tau is at odds with the proposal that its action depends on the oxidation of cysteine residues (249, 250). The ability of LMT to act as a TAI is also consistent with an earlier study showing that dGAE assembly into PHFs is independent of disulphide crosslinking. In addition, circular dichroism has revealed that heparin interacts with MT. This confounds studies aiming to understand how LMT acts, and further supports the use of dGAE for such studies (251). LMT will be discussed in more detail later in the review.

We can only compare dGAE and AD filaments directly once a structure has been resolved for dGAE through the use of cryo-EM or solid-state nuclear magnetic resonance. As previously mentioned, the filaments seen in CTE share similar morphology with AD filaments, but cryo-EM revealed subtle ultrastructural differences, which could be the case for dGAE filaments and PHFs. However, the studies to date highlight the use of dGAE as a reliable *in vitro* model able to replicate tau self-assembly in a controllable manner. It may therefore prove to have broader use as a relevant tool for the investigation of assembly mechanisms and the potential therapeutic targets.

ALZHEIMER'S DISEASE THERAPIES TARGETING TAU SELF-ASSEMBLY

The clear involvement of tau self-assembly as a driving force behind the clinical progression of AD, as well as other tauopathies, has established it as an attractive target for potential therapies. Treatments for AD are extremely limited and offer only temporary symptomatic relief based on neurotransmitter enhancement, without providing disease-modifying activity. Extensive clinical studies have resulted in over 400 failed trials since the last AD treatment was approved in 2003 (47, 252). Targeting tau self-assembly to reduce disease progression has resulted in promising results and may provide much-needed disease-modifying treatment options for AD. Here we discuss some of the treatment options targeting tau self-assembly in AD, utilizing small molecule and immunotherapy approaches.

Tau Aggregation Inhibitors (TAI)

Low molecular weight compounds offer a promising approach for inhibiting the formation of oligomers and fibrils by blocking tau-tau interactions. The microtubule-binding domains have been shown to be responsible for the pathological tau-tau binding, but also facilitating the tau-tubulin binding required for physiological tau function (27, 108). Thus, TAIs should inhibit pathological tau-tau binding whilst not affecting the physiological tau-tubulin interaction, although both occur through the repeat domain. Potential side-effects are extremely important to consider for compounds destined for clinical trials. Wischik and colleagues demonstrated the first TAI to be MT, a diaminophenothiazine. Since then, other compounds have been shown to exhibit TAI activity, such as the potential TAIs identified by the Mandelkow research group, mentioned previously (237–239). None of these potential TAIs have yet reached clinical testing. Both the oxidized MT^{+} and reduced LMT forms of MT have shown promising results in Phase 2 and Phase 3 clinical trials, respectively.

Methylthioninium chloride (MTC, commonly known as methylene blue) was originally synthesized at the end of the nineteenth century for the treatment of malaria, and since then has had a long history of clinical applications. Importantly for AD research, Wischik and colleagues reported MTC as the first TAI, demonstrating its ability to reverse the proteolytic stability of PHFs isolated from AD brain and selectively inhibiting tau self-assembly, without affecting normal tau-tubulin interactions (108). Additional *in vitro* and *in vivo* studies have confirmed MTC as an effective TAI (245, 251, 253). As noted above, it is actually the LMT form which is the active species responsible for TAI activity in the dGAE model of pathological tau assembly. Other studies have suggested that MTC induces degradation of tau aggregates by promoting the proteasomal (254) and macroautophagic (255, 256) clearance systems. The MT moiety has also shown potential for ameliorating mitochondrial dysfunction and reducing oxidative stress, by acting as an alternative electron carrier for the electron transport chain in oxidative phosphorylation (257–264). *In vivo* studies have confirmed that both MTC and a stabilized form of LMT [LMTM, leuco-methylthioninium bis(hydromethanesulfonate)] are able to preserve cognitive function and reducing behavioral deficits associated with pathological tau aggregation in tau transgenic mouse models (253, 265, 266). In cell models, LMTM was shown to block the templated conversion of full-length tau into the truncated 12 kDa form analogous to dGAE (245). Likewise, the tau species used in the Melis and colleagues study to produce tau aggregation pathology in mice depends on the overexpression of a truncated form of tau analogous to dGAE (253).

The pharmacology of MTC is complex, existing as a redox molecule in equilibrium between its reduced LMT form and its oxidized MT species and dependent on the local environment (253). Because its redox potential is close to zero the MT moiety is able to act as an electron carrier between Complex II and Complex IV in the electron transport chain, permitting it to enhance mitochondrial function (257, 267). However, when administered as MTC, the MT moiety needs to be reduced to its uncharged LMT form to permit efficient absorption and distribution to the brain (265). As noted above, MTC is optimally effective as a TAI when converted to LMT in a reducing environment, implying that LMT is the active species required for inhibition of tau aggregation (251). Optimizing the clinical effectiveness of LMT has been a challenge in clinical trials. The MT moiety was first tested clinically in a pure, oxidized MTC form under the name Rember^{TM} (TauRx Theraputics) in Phase 2 clinical trial in mild-to-moderate AD. This showed significant efficacy compared with placebo in terms of reduction in cognitive decline and prevention of decline in brain metabolism as shown by HMPAO-SPECT scan which measures cerebral blood flow (268). However, MTC was found to suffer from dose-linked absorption limitations (268) arising from the need for the body to convert MT^{+} to the active LMT form required for efficient absorption and distribution to the brain (265). A stabilized form of the reduced LMT was developed which permits direct administration of LMT as the mesylate salt, LMTM. LMT has recently been assigned the International Nonproprietary Name "hydromethylthionine." LMTM retains TAI activity *in vitro* and *in vivo* (245, 253), whilst exhibiting superior pharmaceutic properties (265). Results from Phase 3 trials with LMTM have shown promising results with exposure-dependent reduction in cognitive decline and progression of brain atrophy as measured by MRI. LMTM was found to produce better outcomes when given as a monotherapy than as an add-on to symptomatic AD treatments (269, 270). However, a recent report has demonstrated

that there is similar exposure-dependent activity whether LMTM is administered as a monotherapy or as an add-on, but that the overall effect was reduced by about half as add-on (271). This impairment in the activity of LMTM by symptomatic AD drugs could be reproduced in a tau transgenic mouse model for AD, and was found to depend on the brain's homeostatic responses to the activating affects that symptomatic drugs depend on for their ability to boost cognitive function temporarily (267). The concentration-dependent effects showed that LMTM requires a substantially lower dose for pharmacological activity than found for MTC (271). Currently, TauRx is undertaking a new clinical trial (LUCIDITY) which aims to confirm the treatment effects of LMTM on cognitive decline and brain atrophy in mild-to-moderate AD.

Interestingly, LMTM was found to have an almost identical exposure-response profile in a large Phase 3 clinical trial of bvFTD as that seen for AD (272). Fewer than half of bvFTD patients have tau aggregation pathology, with the remainder exhibiting pathological aggregation of TAR DNA binding protein 43, TDP-43. LMTM was found to have activity in mouse model of FTD expressing full-length human tau carrying mutations at P301S and G335D (253). The fact that LMTM has similar pharmacological activity in both AD and bvFTD raises the possibility that the subtle folding differences in the tau filament core in the two diseases may not be important for pharmacological activity for this drug. There is as yet no *in vitro* model for Pick-like tau aggregation as distinct from AD-like tau aggregation to permit examination of this question. However, this example illustrates again the importance of developing reliable and reproducible models that can be used to study distinct mechanisms of aggregation and activity of TAIs.

Curcumin is another compound that has been reported to exhibit TAI properties. Curcumin is a natural polyphenol isolated from the Indian spice turmeric, which has been reported to inhibit assembly of tau (273–275) and Aβ (276). However, curcumin has poor bioavailability and had no effect on cognition or cerebrospinal fluid biomarkers in a Phase 2 trial (277, 278). Therefore, a derivative of curcumin, PE859, has been synthesized that exhibits higher inhibitory activity against tau and Aβ assembly *in vitro*, with high absorption and penetration of the blood brain barrier. Oral administration of PE859 delays onset and progression of motor dysfunction in JNPL3 human P301L tau transgenic mouse (279). Further investigation illustrated the effects of PE859 *in vivo,* demonstrating its dual aggregation inhibitory activity and ameliorating cognitive dysfunction in the senescence-accelerated mouse prone 8 (SAMP8), which show age-related deficits in memory and learning (280). Okuda and colleagues were also able to show that PE859 is protective with respect to Aβ-cytotoxicity *in vitro.* The potential for combined anti-aggregation activity for tau and Aβ makes PE895 an attractive candidate for clinical trials.

Immunotherapy

The potential use of immunotherapy to combat protein self-assembly was initially recognized with studies using antibodies targeting Aβ peptide, which resulted in attenuating Aβ pathology through preventing aggregation and removing existing aggregates (281, 282). The use of successful immunization, passive and active, against pathological tau was first reported using an antibody targeting phospho-tau peptide encompassing the pS396/404 epitope. The passive and active use of this antibody therapy reduced pathological tau levels in mouse models, while attenuating behavioral phenotypes associated with tauopathies (283–285). In addition, the potential of an antibody-based therapy for tau pathology was investigated by Kfoury and colleagues who used a monoclonal antibody against the tau repeat domain, HJ9.3 (epitope 306–321), to inhibit the trans-cellular propagation of tau aggregation (181). Research has continued to investigate the use of antibody therapies to inhibit tau assembly and propagation, which has led to the inclusion of active and passive immunizations in clinical trials.

Active

Active immunization induces the host immune system to stimulate an antibody response and produces an immunological memory with a long-lasting effect (47). However, eliciting an immune response to a native protein poses the risk of adverse immune reactions, which may affect normal tau and its physiological functions (278). Active immunotherapy has been shown to be effective in reducing pathological tau assembly and the production of pathological tau aggregates (17–19, 283, 286, 287). AADvac1 is an active vaccine in clinical trials consisting of a synthetic peptide derived from amino acids tau 294–305 coupled to keyhole limpet hemocyanin with an N-terminal cysteine (19). It originated from *in vitro* studies of monoclonal antibody DC8E8, which binds to a 6 amino acid sequence motif (HXPGGG) present four times within the MTBR, preventing tau oligomerization (20). Transgenic tauopathy rat studies showed that immunization induced tau antibodies that bind to tau in the brain, reduced tau pathology and ameliorated behavioral deficits (19). Phase 1 trials of AADvac1 showed that 29 of 30 patients developed an immunoglobulin G response to the tau peptide component of AADvac1 and against pathological tau 151–391/4R. Two patients withdrew due to adverse events, one from a viral infection that lead to a seizure, thought to be due to the vaccine, and the other an episode of microhemorrhage in a patient with a history of this condition (21). The immunogenicity of AADvac1 has prompted a larger phase 2 trial, which is underway to further assess its safety and effectiveness in alleviating clinical symptoms, and its effect on AD biomarkers in the blood and CNS (278).

Passive

In passive immunization, monoclonal antibodies or immune serum are administered directly to subjects. This tends to offer a more transient effect than active immunization, but can be useful in minimizing risks associated with active immunization (278). The use of passive immunization has been shown not to be limited to targeting extracellular tau. Studies have shown that anti-tau antibodies are able to penetrate the blood brain barrier and also to enter neurons, where they can target intracellular pathological tau and tau aggregates (17, 22, 283, 288–290). Having seen promising results with passive immunization, a number of vaccines targeting various tau epitopes are being

developed. BIIB092 (also known as BMS-986168) is a vaccine currently focused as a PSP treatment, rather than AD. It targets an N-terminal fragment present in extracellular tau (eTau) which was originally observed to be secreted from neurons derived from stem cells of familial AD patients (291). Bright and colleagues showed a correlation between eTau and Aβ *in vitro* and *in vivo*, whereby addition of eTau induced hyperactivity of neurons and increased Aβ production, whereas targeting eTau with an antibody recognizing residues 9–18 of eTau blocked these effects. Two phase 1 trials were completed in patients with PSP and healthy individuals and there is now a Phase 2 trial consisting of 400 patients with PSP. RG7345 is a humanized antibody that targets a specific tau phosphorylated epitope at S422 that has been found enter neurons and reduce tau pathology in transgenic mice (289). However, after a Phase 1 trial it was discontinued by its developer (Biogen), presumably due to an unfavorable pharmacokinetic profile, as it was showing a good safety profile (278).

DISCUSSION

The inability of recombinant full-length tau to self-assemble has limited its experimental use and has created a considerable challenge in tauopathy research. To overcome this problem, research groups have developed different *in vitro* tau models in the hope of replicating the pathological aggregation of tau observed in tauopathies. However, this has produced a number of inconsistent findings, such as the contribution of PTMs to assembly propensity and the importance of intrinsic tau features in the assembly process. This illustrates the problem of the lack of a general *in vitro* tau model tau self-assembly. The recent cryo-EM studies revealing the structures core of filaments associated with tauopathies have further highlighted the complexity of tau self-assembly and the challenge in developing a reliable *in vitro* tau model. Development of a generally agreed model that consistently produces a reliable representation of pathological tau aggregation will aid in identifying and understanding the key features and mechanisms involved in tau self-assembly. This is essential for identifying therapeutic targets for the development of effective disease-modifying treatments.

AUTHOR CONTRIBUTIONS

SO wrote the manuscript. YA-H, MBM, KEM, CRH, and CMW contributed to the manuscript text. KEM and LCS prepared the figures. LCS managed the work. All authors contributed to the article and approved the submitted version.

FUNDING

MBM was funded by Alzheimer's Society (AS-PG-16b-010). SO, KEM, and YA-H are funded by WisTa Laboratories Ltd., (PAR1596). LCS was supported by ARUK South Coast Network.

ACKNOWLEDGMENTS

The authors are grateful to the Alzheimer's Society and Alzheimer's Research UK for support.

REFERENCES

1. Dobson C. Protein folding and misfolding. *Nature.* (2003) 426:884–90. doi: 10.1038/nature02261
2. Kim Y, Hipp M, Bracher A, Hayer-Hartl M, Hartl F. Molecular chaperone functions in protein folding and proteostasis. *Annu Rev Biochem.* (2013) 82:323–55. doi: 10.1146/annurev-biochem-060208-092442
3. Gething M, Sambrook J. Protein folding in the cell. *Nature.* (1992) 355:33–45. doi: 10.1038/355033a0
4. Pace C, Fu H, Lee Fryar K, Landua J, Trevino S, Schell D, et al. Contribution of hydrogen bonds to protein stability. *Protein Sci.* (2014) 23:652–61. doi: 10.1002/pro.2449
5. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature.* (2011) 475:324–32. doi: 10.1038/nature10317
6. Hartl F. Protein misfolding diseases. *Annu Rev Biochem.* (2017) 86:21–6. doi: 10.1146/annurev-biochem-061516-044518
7. Serpell L, Benson M, Liepnieks J, Fraser P. Structural analyses of fibrinogen amyloid fibrils. *Amyloid.* (2007) 14:199–203. doi: 10.1080/13506120701461111
8. Jahn T, Parker M, Homans S, Radford S. Amyloid formation under physiological conditions proceeds via a native-like folding intermediate. *Nat Struc Mol Biol.* (2006) 13:195–201. doi: 10.1038/nsmb1058
9. Eakin C, Berman A, Miranker A. A native to amyloidogenic transition regulated by a backbone trigger. *Nat Struc Mol Biol.* (2006) 13:202–8. doi: 10.1038/nsmb1068
10. Haass C, Selkoe D. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol.* (2007) 8:101–12. doi: 10.1038/nrm2101
11. Gadad B, Britton G, Rao K. Targeting oligomers in neurodegenerative disorders: lessons from α-synuclein, tau, amyloid-β peptide. *J Alzheimer's Dis.* (2011) 24 Suppl 2:223–32. doi: 10.3233/JAD-2011-110182
12. Walsh D, Hartley D, Kusumoto Y, Fezoui Y, Condron M, Lomakin A, et al. Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem.* (1999) 274:25945–52. doi: 10.1074/jbc.274.36.25945
13. O'Malley TT, Witbold WM, Linse S, Walsh DM. The aggregation paths and products of Aβ42 dimers are distinct from Aβ42 monomer. *Biochemistry.* (2016) 55:6150–61. doi: 10.1021/acs.biochem.6b00453
14. Barz B, Liao Q, Strodel B. Pathways of amyloid-β aggregation depend on oligomer shape. *J Am Chem Soc.* (2018) 140:319–27. doi: 10.1021/jacs.7b10343
15. Zhang S, Fox D, Urbanc B. Insights into formation structure of aβ oligomers cross-linked via tyrosines. *J Phys Chem B.* (2017) 121:5523–35. doi: 10.1021/acs.jpcb.7b02495
16. Serpell L, Sunde M, Benson M, Tennent G, Pepys M, Fraser P. The protofilament substructure of amyloid fibrils. *J Mol Biol.* (2000) 300:1033–9. doi: 10.1006/jmbi.2000.3908
17. Troquier L, Caillierez R, Burnouf S, Fernandez-Gomez FJ, Grosjean ME, Zommer N, et al. Targeting phospho-Ser422 by active Tau Immunotherapy in the THYTau22 mouse model: a suitable therapeutic approach. *Curr Alzheimer Res.* (2012) 9:397–405. doi: 10.2174/156720512800492503
18. Ando K, Kabova A, Stygelbout V, Leroy K, Heraud C, Frédérick C, et al. Vaccination with sarkosyl insoluble phf-tau decrease neurofibrillary tangles formation in aged tau transgenic mouse model: a pilot study. *J Alzheimer's Dis.* (2014) 40(Suppl. 1):135–45. doi: 10.3233/JAD-132237

19. Kontsekova E, Zilka N, Kovacech B, Novak P, Novak M. First-in-man tau vaccine targeting structural determinants essential for pathological tau–tau interaction reduces tau oligomerisation and neurofibrillary degeneration in an Alzheimer's disease model. *Alzheimers Res Ther*. (2014) 6:44. doi: 10.1186/alzrt278
20. Kontsekova E, Zilka N, Kovacech B, Skrabana R, Novak M. Identification of structural determinants on tau protein essential for its pathological function: novel therapeutic target for tau immunotherapy in Alzheimer's disease. *Alzheimers Res Ther*. (2014) 6:45. doi: 10.1186/alzrt277
21. Novak P, Schmidt R, Kontsekova E, Zilka N, Kovacech B, Skrabana R, et al. Safety and Immunogenicity of the tau vaccine AADvac1 in patients with Alzheimer's disease: a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet Neurol.* (2017) 16:123–34. doi: 10.1016/S1474-4422(16)30331-3
22. Krishnamurthy PK, Deng Y, Sigurdsson EM. Mechanistic studies of antibody-mediated clearance of tau aggregates using an *ex vivo* brain slice model. *Front Psychiatry.* (2011) 2:59. doi: 10.3389/fpsyt.2011.00059
23. Crowther T, Goedert M, Wischik CM. The repeat region of microtubule-associated protein tau forms part of the core of the paired helical filament of Alzheimer's disease. *Ann Med.* (1989) 21:127–32. doi: 10.3109/07853898909149199
24. 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) 3:519–26. doi: 10.1016/0896-6273(89)90210-9
25. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci USA*. (1975) 72:1858–62. doi: 10.1073/pnas.72.5.1858
26. Cleveland D, Hwo S, Kirschner M. Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly. *J Mol Biol.* (1977) 116:227–47. doi: 10.1016/0022-2836(77)90214-5
27. Goedert M, Jakes R. Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain effects on tubulin polymerization. *EMBO J.* (1990) 9:4225–30. doi: 10.1002/j.1460-2075.1990.tb07870.x
28. Buée L, Bussière T, Buée-Scherrer V, Delacourte A, Hof P. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev*. (2000) 33:95–130. doi: 10.1016/S0165-0173(00)00019-9
29. Wang Y, Loomis P, Zinkowski R, Binder L. A novel tau transcript in cultured human neuroblastoma cells expressing nuclear tau. *J Cell Biol.* (1993) 121:257–67. doi: 10.1083/jcb.121.2.257
30. Nunez J, Fischer I. Microtubule-associated proteins (MAPS) in the peripheral nervous system during development and regeneration. *J Mol Neurosci.* (1997) 8:207–22. doi: 10.1007/BF02736834
31. Shea T, Cressman C. The order of exposure of tau to signal transduction kinases alters the generation of "AD-like" phosphoepitopes. *Cell Mol Neurobiol.* (1999) 19:223–33. doi: 10.1023/A:1006977127422
32. Cross D, Muñoz J, Hernández P, Maccioni, Nuclear R. and cytoplasmic tau proteins from human nonneuronal cells share common structural and functional features with brain tau. *J Cell Biochem.* (2000) 78:305–17. doi: 10.1002/(SICI)1097-4644(20000801)78:2<305::AID-JCB12>3.0.CO;2-W
33. Luo M, Tse S, Memmott J, Andreadis A. novel isoforms of tau that lack the microtubule-binding domain. *J Neurochem.* (2004) 90:340–51. doi: 10.1111/j.1471-4159.2004.02508.x
34. Liu C, Götz J. Profiling murine tau with 0N,1N, and 2N isoform-specific antibodies in brain and peripheral organs reveals distinct subcellular localization, with the 1N isoform being enriched in the nucleus. *PLoS ONE.* (2013) 8:0084849. doi: 10.1371/journal.pone.0084849
35. Uberti D, Rizzini C, Spano P, Memo M. Characterization of tau proteins in human neuroblastoma SH-SY5Y cell line. *Neurosci Lett.* (1997) 235:149–53. doi: 10.1016/S0304-3940(97)00715-5
36. Wei Y, Qu MH, Wang XS, Chen L, Wang DL, Liu Y, et al. Binding to the minor groove of the double-strand, tau protein prevents DNA from damage by peroxidation. *PLoS ONE.* (2008) 3:e2600. doi: 10.1371/journal.pone.0002600
37. Sultan A, Nesslany F, Violet M, Bégard S, Loyens A, Talahari S, et al. Nuclear tau, a key player in neuronal DNA protection. *J Biol Chem.* (2011) 286:4566–75. doi: 10.1074/jbc.M110.199976
38. Bou Samra E, Buhagiar-Labarchède G, Machon C, Guitton J, Onclercq-Delic R, Green M, et al. A role for tau protein in maintaining ribosomal DNA stability and cytidine deaminase-deficient cell survival. *Nat Commun.* (2017) 8:693. doi: 10.1038/s41467-017-00633-1
39. Maina M, Bailey L, Doherty A, Serpell L. The involvement of Aβ42 and tau in nucleolar and protein synthesis machinery dysfunction. *Front Cell Neurosci*. (2018) 12:220. doi: 10.3389/fncel.2018.00220
40. Maina M, Bailey L, Wagih S, Biasetti L, Pollack S, Quinn J, et al. The involvement of tau in nucleolar transcription and the stress response. *Acta Neuropathol Commun*. (2018) 6:70. doi: 10.1186/s40478-018-0565-6
41. Eftekharzadeh B, Daigle J, Kapinos L, Coyne A, Schiantarelli J, Carlomagno Y, et al. Tau protein disrupts nucleocytoplasmic transport in Alzheimer's disease. *Neuron.* (2018) 99:925–40. doi: 10.1016/j.neuron.2018.07.039
42. Paonessa F, Evans L, Solanki R, Larrieu D, Wray S, Hardy J, et al. Microtubules deform the nuclear membrane and disrupt nucleocytoplasmic transport in tau-mediated frontotemporal dementia. *Cell Rep.* (2019) 26:582–93. doi: 10.1016/j.celrep.2018.12.085
43. Frost B, Bardai F, Feany M. Lamin dysfunction mediates neurodegeneration in tauopathies. *Curr Biol.* (2016) 26:129–36. doi: 10.1016/j.cub.2015.11.039
44. Cornelison G, Levy S, Jenson T, Frost B. Tau-induced nuclear envelope invagination causes a toxic accumulation of mRNA in Drosophila. *Aging Cell.* (2019) 18:e12847. doi: 10.1111/acel.12847
45. Frost B, Hemberg M, Lewis J, Feany M. Tau promotes neurodegeneration through global chromatin relaxation. *Nat Neurosci.* (2014) 17:357–66. doi: 10.1038/nn.3639
46. Sun W, Samimi H, Gamez M, Zare H, Frost B. Pathogenic tau-induced piRNA depletion promotes neuronal death through transposable element dysregulation in neurodegenerative tauopathies. *Nat Neurosci.* (2018) 21:1038–48. doi: 10.1038/s41593-018-0194-1
47. Jadhav S, Avila J, Schöll M, Kovacs G, Kövari E, Skrabana R, et al. A walk through tau therapeutic strategies. *Acta Neuropathol Commun.* (2019) 7:22. doi: 10.1186/s40478-019-0664-z
48. Wilcock G, Esiri M. Plaques, tangles and dementia. A quantitative study. *J Neurol Sci.* (1982) 56:343–56. doi: 10.1016/0022-510X(82)90155-1
49. Grundke-Iqbal I, Iqbal K, Tung Y, Quinlan M, Wisniewski H, Binder L. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA*. (1986) 83:4913–7. doi: 10.1073/pnas.83.13.4913
50. Arriagada P, Growdon J, Hedley-Whyte E, Hyman B. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology.* (1992) 42:631–9. doi: 10.1212/WNL.42.3.631
51. Iqbal K, Alonso A, d.C., Chen S, Chohan M, El-Akkad E, et al. Tau pathology in Alzheimer disease and other tauopathies. *Biochim Biophys Acta.* (2005) 1739:198–210. doi: 10.1016/j.bbadis.2004.09.008
52. Maruyama M, Shimada H, Suhara T, Shinotoh H, Ji B, Maeda J, et al. Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. *Neuron.* (2013) 79:1094–108. doi: 10.1016/j.neuron.2013.07.037
53. Gomperts S, Locascio J, Makaretz S, Schultz A, Caso C, Vasdev N, et al. Tau positron emission tomographic imaging in the Lewy body diseases. *JAMA Neurol.* (2016) 73:1334–41. doi: 10.1001/jamaneurol.2016.3338
54. Schwarz A, Yu P, Miller B, Shcherbinin S, Dickson J, Navitsky M, et al. Regional profiles of the candidate tau PET ligand ^{18}F-AV-1451 recapitulate key features of braak histopathological stages. *Brain*. (2016) 139:1539–50. doi: 10.1093/brain/aww023
55. Smith R, Puschmann A, Schöll M, Ohlsson T, van Swieten J, Honer M, et al. ^{18}F-AV-1451 Tau PET imaging correlates strongly with tau neuropathology in MAPT mutation carriers. *Brain*. (2016) 139:2372–9. doi: 10.1093/brain/aww163

56. Mukaetova-Ladinska EB, Garcia-Siera F, Hurt J, Gertz HJ, Xuereb JH, Hills R, et al. Staging of cytoskeletal β-amyloid changes in human isocortex reveals biphasic synaptic protein response during progression of Alzheimer's disease. *Am J Pathol.* (2000) 157:623–36. doi: 10.1016/S0002-9440(10)64573-7
57. Hutton M, Lendon C, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature.* (1998) 393:702–5. doi: 10.1038/31508
58. Poorkaj P, Bird T, Wijsman E, Nemens E, Garruto R, Anderson L, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol.* (1998) 43:815–25. doi: 10.1002/ana.410430617
59. Spillantini M, Crowther R, Jakes R, Hasegawa M, Goedert M. Alpha-synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease dementia with Lewy bodies. *Proc Natl Acad Sci USA.* (1998) 95:6469–73. doi: 10.1073/pnas.95.11.6469
60. Goedert M, Falcon B, Zhang W, Ghetti B, Scheres S. Distinct conformers of assembled tau in Alzheimer's and Pick's diseases. *Cold Spring Harb Symp Quant Biol.* (2018) 83:163–71. doi: 10.1101/sqb.2018.83.037580
61. DeLano WL. *The PyMOL Molecular Graphics System.* San Carlos,CA: Delano Scientific (2002). Available online at: https://www.scirp.org/(S(vtj3fa45qm1ean45vvffcz55))/reference/ReferencesPapers.aspx?ReferenceID=1958992
62. Fitzpatrick AW, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of Tau filaments from Alzheimer's disease brain. *Nature.* (2017) 547:185–90. doi: 10.1038/nature23002
63. Falcon B, Zhang W, Schweighauser M, Murzin A, Vidal R, Garringer H, et al. Tau filaments from multiple cases of sporadic and inherited Alzheimer's disease adopt a common fold. *Acta Neuropathol.* (2018) 136:699–708. doi: 10.1007/s00401-018-1914-z
64. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature.* (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y
65. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature.* (2019) 568:420–3. doi: 10.1038/s41586-019-1026-5
66. Zhang W, Tarutani A, Newell K, Murzin A, Matsubara T, Falcon B, et al. Novel tau filament fold in corticobasal degeneration. *Nature.* (2020) 580:283–7. doi: 10.1038/s41586-020-2043-0
67. Höglinger G, Respondek G, Kovacs G. New classification of tauopathies. *Rev Neurol.* (2018) 174:664–8. doi: 10.1016/j.neurol.2018.07.001
68. Armstrong MJ. Progressive supranuclear palsy: an update. *Curr Neurol Neurosci Rep.* (2018) 18:1–9. doi: 10.1007/s11910-018-0819-5
69. Crary JF, Trojanowski JQ, Schneider JA, Abisambra JF, Abner EL, Alafuzoff I, et al. Primary age-related tauopathy (PART): a common pathology associated with human aging. *Acta Neuropathol.* (2014) 128:755–66. doi: 10.1007/s00401-014-1349-0
70. Arima K. Ultrastructural characteristics of tau filaments in tauopathies: immuno-electron microscopic demonstration of tau filaments in tauopathies. *Neuropathology.* (2006) 26:475–83. doi: 10.1111/j.1440-1789.2006.00669.x
71. Grinberg LT, Heinsen H. Argyrophilic grain disease: an update about a frequent cause of dementia. *Dement Neuropsychol.* (2009) 3:2–7. doi: 10.1590/S1980-57642009DN30100002
72. Rodriguez RD, Grinberg LT. Argyrophilic grain disease: an underestimated tauopathy. *Dement Neuropsychol.* (2015) 9:2–8. doi: 10.1590/S1980-57642015DN91000002
73. Lauretti E, Praticò D. Alzheimer's disease: phenotypic approaches using disease models and the targeting of tau protein. *Expert Opin Ther Targets.* (2020) 24:319–30. doi: 10.1080/14728222.2020.1737012
74. Wischik CM, Novak M, Thøgersen HC, Edwards PC, Runswick MJ, Jakes R, et al. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci USA.* (1988) 85:4506–10. doi: 10.1073/pnas.85.12.4506
75. Goedert M, Wischik C, Crowther R, Walker J, Klug A. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proc Natl Acad Sci USA.* (1988) 85:4051–5. doi: 10.1073/pnas.85.11.4051
76. Goedert M, Spillantini M, Cairns N, Crowther R. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron.* (1992) 8:159–68. doi: 10.1016/0896-6273(92)90117-V
77. Goedert M, Eisenberg D, Crowther R. Propagation of tau aggregates and neurodegeneration. *Annu Rev Neurosci.* (2017) 40:189–210. doi: 10.1146/annurev-neuro-072116-031153
78. Kidd M. Paired helical filaments in electron microscopy of Alzheimer's disease. *Nature.* (1963) 197:192–3. doi: 10.1038/197192b0
79. Kosik K, Joachim C, Selkoe D. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proc Natl Acad Sci USA.* (1986) 83:4044–8. doi: 10.1073/pnas.83.11.4044
80. Kosik K, Orecchio L, Binder L, Trojanowski J, Lee V, Lee G. Epitopes that span the tau molecule are shared with paired helical filaments. *Neuron.* (1988) 1:817–25. doi: 10.1016/0896-6273(88)90129-8
81. Wischik CM, Novak M, Edwards PC, Klug A, Tichelaar W, Crowther RA. Structural characterization of the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci USA.* (1988) 85:4884–8. doi: 10.1073/pnas.85.13.4884
82. Novak M, Kabat J, Wischik CM. Molecular characterization of the minimal protease resistant tau unit of the Alzheimer's disease paired helical filament. *EMBO J.* (1993) 12:365–70. doi: 10.1002/j.1460-2075.1993.tb05665.x
83. Novak M, Wischik C, Edwards P, Pannell R, Milstein C. Characterisation of the first monoclonal antibody against the pronase resistant core of the Alzheimer PHF. *Prog Clin Biol Res.* (1989) 317:755–61.
84. Jakes R, Novak M, Davison M, Wischik CM. Identification of -3 and -4 repeat tau isoforms within the PHF in Alzheimer's disease. *EMBO J.* (1991) 10:2725–9. doi: 10.1002/j.1460-2075.1991.tb07820.x
85. Novak M, Jakes R, Edwards PC, Milstein C, Wischik CM. Difference between the tau protein of Alzheimer paired helical filament core normal tau revealed by epitope analysis of monoclonal antibodies 423 and 7.51. *Proc Natl Acad Sci USA.* (1991) 88:5837–41. doi: 10.1073/pnas.88.13.5837
86. Crowther RA. Straight and paired helical filaments in Alzheimer disease have a common structural unit. *Proc Natl Acad Sci USA.* (1991) 88:2288–92. doi: 10.1073/pnas.88.6.2288
87. von Bergen M, Friedhoff P, Biernat J, Heberle J, Mandelkow EM, Mandelkow E. Assembly of τ protein into Alzheimer paired helical filaments depends on a local sequence motif (^{306}VQIVYK^{311}) forming β structure. *Proc Natl Acad Sci USA.* (2000) 97:5129–34. doi: 10.1073/pnas.97.10.5129
88. von Bergen M, Barghorn S, Li L, Marx A, Biernat J, Mandelkow E, et al. Mutations of tau protein in frontotemporal dementia promote aggregation of paired helical filaments by enhancing local beta-structure. *J Biol Chem.* (2001) 276:48165–74. doi: 10.1074/jbc.M105196200
89. Irwin DJ. Tauopathies as clinicopathological entities. *Parkinsonism Relat Disord.* (2016) 22:S29–33. doi: 10.1016/j.parkreldis.2015.09.020
90. Hof P, Knabe R, Bovier P, Bouras C. Neuropathological observations in a case of autism presenting with self-injury behavior. *Acta Neuropathol.* (1991) 82:321–6. doi: 10.1007/BF00308819
91. Omalu B, DeKosky S, Minster R, Kamboh M, Hamilton R, Wecht C. Chronic traumatic encephalopathy in a national football league player. *Neurosurgery.* (2005) 57:128–34. doi: 10.1227/01.NEU.0000163407.92769.ED
92. Omalu B, Hammers J, Bailes J, Hamilton R, Kamboh M, Webster G, et al. Chronic traumatic encephalopathy in an Iraqi war veteran with posttraumatic stress disorder who committed suicide. *Neurosurg Focus.* (2011) 31:E3. doi: 10.3171/2011.9.FOCUS11178
93. McKee AC, Daneshvar DH, Alvarez VE, Stein TD. The neuropathology of sport. *Acta Neuropathol.* (2014) 127:29–51. doi: 10.1007/s00401-013-1230-6
94. Hof P, Bouras C, Buée L, Delacourte A, Perl D, Morrison J. Differential distribution of neurofibrillary tangles in the cerebral cortex of dementia pugilistica and Alzheimer's disease cases. *Acta Neuropathol.* (1992) 85:23–30. doi: 10.1007/BF00304630
95. Geddes J, Vowles G, Nicoll J, Révész T. Neuronal cytoskeletal changes are an early consequence of repetitive head injury. *Acta Neuropathol.* (1999) 98:171–8. doi: 10.1007/s004010051066
96. Baugh CM, Robbins CA, Stern RA, McKee AC. Current understanding of chronic traumatic encephalopathy. *Curr Treat Options Neurol.* (2014) 16:306. doi: 10.1007/s11940-014-0306-5
97. McKee AC, Cairns NJ, Dickson DW, Folkerth RD, Dirk Keene C, Litvan I, et al. The first NINDS/NIBIB consensus meeting to define neuropathological

criteria for the diagnosis of chronic traumatic encephalopathy. *Acta Neuropathol.* (2016) 131:75–86. doi: 10.1007/s00401-015-1515-z
98. Ling H, Kovacs G, Vonsattel J, Davey K, Mok K, Hardy J, et al. Astrogliopathy predominates the earliest stage of corticobasal degeneration pathology. *Brain*. (2016) 139:3237–52. doi: 10.1093/brain/aww256
99. Ali F, Josephs K. Corticobasal degeneration: key emerging issues. *J Neurol.* (2018) 265:439–45. doi: 10.1007/s00415-017-8644-3
100. Fichou Y, Vigers M, Goring A, Eschmann N, Han S. Heparin-induced tau filaments are structurally heterogeneous and differ from Alzheimer's disease filaments. Heparin-induced tau filaments are structurally heterogeneous and differ from Alzheimer's disease filaments. *Chem Commun*. (2018) 54:4573–6. doi: 10.1039/C8CC01355A
101. González J, Alcántara A, Doadrio A, Sánchez-Montero J. Developments with multi-target drugs for Alzheimer's disease: an overview of the current discovery approaches. *Expert Opin Drug Discov.* (2019) 14:879–91. doi: 10.1080/17460441.2019.1623201
102. Barghorn S, Mandelkow E. Toward a unified scheme for the aggregation of tau into Alzheimer paired helical filaments. *Biochemistry*. (2002) 41:14885–96. doi: 10.1021/bi026469j
103. Mukrasch M, Bibow S, Korukottu J, Jeganathan S, Biernat J, Griesinger C, et al. Structural polymorphism of 441-residue tau at single residue resolution. *PLoS Biol.* (2009) 7:e1000034. doi: 10.1371/journal.pbio.1000034
104. Goedert M, Jakes R, Spillantini M, Hasegawa M, Smith M, Crowther R. Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. *Nature.* (1996) 383:550–3. doi: 10.1038/383550a0
105. Pérez M, Valpuesta J, Medina M, Montejo de Garcini E, Avila J. Polymerization of tau into filaments in the presence of heparin: the minimal sequence required for tau-tau interaction. *J Neurochem.* (1996) 67:1183–90. doi: 10.1046/j.1471-4159.1996.67031183.x
106. Kampers T, Friedhoff P, Biernat J, Mandelkow E, Mandelkow E-M, RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett.* (1996) 399:344–9. doi: 10.1016/S0014-5793(96)01386-5
107. Wilson DM, Binder LI. Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. *In vitro* evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol.* (1997) 150:2181–95.
108. Wischik CM, Edwards PC, Lai RY, Roth M, Harrington CR. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc Natl Acad Sci USA.* (1996) 93:11213–8. doi: 10.1073/pnas.93.20.11213
109. Wischik CM, Lai RYK, Harrington CR. *Modelling Prion-Like Processing of Tau Protein in Alzheimer's Disease for Pharmaceutical Development.* Avila J, Brandt R, Kosik KS, editors. Amsterdam: Harwood Academic Publishers (1997). p. 185–241.
110. Wischik CM, Schelter BO, Wischik DJ, Storey JMD, Harrington CR. Modeling prion-like processing of tau protein in Alzheimer's disease for pharmaceutical development. *J Alzheimers Dis.* (2018) 62:1287–303. doi: 10.3233/JAD-170727
111. Xie C, Soeda Y, Shinzaki Y, In Y, Tomoo K, Ihara Y, et al. Identification of key amino acids responsible for the distinct aggregation properties of microtubule-associated protein 2 tau. *J Neurochem.* (2015) 135:19–26. doi: 10.1111/jnc.13228
112. Seidler P, Boyer D, Rodriguez J, Sawaya M, Cascio D, Murray K, et al. Structure-based inhibitors of tau aggregation. *Nat Chem.* (2018) 10:170–6. doi: 10.1038/nchem.2889
113. Schultz M, Tecedor L, Chang M, Davidson B. Clarifying lysosomal storage diseases. *Trends Neurosci.* (2011) 34:401–10. doi: 10.1016/j.tins.2011.05.006
114. Ihara Y, Morishima-Kawashima M, Nixon R. The ubiquitin-proteasome system and the autophagic-lysosomal system in Alzheimer disease. *Cold Spring Harb Perspect Med*. (2012) 2:a006361. doi: 10.1101/cshperspect.a006361
115. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V, et al. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. *Sci Rep.* (2012) 2:700. doi: 10.1038/srep00700
116. Usenovic M, Niroomand S, Drolet R, Yao L, Gaspar R, Hatcher N, et al. Internalized tau oligomers cause neurodegeneration by inducing accumulation of pathogenic tau in human neurons derived from induced pluripotent stem cells. *J Neurosci.* (2015) 35:14234–50. doi: 10.1523/JNEUROSCI.1523-15.2015
117. Kondo J, Honda T, Mori H, Hamada Y, Miura R, Ogawara M, et al. The carboxyl third of tau is tightly bound to paired helical filaments. *Neuron.* (1988) 1:827–34. doi: 10.1016/0896-6273(88)90130-4
118. Mori H, Hamada Y, Kawaguchi M, Honda T, Kondo J, Ihara Y. A distinct form of tau is selectively incorporated into Alzheimer's paired helical filaments. *Biochem Biophys Res Commun.* (1989) 159:1221–6. doi: 10.1016/0006-291X(89)92240-7
119. Guillozet-Bongaarts A, Garcia-Sierra F, Reynolds M, Horowitz P, Fu Y, Wang T, et al. Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. *Neurobiol Aging.* (2005) 26:1015–22. doi: 10.1016/j.neurobiolaging.2004.09.019
120. Zhang Q, Zhang X, Sun A. Truncated tau at D421 is associated with neurodegeneration and tangle formation in the brain of Alzheimer transgenic models. *Acta Neuropathol.* (2009) 117:687–97. doi: 10.1007/s00401-009-0491-6
121. Cohen T, Guo J, Hurtado D, Kwong L, Mills I, Trojanowski J, et al. The acetylation of tau inhibits its function and promotes pathological tau aggregation. *Nat Commun*. (2011) 2:1255. doi: 10.1038/ncomms1255
122. Arendt T, Stieler J, Holzer M. Tau and tauopathies. *Brain Res Bull.* (2016) 126:238–92. doi: 10.1016/j.brainresbull.2016.08.018
123. Gong C, Lidsky T, Wegiel J, Zuck L, Grundke-Iqbal I, Iqbal K. Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2a in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease. *J Biol Chem*. (2000) 275:5535–44. doi: 10.1074/jbc.275.8.5535
124. Chang H, Sang T, Chiang A. Untangling the tauopathy for Alzheimer's disease and parkinsonism. *J Biomed Sci.* (2018) 25:54. doi: 10.1186/s12929-018-0457-x
125. Mouchlis VD, Melagraki G, Zacharia LC, Afantitis A. Computer-aided drug design of β-secretase, γ-secretase and anti-tau inhibitors for the discovery of novel Alzheimer's therapeutics. *Int J Mol Sci.* (2020) 21:703. doi: 10.3390/ijms21030703
126. Köpke 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) 268:24374–84.
127. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida H, Watanabe A, et al. Hyperphosphorylation of Tau in PHF. *Neurobiol Aging.* (1995) 16:365–71. doi: 10.1016/0197-4580(95)00027-C
128. Derkinderen P, Scales TME, Hanger DP, Leung KY, Byers HL, Ward MA, et al. Tyrosine 394 is phosphorylated in Alzheimer's paired helical filament tau and in fetal tau with c-abl as the candidate tyrosine kinase. *J Neurosci.* (2005) 25:6584–93. doi: 10.1523/JNEUROSCI.1487-05.2005
129. Lindwall G, Cole R. Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J Biol Chem.* (1984) 259:5301–5.
130. Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K. Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. *Proc Natl Acad Sci USA.* (1994) 91:5562–6. doi: 10.1073/pnas.91.12.5562
131. Alonso AdC, Grundke-Iqbal I, Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med.* (1996) 2:783–7. doi: 10.1038/nm0796-783
132. Alonso AdC, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of τ into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci USA.* (2001) 98:6923–8. doi: 10.1073/pnas.121119298
133. Lucas JJ, Hernández F, Gómez-Ramos P, Morán MA, Hen R, Avila J. Decreased nuclear β-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3β conditional transgenic mice. *EMBO J.* (2001) 20:27–39. doi: 10.1093/emboj/20.1.27
134. Fath T, Eidenmüller J, Brandt R. Tau-mediated cytotoxicity in a Pseudohyperphosphorylation model of Alzheimer's disease. *J Neurosci.* (2002) 22:9733–41. doi: 10.1523/JNEUROSCI.22-22-09733.2002
135. Jackson G, Wiedau-Pazos M, Sang T, Wagle N, Brown C, Massachi S, et al. Human wild-type tau interacts with wingless pathway components and

produces neurofibrillary pathology in *Drosophila*. *Neuron.* (2002) 34:509–19. doi: 10.1016/S0896-6273(02)00706-7
136. Pérez M, Hernández F, Gómez-Ramos A, Smith M, Perry G, Avila J. Formation of aberrant phosphotau fibrillar polymers in neural cultured cells. *Eur J Biochem.* (2002) 269:1484–9. doi: 10.1046/j.1432-1033.2002.02794.x
137. Hoover B, Reed M, Su J, Penrod R, Kotilinek L, Grant M, et al. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron.* (2010) 68:1067–81. doi: 10.1016/j.neuron.2010.11.030
138. Hu W, Zhang X, Tung Y, Xie S, Liu F, Iqbal K. Hyperphosphorylation determines both the spread and the morphology of tau pathology. *Alzheimer's Demen J Alzheimer's Assoc.* (2016) 12:1066–77. doi: 10.1016/j.jalz.2016.01.014
139. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, et al. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet.* (2000) 25:402–5. doi: 10.1038/78078
140. Allen B, Ingram E, Takao M, Smith MJ, Jakes R, Virdee K, et al. Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. *J Neurosci.* (2002) 22:9340–51. doi: 10.1523/JNEUROSCI.22-21-09340.2002
141. Cruz J, Tseng H, Goldman J, Shih H, Tsai L. Aberrant cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles. *Neuron.* (2003) 40:471–83. doi: 10.1016/S0896-6273(03)00627-5
142. Noble W, Olm V, Takata K, Casey E, Mary O, Meyerson J, et al. Cdk5 is a key factor in tau aggregation and tangle formation *in vivo*. *Neuron.* (2003) 38:555–65. doi: 10.1016/S0896-6273(03)00259-9
143. Kelleher I, Garwood C, Hanger D, Anderton B, Noble W. Kinase activities increase during the development of tauopathy in htau mice. *J Neurochem.* (2007) 103:2256–67. doi: 10.1111/j.1471-4159.2007.04930.x
144. Lai R, Gertz H, Wischik D, Xuereb J, Mukaetova-Ladinska E, Harrington C, et al. Examination of phosphorylated tau protein as a PHF-precursor at early stage Alzheimer's disease. *Neurobiol Aging.* (1995) 16:433–45. doi: 10.1016/0197-4580(95)00041-C
145. Despres C, Byrne C, Qi H, Cantrelle F, Huvent I, Chambraud B, et al. Identification of the tau phosphorylation pattern that drives its aggregation. *Proc Natl Acad Sci USA.* (2017) 114:9080–5. doi: 10.1073/pnas.1708448114
146. Zhou Y, Shi J, Chu D, Hu W, Guan Z, Gong CX, et al. Relevance of phosphorylation and truncation of tau to the etiopathogenesis of Alzheimer's disease. *Front Aging Neurosci.* (2018) 10:27. doi: 10.3389/fnagi.2018.00027
147. Tepper K, Biernat J, Kumar S, Wegmann S, Timm T, Hübschmann S, et al. Oligomer formation of tau protein hyperphosphorylated in cells. *J Biol Chem.* (2014) 289:34389–407. doi: 10.1074/jbc.M114.611368
148. Wang J, Gong C, Zaidi T, Grundke-Iqbal I, Iqbal K. Dephosphorylation of Alzheimer paired helical filaments by protein phosphatase-2A and −2B. *J Biol Chem.* (1995) 270:4854–60. doi: 10.1074/jbc.270.9.4854
149. Wang JZ, Grundke-Iqbal I, Iqbal K. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. *Eur J Neurosci.* (2007) 25:59–68. doi: 10.1111/j.1460-9568.2006.05226.x
150. Wischik C, Edwards P, Lai R, Gertz H, Xuereb J, Paykel E, et al. Quantitative analysis of tau protein in paired helical filament preparations: implications for the role of tau protein phosphorylation in PHF assembly in Alzheimer's disease. *Neurobiol Aging.* (1995) 16:409–31. doi: 10.1016/0197-4580(95)97327-D
151. Poulter L, Barratt D, Scott C, Caputo C. Locations and immunoreactivities of phosphorylation sites on bovine and porcine tau proteins and a PHF-tau fragment. *J Biol Chem.* (1993) 268:9636–44.
152. Arakhamia T, Lee C, Carlomagno Y, Duong D, Kundinger S, Wang K, et al. Posttranslational modifications mediate the structural diversity of tauopathy strains. *Cell.* (2020) 180:633–44. doi: 10.1016/j.cell.2020.01.027
153. Lu M, Kosik KS. Competition for microtubule-binding with dual expression of tau missense and splice isoforms. *Mol Biol Cell.* (2001) 12:171–84. doi: 10.1091/mbc.12.1.171
154. Lim S, Haque MM, Kim D, Kim DJ, Kim YK. Cell-based models to investigate tau aggregation. *Comput. Struct. Biotechnol. J.* (2014) 12:7–13. doi: 10.1016/j.csbj.2014.09.011
155. Barghorn S, Davies P, Mandelkow E. Tau paired helical filaments from Alzheimer's disease brain and assembled *in vitro* are based on beta-structure in the core domain. *Biochemistry.* (2004) 43:1694. doi: 10.1021/bi0357006
156. Lai RYK, Harrington CR, Wischik CM. Absence of a role for phosphorylation in the tau pathology of Alzheimer's disease. *Biomolecules.* (2016) 6:19. doi: 10.3390/biom6020019
157. Schneider A, Biernat J, von Bergen M, Mandelkow E, Mandelkow EM. Phosphorylation that detaches tau protein from microtubules (Ser262, Ser214). Also protects it against aggregation into Alzheimer paired helical filaments. *Biochemistry.* (1999) 38:3549–58. doi: 10.1021/bi981874p
158. Delobel P, Flament S, Hamdane M, Mailliot C, Sambo A, Bégard S, et al. Abnormal tau phosphorylation of the Alzheimer-type also occurs during mitosis. *J Neurochem.* (2002) 83:412–20. doi: 10.1046/j.1471-4159.2002.01143.x
159. Morozova O, March Z, Robinson A, Colby D. Conformational features of tau fibrils from Alzheimer's disease brain are faithfully propagated by unmodified recombinant protein. *Biochemistry.* (2013) 52:6960–7. doi: 10.1021/bi400866w
160. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, et al. Caspase cleavage of tau: Linking amyloid neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci USA.* (2003) 10032–7. doi: 10.1073/pnas.1630428100
161. Johnson G, Jope R, Binder L. Proteolysis of tau by calpain. *Biochem Biophys Res Commun.* (1989) 163:1505–11. doi: 10.1016/0006-291X(89)91150-9
162. Bednarski E, Lynch G. Cytosolic proteolysis of tau by cathepsin D in hippocampus following suppression of cathepsins B and L. *J Neurochem.* (1996) 67:1846–55. doi: 10.1046/j.1471-4159.1996.67051846.x
163. Arai T, Guo J, McGeer P. Proteolysis of non-phosphorylated and phosphorylated tau by thrombin. *J Biol Chem.* (2005) 280:5145–53. doi: 10.1074/jbc.M409234200
164. Jeganathan S, von Bergen M, Brutlach H, Steinhoff HJ, Mandelkow E. Global hairpin folding of tau in solution. *Biochemistry.* (2006) 45:2283–93. doi: 10.1021/bi0521543
165. Wang YP, Biernat J, Pickhardt M, Mandelkow E, Mandelkow E-M. Stepwise proteolysis liberates tau fragments that nucleate the Alzheimer-like aggregation of full-length tau in a neuronal cell model. *Proc Natl Acad Sci USA.* (2007)104:10252–7. doi: 10.1073/pnas.0703676104
166. Abraha A, Ghoshal N, Gamblin T, Cryns V, Berry R, Kuret J, et al. C-terminal inhibition of tau assembly *in vitro* and in Alzheimer's disease. *J Cell Sci.* (2009) 113:3737–45.
167. Zilka N, Kovacech B, Barath P, Kontsekova E, Novák M. The self-perpetuating tau truncation circle. *Biochem Soc Trans.* (2012) 40:681–6. doi: 10.1042/BST20120015
168. Al-Hilaly Y, Pollack S, Vadukul D, Citossi F, Rickard J, Simpson M, et al. Alzheimer's disease-like paired helical filament assembly from truncated tau protein is independent of disulfide crosslinking. *J Mol Biol.* (2017) 429:3650–65. doi: 10.1016/j.jmb.2017.09.007
169. Al-Hilaly YK, Foster BE, Biasetti L, Lutter L, Pollack SJ, Rickard JE, et al. Tau (297-391) forms filaments that structurally mimic the core of paired helical filaments in Alzheimer's disease brain. *FEBS Lett.* (2020) 594:944–50. doi: 10.1002/1873-3468.13675
170. Mena R, Edwards P, Pérez-Olvera O, Wischik C. Monitoring pathological assembly of tau and beta-amyloid proteins in Alzheimer's disease. *Acta Neuropathol.* (1995) 89:50–6. doi: 10.1007/BF00294259
171. Harrington CR, Mukaetova-Ladinska EB, Hills R, Edwards PC, Montejo de Garcini E, Novak M, et al. Measurement of distinct immunochemical presentations of tau protein in Alzheimer disease. *Proc Natl Acad Sci USA.* (1994) 88:5842–6. doi: 10.1073/pnas.88.13.5842
172. Stadelmann C, Deckwerth T, Srinivasan A, Bancher C, Brück W, Jellinger K, et al. Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death. *Am J Pathol.* (1999) 155:1459–66. doi: 10.1016/S0002-9440(10)65460-0
173. Rohn T, Rissman R, Davis M, Kim Y, Cotman C, Head E. Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. *Neurobiol Dis.* (2002) 11:341–54. doi: 10.1006/nbdi.2002.0549
174. Canu N, Dus L, Barbato C, Ciotti MT, Brancolini C, Rinaldi AM, et al. Tau cleavage dephosphorylation in cerebellar granule neurons undergoing

apoptosis. *J Neurosci.* (1998) 18:7061–74. doi: 10.1523/JNEUROSCI.18-18-07061.1998
175. Rissman R, Poon W, Blurton-Jones M, Oddo S, Torp R, Vitek M, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest.* (2004) 114:121–30. doi: 10.1172/JCI200420640
176. Newman J, Rissman R, Sarsoza F, Kim R, Dick M, Bennett D, et al. Caspase-cleaved tau accumulation in neurodegenerative diseases associated with tau and alpha-synuclein pathology. *Acta Neuropathol.* (2005) 110:135–44. doi: 10.1007/s00401-005-1027-3
177. Guillozet-Bongaarts A, Glajch K, Libson E, Cahill M, Bigio E, Berry R, et al. Phosphorylation and cleavage of tau in non-AD tauopathies. *Acta Neuropathol.* (2007) 113:513–20. doi: 10.1007/s00401-007-0209-6
178. Fasulo L, Ugolini G, Visintin M, Bradbury A, Brancolini C, Verzillo V, et al. The neuronal microtubule-associated protein tau is a substrate for caspase-3 and an effector of apoptosis. *J Neurochem.* (2000) 75:624–33. doi: 10.1046/j.1471-4159.2000.0750624.x
179. Chung C, Song Y, Kim I, Yoon W, Ryu B, Jo D, et al. Proapoptotic effects of tau cleavage product generated by caspase-3. *Neurobiol Dis.* (2001) 8:162–72. doi: 10.1006/nbdi.2000.0335
180. Delobel P, Lavenir I, Fraser G, Ingram E, Holzer M, Ghetti B, et al. Analysis of tau phosphorylation and truncation in a mouse model of human tauopathy. *Am J Pathol.* (2008) 172:123–31. doi: 10.2353/ajpath.2008.070627
181. Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Trans-cellular propagation of tau aggregation by fibrillar species. *J Biol Chem.* (2012) 112:19440–51. doi: 10.1074/jbc.M112.346072
182. Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron.* (2014) 82:1271–88. doi: 10.1016/j.neuron.2014.04.047
183. Frost B, Jacks R, Diamond M. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem.* (2009) 284:12845–52. doi: 10.1074/jbc.M808759200
184. Clavaguera F, Bolmont T, Crowther R, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol.* (2009) 11:909–13. doi: 10.1038/ncb1901
185. Liu L, Drouet V, Wu JW, Witter MP, Small SA, Clelland C, et al. Trans-synaptic spread of tau pathology *in vivo*. *PLoS ONE*. (2012) 7:e31302. doi: 10.1371/journal.pone.0031302
186. Pooler A, Phillips E, Lau D, Noble W, Hanger D. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep.* (2013) 14:389–94. doi: 10.1038/embor.2013.15
187. Wu J, Herman M, Liu L, Simoes S, Acker C, Figueroa H, et al. Small misfolded tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem.* (2013) 288:1856–70. doi: 10.1074/jbc.M112.394528
188. Dujardin S, Lécolle K, Caillierez R, Bégard S, Zommer N, Lachaud C, et al. Neuron-to-neuron wild-type tau protein transfer through a trans-synaptic mechanism: relevance to sporadic tauopathies. *Acta Neuropathol Commun.* (2014) 2:14. doi: 10.1186/2051-5960-2-14
189. Yamada K, Holth J, Liao F, Stewart F, Mahan T, Jiang H, et al. Neuronal activity regulates extracellular tau *in vivo*. *J Exp Med.* (2014) 211:387–93. doi: 10.1084/jem.20131685
190. Calafate S, Buist A, Miskiewicz K, Vijayan V, Daneels G, de Strooper B, et al. Synaptic contacts enhance cell-to-cell tau pathology propagation. *Cell Rep.* (2015) 11:1176–83. doi: 10.1016/j.celrep.2015.04.043
191. Wu J, Hussaini S, Bastille I, Rodriguez G, Mrejeru A, Rilett K, et al. Neuronal activity enhances tau propagation and tau pathology *in vivo*. *Nat Neurosci.* (2016) 19:1085–92. doi: 10.1038/nn.4328
192. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* (1991) 82:809. doi: 10.1007/BF00308809
193. Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem.* (2012) 287:3842–9. doi: 10.1074/jbc.M111.277061
194. Simón D, García-García E, Royo F, Falcón-Pérez J, Avila J. Proteostasis of Tau. tau overexpression results in its secretion via membrane vesicles. *FEBS Lett.* (2012) 586:47–54. doi: 10.1016/j.febslet.2011.11.022
195. Fiandaca M, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz J, et al. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. *Alzheimer's Dementia.* (2015) 11:600–7. doi: 10.1016/j.jalz.2014.06.008
196. Wang Y, Balaji V, Kaniyappan S, Krüger L, Irsen S, Tepper K, et al. The release and trans-synaptic transmission of tau via exosomes. *Mol Neurodegener.* (2017) 12:5. doi: 10.1186/s13024-016-0143-y
197. Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, et al. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nat Neurosci.* (2015) 18:1584–93. doi: 10.1038/nn.4132
198. Fontaine S, Zheng D, Sabbagh J, Martin M, Chaput D, Darling A, et al. DnaJ/Hsc70 chaperone complexes control the extracellular release of neurodegenerative-associated proteins. *EMBO J.* (2016) 35:1537–49. doi: 10.15252/embj.201593489
199. Gómez-Ramos A, Díaz-Hernández M, Rubio A, Díaz-Hernández J, Miras-Portugal M, Avila J. Characteristics and consequences of muscarinic receptor activation by tau protein. *Eur Neuropsychopharmacol.* (2009) 19:708–17. doi: 10.1016/j.euroneuro.2009.04.006
200. Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, et al. Heparan sulfate proteoglycans mediate internalization propagation of specific proteopathic seeds. *Proc Natl Acad Sci USA.* (2013) 110:E3138–47. doi: 10.1073/pnas.1301440110
201. Jackson SJ, Kerridge C, Cooper J, Cavallini A, Falcon B, Cella CV, et al. Short fibrils constitute the major species of seed-competent tau in the brains of mice transgenic for human P301S tau. *J Neurosci.* (2016) 36:762–72. doi: 10.1523/JNEUROSCI.3542-15.2016
202. Pollack S, Trigg J, Khanom T, Biasetti L, Marshall K, Al-Hilaly Y, et al. Paired helical filament forming region of tau (297–391) influences endogenous tau protein and accumulates in acidic compartments in human neuronal cells. *J Mol Biol.* (2020) 20:30448–54. doi: 10.1016/j.jmb.2020.05.027
203. Iba M, McBride J, Guo J, Zhang B, Trojanowski J, Lee V. Tau pathology spread in PS19 tau transgenic mice following locus coeruleus (LC). Injections of synthetic tau fibrils is determined by the LC's afferent and efferent connections. *Acta Neuropathol.* (2015) 130:349–62. doi: 10.1007/s00401-015-1458-4
204. Peeraer E, Bottelbergs A, Van Kolen K, Stancu I, Vasconcelos B, Mahieu M, et al. Intracerebral injection of preformed synthetic tau fibrils initiates widespread tauopathy and neuronal loss in the brains of tau transgenic mice. *Neurobiol Dis.* (2015) 73:83–95. doi: 10.1016/j.nbd.2014.08.032
205. Tanemura K, Akagi T, Murayama M, Kikuchi N, Murayama O, Hashikawa T, et al. Formation of filamentous tau aggregations in transgenic mice expressing V337M human tau. *Neurobiol Dis.* (2001) 8:1036–45. doi: 10.1006/nbdi.2001.0439
206. Wittmann C, Wszolek M, Shulman J, Salvaterra P, Lewis J, Hutton M, et al. Tauopathy in *Drosophila*: neurodegeneration without neurofibrillary tangles. *Science.* (2001) 293:711–4. doi: 10.1126/science.1062382
207. Tatebayashi Y, Miyasaka T, Chui D, Akagi T, Mishima K, Iwasaki K, et al. Tau filament formation associative memory deficit in aged mice expressing mutant (R406W) human Tau. *Proc Natl Acad Sci USA.* (2002) 99:13896–901. doi: 10.1073/pnas.202205599
208. Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker K, Barde Y, et al. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem.* (2003) 86:582–90. doi: 10.1046/j.1471-4159.2003.01879.x
209. SantaCruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, et al. Tau suppression in a neurodegenerative mouse model improves memory function. *Science.* (2005) 309:476–81. doi: 10.1126/science.1113694
210. Spires T, Orne J, SantaCruz K, Pitstick R, Carlson G, Ashe K, et al. Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse model of tauopathy. *Am J Pathol.* (2006) 168:1598–607. doi: 10.2353/ajpath.2006.050840
211. Berger Z, Ravikumar B, Menzies F, Oroz L, Underwood B, Pangalos M, et al. Rapamycin alleviates toxicity of different aggregate-prone proteins. *Hum Mol Genet.* (2006) 15:433–42. doi: 10.1093/hmg/ddi458
212. Yoshiyama Y, Higuchi M, Zhang B, Huang S, Iwata N, Saido T, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* (2007) 53:337–51. doi: 10.1016/j.neuron.2007.01.010
213. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R. Tau oligomers impair memory and induce synaptic and

mitochondrial dysfunction in wild-type mice. *Mol Neurodegener.* (2011) 6:39. doi: 10.1186/1750-1326-6-39
214. Castillo-Carranza DL, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G, et al. Passive immunization with tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J Neurosci.* (2014) 34:4260–72. doi: 10.1523/JNEUROSCI.3192-13.2014
215. Arrasate M, Mitra S, Schweitzer E, Segal M, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature.* (2004) 431:805–10. doi: 10.1038/nature02998
216. Rubinsztein D. The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature.* (2006) 443:780–6. doi: 10.1038/nature05291
217. Pritchard SM, Dolan PJ, Vitkus A, Johnson GV. The toxicity of tau in Alzheimer disease: turnover, targets and potential therapeutics. *J Cell Mol Med.* (2011) 15:1621–35. doi: 10.1111/j.1582-4934.2011.01273.x
218. Nonaka T, Watanabe S, Iwatsubo T, Hasegawa M. Seeded aggregation and toxicity of {alpha}-synuclein and tau: cellular models of neurodegenerative diseases. *J Biol Chem.* (2010) 285:34885–98. doi: 10.1074/jbc.M110.148460
219. Tak H, Haque M, Kim M, Lee J, Baik J, Kim Y, et al. Bimolecular fluorescence complementation; lighting-up tau-tau interaction in living cells. *PLoS ONE.* (2013) 8:e0081682. doi: 10.1371/journal.pone.0081682
220. Chun W, Johnson G. Activation of glycogen synthase kinase 3beta promotes the intermolecular association of tau. The use of fluorescence resonance energy transfer microscopy. *J Biol Chem.* (2007) 282:23410–7. doi: 10.1074/jbc.M703706200
221. Chun W, Waldo G, Johnson G. Split GFP complementation assay: a novel approach to quantitatively measure aggregation of tau *in situ*: effects of gsk3beta activation and caspase 3 cleavage. *J Neurochem.* (2007) 103:2529–39. doi: 10.1111/j.1471-4159.2007.04941.x
222. Hasegawa M, Crowther R, Jakes R, Goedert M. Alzheimer-like changes in microtubule-associated protein tau induced by sulfated glycosaminoglycans. Inhibition of microtubule binding, stimulation of phosphorylation, and filament assembly depend on the degree of sulfation. *J Biol Chem.* (1997) 272:33118–24. doi: 10.1074/jbc.272.52.33118
223. Berriman J, Serpell LC, Oberg KA, Fink AL, Goedert M, Crowther RA. Tau filaments from human brain and from *in vitro* assembly of recombinant protein show cross-β *structure*. (2003) 100:9034–8. doi: 10.1073/pnas.1530287100
224. Arrasate M, Pérez M, Armas-Portela R, Avila J. Polymerization of tau peptides into fibrillar structures. The effect of FTDP-17 mutations. *FEBS Lett.* (1999) 446:199–202. doi: 10.1016/S0014-5793(99)00210-0
225. Goedert M, Jakes R, Crowther R. Effects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau filaments. *FEBS Lett.* (1999) 450:306–11. doi: 10.1016/S0014-5793(99)00508-6
226. Nacharaju P, Lewis J, Easson C, Yen S, Hackett J, Hutton M, et al. Accelerated filament formation from tau protein with specific FTDP-17 missense mutations. *FEBS Lett.* (1999) 447:195–9. doi: 10.1016/S0014-5793(99)00294-X
227. Pickhardt M, Gazova Z, von Bergen M, Khlistunova I, Wang Y, Hascher A, et al. Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments *in vitro* and in cells. *J Biol Chem.* (2005) 280:3628–35. doi: 10.1074/jbc.M410984200
228. Taniguchi S, Suzuki N, Masuda M, Hisanaga S, Iwatsubo T, Goedert M, et al. Inhibition of heparin-induced tau filament formation by phenothiazines, polyphenols, and porphyrins. *J Biol Chem.* (2005) 280:7614–23. doi: 10.1074/jbc.M408714200
229. Xu S, Brunden KR, Trojanowski JQ, Lee VMY. Characterization of tau fibrillization *in vitro*. *Alzheimers Dement.* (2010) 6:110–7. doi: 10.1016/j.jalz.2009.06.002
230. Crespo R, Koudstaal W, Apetri A. *In vitro* assay for studying the aggregation of tau protein drug screening. *J Visual Exp.* (2018). doi: 10.3791/58570
231. Falcon B, Cavallini A, Angers R, Glover S, Murray TK, Barnham L, et al. Conformation determines the seeding potencies of native recombinant tau aggregates. *J Biol Chem.* (2015) 290:1049–65. doi: 10.1074/jbc.M114.589309
232. Zhang W, Falcon B, Murzin AG, Fan J, Crowther RA, Goedert M, et al. Heparin-induced tau filaments are polymorphic and differ from those in Alzheimer's and Pick's diseases. *Elife.* (2019) 8:e43584. doi: 10.7554/eLife.43584.040
233. Fichou Y, Lin Y, Rauch JN, Vigers M, Zeng, Srivastava M, et al. Cofactors are essential constituents of stable and seeding-active tau fibrils. *Proc Natl Acad Sci USA.* (2018) 115:13234–9. doi: 10.1073/pnas.1810058115
234. Gustke N, Trinczek B, Biernat J, Mandelkow EM, Mandelkow E. Domains of tau protein and interactions with microtubules. *Biochemistry.* (1994) 33:9511–22. doi: 10.1021/bi00198a017
235. Mukrasch MD, Biernat J, von Bergen M, Griesinger C, Mandelkow E, Zweckstetter M. Sites of tau important for aggregation populate β-structure and bind to microtubules and polyanions. *J Biol Chem.* (2005) 280:24978–86. doi: 10.1074/jbc.M501565200
236. Friedhoff P, Schneider A, Mandelkow E-M, Mandelkow E. Rapid assembly of Alzheimer-like paired helical filaments from microtubule-associated protein tau monitored by fluorescence in solution. *Biochemistry.* (1998) 37:bi980537d. doi: 10.1021/bi980537d
237. Bulic B, Pickhardt M, Khlistunova I, Biernat J, Mandelkow EM, Mandelkow E, et al. Rhodanine-based tau aggregation inhibitors in cell models of tauopathy. *Angew Chem.* (2007) 46:9215–19. doi: 10.1002/anie.200704051
238. Bulic B, Pickhardt M, Schmidt B, Mandelkow EM, Waldmann H, Mandelkow E. Development of tau aggregation inhibitors for Alzheimer's disease. *Angew Chem.* (2009) 48:2621. doi: 10.1002/anie.200802621
239. Bulic B, Pickhardt M, Mandelkow EM, Mandelkow E. Tau protein and tau aggregation inhibitors. *Neuropharmacology.* (2010) 59:16. doi: 10.1016/j.neuropharm.2010.01.016
240. Crowe A, Ballatore C, Hyde E, Trojanowski JQ, Lee VMY. High throughput screening for small molecule inhibitors of heparin-induced tau fibril formation. *Biochem Biophys Res Commun.* (2007) 358:1–6. doi: 10.1016/j.bbrc.2007.03.056
241. Crowe A, Huang W, Ballatore C, Johnson RL, Hogan AML, Huang R, et al. The identification of aminothienopyridazine inhibitors of tau assembly by quantitative high-throughput screening. *Biochemistry.* (2009) 48:7732–45. doi: 10.1021/bi9006435
242. Bondareff W, Wischik CM, Novak M, Roth M. Sequestration of tau by granulovacuolar degeneration in Alzheimer's disease. *Am J Pathol.* (1991) 139:641–7.
243. Mena R, Wischik CM, Novak M, Milstein C, Cuello AC. A progressive deposition of paired helical filaments (PHF) in the brain characterizes the evolution of dementia in Alzheimer's disease. An immunocytochemical study with a monoclonal antibody against the PHF core. *J Neuropathol Exp Neurol.* (1991) 50:474–90. doi: 10.1097/00005072-199107000-00008
244. Skrabana R, Kontsek P, Mederlyova A, Iqbal K, Novak M. Folding of Alzheimer's core phf subunit revealed by monoclonal antibody 423. *FEBS Lett.* (2004) 568:178–82. doi: 10.1016/j.febslet.2004.04.098
245. Harrington CR, Storey JMD, Clunas S, Harrington KA, Horsley D, Ishaq A, et al. Cellular models of aggregation-dependent template-directed proteolysis to characterize tau aggregation inhibitors for treatment of Alzheimer disease. *J Biol Chem.* (2015) 290:10862–75. doi: 10.1074/jbc.M114.616029
246. Schweers O, Mandelkow EM, Biernat J, Mandelkow E. Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein tau controls the *in vitro* assembly of paired helical filaments. *Proc Natl Acad Sci USA.* (1995) 92:8463–7. doi: 10.1073/pnas.92.18.8463
247. Furukawa Y, Kaneko K, Nukina N. Tau protein assembles into isoform- and disulfide-dependent polymorphic fibrils with distinct structural properties. *J Biol Chem.* (2011) 286:27236–46. doi: 10.1074/jbc.M111.248963
248. Kim D, Lim S, Haque M, Ryoo N, Hong H, Rhim H, et al. Identification of disulfide cross-linked tau dimer responsible for tau propagation. *Sci Rep.* (2015) 5:15231. doi: 10.1038/srep15231
249. Crowe A, James MJ, Lee VMY, Smith AB, Trojanowski JQ, Ballatore C, et al. Aminothienopyridazines methylene blue affect tau fibrillization via cysteine oxidation. *J Biol Chem.* (2013) 112:11024–37. doi: 10.1074/jbc.M112.436006
250. Akoury E, Pickhardt M, Gajda M, Biernat J, Mandelkow E, Zweckstetter M. Mechanistic basis of phenothiazine-driven inhibition of tau aggregation. *Angew Chem.* (2013) 52:3511–5. doi: 10.1002/anie.201208290
251. Al-Hilaly Y, Pollack S, Rickard J, Simpson M, Raulin A, Baddeley T, et al. Cysteine-independent inhibition of Alzheimer's disease-like paired helical filament assembly by leuco-methylthioninium (LMT). *J Mol Biol.* (2018) 430:4119–31. doi: 10.1016/j.jmb.2018.08.010

252. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: *Alzheimers Dement*. (2018) 2018:195–214. doi: 10.1016/j.trci.2018.03.009
253. Melis V, Magbagbeolu M, Rickard JE, Horsley D, Davidson K, Harrington KA, et al. Effects of oxidized and reduced forms of methylthioninium in two transgenic mouse tauopathy models. *Behav Pharmacol.* (2015) 26:353–68. doi: 10.1097/FBP.0000000000000133
254. O'Leary JC, Li Q, Marinec P, Blair LJ, Congdon EE, Johnson AG, et al. Phenothiazine-mediated rescue of cognition in tau transgenic mice requires neuroprotection and reduced soluble tau burden. *Mol Neurodegener.* (2010) 5:45. doi: 10.1186/1750-1326-5-45
255. Congdon EE, Wu JW, Myeku N, Figueroa YH, Herman M, Marinec PS, et al. Methylthioninium chloride (methylene blue) induces autophagy and attenuates tauopathy *in vitro* and *in vivo*. *Autophagy.* (2012) 8:609–22. doi: 10.4161/auto.19048
256. Xie L, Li W, Winters A, Yuan F, Jin K, Yang S. Methylene blue induces macroautophagy through 5′ adenosine monophosphate-activated protein kinase pathway to protect neurons from serum deprivation. *Front Cell Neurosci.* (2013) 7:56. doi: 10.3389/fncel.2013.00056
257. Atamna H, Nguyen A, Schultz C, Boyle K, Newberry J, Kato H, et al. Methylene blue delays cellular senescence and enhances key mitochondrial biochemical pathways. *FASEB J.* (2008) 22:703–12. doi: 10.1096/fj.07-9610com
258. Wen Y, Li W, Poteet EC, Xie L, Tan C, Yan LJ, et al. Alternative mitochondrial electron transfer as a novel strategy for neuroprotection. *J Biol Chem.* (2011) 286:16504–15. doi: 10.1074/jbc.M110.208447
259. Stack C, Jainuddin S, Elipenahli C, Gerges M, Starkova N, Starkov AA, et al. Methylene blue upregulates Nrf2/ARE genes and prevents tau-related neurotoxicity. *Hum Mol Genet.* (2014) 23:3716–32. doi: 10.1093/hmg/ddu080
260. Tretter L, Horvath G, Hölgyesi A, Essek F, Adam-Vizi V. Enhanced hydrogen peroxide generation accompanies the beneficial bioenergetic effects of methylene blue in isolated brain mitochondria. *Free Radical Biol Med.* (2014) 77:317–30. doi: 10.1016/j.freeradbiomed.2014.09.024
261. Li L, Qin L, Lu H, Li P, Song Y, Yang R. Methylene blue improves streptozotocin-induced memory deficit by restoring mitochondrial function in rats. *Brain Res.* (2017) 1657:208–14. doi: 10.1016/j.brainres.2016.12.024
262. Vekaria HJ, Watts LT, Lin AL, Sullivan PG. Targeting mitochondrial dysfunction in cns injury using methylene blue; still a magic bullet? *Neurochem Int.* (2017) 109:117–25. doi: 10.1016/j.neuint.2017.04.004
263. Oz M, Lorke D, Petroianu G. Methylene blue and Alzheimer's disease. *Biochem Pharmacol.* (2009) 78:927–32. doi: 10.1016/j.bcp.2009.04.034
264. Oz M, Lorke DE, Hasan M, Petroianu G A. Cellular and molecular actions of methylene blue in the nervous system. *Med Res Rev*. (2011) 31:93–117. doi: 10.1002/med.20177
265. Baddeley T, McCaffrey J, Storey J, Cheung J, Melis V, Horsley D, et al. Complex disposition of methylthioninium redox forms determines efficacy in tau aggregation inhibitor therapy for Alzheimer's disease. *J Pharmacol Experimental Ther.* (2015) 352:110–8. doi: 10.1124/jpet.114.219352
266. Hochgräfe K, Sydow A, Matenia D, Cadinu D, Könen S, Petrova O, et al. Preventive methylene blue treatment preserves cognition in mice expressing full-length pro-aggregant human Tau. *Acta Neuropathol Commun.* (2015) 25:4. doi: 10.1186/s40478-015-0204-4
267. Riedel G, Klein J, Niewiadomska G, Kondak C, Schwab K, Lauer D, et al. Mechanisms of anticholinesterase interference with tau aggregation inhibitor activity in a tau-transgenic mouse model. *Curr Alzheimer Res.* (2020) 17:285–96. doi: 10.2174/1567205017666200224120926
268. Wischik CM, Staff RT, Wischik DJ, Bentham P, Murray AD, Storey JMD, et al. Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. *J Alzheimer's Dis.* (2015) 44:705–20. doi: 10.3233/JAD-142874
269. Gauthier S, Feldman HH, Schneider LS, Wilcock GK, Frisoni GB, Hardlund JH, et al. Efficacy and safety of tau-aggregation inhibitor therapy in patients with mild or moderate Alzheimer's disease: a randomised, controlled, double-blind, parallel-arm, phase 3 trial. *Lancet.* (2016) 388:2873–84. doi: 10.1016/S0140-6736(16)31275-2
270. Wilcock GK, Gauthier S, Frisoni GB, Jia J, Hardlund JH, Moebius HJ, et al. Potential of low dose leuco-methylthioninium bis(hydromethanesulphonate) (LMTM) monotherapy for treatment of mild Alzheimer's disease: cohort analysis as modified primary outcome in a phase III clinical trial. *J Alzheimers Dis.* (2018) 6:435–57. doi: 10.3233/JAD-170560
271. Schelter BO, Shiells H, Baddeley TC, Rubino CM, Ganesan H, Hammel J, et al. Concentration-dependent activity of hydromethylthionine on cognitive decline brain atrophy in mild to moderate Alzheimer's disease. *J Alzheimers Dis.* (2019) 72:931–46. doi: 10.3233/JAD-190772
272. Shiells H, Schelter BO, Bentham P, Baddeley TC, Rubino CM, Ganesan H, et al. Concentration-dependent activity of hydromethylthionine on clinical decline brain atrophy in a randomized controlled trial in behavioral variant frontotemporal dementia. *J Alzheimers Dis.* (2020) 72:501–9. doi: 10.3233/JAD-191173
273. Hamaguchi T, Ono K, Yamada M. Review: curcumin and Alzheimer's disease. *CNS Neurosci Ther.* (2010) 16:285–97. doi: 10.1111/j.1755-5949.2010.00147.x
274. Ma QL, Zuo X, Yang F, Ubeda OJ, Gant DJ, Alaverdyan M, et al. Curcumin suppresses soluble tau dimers corrects molecular chaperone, synaptic, behavioral deficits in aged human tau transgenic mice. *J Biol Chem.* (2013) 288:4056–65. doi: 10.1074/jbc.M112.393751
275. Bijari N, Balalaie S, Akbari V, Golmohammadi F, Moradi S, Adibi H, et al. Effective suppression of the modified PHF6 peptide/1N4R tau amyloid aggregation by intact curcumin, not its degradation products: another evidence for the pigment as preventive/therapeutic "functional food". *Int J Biol Macromol.* (2018) 120:1009–22. doi: 10.1016/j.ijbiomac.2018.08.175
276. Yang F, Lim G, Begum A, Ubeda O, Simmons M, Ambegaokar S, et al. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. *J Biol Chem.* (2005) 280:5892–901. doi: 10.1074/jbc.M404751200
277. Belkacemi A, Doggui S, Dao L, Ramassamy C. Challenges associated with curcumin therapy in Alzheimer disease. *Expert Rev Mol Med*. (2011) 13:e34. doi: 10.1017/S1462399411002055
278. Congdon EE, Sigurdsson EM. Tau-targeting therapies for Alzheimer disease. *Nat Rev Neurol.* (2018) 14:399–415. doi: 10.1038/s41582-018-0013-z
279. Okuda M, Hijikuro I, Fujita Y, Wu X, Nakayama S, Sakata Y, et al. PE859, a Novel Tau aggregation inhibitor, reduces aggregated tau prevents onset progression of neural dysfunction *in vivo*. *PLoS ONE.* (2015) 10:e0117511. doi: 10.1371/journal.pone.0117511
280. Okuda M, Fujita Y, Hijikuro I, Wada M, Uemura T, Kobayashi Y, et al. PE859, a novel curcumin derivative, inhibits amyloid-β tau aggregation, ameliorates cognitive dysfunction in senescence-accelerated mouse prone 8. *J Alzheimer's Dis.* (2017) 59:313–28. doi: 10.3233/JAD-161017
281. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature.* (1999) 400:173–7. doi: 10.1038/22124
282. Nicoll J, Wilkinson D, Holmes C, Steart P, Markham H, Weller R. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med.* (2003) 9:448–52. doi: 10.1038/nm840
283. Asuni AA, Boutajangout A, Quartermain D, Sigurdsson EM. Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. *J Neurosci.* (2007) 27:9115–29. doi: 10.1523/JNEUROSCI.2361-07.2007
284. Boutajangout A, Quartermain D. Sigurdsson EM. Immunotherapy targeting pathological tau prevents cognitive decline in a new tangle mouse model. *J Neurosci.* (2010) 30:16559–66. doi: 10.1523/JNEUROSCI.4363-10.2010
285. Boutajangout A, Ingadottir J, Davies P, Sigurdsson EM. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *J Neurochem.* (2011) 118:658–67. doi: 10.1111/j.1471-4159.2011.07337.x
286. Boimel M, Grigoriadis N, Lourbopoulos A, Haber E, Abramsky O, Rosenmann H, Efficacy and safety of immunization with phosphorylated tau against neurofibrillary tangles in mice. *Exp Neurol.* (2010) 224:472–85. doi: 10.1016/j.expneurol.2010.05.010

287. Bi M, Ittner A, Ke YD, Götz J, Ittner LM. Tau-Targeted immunization impedes progression of neurofibrillary histopathology in aged P301L tau transgenic mice. *PLoS ONE*. (2011) 6:e26860. doi: 10.1371/journal.pone.0026860
288. Gu J, Congdon EE, Sigurdsson EM. Two novel tau antibodies targeting the 396/404 region are primarily taken up by neurons reduce tau protein pathology. *J Biol Chem*. (2013) 288:33081–95. doi: 10.1074/jbc.M113.494922
289. Collin L, Bohrmann B, Göpfert U, Oroszlan-Szovik K, Ozmen L, Grüninger F. Neuronal uptake of tau/PS422 antibody and reduced progression of tau pathology in a mouse model of Alzheimer's disease. *Brain*. (2014) 137(Pt. 10):2834–46. doi: 10.1093/brain/awu213
290. Kondo A, Shahpasand K, Mannix R, Qiu J, Moncaster J, Chen CH, et al. Antibody against early driver of neurodegeneration cis P-tau blocks brain injury and tauopathy. *Nature*. (2015) 523:431–6. doi: 10.1038/nature14658
291. Bright J, Hussain S, Dang V, Wright S, Cooper B, Byun T, et al. Human secreted tau increases amyloid-beta production. *Neurobiol Aging*. (2015) 36:693–709. doi: 10.1016/j.neurobiolaging.2014.09.007

Conflict of Interest: The authors declare that this study received funding for YA-H, KEM, and SO from TauRx Therapeutics Ltd. an affiliate of WisTa Laboratories Ltd. The funder had the following involvement in the study: authors CRH and CMW hold office at TauRx Therapeutics Ltd. an affiliate of WisTa Laboratories Ltd. These authors contributed to writing and editing of the review manuscript and the decision to submit for publication.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

MINI REVIEW
published: 19 November 2020
doi: 10.3389/fneur.2020.580732

Targeting Tau to Treat Clinical Features of Huntington's Disease

Maria Masnata [1,2†], Shireen Salem [1,3†], Aurelie de Rus Jacquet [1,2], Mehwish Anwer [1,2] and Francesca Cicchetti [1,2,3]*

[1] Centre de Recherche du CHU de Québec, Axe Neurosciences, Québec, QC, Canada, [2] Département de Psychiatrie & Neurosciences, Université Laval, Québec, QC, Canada, [3] Département de Médecine Moléculaire, Université Laval, Québec, QC, Canada

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by severe motor, cognitive and psychiatric impairments. While motor deficits often confirm diagnosis, cognitive dysfunctions usually manifest early in the disease process and are consistently ranked among the leading factors that impact the patients' quality of life. The genetic component of HD, a mutation in the huntingtin (*HTT)* gene, is traditionally presented as the main contributor to disease pathology. However, accumulating evidence suggests the implication of the microtubule-associated tau protein to the pathogenesis and therefore, proposes an alternative conceptual framework where tau and mutant huntingtin (mHTT) act conjointly to drive neurodegeneration and cognitive dysfunction. This perspective on disease etiology offers new avenues to design therapeutic interventions and could leverage decades of research on Alzheimer's disease (AD) and other tauopathies to rapidly advance drug discovery. In this mini review, we examine the breadth of tau-targeting treatments currently tested in the preclinical and clinical settings for AD and other tauopathies, and discuss the potential application of these strategies to HD.

Keywords: tauopathy, tau hyperphosphorylation, cognitive deficits, tau-targeting treatments, tau aggregation inhibitors, tau immunotherapy, microtubule stabilizers, gene silencing

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Rocio Gomez-Pastor, University of Minnesota Twin Cities, United States
Jose Lucas, Consejo Superior de Investigaciones Científicas (CSIC), Spain

***Correspondence:**
Francesca Cicchetti
francesca.cicchetti@crchudequebec.ulaval.ca

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *06 July 2020*
Accepted: *17 September 2020*
Published: *19 November 2020*

Citation:
Masnata M, Salem S, de Rus Jacquet A, Anwer M and Cicchetti F (2020) Targeting Tau to Treat Clinical Features of Huntington's Disease. Front. Neurol. 11:580732. doi: 10.3389/fneur.2020.580732

HUNTINGTON'S DISEASE: A SECONDARY TAUOPATHY

Tau is an important microtubule-associated protein primarily expressed in neurons and known to mediate a plethora of cellular functions such as microtubule dynamics, neurite outgrowth, intracellular trafficking and synaptic plasticity (1–3). These activities are tightly regulated by post-translational modifications of tau, including phosphorylation and dephosphorylation (4–6), acetylation (7, 8), glycosylation (9), O-GlcNAcylation (10), nitration (11), sumoylation (12) and truncation (13). However, while phosphorylation is an essential mechanism regulating the biological activities of tau, abnormally hyperphosphorylated tau (p-tau) can form neurofibrillary tangles (NFTs) and/or neuropil threads (NTs) (14) that interfere with these fundamental mechanisms. This has been classically associated with Alzheimer's disease (AD) or with diseases caused by mutations of the tau gene (*MAPT*) such as Frontotemporal dementia with parkinsonism-17 (15, 16) (**Table 1**). More recently, similar tau dysregulations have been reported in Huntington's disease (HD) (27, 31, 32, 42, 55, 64, 83, 84) [reviewed in (33, 85)]; a disorder driven by an autosomal dominant pattern of inheritance and caused by a pathological CAG repeat expansion exceeding 35 in exon 1 of the huntingtin (*HTT*) gene (86) coding for the huntingtin (HTT) protein. This CAG elongation leads to the production of mutant huntingtin (mHTT) (87–90) which confers

TABLE 1 | Tau pathology and therapeutic strategies in AD and HD.

Evidence of tau dysfunction		Target	Therapeutic approach
Alzheimer's disease	*Huntington's disease*		
• A90V (17), G213R (18), K280del (19), A152T (20), V287I (18), A297V (18), S318L (18), A41T (21). • H1 and H1c haplotypes may be risk factors for AD (22–25); H2 haplotype may be protective against AD (23).	• No *MAPT* mutations identified in GWAS studies (26). • Accelerated cognitive decline in H2 haplotype carriers (27).	*MAPT* polymorphism and mutations	**Modulation of *MAPT* gene expression**
• Tau tangles composed of 3R and 4R tau (28). • Shift from 4R to 3R tau-enriched NFTs in hippocampus (29). • ↑ 3R tau in brainstem with disease progression (30).	• Cortex and striatum of HD patients: ↑ 4R/3R mRNA ratio (31, 32); ↑ 4R/3R tau protein (27, 31–33); ↓ 3R tau protein (27, 31). • Cortex and striatum of R6/1 and HD94 mice: ↑ 4R/3R mRNA ratio; ↑ 4R and ↓ 3R tau protein (31).	Tau isoforms	
• Tau aggregates associated with severity of symptoms and disease progression (34, 35). • Presence of NFTs in several AD brain regions: transentorhinal region, hippocampus, neocortex (36). • Transcellular propagation of tau *in vitro* (37, 38) and spread between brain regions *in vivo* (39–41). • Propagation and deposition of tau inclusions in a sequential pattern in AD patients, from transentorhinal (stage I) to the isocortex (stage V–VI) (36).	• Presence of NFTs in post-mortem HD brain tissue (27, 42–46). • Acquisition of tau inclusions, NFTs, NTs and increased 4R/3R tau in healthy fetal neural allografts in HD recipients (42).	Tau pathological deposits and propagation	**Inhibition of tau aggregation and/or tau immunotherapies**
• GSK-3β (47–49), CDK5 (49, 50), PKA, Erk1/2, JNK1/2/3, p38, CK1 (51), TTBK1, DYRK1A (52, 53) MARK, PKB, PKC, CaMKII, SFK, c-Abl [all reviewed in (54)].	• GSK-3β (55, 56), CDK5 and CaMKII (56).	Tau-targeting kinases	**Targeting hyper-p-tau**
• PP1, PP2A, PP2B, PTEN, PP5 (57–60).	• PP1 and PP2A (56), PP2B (56, 60)	Tau-targeting phosphatases	
• Tau phosphorylation in AD brain tissue: S202, T231, S199, Y18, S262, S356 [For a detailed account on phosphorylation sites, see (61, 62)]. • ↑ phosphorylation at Y18, T231 and S199 in post-mortem brain tissue (63). • ↑ phosphorylation at Y18 and T231 in isocortex and transentorhinal cortex depending on Braak stages (63).	• Tau phosphorylation in HD brain tissue: S396, S404, T205, S199 (32) and S202 (AT8 antibody) (42, 43). • ↑ p-tau in cortex and striatum of HD patients (27, 32). • Detection of hyper-p-tau S202 and T205 in healthy fetal neural allografts in two HD patient recipients (42). • ↑ hyper-p-tau in the brains of R6/2 (56, 64), zQ175 (64) and 140CAG knock-in (56) mouse models of HD.	Disease-associated p-tau sites	**Targeting hyper-p-tau and/or tau immunotherapies**
• Prevention of *in vitro* microtubule assembly (65) and depolymerization (66, 67) by p-tau isolated from human AD brain tissue. • Reduced microtubule density and axonal degeneration in neuronal cultures (68, 69), in Tg mice expressing h-tau (70) or PS19 tau (71), and in AD patients (72). • Contribution of tau-dependent loss of microtubule stability to cognitive deficits in tau 3xTg and rTg4510 mouse models (73). • Tau-related microtubule destabilization is accompanied by Aβ-induced neurodegeneration (74–77).	• Binding of mHTT to microtubules, defects in axonal transport, mitochondrial and vesicular dynamics in primary neurons (78–81). • Reduction of BDNF axonal transport by mHTT in NG108-15 cells, resulting in neuronal loss (82). • Recruitment of mHTT to microtubules by tau (56)	Microtubule dysfunction	**Stabilizing microtubules**

Aβ, Amyloid-beta; AD, Alzheimer's disease; BDNF; brain-derived neurotrophic factor; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CDK5, cyclin-dependent kinase-5; CK1, Casein kinase 1; DYRK1A, Dual specificity tyrosine-phosphorylation-regulated kinase 1A; Erk1/2, extracellular signal-regulated protein kinases 1 and 2; GSK-3ß, glycogen synthase kinase-3; h-tau, human tau; HD, Huntington's disease; hyper-p-tau, hyperphosphorylated tau; JNK1/2/3, c-Jun N-terminal Protein Kinase 1/2/3; MARK, MAP/microtubule affinity-regulating kinase; mHTT, mutant huntingtin; mRNA, messenger RNA; NFTs, neurofibrillary tangles; NTs, neuropil threads; p-tau, phosphorylated tau; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PP2B, protein phosphatase 2B; PP5, serine/threonine-protein phosphatase 5; PTEN, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase; S, serine; SFK, Src Family Kinase; T, threonine; Tg, transgenic; TTBK1, tau-tubulin kinase 1; Y, tyrosine.

a cytotoxic activity to this newly formed protein. This includes sequestration of transcription factors, mitochondrial dysfunction, induction of apoptotic cell death and alteration of the ubiquitin-proteasome system (UPS) (88–90).

While the diagnosis of HD is based on motor features (typically chorea), patients exhibit early and progressive cognitive impairments that impact activities of daily living along with psychiatric disturbances that can evolve to frank psychosis (91, 92). Notably, carriers of the H2 *MAPT* haplotype allegedly experience more rapid cognitive decline than those with an H1 haplotype (27). This is of particular interest since the *MAPT* haplotype has been proposed as a risk factor for

other neurodegenerative disorders such as AD, Parkinson's disease (PD) and PD-associated dementia (22, 23, 93). Along these lines, data collected by Positron Emission Tomography (PET) have allowed to establish correlations between tau and cognitive decline, with tau deposits more closely related with cognitive dysfunction in AD patients than amyloid β (Aβ) (94). Furthermore, both PET and cerebrospinal fluid (CSF) measures of tau, but not Aβ, have been linked to worsening cognition in AD (95). Similarly, the CSF of HD patients contains increasing levels of total tau (t-tau) with disease progression, which correlate with a decline in motor and cognitive functions (96). While a couple of studies have found discrepancies between the levels of CSF t-tau and cognitive decline (96, 97), a correlation between CSF t-tau and mHTT has been reported (97).

In agreement with the concept that HD meets the criteria of a secondary tauopathy is the fact that the cardinal features of tauopathies—misfolding, hyperphosphorylation, NFTs and NTs—have all been identified in post-mortem brain tissue derived from HD patients (27, 43–46, 98–100) [reviewed in (33, 85)]. For example, an increased 4R/3R tau isoform ratio has been observed in *HTT* mutation carriers (31, 32) at late disease stages (3 and 4) (32). In particular, nuclear rod-like tau deposits composed of the 4R tau isoform are more abundant in striatal and cortical tissues of HD patients, while they are virtually undetectable in the brains of control individuals (31). Both abnormal p-tau and mHTT aggregates can be located within neurons (27), although they rarely colocalize (98) or co-precipitate in HD brain homogenates (31).

Collectively, these findings suggest an association between altered tau biology and HD pathology. However, whether tau impairments have a causative effect on the manifestation of certain aspects of the disease, such as cognitive decline, has yet to be established. A closer look at the evidence of tau dysfunction in HD allows us to explore rather uncharted territories in therapeutic development for this condition. Taking advantage of the discoveries and therapeutics designed to attenuate tau dysfunction in AD (101), as a significant number of preclinical studies and clinical trials have already been initiated, may indeed prove to be useful in HD as well. There is a broad diversity of approaches (**Figure 1**), which include decreasing tau phosphorylation, inhibiting tau aggregation and reducing pathological forms of tau using microtubule stabilizing compounds, immunotherapies or silencing of the *MAPT* gene (**Figure 1**), which could all serve treatment purposes. In the following sections, we present the multiple therapeutic approaches to target tau, describe the treatments that have reached clinical trials and discuss their potential application to HD.

THERAPEUTIC STRATEGIES TO TARGET PATHOLOGICAL FORMS OF TAU

Targeting Tau Hyperphosphorylation

Tau function depends on its phosphorylation state. Hyperphosphorylation generates negatively charged repulsive forces which impede functional interactions with microtubules, leading to their destabilization and subsequent cell death (5, 6, 102). Recent studies have reported an increase in p-tau levels in the cortex and striatum of post-mortem brain tissue derived from HD patients (27, 32). More specifically, hyperphosphorylation of tau has been detected at 5 distinct epitopes—S396, S404, T205, S202, and S199 residues—(32, 42, 43), and S202 and T205 were found in neuronal inclusions (27) (**Table 1**). The association of tau hyperphosphorylation in various forms of tauopathies (4, 103, 104), as well as in HD, suggests that approaches aiming at restoring normal levels of p-tau could improve disease outcome (**Figure 1**); approaches that have already been extensively tested in AD models (105–108). One of the strategies to reduce p-tau levels is to modulate the activity of tau-targeting kinases and phosphatases. The physiological function of the tau protein is facilitated by the orchestrated activity of these enzymes through phosphorylation and dephosphorylation at the threonine and serine residues (109). A number of small molecule inhibitors of protein kinases or activators of phosphatases have been studied in pre-clinical and clinical settings and are presented in the following section.

Kinase Inhibitors

Small molecules, which have the ability to reduce tau hyperphosphorylation, have been among the first tau-targeted treatments developed for AD. This evolved from the evidence that kinases, such as cyclin-dependent-like kinase 5 (CDK5) and glycogen synthase kinase 3 beta (GSK-3β), are altered in patients and animal models of the disease (49, 110–114) (**Table 1**). In a transgenic (Tg) HD mouse model that expresses exon 1 of the human *HTT* gene, with approximately 125 CAG repeats (R6/2) (115), a knock-in chimeric HD mouse model, that expresses a human *HTT* exon 1/ mouse *Htt* with 140 CAG repeats (KI140) (116), as well as in patients, the levels and activity of CDK5 and GSK-3β have been found to be dysregulated (55, 56), suggesting that redirecting kinase inhibitors levels/activity could potentially abrogate tau hyperphosphorylation observed in HD.

CDK5 inhibitors, such as Flavopiridol (Alvocidib) and Roscovitine (Seliciclib), have been tested in AD preclinical studies. Flavopiridol efficiently inhibits CDK5 and improves synaptic plasticity as well as motor behavior in mice injected with Aβ oligomers (117). Roscovitine (Seliciclib) prevents tau phosphorylation in a Niemann-Pick Type C disease mouse model (118), which is characterized by CDK5 dysfunction that triggers tau hyperphosphorylation (119). However, CDK5 inhibitors have yet to be tested in the clinical setting and further investigation is therefore needed to determine if they can, in effect, ameliorate tau-associated pathology in either, or both, AD and HD.

GSK-3β inhibitors form another class of compounds, which have been tested in preclinical and clinical contexts. Among the GSK-3β inhibitors, Tideglusib and lithium have shown improvements in AD Tg mouse models that express either four familial AD mutations (FTDP-17 G272V, P301L, and R406W, referred to as VLW mice and VLW mice overexpressing GSK-3β) (120, 121), three familial AD mutations (APP Swedish, MAPT P301L and PSEN1 M146V) (3xTg-AD) (122), AD-related mutations (APP Swedish crossed with Tau VLW) (108) or the *MAPT* mutation P301L alone (123). Tideglusib and

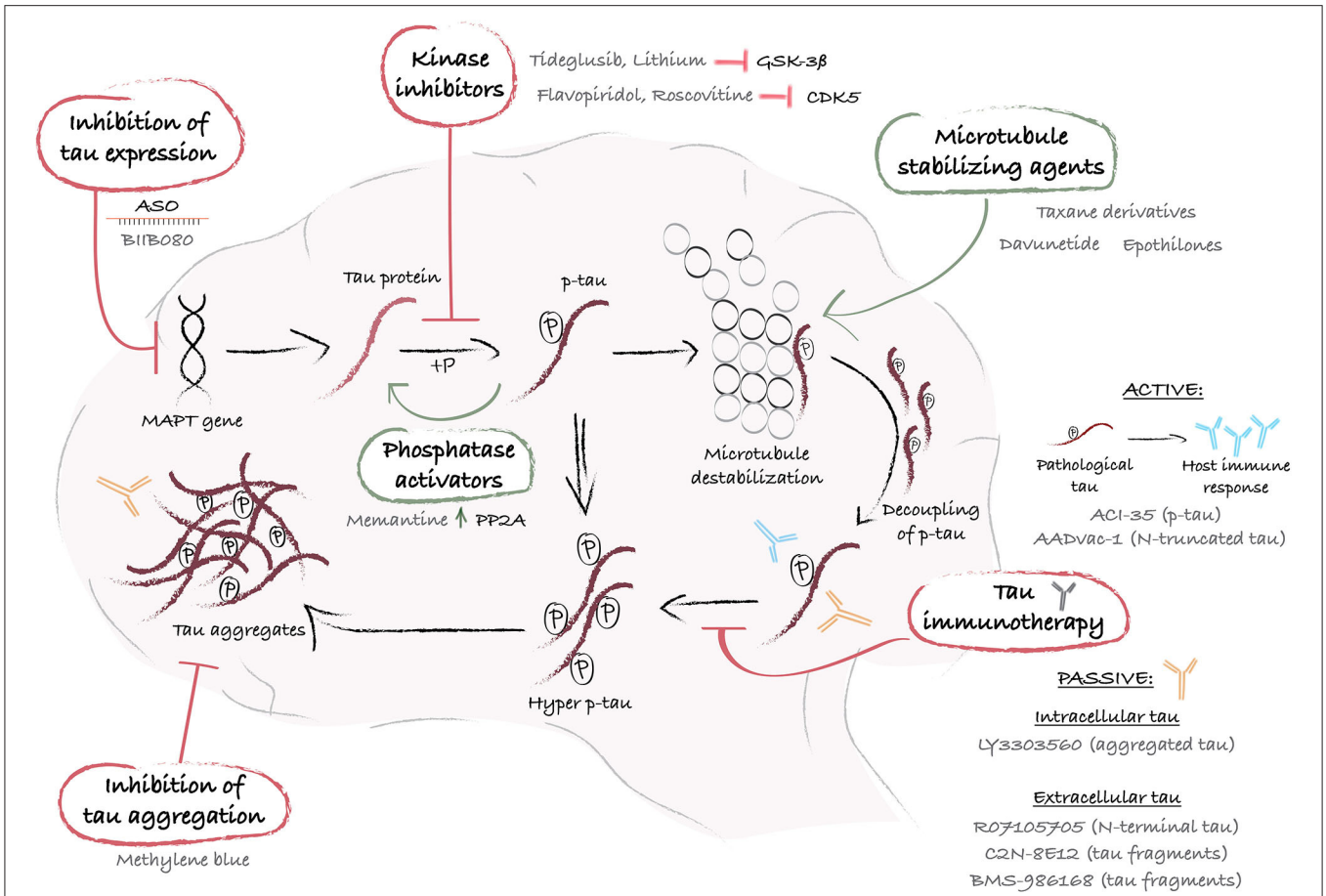


FIGURE 1 | Schematic representation of mechanistic interventions using tau-targeting therapies. The *MAPT* gene encodes for the protein tau, which undergoes post-translational phosphorylation and dephosphorylation that regulate its affinity for microtubules and ensure its functional role as a microtubule stabilizer. When tau undergoes hyperphosphorylation, generally via an abnormal stimulus, it loses its affinity for microtubules and accumulates into the cytoplasm or exits the cells. When released from the microtubules, intracellular hyperphosphorylated tau proteins self-assemble into aggregates of increasing complexity, and ultimately produce large pathological aggregates and NFTs. At each step of the process, drug targets have been identified and a variety of therapeutic approaches have been designed and tested in preclinical and/or clinical studies. A first strategy leverages the use of ASO (e.g. BIIB080) to control *MAPT* gene expression and reduce tau synthesis. Alternatively, pathological tau hyperphosphorylation can be reversed with kinase inhibitors (e.g. Tideglusib or lithium) or phosphatases activators (e.g. Memantine), and tau loss of function can be counterbalanced with microtubule stabilizers (e.g. Taxane derivatives, Davunetide, or Epothilones). Furthermore, immunization-based strategies offer an interesting approach to sequester and degrade p-tau before aggregation, using peptides that mimic a specific p-tau amino acid sequence (e.g. ACI-35 or AADvac-1), active or passive immunization to target either intracellular (e.g. LY3303560) or extracellular tau (e.g. RO7105705, C2N-8E12 or BMS-986168). Lastly, tau aggregation inhibitors, such as Methylene blue, provide an additional means by which to eliminate tau aggregates and restore cellular health. ASO, antisense oligonucleotide; CDK5, cyclin-dependent kinase-5; GSK-3ß, glycogen synthase kinase-3; hyper p-tau, hyperphosphorylated tau; MAPT, microtubule-associated protein tau; P, phosphate; PP2A, protein phosphatase 2A; p-tau, phosphorylated tau.

lithium considerably reduce phosphorylation and aggregation of tau, decrease neuroinflammation and neuronal death and improve learning and memory abilities (108, 120–123). Both drugs have also been tested in phase I and II clinical trials for AD with overall positive results, demonstrating safety, tolerability and improved cognition in comparison to placebo-treated individuals (124) [reviewed in (125)]. The therapeutic potential of lithium has further been evaluated in the R6/2 mouse model and in HD patients, and studies have reported encouraging results (126–128) [reviewed in (129)]. Lithium was found to regulate HD-associated pathological processes such as glutamate excitotoxicity, altered levels of neurotrophic and growth factors and transcriptional dysregulation (130–133) [reviewed in (129)]. For example, lithium increased levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in several brain regions of an animal model of mania generated with ouabain or wild-type animals, as well as in wild-type cultured neurons (133–136). Furthermore, exposure to lithium mediated significant transcriptional changes connected to signal transduction (e.g., mTOR and Wnt-related signaling) in the corpus callosum of wild-type rats (137), as well as transcription factors and genes related to metabolism in the substantia nigra pars compacta of parkinsonian mice (induced by the neurotoxin MPTP) (138). Clinical studies showed that motor abnormalities were not improved in most HD patients treated with lithium, but some reports suggest an amelioration

of cognitive disturbances and mood disorder (139, 140). The positive outcomes on non-motor impairments suggest a potential benefit of lithium treatment in HD individuals, especially for patients in early stages of disease, when cognitive and psychiatric symptoms are predominant. However, it is unclear whether the administration of lithium diminishes GSK-3β activity and p-tau levels, as conflicting findings on GSK-3β levels in post-mortem tissues of HD patients have been reported. Indeed, GSK-3β levels and activity have been shown to be intrinsically decreased in the striatum and cortex of HD individuals (83, 141), while increased GSK-3β levels were measured in the hippocampus (55). These discrepancies may reflect a dynamic molecular response at different disease stages, or suggest the differential alteration of GSK-3β levels in specific brain regions. Evaluation of p-tau in the CSF or imaging with p-tau PET tracers could help identify whether the beneficial effects of lithium are directly related to a reduction of p-tau levels.

Phosphatase Activators

Since tau phosphorylation is a reversible process, triggering tau dephosphorylation at serine and threonine residues could reduce hyperphosphorylation. Phosphatases [described extensively in (57)] catalyze the interconversion reactions of tau, from phosphorylated to dephosphorylated states, thereby regulating the degree of tau phosphorylation. Several studies have demonstrated that the activity of protein phosphatase 2A (PP2A) and serine/threonine-protein phosphatase (PP2B) is decreased in AD mouse models and patients (58, 142) [reviewed in (59)], as well as in both Tg and knock-in HD mouse models (56, 64, 143) (**Table 1**). The Tg mouse line used in the aforementioned studies was the R6/1, which expresses the exon 1 of the human *HTT* gene containing approximately 114 CAG repeats (115) and the R6/2 model, described above. The knock-in mouse lines discussed above were KI140 and zQ175, which respectively express human *HTT* exon 1 with approximately 140 and 188 CAG repeats inserted in the mouse *Htt* (116, 144). Furthermore, PP2A is an important phosphatase known to regulate the activity of the GSK-3β and other kinases implicated in tau pathological modifications (145), and dysregulation of PP2A activity leads to cellular dysfunction including cytoskeletal alterations, impairment of synaptic function and tau mislocalization (146–149). Activation of the phosphatase PP2A has been shown to reduce abnormal phosphorylation of tau in the brain and ameliorate AD pathology (150, 151), suggesting that PP2A phosphatase activators could likely restore physiological levels of tau phosphorylation in patients.

Memantine is a small molecule that has been found to reverse tau hyperphosphorylation. It enhances PP2A activity, improves neuronal viability (152) and reduces glutamate excitotoxicity in GABAergic neurons by acting as a N-methyl-D-aspartate (NMDA) receptor antagonist (153). Furthermore, Memantine mitigates lipopolysaccharide-induced neurodegeneration in mixed rat primary cultures by attenuating the microglial inflammatory response (154). It therefore appears that Memantine is a multifunctional molecule that acts on several cell types and on tau-independent molecular targets, although additional studies are needed to determine if tau directly promotes the beneficial effects of Memantine. Clinical studies indicated that Memantine improves attention, agitation, delusion, global well-being, daily functions and independence in patients with mild cognitive decline (106, 107). There are 87 Memantine clinical trials listed on clinicaltrials.gov, all aiming to treat dementia associated with neurodegenerative diseases, including HD. Thus far, a small pilot (155) and one case study (156) have reported that Memantine improves motor, but not cognitive deficits, in HD patients. However, it may be premature to conclude from these small-scale studies, especially given the fact that both trials were based on the selection of patients in advanced stages of disease that may have further tainted the true potential of Memantine. Hence, Memantine is currently under investigation in the clinical trial MITIGATE-HD, which recruited a large number of pre-manifest HD, early HD and control individuals providing a better opportunity to investigate this compound as a treatment for cognitive impairments. However, the secondary outcome measures of the MITIGATE-HD study do not include CSF t-tau or p-tau nor tau PET imaging evaluation and it will therefore not be possible to determine whether the improvement in cognition, if any, is due to the ability of Memantine to reduce tau hyperphosphorylation.

Views on Targeting Pathological Forms of P-Tau

Although targeting the mechanisms of phosphorylation/dephosphorylation of tau is a seemingly logical strategy, several limitations should be considered. Based on the outcomes of AD clinical trials, the major drawback is the dysregulation of essential kinases and phosphatases responsible for the post-translational modifications of unrelated tau substrates. For instance, GSK-3β phosphorylates more than 100 substrates, while PP2A has more than 300 known targets. Furthermore, both enzymes are themselves regulated by a number of signaling pathways (157, 158). Pharmacological alterations of these complex signaling pathways may lead to activation of compensatory mechanisms, which would in turn generate unpredictable outcomes (158). For example, pharmacological activation of the PP2B phosphatase could promote dephosphorylation of mHTT at serine 421, leading to the disruption of axonal transport and cellular distribution of important neurotrophic factors (159). Small molecules aimed at specifically targeting the multi-substrate enzymes involved in tau phosphorylation are accompanied by decisive limitations, which constrain their use in the treatment of HD and other tauopathies. However, the promising clinical results obtained with lithium and Memantine do warrant further investigation in the context of HD.

Inhibiting the Formation of Tau Aggregates

When hyperphosphorylated tau detaches from the microtubules, it relocates to the somatodendritic compartment and self-assembles into increasingly complex aggregate species. This process begins with soluble oligomers which grow into pre-fibrils and ultimately form insoluble NFTs (66) [reviewed in (160)]. These NFTs accumulate with disease progression in AD (34, 161, 162) as well as in HD (44–46, 99) (**Table 1**), and therapeutic strategies to prevent tau aggregation have therefore become a

major focus of research (**Figure 1**). However, the perspective that accumulation of NFTs causes neurodegeneration has been more recently challenged (163, 164) [reviewed in (165)]. Indeed, mislocalized, soluble misfolded, soluble hyperphosphorylated forms of tau and tau oligomers are emerging as candidate neurotoxic entities (166–168). For example, the mislocalization of tau to dendritic spines has been established in AD and seemingly induces synaptic impairments in rTg4510 and P301S tau mouse models (167, 169–171) as well as the loss of dendritic spines in AD patients (172). Spinal alterations have been observed in R6/2 mice (173), but the relationship between tau and spine instability in HD remains to be elucidated. Additionally, as observed in AD and HD, pre-tangles and NFTs have been identified in several brain regions, predominantly in the putamen, cortex and hippocampus (22, 27, 43, 44, 46, 98–100). It is therefore reasonable to anticipate that inhibiting tau aggregation at early and late stages of fibrillization may improve HD-related neurotoxicity and, on a larger scale, cognitive deficits.

The design of inhibitors of tau aggregation has already generated a significant number of small molecules for drug screening. Among 3,000 anti-tau aggregation molecules studied, fibrillization inhibitors have been identified as the most effective in preventing the formation of NFTs in Tg animals expressing mutated human tau [reviewed in (174)]. For example, Methylene Blue and its derivatives have been shown to stabilize tau in a conformation that prevents its fibrillization (175), but other neurotoxic forms of tau, such as oligomers, do not appear to be affected (164, 176). Nonetheless, treatment of JNPL3 Tg mice expressing the P301L tau mutation results in the amelioration of cognitive deficits (177) and Methylene Blue is the only tau-targeting treatment that has reached phase III in AD. Importantly, this drug can inhibit the aggregation of other self-assembling proteins including mHTT (178), decrease the formation of mHTT inclusion bodies in primary mouse neurons and R6/2 mice, and improve motor deficits in these animals (178).

Views on Inhibiting the Formation of Tau Aggregates

The current state of the field offers histological evidence of tau aggregation in post-mortem HD brain tissue, but available experimental animal models do not recapitulate pathological tau inclusions. Establishing alternative models that reproduce key features and molecular dysfunctions of HD is a prerequisite to evaluate the contributions of tau aggregates to pathology as well as the potential of anti-tau aggregation therapies. Additionally, it is becoming increasingly clear that pathological forms do not solely consist of NFTs, but include aggregate intermediates and soluble post-translationally modified tau. Considering the similarities among tauopathies, an efficient therapeutic approach for HD should therefore target multiple tau species, including oligomers and hyperphosphorylated forms of the protein (27).

Tau Immunotherapies

Tau-based immunotherapies refer to the neutralization and clearance of the tau protein by host-generated antibodies (active immunization) or by the administration of tau-specific antibodies (passive immunization). Immunotherapies can be designed to target a variety of tau species, including t-tau, hyperphosphorylated tau, extracellular tau, oligomeric tau or tau fragments (**Figure 1**) (165, 179–181). This approach provides greater precision and flexibility to therapeutic designs, but a successful clinical outcome relies on the identification of the exact pathological forms of tau responsible for a specific phenotype. Multiple forms of tau are potential candidates for immunotherapies in HD, including tau aggregates (27), p-tau (27, 32), tau oligomers (27) and caspase-2 cleaved Δtau314 (84). Each form appears to be associated with distinct disease phenotypes/stages, and tau hyperphosphorylation at S396, S404, T205, and S199 epitopes has been observed in stage 4 HD patients (32). The presence of tau oligomers (T22 and TOMA positive staining) has also been detected in the putamen of stage 4 HD patients (27). Caspase-2 cleaved Δtau314 protein is a form of tau associated with dementia in Lewy body disease (182) and is found in greater concentrations in the caudate nucleus and prefrontal cortex of HD subjects when compared to healthy controls (84). The following paragraphs will survey the available literature to propose additional therapeutic frameworks for the next generation of HD drugs.

Active Immunization

Active immunization by exposure of the host immune system to pathological forms of tau has also been considered to induce a long-lasting anti-tau immunity. Several peptides have been designed using tau pathological forms such as p-tau (181, 183), oligomers (180, 184) and truncated tau (185). Immunization of animals recapitulating AD features can result in pathophysiological and behavioral improvements (181), with the host-generated antibodies depict specific recognition of p-tau (181). Based on these findings, two anti-tau vaccines have been tested in AD clinical trials; one targeting S396 and S404 phosphorylated tau (ACI-35) (186) and the other targeting the pathological N-truncated form of tau (AADvac-1 or Axon peptide 108 conjugated to keyhole limpet hemocyanin) (187). Importantly, the ACI-35 vaccine was designed against pathological forms of tau also found in HD patients and further investigations could establish its potential as a candidate immunotherapy to ameliorate cognitive deficits in this patient population (27, 99).

Passive Immunization

Passive immunization is achieved by administering pre-formed antibodies to recognize a specific antigen and constitutes an alternative immunization approach that does not solely rely on the immune system of the host. Antibodies can further be engineered to express desirable properties and bind tau within intracellular and/or extracellular compartments. Several antibodies have been designed to target various forms of tau and the promising antibodies that have reached clinical trials are discussed below.

Passive immunization to target intracellular tau

Following peripheral administration, anti-tau antibodies are able to cross the blood brain barrier to reach neuronal and non-neuronal elements. The antibodies are internalized by neurons via receptor-mediated endocytosis (188), bind tau and the emerging tau-antibody complex is subsequently degraded

through the proteasomal system (189). A significant number of tau passive immunotherapies have been designed to target p-tau, and the anti-tau pS202 monoclonal antibody (named CP13) has thus far demonstrated a superior efficacy compared to other anti-p-tau antibodies (190). Indeed, CP13 reduces soluble and insoluble total and p-tau levels in the cortex and hindbrain of a Tg mouse model expressing the human P301L mutation and which is characterized by a severe tauopathy (190). In HD, increased pS202 levels correlate with alterations in tau phosphorylation in both R6/2 and zQ175 mouse models (56, 64), as well as in patients (27, 42, 43). In addition to p-tau, antibodies have been designed to target other pathological forms of the protein. The humanized antibody LY3303560 (also referred to as Zagotenemab) is derived from a monoclonal antibody used in histology (MC1) to identify pathological conformations of tau (191) in both AD (191) and HD human brain tissue (33). It has also demonstrated greater affinity toward soluble tau aggregates compared to tau monomers *in vitro* (179). This immunotherapy is safe and well-tolerated in humans, as established by the successful completion of a phase I and an ongoing phase II clinical trial evaluating its efficacy in AD (192). CP13 and LY3303560 are therefore attractive immunotherapy candidates that could be tested for efficacy in preclinical models of HD. Animal models such as zQ175 or KI140 mice recapitulate a slow disease progression and the gradual acquisition of molecular and behavioral HD phenotypes, and could therefore provide a suitable experimental system to evaluate the benefits of immunotherapies on HD-related cognitive as well as motor deficits (116, 144).

Passive immunization to target extracellular tau

In addition to neurotoxic effects resulting from post-translational modifications, tau has been suggested to adopt prion-like properties that result in its transcellular propagation through the extracellular milieu, to ultimately seed pathology in healthy recipient cells (193). Evidence from cellular (194, 195) and animal models (39–41), as well as post-mortem tissue (36, 161, 162), support this hypothesis (**Table 1**). For example, a single injection of AD brain homogenate in the cortex or hippocampus of a naïve mouse induces endogenous tau misfolding and aggregation with detrimental consequences on physiological functions and behavior (41). Extracellular tau is particularly efficient in corrupting functional endogenous tau and initiating pathological aggregates that lead to the formation of NFTs (196). A number of mechanisms of tau spreading have been identified and include cytoplasmic exchange by tunneling nanotubes (197), as well as transsynaptic (198) or transcellular propagation via exocytosis and endocytosis (194, 195, 199). Several antibodies targeting extracellular tau have been designed to prevent transcellular propagation and seeding of tau-related pathology. Antibodies are now available to recognize a diversity of extracellular tau isoforms and fragments. The antibody C2N-8E12 (also referred to as ABBV-8E12) recognizes the isoform tau-F (441 aa) (200), while the antibody RO7105705 (also referred to as Semorinemab) binds the N-terminus of all six tau isoforms (201). Gosuranemab (also referred to as BMS-986168) was engineered using a tau fragment released into the conditioned media prepared from AD patient-derived cortical neurons (202, 203). Despite differences in the targeted epitopes, all of these antibodies have been shown to ameliorate tau pathology and behavioral deficits in mouse models that express *MAPT* mutations (JNPL3, P301L and P301S) (203–206). These observations suggest that reducing extracellular levels of tau is a promising strategy to improve AD pathology. Favorable to their use is the fact that antibodies tested in phase I clinical trial have met safety and tolerability criteria (207) [reviewed in (208)]. Similar routes of tau propagation have been suggested to occur in HD patients who received fetal grafts to replace cell loss generated by the disease process (42). However, these observations originate from post-mortem analyses on a few rare cases and more definitive evidence is needed to conclude that tau can indeed propagate in the HD brain. Furthermore, HD patients have lower concentrations of t-tau in the CSF in comparison to AD affected individuals, (96, 97, 209–212), and whether CSF t-tau levels truly reflect extracellular tau load in the central nervous system (CNS) of HD patients remains to be elucidated.

Views on Tau Immunotherapies

Research in the field of immunotherapy has made significant progress in the development of anti-tau treatments for AD and is gaining momentum in HD [reviewed in (101, 213)]. Antibody-based therapies have the distinct advantage of being highly specific to the selected epitopes and can target intracellular or extracellular tau both in the periphery and in the CNS. Antibodies have a low molecular weight and are therefore suitable for nanocarrier-based delivery approaches to reduce their degradation, mitigate the host immune response and control the rate of antibody release (214). Furthermore, administration of tau-targeting antibodies by injection is a straightforward and safe procedure (207). Based on the concept that mHTT can propagate between cells and template pathology in a prion-like fashion, active and passive immunization strategies targeting extracellular mHTT are attracting interest (215–222) [extensively reviewed in (213)], and could be tested in parallel to a tau, or mHTT/tau combined immunization. Furthermore, halting accumulation and propagation of pathogenic proteins in HD patients at early stages of the disease, before they are afflicted by neurodegeneration and cognitive dysfunction, may provide the most effective protection.

However, a major limitation of current studies is that the majority of tau-related dysregulations have not been reported in premanifest or early-stage diseased patients. As a result, the association of pathological forms of tau with late stage disease could cast doubts on the validity of targeting tau in HD (32). A broad investigation on a larger population of HD patients, matched for age, sex and CAG repeat length, would provide more accurate information on the progression of tau pathology. In particular, future studies could take advantage of the technological advances achieved with PET scans and specific anti-tau ligands (223), to enable the analysis of pathological tau in the brain of living patients with a degree of specificity that is not possible with post-mortem analyses. By doing so, tau abnormalities could be directly compared to cognitive performance and importantly, changes over time. For instance,

the PET ligand [^{18}F]MK-6240, which has already been validated in AD, is a new generation tau ligand with high sensitivity for NFTs and negligible off-target binding (224), which makes it ideal for defining the extent of tau pathology in HD brains and investigating its relationship with cognitive profile.

MITIGATION OF TAU-INDUCED CELLULAR ALTERATIONS BY STABILIZING MICROTUBULES

Structural and functional damage to the neuronal cytoskeleton constitutes a key event in the pathogenesis of tauopathies (225–227). Therefore, microtubule-stabilizing small molecules have been developed and studied as a therapeutic approach to treat tauopathies (**Figure 1**). Alterations of microtubule-dependent axonal transport is a characteristic of HD pathology (78, 79) and could result from the independent, but coordinated effects of mHTT and tau (**Table 1**). mHTT has been shown to interact with and destabilize microtubules, resulting in alterations of axonal transport and loss of neuronal viability (78, 79, 228). Tau has further been found to recruit mHTT to the microtubule network (56), which could precipitate the destabilization of microtubules. For example, microtubule stabilization using the small molecule Taxol, a semisynthetic taxane derivative, inhibits the entry of mHTT into the nucleus and increases neuronal survival in HD primary striatal and cortical neurons (78). These observations suggest that tau and mHTT can both induce microtubule destabilization, which support the restoration of microtubule functions as a therapeutic approach for HD.

The cytoskeleton stabilizing agents Epothilone and Taxane derivatives have been found to interact with tubulin, restore rapid axonal transport, and alleviate cognitive and motor impairments in mouse models of tauopathies (T44 tau Tg mice; PS19 tau Tg mice) (71, 229). However, the Epothilone D phase I clinical trial was discontinued due to toxicity and severe side-effects were reported for taxoid TPI 287 (abeotaxane) (230). On the other hand, the microtubule-stabilizing peptide Davunetide (NAP) (231, 232) was well-tolerated in preclinical toxicology and clinical safety studies (233). NAP demonstrated highly potent neuroprotective properties by reducing the activity of the microtubule-severing protein katanin (234), inhibiting programmed cell death and restoring mitochondrial function in the PD-related A53T α-synuclein SH-SY5Y cell culture model (235). In a Tg schizophrenia mouse model (activity-dependent neuroprotective protein (ADNP)$^{+/-}$ Tg mice), intranasal administration of NAP decreased brain levels of p-tau and improved cognition (236). The treatment of patients suffering from mild cognitive impairments with NAP showed improved attention and working memory (233). However, no improvement of cognitive deficits was observed in Progressive Supranuclear Palsy (PSP) patients treated with NAP, which could be partly explained by an ineffective dosage (237).

Views on Stabilizing Microtubules

The regulation and restoration of microtubule function is an attractive strategy substantiated by promising preclinical results. Epothilone and Taxane derivatives were approved by the FDA in the early 1990s [history reviewed in (238)] as chemotherapies to treat aggressive cancers and despite significant side-effects, they are among the most relied upon therapies to treat solid tumors (239, 240). In the case of neurodegenerative diseases, the toxicity of microtubule-stabilizing agents is a major drawback that may limit their clinical use, altogether. However, new microtubules stabilizing agents such as Davunetide and others [reviewed in (241)] exhibit promising disease-mitigating outcomes in animal models, with more manageable side-effects in clinical trials, and could therefore be the new frontier in the search for microtubules stabilizing strategies to ameliorate tau pathology in neurodegenerative diseases, including HD.

MODULATION OF *MAPT* GENE EXPRESSION

Mutations in the *MAPT* gene have been associated with neurodegenerative diseases such as Frontotemporal dementia (20, 242–245), PSP (245) and AD (17–21); diseases characterized by abnormal accumulation of pathological forms of tau and that further correlate with cognitive deficits. Impaired alternative splicing of exon 10 of the *MAPT* gene leads to an imbalance in the amount of 3R and 4R tau isoforms within cells, and a change in 3R to 4R ratio has been associated with specific tauopathies. For instance, NFTs are found to be immunopositive for both 4R and 3R in AD (28), while patients with Pick's disease predominantly express the 3R tau isoform (246). As a result, temporarily altering *MAPT* expression or splicing using antisense oligonucleotides (ASO) has been considered (**Figure 1**) (247). To our knowledge, no *MAPT* mutations have been associated with HD, but patients express lower 3R and increased 4R tau mRNA and protein levels (32) and the 4R isoform is enriched in tau nuclear rods found in the striatum (31). Therefore, selectively reducing the levels of 4R tau without altering the overall expression of tau protein in HD could restore protein homeostasis (31, 32).

Tau is involved in essential cellular functions (248) and as a result, tau silencing could raise safety concerns that would limit its translation to the clinic. Studies in tau knock-out animal models have demonstrated that loss of *Mapt* causes cognitive and motor deficits in an age- and strain-dependent manner (249). A marked reduction in the number of dopaminergic neurons was observed in the substantia nigra pars compacta of *Mapt* $^{-/-}$ mice, which correlated with PD-like motor deficits in 12-month old animals (249, 250). However, the partial or total downregulation of the *Mapt* gene in the R6/1 Tg (115 CAG repeats) HD mouse model improves motor behavior and does not induce significant side-effects (31). Furthermore, the reduction of tau protein levels using the ASO BIIB080 prevents NFTs deposition and clears pre-existing tau aggregates, mitigates neuronal loss, ameliorates behavior (nesting) and extends the survival of PS19 Tg mice expressing the disease-associated P301S tau mutation (251). In non-human primates, ASO BIIB080 reduces tau mRNA and protein levels in the brain, spinal cord and CSF (251). These promising results enabled the advancement of ASO BIIB080 to phase I clinical trial that is currently ongoing (252).

Views on Modulating *MAPT* Gene Expression

Modulation of gene expression is a novel therapeutic avenue to treat tauopathies and mRNA-targeting strategies using ASO have recently been proposed as a potential treatment for HD (253). The preliminary results of a clinical trial on IONIS-HTTRx, an ASO drug that targets *HTT*, reported a sharp decrease in mHTT CSF levels with no serious adverse events (254, 255). To our knowledge, silencing more than one gene via ASOs has never been tested, but lowering both HTT and tau expression levels in HD patients has recently been proposed (256). However, gene silencing and modification of gene expression are novel technologies with only a few treatments approved for clinical use. For instance Fomivirsen, an antisense antiviral drug for the treatment of retinitis cytomegalovirus, was the first antisense drug approved by the FDA in 1998 (257), and the second ASO therapy to reach the market (Mipomersen, to treat familial hypercholesterolemia) was FDA approved only 15 years later. As of today, little is known about the possible long-term consequences of modulating protein levels with ASOs, and it is essential to extensively investigate whether modulation of both *MAPT* and *HTT* expression could ameliorate disease outcome in HD patients without inducing long-lasting side-effects.

ADDITIONAL TAU-TARGETING APPROACHES

In addition to phosphorylation, tau can undergo a number of post-translational modifications such as glycosylation (9), acetylation (7), truncation (258), glycation (259), nitration (260) and ubiquitination (261) [reviewed in (262)]. These modifications have been associated with tauopathies and mainly investigated in AD brains, with observation of increases in acetylated (7), caspase-truncated (263) and N-glycosylated forms of tau (9). Several molecules have been tested in preclinical and clinical studies to target these different disease-associated post-translational modifications. For example, Salsalate is a nonsteroidal anti-inflammatory drug that decreases t-tau levels and acetylated tau at the K174 residue, ameliorates hippocampal atrophy and memory deficits in PS19 Tg mice (8), and has consequently advanced to phase 1 PSP clinical trial (264). It remains undetermined whether tau post-translational modifications, such as acetylation or glycosylation, occur in HD and only a broader understanding of all forms of tau alterations in the disease would support the exploration of these additional tau-targeting approaches.

Another aspect of pathology is the impairment of protein degradation via alterations of the UPS, which has been observed and associated with tauopathies, and proposed to mediate the accumulation of tau within cells (265, 266) [reviewed in (267)]. The search for drugs that increase the degradation of tau by activating the UPS led to the identification of Rolipram (268). Rolipram has been found to activate the UPS by stimulating the protein kinase A (PKA)/cAMP pathway in *ex vivo* cortical brain slices of rTg4510 mice and to reduce both t-tau and insoluble tau levels (266). Rolipram also decreases t-tau and p-tau levels in a Tg mouse model of early-stage tauopathy expressing the proteasome 26S-targeted fragment (rTg4510:Ub-G76V-GFP) and improves reversal learning behavior in experimental animals (266).

Disease-associated post-translational modifications of tau, as well as dysfunction of the UPS, are compelling alternatives to explore for the treatment of tauopathies. In contrast to the therapeutic strategies discussed in sections *Therapeutic Strategies to Target Pathological Forms of Tau, Mitigation of tau-induced cellular alterations by stabilizing microtubules, Modulation of MAPT Gene Expression* of this review, the relevance of these pathways remains to be demonstrated in models of HD, and the functional and mechanistic contributions of UPS dysfunction to HD pathology are unclear (269). HD-related alterations of the UPS have been hinted at, with early studies showing sequestration of UPS components within mHTT inclusion bodies (87, 270) in Tg HD mouse models (R6/1, R6/2 and R6/5 Tg - 130-155 CAG repeats) (87) as well as in human brain tissue (270). However, further characterization suggested that UPS impairments could be a transient phenotype, as global UPS activity was not significantly impaired in the R6/2 model and in the HD94 mouse model that expresses mHTT fragments under a tetracycline-responsive system (271, 272). An explanation for these seemingly contradictory findings was proposed by Schipper-Krom et al. who suggested that proteasome recruitment into inclusion bodies is a dynamic and reversible process, that does not inhibit the catalytic activity of the sequestered proteasome units (273), thus supporting observations that the UPS activity may not be impaired in HD mouse models. Nonetheless, pharmacological and genetic modulation of the proteasome activity suggest its implication in mHTT aggregation and cell survival, as a reduced activity is associated with increased aggregation (88, 274) and increased activity promotes the survival of HD striatal neurons (275).

CONCLUSION

HD patients and their families carry a heavy emotional and financial burden as they face the challenges of a very complex disease, which manifests with a blend of motor, neuropsychiatric and cognitive deficits. Efficient symptomatic treatments, and more importantly disease-modifying therapies, are urgently needed. The diversity of symptoms and difficulty to target the root of the disease, a mutation in the *HTT* gene, suggests that combinatorial therapies that attenuate multiple dysfunctional proteins and pathways may ultimately be the best strategy to tackle the complex portrait of HD (**Table 1**). Here, we have reviewed compelling evidence suggesting that HD patients develop features of tauopathies and tau-related dysfunctions. These include the (i) altered exon 10 splicing of *MAPT* (31, 32), (ii) correlations between H2 *MAPT* haplotype and severity of cognitive decline (27), (iii) dysfunction of tau-targeting kinases and phosphatases (55, 56, 64, 83), (iv) increased soluble hyperphosphorylated tau (32), (v) altered levels of t-tau in the brain and CSF (32, 96, 97, 209), and

(vi) presence of brain NFTs (27, 98, 99). The accumulating evidence of tau pathology encompasses broad dysregulations at the genetic and molecular levels, in CNS tissue and associated biological fluids, and therefore argue in favor of the classification of HD as a secondary tauopathy. In-depth studies are now needed to determine if tau dysfunction directly causes some of the features associated with HD, and in particular cognitive deficits.

Therapeutic approaches targeting tau pathology have been initially designed to treat AD (**Figure 1**) (101) or primary tauopathies such as PSP (276), based on a large body of evidence suggesting a strong connection between alterations of tau function and cognitive decline [extensively reviewed in (277)]. HD pathology is also associated with both pathological forms of tau and cognitive impairments, thus tau-targeting therapies may offer a completely new angle to treat HD-associated cognitive dysfunction. A diversity of tau-targeting methodologies has been developed, from small molecules to immunotherapies and modulation of gene expression using ASO. These therapies are at various stages of drug development, providing exciting and hopeful prospects toward the betterment of the HD condition.

AUTHOR CONTRIBUTIONS

MM and SS contributed equally to reviewing the literature and writing the first draft of the manuscript. ARJ, MA, and FC contributed to discussions regarding the manuscript, revised, and finalized the review. All authors contributed to the article and approved the submitted version.

FUNDING

FC is a recipient of a Researcher Chair from the Fonds de Recherche du Québec en Santé (FRQS, 35059) providing salary support and operating funds, and receives funding from the Canadian Institutes of Health Research (CIHR, PJT-162164 and PJT-168865) to conduct her HD-related research. At the time of the study, MM was supported by a *Desjardins bourse de prestige Didier Mouginot* from the Fondation du CHU de Québec and a *bourse d'excellence du Centre Thématique de Recherche en Neurosciences* also from CHU de Québec. SS holds a *Bourse de formation Desjardins pour la recherche et innovation* from CHU de Québec, and MA is a recipient of a postdoctoral fellowship from FRQS.

REFERENCES

1. Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, et al. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature.* (1994) 369:488–91. doi: 10.1038/369488a0
2. Sydow A, Van der Jeugd A, Zheng F, Ahmed T, Balschun D, Petrova O, et al. Tau-induced defects in synaptic plasticity, learning, and memory are reversible in transgenic mice after switching off the toxic tau mutant. *J Neurosci.* (2011) 31:2511–25. doi: 10.1523/JNEUROSCI.5245-10.2011
3. Wang J-Z, Liu F. Microtubule-associated protein tau in development, degeneration and protection of neurons. *Prog Neurobiol.* (2008) 85:148–75. doi: 10.1016/j.pneurobio.2008.03.002
4. Alonso AD, Cohen LS, Corbo C, Morozova V, ElIdrissi A, Phillips G, et al. Hyperphosphorylation of tau associates with changes in its function beyond microtubule stability. *Front Cell Neurosci.* (2018) 12:338. doi: 10.3389/fncel.2018.00338
5. Jameson L, Frey T, Zeeberg B, Dalldorf F, Caplow M. Inhibition of microtubule assembly by phosphorylation of microtubule-associated proteins. *Biochemistry.* (1980) 19:2472–9. doi: 10.1021/bi00552a027
6. Lindwall G, Cole RD. Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J Biol Chem.* (1984) 259:5301–5.
7. Min S-W, Cho S-H, Zhou Y, Schroeder S, Haroutunian V, Seeley WW, et al. Acetylation of tau inhibits its degradation and contributes to tauopathy. *Neuron.* (2010) 67:953–66. doi: 10.1016/j.neuron.2010.08.044
8. Min S-W, Chen X, Tracy TE, Li Y, Zhou Y, Wang C, et al. Critical role of acetylation in tau-mediated neurodegeneration and cognitive deficits. *Nat Med.* (2015) 21:1154–62. doi: 10.1038/nm.3951
9. Wang JZ, Grundke-Iqbal I, Iqbal K. Glycosylation of microtubule-associated protein tau: an abnormal posttranslational modification in Alzheimer's disease. *Nat Med.* (1996) 2:871–5. doi: 10.1038/nm0896-871
10. Arnold CS, Johnson GV, Cole RN, Dong DL, Lee M, Hart GW. The microtubule-associated protein tau is extensively modified with O-linked N-acetylglucosamine. *J Biol Chem.* (1996) 271:28741–4. doi: 10.1074/jbc.271.46.28741
11. Horiguchi T, Uryu K, Giasson BI, Ischiropoulos H, LightFoot R, Bellmann C, et al. Nitration of tau protein is linked to neurodegeneration in tauopathies. *Am J Pathol.* (2003) 163:1021–31. doi: 10.1016/S0002-9440(10)63462-1
12. Dorval V, Fraser PE. Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and α-synuclein. *J Biol Chem.* (2006) 281:9919–24. doi: 10.1074/jbc.M510127200
13. Novak M, Kabat J, Wischik CM. Molecular characterization of the minimal protease resistant tau unit of the Alzheimer's disease paired helical filament. *EMBO J.* (1993) 12:365–70. doi: 10.1002/j.1460-2075.1993.tb05665.x
14. Mudher A, Brion J-P, Avila J, Medina M, Buée L. EuroTau: towing scientists to tau without tautology. *Acta Neuropathol Commun.* (2017) 5:90. doi: 10.1186/s40478-017-0491-z
15. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature.* (1998) 393:702–5. doi: 10.1038/31508
16. Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci USA.* (1998) 95:7737–41. doi: 10.1073/pnas.95.13.7737
17. Sala Frigerio C, Lau P, Troakes C, Deramecourt V, Gele P, Van Loo P, et al. On the identification of low allele frequency mosaic mutations in the brains of Alzheimer's disease patients. *Alzheimers Dement.* (2015) 11:1265–76. doi: 10.1016/j.jalz.2015.02.007
18. Jin SC, Pastor P, Cooper B, Cervantes S, Benitez BA, Razquin C, et al. Pooled-DNA sequencing identifies novel causative variants in PSEN1, GRN and MAPT in a clinical early-onset and familial Alzheimer's disease Ibero-American cohort. *Alzheimers Res Ther.* (2012) 4:34. doi: 10.1186/alzrt137
19. Momeni P, Pittman A, Lashley T, Vandrovcova J, Malzer E, Luk C, et al. Clinical and pathological features of an Alzheimer's disease patient with the MAPT Delta K280 mutation. *Neurobiol Aging.* (2009) 30:388–93. doi: 10.1016/j.neurobiolaging.2007.07.013
20. Coppola G, Chinnathambi S, Lee JJ, Dombroski BA, Baker MC, Soto-Ortolaza AI, et al. Evidence for a role of the rare p.A152T variant in MAPT in increasing the risk for FTD-spectrum and Alzheimer's diseases. *Hum Mol Genet.* (2012) 21:3500–12. doi: 10.1093/hmg/dds161
21. Schulte EC, Fukumori A, Mollenhauer B, Hor H, Arzberger T, Perneczky R, et al. Rare variants in β-Amyloid precursor protein (APP) and Parkinson's disease. *Eur J Hum Genet.* (2015) 23:1328–33. doi: 10.1038/ejhg.2014.300
22. Myers AJ, Kaleem M, Marlowe L, Pittman AM, Lees AJ, Fung HC, et al. The H1c haplotype at the MAPT locus is associated with Alzheimer's disease. *Hum Mol Genet.* (2005) 14:2399–404. doi: 10.1093/hmg/ddi241

23. Zhang C-C, Zhu J-X, Wan Y, Tan L, Wang H-F, Yu J-T, et al. Meta-analysis of the association between variants in MAPT and neurodegenerative diseases. *Oncotarget.* (2017) 8:44994–5007. doi: 10.18632/oncotarget.16690
24. Pastor P, Moreno F, Clarimón J, Ruiz A, Combarros O, Calero M, et al. MAPT H1 haplotype is associated with late-onset Alzheimer's Disease risk in APOEε4 noncarriers: results from the dementia genetics Spanish consortium. *J Alzheimers Dis.* (2016) 49:343–52. doi: 10.3233/JAD-150555
25. Sánchez-Juan P, Moreno S, de Rojas I, Hernández I, Valero S, Alegret M, et al. The MAPT H1 haplotype is a risk factor for Alzheimer's Disease in APOE ε4 non-carriers. *Front Aging Neurosci.* (2019) 11:327. doi: 10.3389/fnagi.2019.00327/full
26. Moss DJH, Pardiñas AF, Langbehn D, Lo K, Leavitt BR, Roos R, et al. Identification of genetic variants associated with Huntington's disease progression: a genome-wide association study. *Lancet Neurol.* (2017) 16:701–11. doi: 10.1016/S1474-4422(17)30161-8
27. Vuono R, Winder-Rhodes S, de Silva R, Cisbani G, Drouin-Ouellet J, REGISTRY Investigators of the European Huntington's Disease Network, et al. The role of tau in the pathological process and clinical expression of Huntington's disease. *Brain.* (2015) 138(Pt 7):1907–18. doi: 10.1093/brain/awv107
28. Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron.* (1992) 8:159–68. doi: 10.1016/0896-6273(92)90117-V
29. Uchihara T, Hara M, Nakamura A, Hirokawa K. Tangle evolution linked to differential 3- and 4-repeat tau isoform deposition: a double immunofluorolabeling study using two monoclonal antibodies. *Histochem Cell Biol.* (2012) 137:261–7. doi: 10.1007/s00418-011-0891-2
30. Uematsu M, Nakamura A, Ebashi M, Hirokawa K, Takahashi R, Uchihara T. Brainstem tau pathology in Alzheimer's disease is characterized by increase of three repeat tau and independent of amyloid β. *Acta Neuropathol Commun.* (2018) 6:1. doi: 10.1186/s40478-017-0501-1
31. Fernández-Nogales M, Cabrera JR, Santos-Galindo M, Hoozemans JJM, Ferrer I, Rozemuller AJM, et al. Huntington's disease is a four-repeat tauopathy with tau nuclear rods. *Nat Med.* (2014) 20:881–5. doi: 10.1038/nm.3617
32. St-Amour I, Turgeon A, Goupil C, Planel E, Hébert SS. Co-occurrence of mixed proteinopathies in late-stage Huntington's disease. *Acta Neuropathol.* (2018) 135:249–65. doi: 10.1007/s00401-017-1786-7
33. Gratuze M, Cisbani G, Cicchetti F, Planel E. Is Huntington's disease a tauopathy? *Brain.* (2016) 139(Pt 4):1014–25. doi: 10.1093/brain/aww021
34. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology.* (1992) 42(3 Pt 1):631–9. doi: 10.1212/WNL.42.3.631
35. Ossenkoppele R, Schonhaut DR, Schöll M, Lockhart SN, Ayakta N, Baker SL, et al. Tau PET patterns mirror clinical and neuroanatomical variability in Alzheimer's disease. *Brain.* (2016) 139(Pt 5):1551–67. doi: 10.1093/brain/aww027
36. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* (1991) 82:239–59. doi: 10.1007/BF00308809
37. Hallinan GI, Vargas-Caballero M, West J, Deinhardt K. Tau misfolding efficiently propagates between individual intact hippocampal neurons. *J Neurosci.* (2019) 39:9623–32. doi: 10.1523/JNEUROSCI.1590-19.2019
38. Wu JW, Hussaini SA, Bastille IM, Rodriguez GA, Mrejeru A, Rilett K, et al. Neuronal activity enhances tau propagation and tau pathology *in vivo*. *Nat Neurosci.* (2016) 19:1085–92. doi: 10.1038/nn.4328
39. de Calignon A, Polydoro M, Suárez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron.* (2012) 73:685–97. doi: 10.1016/j.neuron.2011.11.033
40. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol.* (2009) 11:909–13. doi: 10.1038/ncb1901
41. Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. *Proc Natl Acad Sci USA.* (2013) 110:9535–40. doi: 10.1073/pnas.1301175110
42. Cisbani G, Maxan A, Kordower JH, Planel E, Freeman TB, Cicchetti F. Presence of tau pathology within foetal neural allografts in patients with Huntington's and Parkinson's disease. *Brain.* (2017) 140:2982–92. doi: 10.1093/brain/awx255
43. Jellinger KA. Alzheimer-type lesions in Huntington's disease. *J Neural Transm.* (1998) 105:787–99. doi: 10.1007/s007020050095
44. McIntosh GC, Jameson HD, Markesbery WR. Huntington disease associated with Alzheimer disease. *Ann Neurol.* (1978) 3:545–8. doi: 10.1002/ana.410030616
45. Myers RH, Sax DS, Schoenfeld M, Bird ED, Wolf PA, Vonsattel JP, et al. Late onset of Huntington's disease. *J Neurol Neurosurg Psychiatry.* (1985) 48:530–4. doi: 10.1136/jnnp.48.6.530
46. Reyes MG, Gibbons S. Dementia of the Alzheimer's type and Huntington's disease. *Neurology.* (1985) 35:273–7. doi: 10.1212/WNL.35.2.273
47. Hye A, Kerr F, Archer N, Foy C, Poppe M, Brown R, et al. Glycogen synthase kinase-3 is increased in white cells early in Alzheimer's disease. *Neurosci Lett.* (2005) 373:1–4. doi: 10.1016/j.neulet.2004.10.031
48. Pei JJ, Tanaka T, Tung YC, Braak E, Iqbal K, Grundke-Iqbal I. Distribution, levels, and activity of glycogen synthase kinase-3 in the Alzheimer disease brain. *J Neuropathol Exp Neurol.* (1997) 56:70–8. doi: 10.1097/00005072-199701000-00007
49. Swatton JE, Sellers LA, Faull RLM, Holland A, Iritani S, Bahn S. Increased MAP kinase activity in Alzheimer's and Down syndrome but not in schizophrenia human brain. *Eur J Neurosci.* (2004) 19:2711–9. doi: 10.1111/j.0953-816X.2004.03365.x
50. Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P, Tsai LH. Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature.* (1999) 402:615–22. doi: 10.1038/45159
51. Yasojima K, Kuret J, DeMaggio AJ, McGeer E, McGeer PL. Casein kinase 1 delta mRNA is upregulated in Alzheimer disease brain. *Brain Res.* (2000) 865:116–20. doi: 10.1016/S0006-8993(00)02200-9
52. Kimura R, Kamino K, Yamamoto M, Nuripa A, Kida T, Kazui H, et al. The DYRK1A gene, encoded in chromosome 21 Down syndrome critical region, bridges between beta-amyloid production and tau phosphorylation in Alzheimer disease. *Hum Mol Genet.* (2007) 16:15–23. doi: 10.1093/hmg/ddl437
53. Ryoo S-R, Cho H-J, Lee H-W, Jeong HK, Radnaabazar C, Kim Y-S, et al. Dual-specificity tyrosine(Y)-phosphorylation regulated kinase 1A-mediated phosphorylation of amyloid precursor protein: evidence for a functional link between down syndrome and Alzheimer's disease. *J Neurochem.* (2008) 104:1333–44. doi: 10.1111/j.1471-4159.2007.05075.x
54. Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin M-L, Yardin C, et al. Tau protein kinases: involvement in Alzheimer's disease. *Ageing Res Rev.* (2013) 12:289–309. doi: 10.1016/j.arr.2012.06.003
55. L'Episcopo F, Drouin-Ouellet J, Tirolo C, Pulvirenti A, Giugno R, Testa N, et al. GSK-3β-induced Tau pathology drives hippocampal neuronal cell death in Huntington's disease: involvement of astrocyte-neuron interactions. *Cell Death Dis.* (2016) 7:e2206. doi: 10.1038/cddis.2016.104
56. Blum D, Herrera F, Francelle L, Mendes T, Basquin M, Obriot H, et al. Mutant huntingtin alters tau phosphorylation and subcellular distribution. *Hum Mol Genet.* (2015) 24:76–85. doi: 10.1093/hmg/ddu421
57. Gong CX, Singh TJ, Grundke-Iqbal I, Iqbal K. Phosphoprotein phosphatase activities in Alzheimer disease brain. *J Neurochem.* (1993) 61:921–7. doi: 10.1111/j.1471-4159.1993.tb03603.x
58. Liu F, Grundke-Iqbal I, Iqbal K, Gong C-X. Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur J Neurosci.* (2005) 22:1942–50. doi: 10.1111/j.1460-9568.2005.04391.x
59. Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin M-L, Terro F. Tau protein phosphatases in Alzheimer's disease: the leading role of PP2A. *Ageing Res Rev.* (2013) 12:39–49. doi: 10.1016/j.arr.2012.06.008
60. Rahman A, Grundke-Iqbal I, Iqbal K. Phosphothreonine-212 of Alzheimer abnormally hyperphosphorylated tau is a preferred substrate of protein phosphatase-1. *Neurochem Res.* (2005) 30:277–87. doi: 10.1007/s11064-005-2483-9
61. Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med.* (2009) 3:112–9. doi: 10.1016/j.molmed.2009.01.003

62. Hanger DP, Byers HL, Wray S, Leung KY, Saxton MJ, Seereeram A, et al. Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis. *J Biol Chem.* (2007) 32:23654. doi: 10.1074/jbc.m703269200
63. Neddens J, Temmel M, Flunkert S, Kerschbaumer B, Hoeller C, Loeffler T, et al. Phosphorylation of different tau sites during progression of Alzheimer's disease. *Acta Neuropathol Commun.* (2018) 6:52. doi: 10.1186/s40478-018-0557-6
64. Gratuze M, Noël A, Julien C, Cisbani G, Milot-Rousseau P, Morin F, et al. Tau hyperphosphorylation and deregulation of calcineurin in mouse models of Huntington's disease. *Hum Mol Genet.* (2015) 24:86–99. doi: 10.1093/hmg/ddu456
65. Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K. Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. *PNAS.* (1994) 12:5562–6. doi: 10.1073/pnas.91.12.5562
66. Alonso AC, Grundke-Iqbal I, Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med.* (1996) 2:783–7. doi: 10.1038/nm0796-783
67. Alonso AD, Grundke-Iqbal I, Barra HS, Iqbal K. Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. *PNAS.* (1997) 1:298–303. doi: 10.1073/pnas.94.1.298
68. Merrick SE, Trojanowski JQ, Lee VM-Y. Selective destruction of stable microtubules and axons by inhibitors of protein serine/threonine phosphatases in cultured human neurons (NT2N Cells). *J Neurosci.* (1997) 15:5726–37. doi: 10.1523/JNEUROSCI.17-15-05726.1997
69. Sherman MA, LaCroix M, Amar F, Larson ME, Forster C, Aguzzi A, et al. Soluble conformers of Aβ and tau alter selective proteins governing axonal transport. *J Neurosci.* (2016) 37:9647–58. doi: 10.1523/JNEUROSCI.1899-16.2016
70. Ishihara T, Hong M, Zhang B, Nakagawa Y, Lee MK, Trojanowski JQ, et al. Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron.* (1999) 3:751—62. doi: 10.1016/s0896-6273(00)81127-7
71. Brunden KR, Zhang B, Carroll J, Yao Y, Potuzak JS, Hogan A-ML, et al. Epothilone D improves microtubule density, axonal integrity, and cognition in a transgenic mouse model of tauopathy. *J Neurosci.* (2010) 30:13861–6. doi: 10.1523/JNEUROSCI.3059-10.2010
72. Cash AD, Aliev G, Siedlak SL, Nunomura A, Fujioka H, Zhu X, et al. Microtubule reduction in Alzheimer's disease and aging is independent of tau filament formation. *Am J Pathol.* (2003) 5:1623–7. doi: 10.1016/s0002-9440(10)64296-4
73. Barten DM, Fanara P, Andorfer C, Hoque N, Wong PYA, Husted KH, et al. Hyperdynamic microtubules, cognitive deficits, and pathology are improved in tau transgenic mice with low doses of the microtubule-stabilizing agent BMS-241027. *J Neurosci.* (2012) 21:7137–45. doi: 10.1523/JNEUROSCI.0188-12.2012
74. King ME, Kan H-M, Baas PW, Erisir A, Glabe CG, Bloom GS. Tau-dependent microtubule disassembly initiated by prefibrillar β-amyloid. *J Cell Biol.* (2006) 4:541–6. doi: 10.1083/jcb.200605187
75. Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to β-amyloid-induced neurotoxicity. *PNAS.* (2002) 9:6364–9. doi: 10.1073/pnas.092136199
76. Zempel H, Luedtke J, Kumar Y, Biernat J, Dawson H, Mandelkow E, et al. Amyloid-β oligomers induce synaptic damage via Tau-dependent microtubule severing by TTLL6 and spastin. *EMBO J.* (2013) 22:2920–37. doi: 10.1038/emboj.2013.207
77. Zempel H, Thies E, Mandelkow E, Mandelkow EM. Aβ oligomers cause localized Ca^{2+} elevation, missorting of endogenous tau into dendrites, tau phosphorylation, and destruction of microtubules and spines. *J Neurosci.* (2010) 36:11938–50. doi: 10.1523/JNEUROSCI.2357-10.2010
78. Trushina E, Heldebrant MP, Perez-Terzic CM, Bortolon R, Kovtun IV, Badger JD, et al. Microtubule destabilization and nuclear entry are sequential steps leading to toxicity in Huntington's disease. *Proc Natl Acad Sci USA.* (2003) 100:12171–6. doi: 10.1073/pnas.2034961100
79. Trushina E, Dyer RB, Badger JD, Ure D, Eide L, Tran DD, et al. Mutant huntingtin impairs axonal trafficking in mammalian neurons *in vivo* and *in vitro*. *Mol Cell Biol.* (2004) 24:8195–209. doi: 10.1128/MCB.24.18.8195-8209.2004
80. Chang DTW, Rintoul GL, Pandipati S, Reynolds IJ. Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. *Neurobiol Dis.* (2006) 2:388–400. doi: 10.1016/j.nbd.2005.12.007
81. Orr AL, Li S, Wang C-E, Li H, Wang J, Rong J, et al. N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. *J Neurosci.* (2008) 11:2783–92. doi: 10.1523/JNEUROSCI.0106-08.2008
82. Gauthier LR, Charrin BC, Borrell-Pagès M, Dompierre JP, Rangone H, Cordelières FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell.* (2004) 1:127–38. doi: 10.1016/j.cell.2004.06.018
83. Fernández-Nogales M, Hernández F, Miguez A, Alberch J, Ginés S, Pérez-Navarro E, et al. Decreased glycogen synthase kinase-3 levels and activity contribute to Huntington's disease. *Hum Mol Genet.* (2015) 24:5040–52. doi: 10.1093/hmg/ddv224
84. Liu P, Smith BR, Huang ES, Mahesh A, Vonsattel JPG, Petersen AJ, et al. A soluble truncated tau species related to cognitive dysfunction and caspase-2 is elevated in the brain of Huntington's disease patients. *Acta Neuropathol Commun.* (2019) 7:111. doi: 10.1186/s40478-019-0764-9
85. Maxan A, Cicchetti F. Tau: a common denominator and therapeutic target for neurodegenerative disorders. *J Exp Neurosci.* (2018) 12:1179069518772380. doi: 10.1177/1179069518772380
86. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's disease collaborative research group. *Cell.* (1993) 72:971–83. doi: 10.1016/0092-8674(93)90585-E
87. Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell.* (1997) 90:537–48. doi: 10.1016/S0092-8674(00)80513-9
88. Jana NR, Zemskov EA, Wang GH, Nukina N. Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Hum Mol Genet.* (2001) 10:1049–59. doi: 10.1093/hmg/10.10.1049
89. Venkatraman P, Wetzel R, Tanaka M, Nukina N, Goldberg AL. Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Mol Cell.* (2004) 14:95–104. doi: 10.1016/S1097-2765(04)00151-0
90. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science.* (2001) 293:493–8. doi: 10.1126/science.1059581
91. Ha AD, Fung VSC. Huntington's disease. *Curr Opin Neurol.* (2012) 25:491–8. doi: 10.1097/WCO.0b013e3283550c97
92. Tang C, Feigin A. Monitoring Huntington's disease progression through preclinical and early stages. *Neurodegener Dis Manag.* (2012) 2:421–435. doi: 10.2217/nmt.12.34
93. Winder-Rhodes SE, Hampshire A, Rowe JB, Peelle JE, Robbins TW, Owen AM, et al. Association between MAPT haplotype and memory function in patients with Parkinson's disease and healthy aging individuals. *Neurobiol Aging.* (2015) 36:1519–28. doi: 10.1016/j.neurobiolaging.2014.12.006
94. Brier MR, Gordon B, Friedrichsen K, McCarthy J, Stern A, Christensen J, et al. Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. *Sci Transl Med.* (2016) 8:338ra66. doi: 10.1126/scitranslmed.aaf2362
95. Koychev I, Gunn RN, Firouzian A, Lawson J, Zamboni G, Ridha B, et al. PET tau and amyloid-β burden in mild Alzheimer's Disease: divergent relationship with age, cognition, and cerebrospinal fluid biomarkers. *J Alzheimers Dis.* (2017) 60:283–93. doi: 10.3233/JAD-170129
96. Rodrigues FB, Byrne L, McColgan P, Robertson N, Tabrizi SJ, Leavitt BR, et al. Cerebrospinal fluid total tau concentration predicts clinical phenotype in Huntington's disease. *J Neurochem.* (2016) 139:22–5. doi: 10.1111/jnc.13719
97. Wild EJ, Boggio R, Langbehn D, Robertson N, Haider S, Miller JRC, et al. Quantification of mutant huntingtin protein in cerebrospinal fluid

from Huntington's disease patients. *J Clin Invest.* (2015) 125:1979–86. doi: 10.1172/JCI80743
98. Caparros-Lefebvre D, Kerdraon O, Devos D, Dhaenens CM, Blum D, Maurage CA, et al. Association of corticobasal degeneration and Huntington's disease: can tau aggregates protect huntingtin toxicity? *Mov Disord.* (2009) 24:1089–90. doi: 10.1002/mds.22204
99. Davis MY, Keene CD, Jayadev S, Bird T. The co-occurrence of Alzheimer's disease and Huntington's disease: a neuropathological study of 15 elderly Huntington's disease subjects. *J Huntingtons Dis.* (2014) 3:209–17. doi: 10.3233/JHD-140111
100. Moss RJ, Mastri AR, Schut LJ. The coexistence and differentiation of late onset Huntington's Disease and Alzheimer's Disease: a case report and review of the literature. *J Am Geriatr Soc.* (1988) 36:237–42. doi: 10.1111/j.1532-5415.1988.tb01807.x
101. Congdon EE, Sigurdsson EM. Tau-targeting therapies for Alzheimer disease. *Nat Rev Neurol.* (2018) 14:399–415. doi: 10.1038/s41582-018-0013-z
102. Iqbal K, Grundke-Iqbal I, Zaidi T, Merz PA, Wen GY, Shaikh SS, et al. Defective brain microtubule assembly in Alzheimer's disease. *Lancet.* (1986) 2:421–6. doi: 10.1016/S0140-6736(86)92134-3
103. Buée L, Bussière T, Buée-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev.* (2000) 33:95–130. doi: 10.1016/S0165-0173(00)00019-9
104. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA.* (1986) 83:4913–7. doi: 10.1073/pnas.83.13.4913
105. Domínguez JM, Fuertes A, Orozco L, del Monte-Millán M, Delgado E, Medina M. Evidence for irreversible inhibition of glycogen synthase kinase-3β by tideglusib. *J Biol Chem.* (2012) 287:893–904. doi: 10.1074/jbc.M111.306472
106. Reisberg B, Doody R, Stöffler A, Schmitt F, Ferris S, Möbius HJ, et al. Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med.* (2003) 348:1333–41. doi: 10.1056/NEJMoa013128
107. Reisberg B, Doody R, Stöffler A, Schmitt F, Ferris S, Möbius HJ. A 24-week open-label extension study of memantine in moderate to severe Alzheimer disease. *Arch Neurol.* (2006) 63:49–54. doi: 10.1001/archneur.63.1.49
108. Serenó L, Coma M, Rodríguez M, Sánchez-Ferrer P, Sánchez MB, Gich I, et al. A novel GSK-3beta inhibitor reduces Alzheimer's pathology and rescues neuronal loss *in vivo*. *Neurobiol Dis.* (2009) 35:359–67. doi: 10.1016/j.nbd.2009.05.025
109. Noble W, Hanger DP, Miller CCJ, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. *Front Neurol.* (2013) 4:83. doi: 10.3389/fneur.2013.00083
110. Lee KY, Clark AW, Rosales JL, Chapman K, Fung T, Johnston RN. Elevated neuronal Cdc2-like kinase activity in the Alzheimer disease brain. *Neurosci Res.* (1999) 34:21–9. doi: 10.1016/S0168-0102(99)00026-7
111. Leroy K, Yilmaz Z, Brion J-P. Increased level of active GSK-3beta in Alzheimer's disease and accumulation in argyrophilic grains and in neurones at different stages of neurofibrillary degeneration. *Neuropathol Appl Neurobiol.* (2007) 33:43–55. doi: 10.1111/j.1365-2990.2006.00795.x
112. Lucas JJ, Hernández F, Gómez-Ramos P, Morán MA, Hen R, Avila J. Decreased nuclear β-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3β conditional transgenic mice. *EMBO J.* (2001) 20:27–39. doi: 10.1093/emboj/20.1.27
113. Spittaels K, Haute CV den, Dorpe JV, Geerts H, Mercken M, Bruynseels K, et al. Glycogen synthase kinase-3β phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice. *J Biol Chem.* (2000) 275:41340–9. doi: 10.1074/jbc.M006219200
114. Terwel D, Muyllaert D, Dewachter I, Borghgraef P, Croes S, Devijver H, et al. Amyloid activates GSK-3β to aggravate neuronal tauopathy in bigenic mice. *Am J Pathol.* (2008) 172:786–98. doi: 10.2353/ajpath.2008.070904
115. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell.* (1996) 87:493–506. doi: 10.1016/S0092-8674(00)81369-0
116. Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet M-F. Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. *J Comp Neurol.* (2003) 465:11–26. doi: 10.1002/cne.10776
117. Leggio GM, Catania MV, Puzzo D, Spatuzza M, Pellitteri R, Gulisano W, et al. The antineoplastic drug flavopiridol reverses memory impairment induced by Amyloid-ß1-42 oligomers in mice. *Pharmacol Res.* (2016) 106:10–20. doi: 10.1016/j.phrs.2016.02.007
118. Zhang M, Li J, Chakrabarty P, Bu B, Vincent I. Cyclin-dependent kinase inhibitors attenuate protein hyperphosphorylation, cytoskeletal lesion formation, and motor defects in Niemann-Pick type C mice. *Am J Clin Pathol.* (2004) 165:843–53. doi: 10.1016/S0002-9440(10)63347-0
119. Bu B, Li J, Davies P, Vincent I. Deregulation of cdk5, hyperphosphorylation, and cytoskeletal pathology in the Niemann–Pick Type C murine model. *J Neurosci.* (2002) 22:6515–25. doi: 10.1523/JNEUROSCI.22-15-06515.2002
120. Engel T, Goñi-Oliver P, Lucas J, Avila J, Hernández F. Chronic lithium administration to FTDP-17 tau and GSK-3β overexpressing mice prevents tau hyperphosphorylation and neurofibrillary tangle formation, but pre-formed neurofibrillary tangles do not revert. *J Neurochem.* (2006) 99:1445–55. doi: 10.1111/j.1471-4159.2006.04139.x
121. Pérez M, Hernández F, Lim F, Díaz-Nido J, Avila J. Chronic lithium treatment decreases mutant tau protein aggregation in a transgenic mouse model. *J Alzheimers Dis.* (2003) 5:301–8. doi: 10.3233/JAD-2003-5405
122. Caccamo A, Oddo S, Tran LX, LaFerla FM. Lithium reduces tau phosphorylation but not Aβ or working memory deficits in a transgenic model with both plaques and tangles. *Am J Pathol.* (2007) 170:1669–75. doi: 10.2353/ajpath.2007.061178
123. Noble W, Planel E, Zehr C, Olm V, Meyerson J, Suleman F, et al. Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration *in vivo*. *Proc Natl Acad Sci USA.* (2005) 102:6990–5. doi: 10.1073/pnas.0500466102
124. Lovestone S, Boada M, Dubois B, Hüll M, Rinne JO, Huppertz H-J, et al. A phase II trial of tideglusib in Alzheimer's disease. *J Alzheimers Dis.* (2015) 45:75–88. doi: 10.3233/JAD-141959
125. Matsunaga S, Kishi T, Annas P, Basun H, Hampel H, Iwata N. Lithium as a Treatment for Alzheimer's Disease: a systematic review and meta-analysis. *J Alzheimers Dis.* (2015) 48:403–10. doi: 10.3233/JAD-150437
126. Andén NE, Dalén P, Johansson B. Baclofen and lithium in Huntington's chorea. *Lancet.* (1973) 2:93. doi: 10.1016/S0140-6736(73)93285-6
127. Vestergaard P, Baastrup PC, Petersson H. Lithium treatment of Huntington's chorea. A placebo-controlled clinical trial. *Acta Psychiatr Scand.* (1977) 56:183–8. doi: 10.1111/j.1600-0447.1977.tb03561.x
128. Wood NI, Morton AJ. Chronic lithium chloride treatment has variable effects on motor behaviour and survival of mice transgenic for the Huntington's disease mutation. *Brain Res Bull.* (2003) 61:375–83. doi: 10.1016/S0361-9230(03)00141-2
129. Scheuing L, Chiu C-T, Liao H-M, Linares GR, Chuang D-M. Preclinical and clinical investigations of mood stabilizers for Huntington's disease: what have we learned? *Int J Biol Sci.* (2014) 10:1024–38. doi: 10.7150/ijbs.9898
130. Ishii T, Hashimoto E, Ukai W, Tateno M, Yoshinaga T, Saito S, et al. Lithium-induced suppression of transcription repressor NRSF/REST: effects on the dysfunction of neuronal differentiation by ethanol. *Eur J Pharmacol.* (2008) 593:36–43. doi: 10.1016/j.ejphar.2008.07.021
131. Kopnisky KL, Chalecka-Franaszek E, Gonzalez-Zulueta M, Chuang D-M. Chronic lithium treatment antagonizes glutamate-induced decrease of phosphorylated CREB in neurons via reducing protein phosphatase 1 and increasing MEK activities. *Neuroscience.* (2003) 116:425–35. doi: 10.1016/S0306-4522(02)00573-0
132. Nonaka S, Hough CJ, Chuang D-M. Chronic lithium treatment robustly protects neurons in the central nervous system against excitotoxicity by inhibiting N-methyl-d-aspartate receptor-mediated calcium influx. *Proc Natl Acad Sci USA.* (1998) 95:2642–7. doi: 10.1073/pnas.95.5.2642
133. Fukumoto T, Morinobu S, Okamoto Y, Kagaya A, Yamawaki S. Chronic lithium treatment increases the expression of brain-derived neurotrophic factor in the rat brain. *Psychopharmacol.* (2001) 158:100–6. doi: 10.1007/s002130100871
134. Hellweg R, Lang UE, Nagel M, Baumgartner A. Subchronic treatment with lithium increases nerve growth factor content in distinct brain

regions of adult rats. *Mol Psychiatry*. (2002) 7:604–8. doi: 10.1038/sj.mp.4001042
135. Jornada LK, Moretti M, Valvassori SS, Ferreira CL, Padilha PT, Arent CO, et al. Effects of mood stabilizers on hippocampus and amygdala BDNF levels in an animal model of mania induced by ouabain. *J Psychiatr Res.* (2010) 44:506–10. doi: 10.1016/j.jpsychires.2009.11.002
136. Yasuda S, Liang M-H, Marinova Z, Yahyavi A, Chuang D-M. The mood stabilizers lithium and valproate selectively activate the promoter IV of brain-derived neurotrophic factor in neurons. *Mol Psychiatry*. (2009) 14:51–9. doi: 10.1038/sj.mp.4002099
137. Akkouh IA, Skrede S, Holmgren A, Ersland KM, Hansson L, Bahrami S, et al. Exploring lithium's transcriptional mechanisms of action in bipolar disorder: a multi-step study. *Neuropsychopharmacology*. (2020) 45:947–55. doi: 10.1038/s41386-019-0556-8
138. Farah R, Khamisy-Farah R, Amit T, Youdim MBH, Arraf Z. Lithium's gene expression profile, relevance to neuroprotection a cDNA microarray study. *Cell Mol Neurobiol.* (2013) 33:411–20. doi: 10.1007/s10571-013-9907-x
139. Danivas V, Moily NS, Thimmaiah R, Muralidharan K, Purushotham M, Muthane U, et al. Off label use of lithium in the treatment of Huntington's disease: a case series. *Indian J Psychiatry*. (2013) 55:81–3. doi: 10.4103/0019-5545.105522
140. Leonard DP, Kidson MA, Brown JG, Shannon PJ, Taryan S. A double blind trial of lithium carbonate and haloperidol in Huntington's chorea. *Aust N Z J Psychiatry*. (1975) 9:115–8. doi: 10.3109/00048677509159834
141. Lim NKH, Hung LW, Pang TY, Mclean CA, Liddell JR, Hilton JB, et al. Localized changes to glycogen synthase kinase-3 and collapsin response mediator protein-2 in the Huntington's disease affected brain. *Hum Mol Genet.* (2014) 23:4051–63. doi: 10.1093/hmg/ddu119
142. Tanimukai H, Grundke-Iqbal I, Iqbal K. Up-regulation of inhibitors of protein phosphatase-2A in Alzheimer's disease. *Am J Pathol.* (2005) 166:1761–71. doi: 10.1016/S0002-9440(10)62486-8
143. Xifró X, Giralt A, Saavedra A, García-Martínez JM, Díaz-Hernández M, Lucas JJ, et al. Reduced calcineurin protein levels and activity in exon-1 mouse models of Huntington's disease: Role in excitotoxicity. *Neurobiol Dis.* (2009) 36:461–9. doi: 10.1016/j.nbd.2009.08.012
144. Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, et al. Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175. *PLoS ONE.* (2012) 7:e49838. doi: 10.1371/journal.pone.0049838
145. Millward TA, Zolnierowicz S, Hemmings BA. Regulation of protein kinase cascades by protein phosphatase 2A. *Trends Biochem Sci.* (1999) 24:186–91. doi: 10.1016/S0968-0004(99)01375-4
146. Kins S, Crameri A, Evans DR, Hemmings BA, Nitsch RM, Gotz J. Reduced protein phosphatase 2A activity induces hyperphosphorylation and altered compartmentalization of tau in transgenic mice. *J Biol Chem.* (2001) 276:38193–200. doi: 10.1074/jbc.M102621200
147. Rui M, Ng KS, Tang Q, Bu S, Yu F. Protein phosphatase PP2A regulates microtubule orientation and dendrite pruning in Drosophila. *EMBO Rep.* (2020) 21:e48843. doi: 10.15252/embr.201948843
148. Viquez NM, Li CR, Wairkar YP, DiAntonio A. The B′ protein phosphatase 2A regulatory subunit well-rounded regulates synaptic growth and cytoskeletal stability at the drosophila neuromuscular junction. *J Neurosci.* (2006) 26:9293–303. doi: 10.1523/JNEUROSCI.1740-06.2006
149. Wang J, Xie R, Kou X, Liu Y, Qi C, Liu R, et al. A protein phosphatase 2A deficit in the hippocampal CA1 area impairs memory extinction. *Mol Brain.* (2019) 12:51. doi: 10.1186/s13041-019-0469-9
150. Kickstein E, Krauss S, Thornhill P, Rutschow D, Zeller R, Sharkey J, et al. Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. *Proc Natl Acad Sci USA*. (2010) 107:21830–5. doi: 10.1073/pnas.0912793107
151. Wei H, Zhang H, Wang X, Xie J, An D, Wan L, et al. Direct activation of protein phosphatase 2A (PP2A) by tricyclic sulfonamides ameliorates Alzheimer's disease pathogenesis in cell and animal models. *Neurotherapeutics*. (2020) doi: 10.1007/s13311-020-00841-6
152. Li L, Sengupta A, Haque N, Grundke-Iqbal I, Iqbal K. Memantine inhibits and reverses the Alzheimer type abnormal hyperphosphorylation of tau and associated neurodegeneration. *FEBS Lett.* (2004) 566:261–9. doi: 10.1016/j.febslet.2004.04.047
153. Milnerwood AJ, Gladding CM, Pouladi MA, Kaufman AM, Hines RM, Boyd JD, et al. Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron.* (2010) 65:178–90. doi: 10.1016/j.neuron.2010.01.008
154. Wu H-M, Tzeng N-S, Qian L, Wei S-J, Hu X, Chen S-H, et al. Novel neuroprotective mechanisms of memantine: increase in neurotrophic factor release from astroglia and anti-inflammation by preventing microglial activation. *Neuropsychopharmacology*. (2009) 34:2344–57. doi: 10.1038/npp.2009.64
155. Beister A, Kraus P, Kuhn W, Dose M, Weindl A, Gerlach M. The N-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. *J Neural Transm Suppl.* (2004) 68:117–22. doi: 10.1007/978-3-7091-0579-5_14
156. Cankurtaran ES, Ozalp E, Soygur H, Cakir A. Clinical experience with risperidone and memantine in the treatment of Huntington's disease. *J Natl Med Assoc.* (2006) 98:1353–5.
157. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther.* (2015) 148:114–31. doi: 10.1016/j.pharmthera.2014.11.016
158. Wlodarchak N, Xing Y. PP2A as a master regulator of the cell cycle. *Crit Rev Biochem Mol Biol.* (2016) 51:162–84. doi: 10.3109/10409238.2016.1143913
159. Pineda JR, Pardo R, Zala D, Yu H, Humbert S, Saudou F. Genetic and pharmacological inhibition of calcineurin corrects the BDNF transport defect in Huntington's disease. *Mol Brain.* (2009) 2:33. doi: 10.1186/1756-6606-2-33
160. Cowan CM, Mudher A. Are tau aggregates toxic or protective in tauopathies? *Front Neurol.* (2013) 4:114. doi: 10.3389/fneur.2013.00114
161. Braak H, Braak E. Staging of alzheimer's disease-related neurofibrillary changes. *Neurobiol Aging.* (1995) 16:271–8. doi: 10.1016/0197-4580(95)00021-6
162. Braak H, Braak E. Evolution of neuronal changes in the course of Alzheimer's disease. In: Jellinger K, Fazekas F, Windisch M, editors. *Ageing and Dementia*. Vienna: Springer. (1998). p. 127–40. doi: 10.1007/978-3-7091-6467-9_11
163. Iqbal K, Alonso A del C, Grundke-Iqbal I. Cytosolic abnormally hyperphosphorylated tau but not paired helical filaments sequester normal MAPs and inhibit microtubule assembly. *J Alzheimers Dis.* (2008) 14:365–70. doi: 10.3233/JAD-2008-14402
164. Takashima A, Soeda Y. Granular tau oligomer as toxic tau aggregation form in AD. *Alzheimers Dement.* (2014) 10:P161. doi: 10.1016/j.jalz.2014.04.150
165. Kopeikina KJ, Hyman BT, Spires-Jones TL. Soluble forms of tau are toxic in Alzheimer's disease. *Transl Neurosci.* (2012) 3:223–33. doi: 10.2478/s13380-012-0032-y
166. Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, et al. Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *J Neurosci.* (2007) 27:3650–62. doi: 10.1523/JNEUROSCI.0587-07.2007
167. Hoffmann NA, Dorostkar MM, Blumenstock S, Goedert M, Herms J. Impaired plasticity of cortical dendritic spines in P301S tau transgenic mice. *Acta Neuropathol Commun.* (2013) 1:82. doi: 10.1186/2051-5960-1-82
168. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener.* (2011) 6:39. doi: 10.1186/1750-1326-6-39
169. Kopeikina KJ, Polydoro M, Tai H-C, Yaeger E, Carlson GA, Pitstick R, et al. Synaptic alterations in the rTg4510 mouse model of tauopathy. *J Comp Neurol.* (2013) 521:1334–53. doi: 10.1002/cne.23234
170. Rocher AB, Crimins JL, Amatrudo JM, Kinson MS, Todd-Brown MA, Lewis J, et al. Structural and functional changes in tau mutant mice neurons are not linked to the presence of NFTs. *Exp Neurol.* (2010) 223:385–93. doi: 10.1016/j.expneurol.2009.07.029
171. Yoshiyama Y, Higuchi M, Zhang B, Huang S-M, Iwata N, Saido TC, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* (2007) 53:337–51. doi: 10.1016/j.neuron.2007.01.010
172. Merino-Serrais P, Benavides-Piccione R, Blazquez-Llorca L, Kastanauskaite A, Rábano A, Avila J, et al. The influence of phospho-τ on dendritic spines

of cortical pyramidal neurons in patients with Alzheimer's disease. *Brain.* (2013) 136(Pt 6):1913–28. doi: 10.1093/brain/awt088
173. Murmu RP, Li W, Holtmaat A, Li J-Y. Dendritic spine instability leads to progressive neocortical spine loss in a mouse model of Huntington's Disease. *J Neurosci.* (2013) 33:12997–3009. doi: 10.1523/JNEUROSCI.5284-12.2013
174. Bulic B, Pickhardt M, Mandelkow E-M, Mandelkow E. Tau protein and tau aggregation inhibitors. *Neuropharmacology.* (2010) 59:276–89. doi: 10.1016/j.neuropharm.2010.01.016
175. Crowe A, James MJ, Lee VM-Y, Smith AB, Trojanowski JQ, Ballatore C, et al. Aminothienopyridazines and methylene blue affect tau fibrillization via cysteine oxidation. *J Biol Chem.* (2013) 288:11024–37. doi: 10.1074/jbc.M112.436006
176. Soeda Y, Saito M, Maeda S, Ishida K, Nakamura A, Kojima S, et al. Methylene blue inhibits formation of tau fibrils but not of granular tau oligomers: a plausible key to understanding failure of a clinical trial for Alzheimer's disease. *J Alzheimers Dis.* (2019) 68:1677–86. doi: 10.3233/JAD-181001
177. Hosokawa M, Arai T, Masuda-Suzukake M, Nonaka T, Yamashita M, Akiyama H, et al. Methylene blue reduced abnormal tau accumulation in P301L tau transgenic mice. *PLoS ONE.* (2012) 7:e52389. doi: 10.1371/journal.pone.0052389
178. Sontag EM, Lotz GP, Agrawal N, Tran A, Aron R, Yang G, et al. Methylene blue modulates huntingtin aggregation intermediates and is protective in Huntington's disease models. *J Neurosci.* (2012) 32:11109–19. doi: 10.1523/JNEUROSCI.0895-12.2012
179. Alam R, Driver D, Wu S, Lozano E, Key SL, Hole JT, et al. Preclinical characterization of an antibody [LY3303560] targeting aggregated tau. *Alzheimers Dement.* (2017) 13:P592–3. doi: 10.1016/j.jalz.2017.07.227
180. Castillo-Carranza DL, Gerson JE, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Kayed R. Specific targeting of tau oligomers in Htau mice prevents cognitive impairment and tau toxicity following injection with brain-derived tau oligomeric seeds. *J Alzheimers Dis.* (2014) 40(Suppl. 1):S97–111. doi: 10.3233/JAD-132477
181. Theunis C, Crespo-Biel N, Gafner V, Pihlgren M, López-Deber MP, Reis P, et al. Efficacy and safety of a liposome-based vaccine against protein Tau, assessed in tau.P301L mice that model tauopathy. *PLoS ONE.* (2013) 8:e72301. doi: 10.1371/journal.pone.0072301
182. Smith BR, Nelson KM, Kemper LJ, Leinonen-Wright K, Petersen A, Keene CD, et al. A soluble tau fragment generated by caspase-2 is associated with dementia in Lewy body disease. *Acta Neuropathol Commun.* (2019) 7:124. doi: 10.1186/s40478-019-0765-8
183. Selenica M-LB, Davtyan H, Housley SB, Blair LJ, Gillies A, Nordhues BA, et al. Epitope analysis following active immunization with tau proteins reveals immunogens implicated in tau pathogenesis. *J Neuroinflammation.* (2014) 11:152. doi: 10.1186/s12974-014-0152-0
184. Castillo-Carranza DL, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G, et al. Passive immunization with tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J Neurosci.* (2014) 34:4260–72. doi: 10.1523/JNEUROSCI.3192-13.2014
185. Paholikova K, Salingova B, Opattova A, Skrabana R, Majerova P, Zilka N, et al. N-terminal truncation of microtubule associated protein tau dysregulates its cellular localization. *J Alzheimers Dis.* (2015) 43:915–26. doi: 10.3233/JAD-140996
186. AC Immune SA. *A Study to Evaluate the Safety, Tolerability and Immunogenicity of Tau Targeted Vaccines in Participants With Early Alzheimer's Disease.* Report No. NCT04445831 (2020). Available online at: www.clinicaltrials.gov (accessed October 7, 2020).
187. Axon Neuroscience SE. *Safety Study of AADvac1, a Tau Peptide-KLH-Conjugate Active Vaccine to Treat Alzheimer's Disease.* Report No. NCT01850238 (2013). Available online at: www.clinicaltrials.gov (accessed October 7, 2020).
188. Fuller JP, Stavenhagen JB, Teeling JL. New roles for Fc receptors in neurodegeneration-the impact on Immunotherapy for Alzheimer's Disease. *Front Neurosci.* (2014) 8:235. doi: 10.3389/fnins.2014.00235
189. McEwan WA, Falcon B, Vaysburd M, Clift D, Oblak AL, Ghetti B, et al. Cytosolic Fc receptor TRIM21 inhibits seeded tau aggregation. *Proc Natl Acad Sci USA.* (2017) 114:574–9. doi: 10.1073/pnas.1607215114
190. d'Abramo C, Acker CM, Jimenez H, Davies P. Passive immunization in JNPL3 transgenic mice using an array of phospho-tau specific antibodies. *PLoS ONE.* (2015) 10:e0135774. doi: 10.1371/journal.pone.0135774
191. Jicha GA, Bowser R, Kazam IG, Davies P. Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. *J Neurosci Res.* (1997) 48:128–32. doi: 10.1002/(SICI)1097-4547(19970415)48:2<128::AID-JNR5>3.0.CO;2-E
192. Eli Lilly and Company. *Assessment of Safety, Tolerability, and Efficacy of LY3303560 in Early Symptomatic Alzheimer's Disease.* Report No. NCT03518073. (2020). Available online at: clinicaltrials.gov (accessed Jun 23, 2020).
193. Evans LD, Wassmer T, Fraser G, Smith J, Perkinton M, Billinton A, et al. Extracellular monomeric and aggregated tau efficiently enter human neurons through overlapping but distinct pathways. *Cell Rep.* (2018) 22:3612–24. doi: 10.1016/j.celrep.2018.03.021
194. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem.* (2009) 284:12845–52. doi: 10.1074/jbc.M808759200
195. Mirbaha H, Holmes BB, Sanders DW, Bieschke J, Diamond MI. Tau trimers are the minimal propagation unit spontaneously internalized to seed intracellular aggregation. *J Biol Chem.* (2015) 290:14893–903. doi: 10.1074/jbc.M115.652693
196. Kaufman SK, Del Tredici K, Thomas TL, Braak H, Diamond MI. Tau seeding activity begins in the transentorhinal/entorhinal regions and anticipates phospho-tau pathology in Alzheimer's disease and PART. *Acta Neuropathol.* (2018) 136:57–67. doi: 10.1007/s00401-018-1855-6
197. Abounit S, Wu JW, Duff K, Victoria GS, Zurzolo C. Tunneling nanotubes: a possible highway in the spreading of tau and other prion-like proteins in neurodegenerative diseases. *Prion.* (2016) 10:344–51. doi: 10.1080/19336896.2016.1223003
198. Calafate S, Buist A, Miskiewicz K, Vijayan V, Daneels G, de Strooper B, et al. Synaptic contacts enhance cell-to-cell tau pathology propagation. *Cell Rep.* (2015) 11:1176–83. doi: 10.1016/j.celrep.2015.04.043
199. Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, et al. Small misfolded tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem.* (2013) 288:1856–70. doi: 10.1074/jbc.M112.394528
200. Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Trans-cellular propagation of tau aggregation by fibrillar species. *J Biol Chem.* (2012) 287:19440–51. doi: 10.1074/jbc.M112.346072
201. Plotkin SS, Cashman NR. Passive immunotherapies targeting Aβ and tau in Alzheimer's disease. *Neurobiol Dis.* (2020) 144:e105010. doi: 10.1016/j.nbd.2020.105010
202. Mullard A. Stem-cell discovery platforms yield first clinical candidates. *Nat Rev Drug Disc.* (2015) 14:589–91. doi: 10.1038/nrd4708
203. Bright J, Hussain S, Dang V, Wright S, Cooper B, Byun T, et al. Human secreted tau increases amyloid-beta production. *Neurobiol Aging.* (2015) 36:693–709. doi: 10.1016/j.neurobiolaging.2014.09.007
204. Lee S-H, Le Pichon CE, Adolfsson O, Gafner V, Pihlgren M, Lin H, et al. Antibody-mediated targeting of tau *in vivo* does not require effector function and microglial engagement. *Cell Rep.* (2016) 16:1690–700. doi: 10.1016/j.celrep.2016.06.099
205. Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, Maloney SE, et al. Anti-tau antibodies that block tau aggregate seeding *in vitro* markedly decrease pathology and improve cognition *in vivo*. *Neuron.* (2013) 80:402–14. doi: 10.1016/j.neuron.2013.07.046
206. Yanamandra K, Jiang H, Mahan TE, Maloney SE, Wozniak DF, Diamond MI, et al. Anti-tau antibody reduces insoluble tau and decreases brain atrophy. *Ann Clin Transl Neurol.* (2015) 2:278–88. doi: 10.1002/acn3.176
207. Boxer AL, Qureshi I, Ahlijanian M, Grundman M, Golbe LI, Litvan I, et al. Safety of the tau-directed monoclonal antibody BIIB092 in progressive supranuclear palsy: a randomised, placebo-controlled, multiple ascending dose phase 1b trial. *Lancet Neurol.* (2019) 18:549–58. doi: 10.1016/S1474-4422(19)30139-5

208. Jadhav S, Avila J, Schöll M, Kovacs GG, Kövari E, Skrabana R, et al. A walk through tau therapeutic strategies. *Acta Neuropathol Commun.* (2019) 7:22. doi: 10.1186/s40478-019-0664-z
209. Constantinescu R, Romer M, Zetterberg H, Rosengren L, Kieburtz K. Increased levels of total tau protein in the cerebrospinal fluid in Huntington's disease. *Parkinsonism Relat Disord.* (2011) 17:714–5. doi: 10.1016/j.parkreldis.2011.06.010
210. Niemelä V, Landtblom A-M, Blennow K, Sundblom J. Tau or neurofilament light-Which is the more suitable biomarker for Huntington's disease? *PLoS ONE.* (2017) 12:e0172762. doi: 10.1371/journal.pone.0172762
211. Rodrigues FB, Byrne LM, McColgan P, Robertson N, Tabrizi SJ, Zetterberg H, et al. Cerebrospinal fluid inflammatory biomarkers reflect clinical severity in Huntington's disease. *PLoS ONE.* (2016) 11:e0163479. doi: 10.1371/journal.pone.0163479
212. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol.* (2016) 15:673–84. doi: 10.1016/S1474-4422(16)00070-3
213. Denis HL, David LS, Cicchetti F. Antibody-based therapies for Huntington's disease: current status and future directions. *Neurobiol Dis.* (2019) 132:104569. doi: 10.1016/j.nbd.2019.104569
214. Wagh A, Law B. Methods for conjugating antibodies to nanocarriers. *Methods Mol Biol.* (2013) 1045:249–66. doi: 10.1007/978-1-62703-541-5_15
215. Bartl S, Oueslati A, Southwell AL, Siddu A, Parth M, David LS, et al. Inhibiting cellular uptake of mutant huntingtin using a monoclonal antibody: implications for the treatment of Huntington's disease. *Neurobiol Dis.* (2020) 141:104943. doi: 10.1016/j.nbd.2020.104943
216. Cicchetti F, Lacroix S, Cisbani G, Vallières N, Saint-Pierre M, St-Amour I, et al. Mutant huntingtin is present in neuronal grafts in Huntington disease patients. *Ann Neurol.* (2014) 76:31–42. doi: 10.1002/ana.24174
217. Gosset P, Maxan A, Alpaugh M, Breger L, Dehay B, Tao Z, et al. Evidence for the spread of human-derived mutant huntingtin protein in mice and non-human primates. *Neurobiol Dis.* (2020) 141:104941. doi: 10.1016/j.nbd.2020.104941
218. Jeon I, Cicchetti F, Cisbani G, Lee S, Li E, Bae J, et al. Human-to-mouse prion-like propagation of mutant huntingtin protein. *Acta Neuropathol.* (2016) 132:577–92. doi: 10.1007/s00401-016-1582-9
219. Masnata M, Cicchetti F. The evidence for the spread and seeding capacities of the mutant huntingtin protein in *in vitro* systems and their therapeutic implications. *Front Neurosci.* (2017) 11:647. doi: 10.3389/fnins.2017.00647
220. Masnata M, Sciacca G, Maxan A, Bousset L, Denis HL, Lauruol F, et al. Demonstration of prion-like properties of mutant huntingtin fibrils in both *in vitro* and *in vivo* paradigms. *Acta Neuropathol.* (2019) 137:981–1001. doi: 10.1007/s00401-019-01973-6
221. Maxan A, Sciacca G, Alpaugh M, Tao Z, Breger L, Dehay B, et al. Use of adeno-associated virus-mediated delivery of mutant huntingtin to study the spreading capacity of the protein in mice and non-human primates. *Neurobiol Dis.* (2020) 141:104951. doi: 10.1016/j.nbd.2020.104951
222. Rieux M, Alpaugh M, Sciacca G, Saint-Pierre M, Masnata M, Denis HL, et al. Shedding a new light on Huntington's disease: how blood can both propagate and ameliorate disease pathology. *Mol Psychiatry.* (2020) doi: 10.1038/s41380-020-0787-4
223. Giehl K, Reetz K, Dogan I, Werner CJ, Schulz JB, Hammes J, et al. Tau pathology in Huntington's disease: a brief *in vivo* PET-imaging report. *Basal Ganglia.* (2017) 8:13. doi: 10.1016/j.baga.2017.02.038
224. Pascoal TA, Shin M, Kang MS, Chamoun M, Chartrand D, Mathotaarachchi S, et al. *In vivo* quantification of neurofibrillary tangles with [18F]MK-6240. *Alzheimer's Res Ther.* (2018) 10:74. doi: 10.1186/s13195-018-0402-y
225. Gong N-J, Chan C-C, Leung L-M, Wong C-S, Dibb R, Liu C. Differential microstructural and morphological abnormalities in mild cognitive impairment and Alzheimer's disease: evidence from cortical and deep gray matter. *Hum Brain Mapp.* (2017) 38:2495–508. doi: 10.1002/hbm.23535
226. Praprotnik D, Smith MA, Richey PL, Vinters HV, Perry G. Filament heterogeneity within the dystrophic neurites of senile plaques suggests blockage of fast axonal transport in Alzheimer's disease. *Acta Neuropathol.* (1996) 91:226–35. doi: 10.1007/s004010050420
227. Scheff SW, DeKosky ST, Price DA. Quantitative assessment of cortical synaptic density in Alzheimer's disease. *Neurobiol Aging.* (1990) 11:29–37. doi: 10.1016/0197-4580(90)90059-9
228. Szebenyi G, Morfini GA, Babcock A, Gould M, Selkoe K, Stenoien DL, et al. Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. *Neuron.* (2003) 40:41–52. doi: 10.1016/S0896-6273(03)00569-5
229. Zhang B, Maiti A, Shively S, Lakhani F, McDonald-Jones G, Bruce J, 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 USA.* (2005) 102:227–31. doi: 10.1073/pnas.0406361102
230. Tsai RM, Miller Z, Koestler M, Rojas JC, Ljubenkov PA, Rosen HJ, et al. Reactions to multiple ascending doses of the microtubule stabilizer TPI-287 in patients with Alzheimer disease, progressive supranuclear palsy, and corticobasal syndrome: a randomized clinical trial. *JAMA Neurol.* (2019) 77:215–24. doi: 10.1001/jamaneurol.2019.3812
231. Gozes I, Divinski I. NAP, a neuroprotective drug candidate in clinical trials, stimulates microtubule assembly in the living cell. *Curr Alzheimer Res.* (2007) 4:507–9. doi: 10.2174/156720507783018208
232. Sari Y. Activity-dependent neuroprotective protein-derived peptide, NAP, preventing alcohol-induced apoptosis in fetal brain of C57BL/6 mouse. *Neuroscience.* (2009) 158:1426–35. doi: 10.1016/j.neuroscience.2008.11.021
233. Morimoto BH, Schmechel D, Hirman J, Blackwell A, Keith J, Gold M, et al. A double-blind, placebo-controlled, ascending-dose, randomized study to evaluate the safety, tolerability and effects on cognition of AL-108 after 12 weeks of intranasal administration in subjects with mild cognitive impairment. *Dement Geriatr Cogn Disord.* (2013) 35:325–36. doi: 10.1159/000348347
234. Sudo H, Baas PW. Strategies for diminishing katanin-based loss of microtubules in tauopathic neurodegenerative diseases. *Hum Mol Genet.* (2011) 20:763–78. doi: 10.1093/hmg/ddq521
235. Melo TQ, van Zomeren KC, Ferrari MFR, Boddeke HWGM, Copray JCVM. Impairment of mitochondria dynamics by human A53T α-synuclein and rescue by NAP (davunetide) in a cell model for Parkinson's disease. *Exp Brain Res.* (2017) 235:731–42. doi: 10.1007/s00221-016-4836-9
236. Vulih-Shultzman I, Pinhasov A, Mandel S, Grigoriadis N, Touloumi O, Pittel Z, et al. Activity-dependent neuroprotective protein snippet NAP reduces tau hyperphosphorylation and enhances learning in a novel transgenic mouse model. *J Pharmacol Exp Ther.* (2007) 323:438–49. doi: 10.1124/jpet.107.129551
237. Boxer AL, Lang AE, Grossman M, Knopman DS, Miller BL, Schneider LS, et al. Davunetide in patients with progressive supranuclear palsy: a randomised, double-blind, placebo-controlled phase 2/3 trial. *Lancet Neurol.* (2014) 13:676–85. doi: 10.1016/S1474-4422(14)70088-2
238. Weaver BA. How taxol/paclitaxel kills cancer cells. *Mol Biol Cell.* (2014) 25:2677–81. doi: 10.1091/mbc.e14-04-0916
239. Caparica R, Lambertini M, Azambuja E de. How I treat metastatic triple-negative breast cancer. *ESMO Open.* (2019) 4(Suppl. 2):e000504. doi: 10.1136/esmoopen-2019-000504
240. Quick QA. Epothilone B induces glioblastoma cell death via survivin down-regulation. *Exp Oncol.* (2008) 30:195–201.
241. Varidaki A, Hong Y, Coffey ET. Repositioning microtubule stabilizing drugs for brain disorders. *Front Cell Neurosci.* (2018) 12:226. doi: 10.3389/fncel.2018.00226
242. Hayashi S, Toyoshima Y, Hasegawa M, Umeda Y, Wakabayashi K, Tokiguchi S, et al. Late-onset frontotemporal dementia with a novel exon 1 (Arg5His) tau gene mutation. *Ann Neurol.* (2002) 51:525–30. doi: 10.1002/ana.10163
243. Iyer A, Lapointe NE, Zielke K, Berdynski M, Guzman E, Barczak A, et al. A novel MAPT mutation, G55R, in a frontotemporal dementia patient leads to altered tau function. *PLoS ONE.* (2013) 8:e76409. doi: 10.1371/journal.pone.0076409
244. Spillantini MG, Yoshida H, Rizzini C, Lantos PL, Khan N, Rossor MN, et al. A novel tau mutation (N296N) in familial dementia with swollen achromatic neurons and corticobasal inclusion bodies. *Ann Neurol.* (2000) 48:939–43. doi: 10.1002/1531-8249(200012)48:6<939::AID-ANA17>3.0.CO;2-1

245. Stanford PM, Brooks WS, Teber ET, Hallupp M, McLean C, Halliday GM, et al. Frequency of tau mutations in familial and sporadic frontotemporal dementia and other tauopathies. *J Neurol.* (2004) 251:1098–104. doi: 10.1007/s00415-004-0489-x
246. Caffrey TM, Wade-Martins R. Functional MAPT haplotypes: Bridging the gap between genotype and neuropathology. *Neurobiol Dis.* (2007) 27:1–10. doi: 10.1016/j.nbd.2007.04.006
247. Schoch KM, DeVos SL, Miller RL, Chun SJ, Norrbom M, Wozniak DF, et al. Increased 4R-tau induces pathological changes in a human-tau mouse model. *Neuron.* (2016) 90:941–7. doi: 10.1016/j.neuron.2016.04.042
248. Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. *Acta Neuropathol.* (2017) 133:665–704. doi: 10.1007/s00401-017-1707-9
249. Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK, et al. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nat Med.* (2012) 18:291–5. doi: 10.1038/nm.2613
250. Ikegami S, Harada A, Hirokawa N. Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice. *Neurosci Lett.* (2000) 279:129–32. doi: 10.1016/S0304-3940(99)00964-7
251. DeVos SL, Miller RL, Schoch KM, Holmes BB, Kebodeaux CS, Wegener AJ, et al. Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. *Sci Transl Med.* (2017) 9:eaag0481. doi: 10.1126/scitranslmed.aag0481
252. Ionis Pharmaceuticals, Inc. *A Randomized, Double-Blind, Placebo-Controlled Study, Followed by an Open-Label Extension, to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Ascending Doses of Intrathecally Administered ISIS 814907 in Patients With Mild Alzheimer's Disease.* Report No.: NCT03186989 (2020). Available online at: clinicaltrials.gov (accessed June 23, 2020).
253. Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, Wild EJ, Saft C, Barker RA, et al. Targeting huntingtin expression in patients with Huntington's Disease. *N Engl J Med.* (2019) 380:2307–16. doi: 10.1056/NEJMoa1900907
254. Mullard A. Pioneering antisense drug heads into pivotal trials for Huntington disease. *Nat Rev Drug Discov.* (2019) 18:161–3. doi: 10.1038/d41573-019-00018-7
255. Smith AV, Tabrizi SJ. Therapeutic antisense targeting of huntingtin. *DNA Cell Biol.* (2019) 39:154–8. doi: 10.1089/dna.2019.5188
256. Fernández-Nogales M, Lucas JJ. Altered levels and isoforms of tau and nuclear membrane invaginations in Huntington's disease. *Front Cell Neurosci.* (2019) 13:574. doi: 10.3389/fncel.2019.00574
257. Oberemok VV, Laikova KV, Repetskaya AI, Kenyo IM, Gorlov MV, Kasich IN, et al. A Half-century history of applications of antisense oligonucleotides in medicine, agriculture and forestry: we should continue the journey. *Molecules.* (2018) 23:1302. doi: 10.3390/molecules23061302
258. Mena R, Edwards PC, Harrington CR, Mukaetova-Ladinska EB, Wischik CM. Staging the pathological assembly of truncated tau protein into paired helical filaments in Alzheimer's disease. *Acta Neuropathol.* (1996) 91:633–41. doi: 10.1007/s004010050477
259. Nacharaju P, Ko L, Yen SH. Characterization of in vitro glycation sites of tau. *J Neurochem.* (1997) 69:1709–19. doi: 10.1046/j.1471-4159.1997.69041709.x
260. Reynolds MR, Berry RW, Binder LI. Site-specific nitration and oxidative dityrosine bridging of the tau protein by peroxynitrite: implications for Alzheimer's disease. *Biochemistry.* (2005) 44:1690–700. doi: 10.1021/bi047982v
261. Cripps D, Thomas SN, Jeng Y, Yang F, Davies P, Yang AJ. Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. *J Biol Chem.* (2006) 281:10825–38. doi: 10.1074/jbc.M512786200
262. Martin L, Latypova X, Terro F. Post-translational modifications of tau protein: Implications for Alzheimer's disease. *Neurochem Int.* (2011) 58:458–71. doi: 10.1016/j.neuint.2010.12.023
263. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest.* (2004) 114:121–30. doi: 10.1172/JCI200420640
264. Boxer A. *A 6 Month, Open-Label, Pilot Futility Clinical Trial of Oral Salsalate for Progressive Supranuclear Palsy.* Report No.: NCT02422485 (2018). Available online at: clinicaltrials.gov (accessed July 1, 2020).
265. Keller JN, Hanni KB, Markesbery WR. Impaired proteasome function in Alzheimer's disease. *J Neurochem.* (2000) 75:436–9. doi: 10.1046/j.1471-4159.2000.0750436.x
266. Myeku N, Clelland CL, Emrani S, Kukushkin NV, Yu WH, Goldberg AL, et al. Tau-driven 26S proteasome impairment and cognitive dysfunction can be prevented early in disease by activating cAMP-PKA signaling. *Nat Med.* (2016) 22:46–53. doi: 10.1038/nm.4011
267. van Leeuwen FW, Hol EM, Fischer DF. Frameshift proteins in Alzheimer's disease and in other conformational disorders: time for the ubiquitin-proteasome system. *J Alzheimers Dis.* (2006) 9(3 Suppl):319–25. doi: 10.3233/JAD-2006-9S336
268. Lokireddy S, Kukushkin NV, Goldberg AL. cAMP-induced phosphorylation of 26S proteasomes on Rpn6/PSMD11 enhances their activity and the degradation of misfolded proteins. *Proc Natl Acad Sci USA.* (2015) 112:E7176–85. doi: 10.1073/pnas.1522332112
269. Ortega Z, Lucas JJ. Ubiquitin–proteasome system involvement in Huntington's disease. *Front Mol Neurosci.* (2014) 7:77. doi: 10.3389/fnmol.2014.00077
270. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science.* (1997) 277:1990–3. doi: 10.1126/science.277.5334.1990
271. Bett JS, Cook C, Petrucelli L, Bates GP. The ubiquitin-proteasome reporter GFPu does not accumulate in neurons of the R6/2 transgenic mouse model of Huntington's Disease. *PLoS ONE.* (2009) 4:e5128. doi: 10.1371/journal.pone.0005128
272. Ortega Z, Díaz-Hernández M, Maynard CJ, Hernández F, Dantuma NP, Lucas JJ. Acute polyglutamine expression in inducible mouse model unravels ubiquitin/proteasome system impairment and permanent recovery attributable to aggregate formation. *J Neurosci.* (2010) 30:3675–88. doi: 10.1523/JNEUROSCI.5673-09.2010
273. Schipper-Krom S, Juenemann K, Jansen AH, Wiemhoefer A, Nieuwendijk R van den, Smith DL, et al. Dynamic recruitment of active proteasomes into polyglutamine initiated inclusion bodies. *FEBS Lett.* (2014) 588:151–9. doi: 10.1016/j.febslet.2013.11.023
274. Nekrasov ED, Vigont VA, Klyushnikov SA, Lebedeva OS, Vassina EM, Bogomazova AN, et al. Manifestation of Huntington's disease pathology in human induced pluripotent stem cell-derived neurons. *Mol Neurodegener.* (2016) 11:27. doi: 10.1186/s13024-016-0092-5
275. Seo H, Sonntag K-C, Kim W, Cattaneo E, Isacson O. Proteasome activator enhances survival of Huntington's disease neuronal model cells. *PLoS ONE.* (2007) 2:e238. doi: 10.1371/journal.pone.0000238
276. Boxer AL, Yu J-T, Golbe LI, Litvan I, Lang AE, Höglinger GU. Advances in progressive supranuclear palsy: new diagnostic criteria, biomarkers, and therapeutic approaches. *Lancet Neurol.* (2017) 16:552–63. doi: 10.1016/S1474-4422(17)30157-6
277. Bejanin A, Schonhaut DR, La Joie R, Kramer JH, Baker SL, Sosa N, et al. Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. *Brain.* (2017) 140:3286–300. doi: 10.1093/brain/awx243

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 23 November 2020
doi: 10.3389/fneur.2020.598907

Alterations in Tau Metabolism in ALS and ALS-FTSD

Michael J. Strong[1,2]*, *Neil S. Donison*[1,3] *and Kathryn Volkening*[1,2]

[1] *Molecular Medicine, Schulich School of Medicine and Dentistry, Robarts Research Institute, Western University, London, ON, Canada,* [2] *Department of Clinical Neurological Sciences, Schulich School of Medicine and Dentistry, Western University, London, ON, Canada,* [3] *Neuroscience Graduate Program, Western University, London, ON, Canada*

There is increasing acceptance that amyotrophic lateral sclerosis (ALS), classically considered a neurodegenerative disease affecting almost exclusively motor neurons, is syndromic with both clinical and biological heterogeneity. This is most evident in its association with a broad range of neuropsychological, behavioral, speech and language deficits [collectively termed ALS frontotemporal spectrum disorder (ALS-FTSD)]. Although the most consistent pathology of ALS and ALS-FTSD is a disturbance in TAR DNA binding protein 43 kDa (TDP-43) metabolism, alterations in microtubule-associated tau protein (tau) metabolism can also be observed in ALS-FTSD, most prominently as pathological phosphorylation at Thr^{175} ($pThr^{175}$tau). $pThr^{175}$ has been shown to promote exposure of the phosphatase activating domain (PAD) in the tau N-terminus with the consequent activation of GSK3β mediated phosphorylation at Thr^{231} ($pThr^{231}$tau) leading to pathological oligomer formation. This pathological cascade of tau phosphorylation has been observed in chronic traumatic encephalopathy with ALS (CTE-ALS) and in both *in vivo* and *in vitro* experimental paradigms, suggesting that it is of critical relevance to the pathobiology of ALS-FTSD. It is also evident that the co-existence of alterations in the metabolism of TDP-43 and tau acts synergistically in a rodent model to exacerbate the pathology of either.

Keywords: TDP-43, phosphorylation, traumatic encephalopathy, motor neuron, frontotemporal

OPEN ACCESS

Edited by:
Sonia Do Carmo,
McGill University, Canada

Reviewed by:
Antonio Giuliano Zippo,
Institute of Neuroscience, Italy
Shinsuke Ishigaki,
Nagoya University, Japan
Lezanne Ooi,
University of Wollongong, Australia

***Correspondence:**
Michael J. Strong
mstrong@uwo.ca

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *25 August 2020*
Accepted: *27 October 2020*
Published: *23 November 2020*

Citation:
Strong MJ, Donison NS and Volkening K (2020) Alterations in Tau Metabolism in ALS and ALS-FTSD. Front. Neurol. 11:598907. doi: 10.3389/fneur.2020.598907

INTRODUCTION

Our understanding of the pathobiology of amyotrophic lateral sclerosis (ALS) has evolved dramatically since its first description as a clinicopathological entity in 1874 by Charcot as a disorder of progressive muscular atrophy associated with spasticity (1). ALS was once considered a disorder restricted to the degeneration of the descending supraspinal motor neurons and those lower motor neurons subserving bulbar and spinal motor functions, with specific sparing of a number of functions (including cognition). This concept, however, has been replaced by the understanding that ALS is a multisystems disorder for which motor neuron dysfunction is one aspect of a much larger picture (2, 3).

Given this, it is not surprising that ALS is now considered to be syndromic with clinical manifestations that reflect not only a range of underlying genetic and biochemical impairments consistent with prominent disruptions in RNA metabolism and protein degradation pathways, but also with considerable non-motor involvement (4). This latter aspect has become increasingly evident as our recognition and understanding of the neuropsychological manifestations of ALS has grown. While there has also been remarkable growth in our understanding of the perturbed

biochemistry underlying this aspect of the disorder, there remains a tremendous amount that is unknown. In this review, we will discuss the nature of the phenotypic heterogeneity of ALS as it applies to associated syndromes of frontotemporal dysfunction that can be associated with ALS, explore the nature of the proteinopathies that are thought to underlie the process including a critical analysis of perturbations in microtubule associated protein tau (tau) metabolism, and consider a conceptual framework in which synergism amongst co-expressed toxic proteins can drive disease phenotype.

CONTEMPORARY CONCEPTUALIZATION OF THE CLINICAL AND NEUROPATHOLOGICAL FEATURES OF ALS

The classic clinical and neuropathological description of ALS hinges on the evidence of motor system degeneration in which the loss of lower motor neurons drives progressive muscle atrophy while the loss of upper motor neurons is predominantly, but not exclusively, manifested as spasticity. The loss of motor function culminates in respiratory failure in the majority of individuals within 3 and 5 years of symptom onset, although there is wide variability in survivorship (5). Degenerating motor neurons in ALS classically bear the neuropathological hallmark of neuronal cytoplasmic inclusions (NCIs) composed largely of cytoskeletal proteins, predominantly of the neuronal intermediate filament family (6). Ubiquitination of these proteins is consistent with the concept of impaired proteasomal degradation in ALS as a key pathological process.

This classical conceptualization of ALS has been significantly altered through two fundamental observations: firstly, that widespread frontotemporal dysfunction can occur in a significant proportion of patients (7–11); and secondly, that a fundamental alteration in RNA metabolism, at multiple levels, is a core biological process for the majority of ALS cases (12). This latter postulate is supported by the observation of NCIs composed of a broad range of RNA binding proteins not only within degenerating motor neurons, but within cortical and subcortical neurons in ALS. It was these observations that have now provided a unifying linkage between the two major clinical aspects of ALS–progressive motor neuron degeneration and frontotemporal dysfunction. The key breakthrough that led to this transformative understanding of ALS was the discovery that TDP-43 (TAR DNA binding protein, or transactive response DNA binding protein 43 kDa) accumulates in degenerating motor neurons and within degenerating cortical neurons in frontotemporal lobar degeneration (FTLD) (13, 14). This protein is a DNA/RNA binding protein that is vital to stress and injury responses and is intricately involved in the regulation of RNA metabolism (15, 16). While pathological neuronal and glial cytoplasmic and nuclear inclusions of TDP-43 are now considered to be the neuropathological hallmark of ALS, multiple RNA binding proteins can aggregate as pathological NCIs in ALS, often within the same motor neurons, and often colocalizing to the same aggregates (17–24). This lends support to the broader concept of ALS as a disorder of RNA metabolism in which a diverse array of RNA binding proteins can be involved, likely in several different mechanisms ranging from mutations resulting in gain- or loss-of-function of RNA binding proteins [including fused in sarcoma (FUS) and TDP-43] to also including those proteins in which ALS-associated mutations confer novel RNA interacting capacity [for instance as observed with mutations in copper/zinc superoxide dismutase (mtSOD1)] (25–27). This is consistent with the conceptualization that ALS is syndromic and reflective of a significant biological heterogeneity underlying not just its pathobiology, but also its phenotypic expression.

Nowhere is this more evident than in our understanding and acceptance that individuals with otherwise classical ALS can be affected by a range of neuropsychological, behavioral, speech, and language deficits. These can range from mild to severe impairment, including frontotemporal dementia (FTD) (28, 29). These deficits are encapsulated in the most recent diagnostic criteria under the rubric of the "frontotemporal spectrum disorder" of ALS (ALS-FTSD) (7). Applying these criteria, less than half of ALS patients have a pure motor neuron disorder in which there is no evidence of degeneration outside of the motor system, and for whom the El Escorial or Awaji criteria are fully met (30, 31). Amongst the remaining patients, ∼15% will also suffer from, or will have presented with, a FTD that meets the Rascovsky et al. criteria for diagnosis, including progression (32, 33). The remaining patients will exhibit one or more features of behavioral impairment, cognitive or executive dysfunction, speech, and/or language impairments, and in some cases, a mixture thereof but at levels insufficient for a diagnosis of FTD. Approximately 2% of ALS patients can also be affected by a non-FTD dementia such as Alzheimer's disease or vascular cognitive impairment. Finally, there is also a subgroup of patients with frontotemporal lobar degeneration (FTLD, the neuropathological correlate of the FTD) in whom motor neuron degeneration, typically marked by pathological TDP-43 inclusions, is only obvious postmortem.

It is this broad range of clinical deficits that has led to the current thinking that ALS and FTD are but two points on a disease continuum, with ALS-FTSD residing between these two extremes (34). Where along this continuum a patient will manifest is simply the clinical reflection of where the greatest burden of disease is impacting at that moment in time, both in terms of the extent of pathology present but also on the nature of disruption of the affected neural networks. This conceptualization is supported by the observation that the neuropathological hallmark of the vast majority of ALS cases, regardless of the presence or absence of ALS-FTSD, is the presence of pathological cytoplasmic TDP-43 inclusions reflective of disruption in the metabolism of TDP-43 and thus sharing a feature in common with a subset of FTD patients (35, 36).

To this end, the pathological characterization of FTLD is based on the primary protein associated with the neuropathology. Using this classification, approximately half of which are FTLD-TDP (37) and slightly less than half are tau predominant pathology (FTLD-tau) (36). The remaining minority of cases

demonstrate neither, but rather pathology driven by alterations in the FET family of proteins [FUS; Ewing's Sarcoma (EWS) and TATA-binding protein-associated factor 15 (TAF15)] metabolism or with evidence for impaired ubiquitin-conjugated of proteins (FTLD-UPS) (37). The challenge with this categorization is that there is increasing evidence to support overlaps amongst these pathologies, and in particular between TDP-43 and tau (38). As will be discussed, there is evidence to suggest that this underlies the pathobiology of ALS-FTSD.

PATHOLOGICAL TAU METABOLISM IN ALS-FTSD

Tau is an intrinsically disordered, highly conserved protein that is primarily enriched in axons of central neurons and whose primary function is to stabilize and promote microtubule stability (39–42). The *MAPT* gene located on chromosome 17q21 that encodes for human tau contains 16 exons, of which exons 2 and 3 encode for N-terminus insertions of 29 amino acids in length while exon 10 encodes for one of four microtubule binding repeats (**Figure 1**). Through alternative splicing of the tau mRNA, tau can therefore exist as 6 isoforms ranging from 352 to 441 amino acid residues with apparent molecular weights of 48–67 kDa (predicted 36–45 kDa without modifications) and characterized by the presence or absence of one or two N-terminus inserts (yielding 0N, 1N, or 2N isoforms) accompanied by the expression of either 3 or 4 microtubule binding repeats (3R or 4R) (48). Tau is a largely unstructured soluble protein that *in vitro* takes on a "paperclip" conformation such that the N-terminus, C-terminus, and microtubule binding region (MTBR) are in close approximation (**Figure 1C**) (43–46, 49, 50). The relative expression of 3R to 4R isoforms is highly regulated and when altered is associated with a range of neurodegenerative disease states characterized by FTLD with pathological tau deposition (36). Tau undergoes extensive post-translational modification, with phosphorylation arguably being the most crucial to its interaction with microtubules (48, 51). Site specific tau phosphorylation modulates the interaction between tau and microtubules, with pathological phosphorylation dissociating the two and giving rise to elevated soluble tau isoforms which are then free to dimerize into stable tau oligomers, which then continue to polymerize into protomers and lead to fibril formation.

While the primary function of tau is to promote microtubule stability, tau also appears to be involved in several other non-cytoskeletal related activities within cells. Phosphorylation of tau plays a role in determining the localization of tau throughout neurons, with soma localized tau exhibiting high levels of phosphorylation, and a gradual loss of phosphorylation occurring as tau is located closer to the growth cone of an axon (52). It was originally thought that tau phosphorylation was altered as tau was transported through the cell; however, evidence also supports a model in which new tau is synthesized and immediately phosphorylated, with this pattern of phosphorylation dictating where in the cell that tau will ultimately be localized (49, 53, 54).

A tau isoform that lacks phosphorylation at S^{195}-S^{202} is localized to the nucleus, where it is involved in DNA binding (either through association with AT-rich DNA, or with the minor groove of AT-rich DNA sequences) (55, 56), heterochromatin stability, transcription repression of ribosomal DNA, long non-coding RNA production, or possibly in repressing gene expression (55, 57, 58). The isoform of tau expressed is also important in that compartmentalization appears to prefer one isoform type to another, with isoforms bearing 0N or 2N preferentially located outside of the nucleus, while 1N isoforms are found preferentially in the nucleus (59).

In response to cell stress, nuclear tau translocates to the nucleolus, where it is postulated to increase RNA binding protein interactions with TIA1 (T-cell intracellular antigen) (60). Cell stress also elicits the formation of stress granules (SG) within neurons. TIA1 translocates to SG in the cytosol, where these TIA1 positive SG have been detected to localize with tau that is highly phosphorylated, or "hyper" phosphorylated. However, not all SG are TIA1 positive, and other populations of SG do not appear to colocalize with hyperphosphorylated tau, for example the G3BP1-positive SG (61). SG formation has been noted to be upregulated in ALS, with both TDP-43 and FUS proteins playing important roles in the formation and maintenance of SG [reviewed in (62)]. Proteins with low complexity/highly disordered domains appear to be integral to SG formation, and dysregulation of the expression of these proteins, the presence of mutations, or alterations in the localization of these proteins appear to have a profound effects on SG dynamics in neurodegenerative disease including ALS and FTLD [reviewed in (63, 64)]. The role(s) of tau in SG formation is unclear; however evidence shows that if tau is overexpressed there is an increase in SG formation (65–67). There is also evidence suggesting that the deposition of tau can induce the condensation of RNA binding proteins through liquid-liquid phase separation (LLPS). LLPS has been shown to involve highly disordered protein domains [including the DNA/RNA binding protein TDP-43 (68)] that tend to fall into an insoluble state spontaneously when in high enough concentrations. Tau can experience LLPS and as such may induce the formation of toxic tau conformations (69–72). It would not be unreasonable to postulate that tau may help to initiate LLPS of RNA binding proteins that are contained within the same SG. Consistent with this concept, there is evidence that the RNA binding proteins Musashi 1 and 2 (MSI 1 and MSI 2, respectively), both highly expressed in mature neurons, form intranuclear complexes with oligomeric tau proteins in a concentration dependant manner (73). In doing so, these complexes significantly impair nucleocytoplasmic transport of NLS-containing cargo, a process that has been postulated to fundamental to the pathobiology of ALS and FTD (74). It is of direct relevance therefore that both intracellular and extracellular MSI 1 and MSI 2 complexes are present in both ALS and FTD cortical tissue, and that this occurs in the presence of increased oligomeric tau (73). It is often inferred that the metabolism of tau protein is altered in any given disease process based largely on the presence of tau immunoreactive NCIs; the finding that this immunoreactivity is also for specific tau phospho epitopes normally observed in the presence of a disease state supports this.

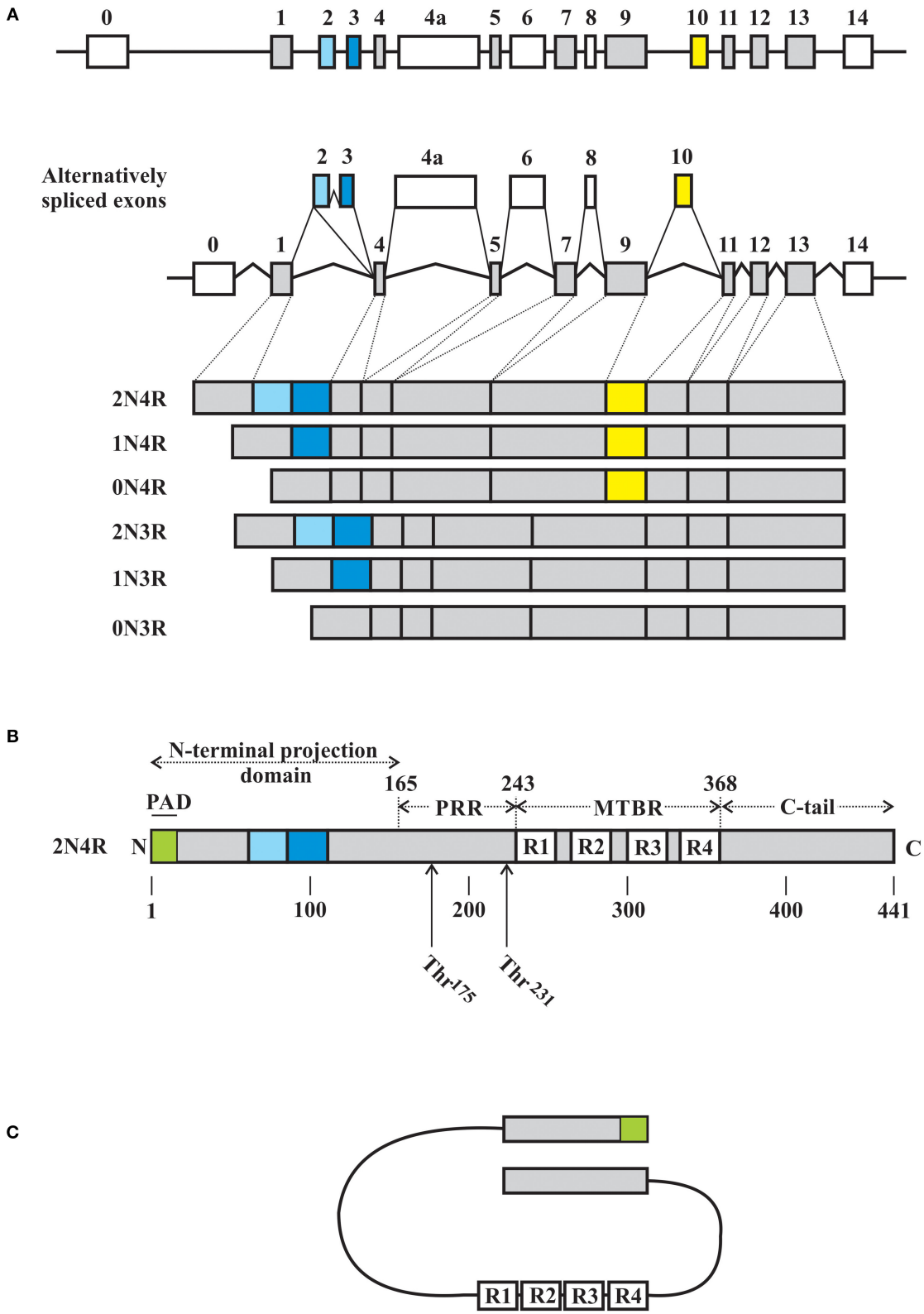


FIGURE 1 | Schematic representations of *MAPT* gene and tau. **(A)** Tau is encoded by a single gene on chromosome 17q.31, with 16 exons, including the alternatively spliced exons 2, 3, 4a, 6, 8, and 10. Start and stop codons are located in exons 1 and 13, respectively. Exons 2, 3, and 10 are alternatively expressed in the adult human brain, giving rise to 6 tau isoforms based on the inclusion of 0, 1, or 2 N-terminus inserts encoded by exons 2 or 3 (exon 2 can be alternatively

(Continued)

FIGURE 1 | spliced independent of exon 3, while exon 3 is always expressed with an exon 2 encoded insert) with 3 or 4 microtubule binding repeats (MTBR) depending on the expression of an exon 10 encoded repeat. As such, the 6 isoforms are described as 0N, 1N, or 2N with either 3R or 4R MTBRs. Exon 4A and 6 are expressed in the peripheral nervous system giving rise to additional tau isoforms. To date, exon 8 does not appear to be expressed. **(B)** Using numbering based on the 2N4R isoform of tau of 441 amino acid residues, tau has four major domains, including the N-terminus domain (residues 1–165) with the phosphatase activating domain (PAD) localized to the extreme N-terminus region, a proline rich domain (PRR, residues 166–242), a microtubule assembly domain (residues 243–367) consisting of either 3 or 4 microtubule binding repeats (R1 thru R4, each of ∼31 amino acid residues), and a C-terminus domain (residues 368–441). **(C)** Schematic representation of the proposed paperclip conformation of soluble tau in which the N-terminus domain, C-terminus domain, and MTBR are in close approximation. Schematic illustrations based on the following references: (43–47).

This is further augmented by the biochemical characterization of the tau protein following separation into detergent soluble or insoluble fractions, with characterization then based on the relative preponderance of either or both the 3R or 4R isoforms. However, the deposition of tau protein can be either a primary process as in neurodegeneration or a secondary process as in a stress response (60, 61, 75).

In the following, we will examine the neuropathological, biochemical, and experimental evidence in support of pathological tau metabolism amongst a subset of ALS patients.

Neuropathological and Biochemical Evidence of Altered Tau Metabolism in ALS

Tau dysmetabolism has been most clearly documented in the Western Pacific variant of ALS in which affected individuals developed either a Parkinson-Dementia-ALS complex or ALS alone, at rates several thousand fold greater than observed amongst the rest of the world (76). The characteristic neuropathological feature was the widespread deposition of tau protein throughout the neuroaxis, including spinal motor neurons (77, 78). This latter feature is not traditionally observed in other ALS variants with the exception of those individuals with chronic traumatic encephalopathy (CTE) who also develop clinical and electrophysiological features consistent with a diagnosis of ALS (CTE-ALS) (79–81).

Beyond this, the evidence for alterations in tau metabolism in ALS has been scant and largely limited to individual case reports or unique inherited disorders in which ALS has been described amongst a broader range of neurological and neuropathological deficits or in association with rare *MAPT* mutations (82–87). However, two instructive exceptions drawn from the spectrum of genetic mutations associated with ALS, ALS-FTD and FTD are the pathological hexanucleotide expansions of C9*orf*72 and the ALS-associated mutations of FUS (88–90). Both provide further support for the concept of a biological continuum underlying the pathogenesis of ALS and FTD in addition to insights into mechanisms of altered tau metabolism.

Pathological hexanucleotide repeat $(GGGGCC)_n$ expansion of C9*orf*72 is the most common hereditary molecular cause of ALS, ALS-FTD and FTD. The neuropathological hallmark of the associated FTLD includes foci of dinucleotide-peptide repeats (DPRs) in specific neuronal subpopulations in association with more widespread pathological TDP-43 deposition [reviewed in (36, 91)] in the absence of an associated tauopathy or when present, as a non-specific finding (92). Although the exact mechanism by which these pathological expansions give rise to a toxic variant of C9*orf*72, there is evidence to support a role in fundamentally altering RNA metabolism through a variety of avenues [reviewed in (91)]. However, there is also emerging evidence that pathological C9*orf*72 expansion may act in a synergistic manner to increase the propensity for tau pathology by increasing cdk5-mediated tau phosphorylation, including pathological phosphorylation (93–95).

In contrast, mutations in FUS are an uncommon molecular cause of familial ALS, rarely observed in sporadic ALS, and found in only a single case report in consanguineous twins with FTD (17, 18, 90, 96–98). While FUS has an array of both cytoplasmic and nuclear functions, it plays a critical role in regulating alternative splicing through its association with the spliceosome, including in regulating the physiological splicing of *MAPT* exon 10 (99). The loss of FUS activity results in the preferential inclusion of exon 10 and an increased expression of 4R tau (100). FUS forms a complex with splicing factor, proline- and glutamine-rich (SFPQ) through an RNA-dependant interaction such that the loss of either FUS or SFPQ results in the preferential expression of 4R tau and an accompanying increase in ptau expression, including pathological tau isoforms (101–103). The importance of this is highlighted by the finding of reduced interactions between FUS and SFPQ across a broad range of FTLDs, including ALS-FTD (102). The recent report of a single atypical case of sporadic ALS manifesting with predominantly upper motor neuron dysfunction with extrapyramidal features who at autopsy demonstrated pyramidal neuron FUS basophilic inclusions that colocalized with AT8 tau immunoreactivity in motor neurons, in addition to a 4R predominant tauopathy, provides support for such a proposed role of FUS in contributing to alterations in tau metabolism in ALS (104).

More recently, a 17 kDa neurotoxic tau fragment (tau^{45-230}) has been observed in extracts of both the brain and ventral spinal cord of sporadic ALS patients, but not in controls (105, 106). This fragment is generated by calpain cleavage of full length tau and has been observed both *in vitro* and *in vivo* across a broad range of tauopathies (107, 108).

Both tau immunoreactive inclusions and pathological tau phosphorylation disproportionate for age have been observed in ALS patients affected with cognitive or executive dysfunction (ALSci), typically in the form of dystrophic neurites, neurofibrillary tangle-like structures and pre-tangles, neuritic granules, and tau-immunoreactive tufted astrocytes (109–111). Tau isolated from mesial frontal cortex and subcortical at postmortem demonstrates a significantly greater propensity for fibril formation in a thioflavin S assay, partitions into the sarkosyl-insoluble fraction as all 6 isoforms (distinguishing it from Alzheimer's disease tau, 3R or 4R tau disorders), and is

pathologically phosphorylated at Thr^{175} ($pThr^{175}$tau) (112). Using a polyclonal antibody recognizing $pThr^{175}$tau, widespread tau deposition, sparing the motor neurons, was observed to be in association with a diffuse increase in TDP-43 immunoreactivity (110). In the same study, it was also observed that of those patients without evidence of neuropsychological impairment, approximately half demonstrated $pThr^{175}$tau immunoreactive pathology although significantly less pronounced than observed in those with evidence of ALS-FTSD. Of note, the active isoform of GSK3β (pGSK3β) was also upregulated in those regions most extensively affected with tau pathology (113). The observation of $pThr^{175}$tau pathology was subsequently confirmed in a separate cohort of motor neuron disease (MND) patients with concomitant FTD, including approximately 10% with non-descript neuronal aggregates and as with the earlier study, associated with pathological TDP-43 expression (114). Although not examined for the $pThr^{175}$tau phospho epitope, ptau ($pSer^{396}$, $pSer^{214}$, and $pSer^{404}$) has been described in ALS cervical spinal cord and motor cortex in the cytosol and nuclei of motor neurons as diffuse immunoreactivity in the absence of fibril formation (115). The presence of nuclear tau in this latter series may be reflective of a neuronal stress response although no comment was made with respect to the presence or absence of nucleolar tau staining. To date, no other tau phospho-epitopes have been examined or characterized in either ALS or ALS-FTSD to this extent.

In addition to this evidence supporting alterations in tau metabolism in sporadic ALS, a subset of CTE patients (in whom a tauopathy is the neuropathological hallmark) will have evidence of ALS either antemortem with clinical or electrophysiological evidence of diffuse motor neuron degeneration, or at autopsy (80, 81, 116). Amongst these patients, $pThr^{175}$tau, $pThr^{231}$tau, and oligomeric tau (T22) immunoreactive inclusions are readily observed in both cortical and spinal motor neurons (79).

Given the broad array of pathological site specific tau phospho epitopes associated with a range of neurodegenerative disorders, the observation of $pThr^{175}$tau immunoreactive inclusions suggests but does not prove that phosphorylation at this epitope is also pathologically important amongst a number of tauopathies (117). However, $pThr^{175}$tau is not observed in fetal human tissue although tau exhibits significantly higher molar phosphorylation in the fetus, nor is it observed in the healthy aged (117, 118). Hence, its presence suggests a role in the genesis of pathological tau fibrils.

Elevations in cerebrospinal fluid tau and/or TDP-43 have been widely documented and suggested as potential biomarkers for ALS although for the former these have in general reflected total tau (119, 120). However, others have failed to find similar increases in either CSF total tau or ptau levels in ALS patients (121–124). Thus, it remains unclear if CSF tau can be used as a surrogate marker for a concomitant tauopathy in ALS.

Experimental Evidence in Support of Alterations of Tau Metabolism in ALS

Supporting the hypothesis of pathological significance of $pThr^{175}$tau, the introduction of a pseudophosphorylated 2N4R human tau construct mimicking $pThr^{175}$tau (Thr^{175}Asp-tau) induces pathological tau fibril formation in both HEK293T and Neuro2a cells, significantly more so than when either wild-type human tau or tau in which phosphorylation at Thr^{175} was inhibited (Thr^{175}Ala-tau) were expressed (125). The induction of pathological tau fibril formation *in vitro* was dependent on up-regulation of pGSK3β activity, which in turn induced phosphorylation at Thr^{231}tau ($pThr^{231}$tau)—one of several key phosphorylation sites in the cascade of driving tau oligomer formation (**Figure 2**) (126). The activation of GSK3β is dependent on the dephosphorylation of $pSer^{9}$ which removes inhibition, allowing for GSK3β activity on its substrate (127, 128). We have shown that the phosphorylation of Thr^{175} of tau is associated with an enhanced exposure of the N-terminus of tau, increasing access to the tau phosphatase activating domain (PAD) located at amino acids 2–18 (129, 130). PAD exposure activates protein phosphatase-1 (PP1) which dephosphorylates $pSer^{9}$ of GSK3β enhancing GSK3β activity through a relative increase in exposure of $pTyr^{216}$ while phosphorylation of tau residue 18 (Y18) by the non-receptor tyrosine kinase fyn inhibits the PP1-GSK3β cascade (129, 131). While it remains unknown how tau phosphorylation at Thr^{175} leads to an increased PAD exposure, Thr^{175} resides within the proline-rich domain of tau in which the phosphorylation of other amino acids induces an opening of the tau paperclip conformation, leading to increased PAD exposure (43, 46, 47, 132). It is reasonable to anticipate that $pThr^{175}$ would act similarly.

PAD exposure has been observed to be a common feature of the pathological inclusions across a broad range of tauopathies (133) and has been associated with neurotoxicity through the inhibition of fast axonal transport as an early contribution to the process of neurodegeneration (130, 133–135). In this mechanism, PAD exposure and activation of GSK3β results in phosphorylation of the kinesin light chain which promotes the release of cargo from kinesin 1 (130).

In addition, $pThr^{231}$tau can exist in either *trans* or *cis* conformation (*trans*-$pThr^{231}$ and *cis*-$pThr^{231}$, respectively), in which *cis*-$pThr^{231}$ is specific to the induction of tau pathology and cannot be readily degraded, in contrast to *trans*-$pThr^{231}$ (136). Under normal physiological conditions, the isomerase peptidyl-propyl *cis-trans* isomerase NIMA-interacting 1 (PIN1) catalyzes the isomerization of *cis*-$pThr^{231}$ to *trans*-$pThr^{231}$, allowing for the dephosphorylation of this site by PP2A (protein phosphatase 2) and the subsequent ability for tau to exert its physiological activity in microtubule polymerization (137, 138). The pathological relevance of *cis*-$pThr^{231}$ is highlighted by its observation in association with pathological tau deposition in AD and CTE (both experimental and human) (139). In accordance to this, it has also been demonstrated that there are reduced levels of PIN1 in human AD brains, resulting in decreased activity and thus the inability to isomerize *cis*-tau (138, 140). Additionally, genetic polymorphisms resulting in a decrease in PIN1 levels are associated with an increased risk of late-onset AD, whereas another polymorphism which leads to increased PIN1 expression has a neuroprotective effect (141, 142). The relative expression of *cis* vs. *trans*-tau and PIN1 activity in ALS or ALS-FTSD has not been examined to date.

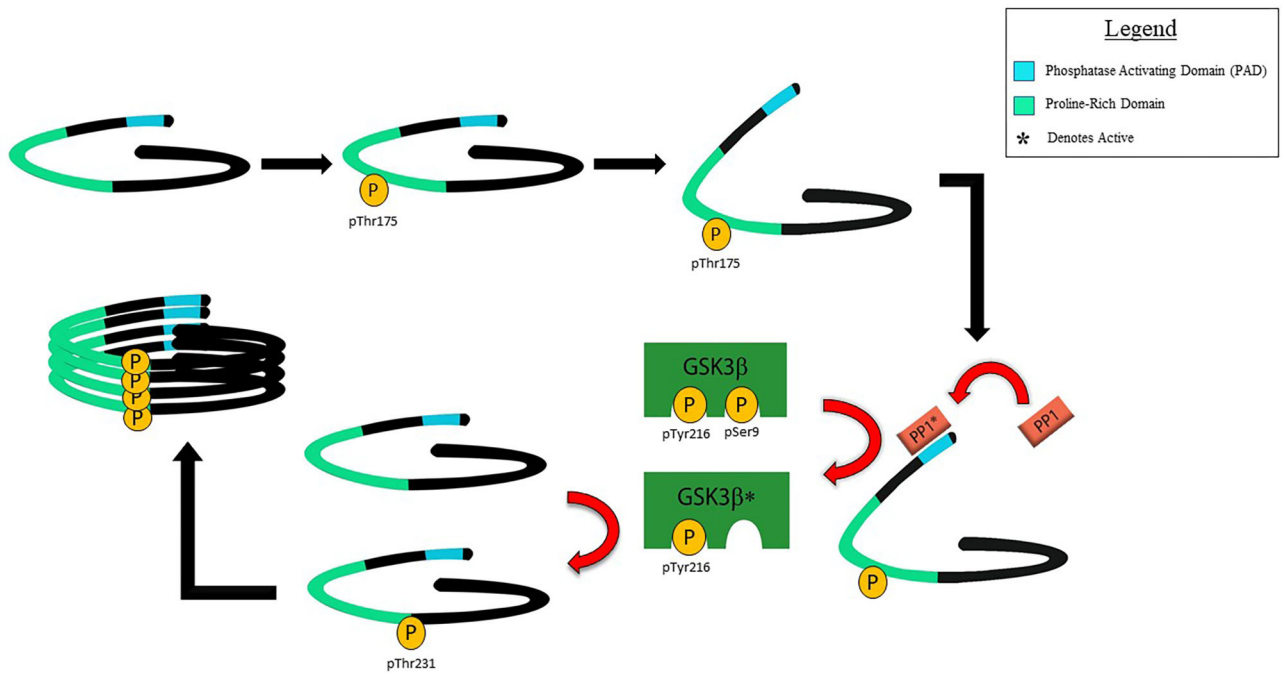


FIGURE 2 | Schematic of proposed mechanism for the toxicity of pThr^{175}tau. Under normal physiological conditions, tau is thought to exist in a closed paperclip conformation. Following phosphorylation of Thr^{175} by a kinase or kinases yet to be defined, the closed paperclip structure opens sufficiently to allow for exposure of the PAD which in turn activates protein phosphatase 1 (PP1). PP1 in turn dephosphorylates Ser^{9} of GSK3β, allowing for increased pGSK3β by unmasking pTyr^{216}. pGSK3β in turn phosphorylates Thr^{231}tau in a process that does not require priming by phosphorylation of Ser^{235}tau. It is not known whether pThr^{231} exists in either the *cis* or *trans* isoform, but our evidence suggests that the presence of pThr^{231} is associated with increased tau oligomer formation and its subsequent polymerization into tau fibrils.

In vivo Evidence of pThr^{175} Toxicity

It had been previously observed that the use of somatic gene transfer of tau harboring the P301L mutation that is causal for frontotemporal dementia with parkinsonism (FTDP-17) using a recombinant adeno-associated virus (rAAV9) injected into the hippocampus of rats induced both tau pathology and impairments in spatial working memory (143). Given this, we examined whether Thr^{175}Asp-tau, when inoculated using somatic gene transfer with rAAV9 could similarly induce both pathology and behavioral deficits in rats. We observed that when Thr^{175}-Asp tau was inoculated into the hippocampus of young adult female Sprague-Dawley rats, tau pathology was evident as early as 1 month post inoculation and consistently so by 1 year post-inoculation (144). While this was not associated with a behavioral or motor phenotype, it was observed that the burden of neuropathology was localized to the CA2 region, a region for which the testing paradigm employed was insensitive. It was of note however that the induction of tau pathology was accompanied by an upregulation of TDP-43 expression in this model.

Given the observation of pThr^{175}tau, pGSK3β, pThr^{231}tau, and oligomeric tau in affected neurons of individuals with CTE-ALS, we also examined whether a single subconcussive injury administered to Sprague-Dawley rats would also induce pathological tau fibril formation. Indeed this was the case, suggesting that this specific pathway of pathological tau fibril formation was a primary event (79).

As has been alluded to, concomitant pathologies of both TDP-43 and tau are increasingly recognized, including AD and the aforementioned ALS, CTE-ALS and *in vivo* models of pThr^{175} tau pathology (145–147). To begin to understand how these two pathologies inter-relate, we repeated the somatic gene transfer AAV9 hippocampal inoculums in rats also expressing the ALS-associated mutant human TDP-43 (TDP-43^{M337V}). In these rats, constitutive cholinergic neuronal expression was driven by a ChAT promoter with a tTA-dependant tetracycline response element driver (148, 149). In the presence of doxycycline, no mtTDP-43 expression occurred whereas ChAT-tTA/TRE-TDP-43^{M337V} rats, in the absence of doxycycline, developed a fulminant motor phenotype with prominent motor neuron NCI formation. Six months following stereotactic hippocampal inoculums of rAAV9 GFP-tagged tau^{T175D}2N4R, a 50% withdrawal of doxycycline was undertaken. Rats were sacrificed 30 days later. Within 3 weeks, all ChAT-tTA/TRE-TDP-43^{M337V} rats had developed motor deficits. At 30 days, a 2-fold increase in the extent of tau pathology was observed in the hippocampus of those rats inoculated with the pseudophosphorylated tau. This was associated with immunoreactivity to a monoclonal antibody recognizing tau phosphorylated at Ser^{202} and Thr^{205} (AT8) (150), suggesting that

the tau construct had been further modified to a pathological tau phospho-isoform in the presence of the mutant TDP-43. There was also a trend toward an increase in the extent of spinal motor neuron pathology, a finding that may serve to explain the shortened survival in ALS-FTSD patients when contrasted to patients with ALS alone (151).

This inter-relationship between tau and TDP-43 has been further characterized in a murine model of CTE using a repetitive mild traumatic brain injury (rmTBI) in C57Bl6 mice using a repeated weight drop. In this model, a prominent tauopathy developed, including *cis*-ptau accumulations at 6 months post injury in association with tau oligomers, tangles, and TDP-43 immunoreactive NCIs (139). As described earlier, the *cis* conformation of pThr^{231}tau prevents pThr^{231} dephosphorylation and the ability of tau to stabilize microtubules. The intraperitoneal administration of a monoclonal neutralizing antibody to *cis*-ptau eliminated the pathological tau accumulation and subsequent TDP-43 pathology, suggesting a linkage between the two pathologies. However, the induction of TDP-43 pathology may in this case represent a physiological stress response marked by an upregulation in its expression as previously observed following axonal injury, and thus only indirectly linked to the induction of the tau pathology (15).

Of note, both tau tubulin kinase 1 (TTBK1) and tau tubulin kinase 2 (TTBK2) have been shown to extensively phosphorylate both TDP-43 (152) and tau (153, 154). Both kinases have been observed to co-localize with TDP-43 and tau aggregates in FTLD-tau and FTLD-TDP cases (152, 155). These observations provide further support for the concept of a pathological commonality underlying these two proteinopathies and a mechanism to drive both pathological tau and TDP-43 phosphorylation concomitantly (156).

CONCLUSIONS

The conventional concept of ALS as a pure motor neuron disorder and a discrete disease has long been replaced by one of a multisystem disorder that is syndromic in nature and for which progressive neuropsychological deficits, including a frontotemporal spectrum disorder, are common. As this conceptualization of ALS has evolved, so too has our understanding of its underlying pathobiology as reflected in the broad range of not only causative gene mutations associated with both familial and sporadic variants of the disorder, but the broad range of protein aggregates which typify its pathology (4). Embedded within this has been the recognition that FTD and ALS are increasingly recognized to share not only a clinical phenotypic spectrum, but also an underlying pathobiology as reflected not only in alterations in the metabolism of TDP-43, but increasingly in the metabolism of tau.

While the classical role of tau is the stabilization of microtubules through interactions mediated by its MBTR, there is increasing evidence to suggest a much broader repertoire of function of tau under both normal physiological states as well as in response to stress. These functions are critically dependant on a complex array of determinants including the somatopically-specific subcellular distribution of individual tau isoforms that includes an emerging role in gene expression and inter-relationships with RNA binding proteins, the impact of cellular stress upon tau expression, the conformational structure of tau and the extent of its post-translational modification (44, 49, 157). Although a broad array of post-translational modifications and their relationship to specific disease states have been well-characterized [reviewed in (44)], we have focused on the specific role of pathological phosphorylation at Thr^{175} and the mechanisms by which this culminates in the generation of oligomeric tau and a subsequent tauopathy, including a synergistic role with pathological TDP-43 deposition.

However, several pivotal questions remain in order to further clarify the role of alterations in tau metabolism in ALS, including:

- In the context of understanding that ALS-FTSD represents a broad array of neuropsychological, behavioral, speech, and language deficits, and that our initial studies characterizing pThr^{175}-tau expression were undertaken in ALS patients with cognitive impairment (ALSci) while those of Behrouzi et al. were undertaken of individuals with FTD and ALS (114), clarifying the spectrum of clinicopathological correlates to ALS-FTSD and whether alterations in tau metabolism are specific to one or more variants within the spectrum;
- Linked to this, further defining the nature of TDP-43 deposition in ALS-FTSD in tau NCI-bearing neurons with a view to understanding whether the TDP-43 accumulation bears hallmarks of pathological tau deposition or that observed in a stress response;
- Identifying the kinase or kinases responsible for the induction of phosphorylation at Thr^{175}, the conditions under which the pathway becomes activated, and whether these differ amongst the range of FTLDs in which pThr^{175}tau has been observed;
- Determining whether the pThr^{231}tau exists in either *cis* or *trans* conformation and the associated activity of PIN1 in ALS-FTSD and its *in vivo* model as demonstrated using somatic gene transfer with rAAV9, including in the presence or absence of pathological ALS-associated TDP-43 mutations;
- Further understanding the inter-relationships between alterations in the expression of specific tau isoforms and alterations in TDP-43 metabolism, in particular in relationship to the role of TDP-43 in mediating gene expression through its nuclear functions as an RNA binding protein; and
- Determining whether therapeutic strategies targeted toward restoring physiological tau/microtubule interactions would be of therapeutic benefit in ALS-FTSD.

AUTHOR CONTRIBUTIONS

MS: conceptualization and original draft preparation. MS, ND, and KV: writing, review, and editing. All authors have read and agreed to the published version of the manuscript.

FUNDING

Portions of this research were funded by the Ontario Neurodegeneration Research Initiative (ONDRI) funding and the National Hockey League Players Association (NHLPA).

REFERENCES

1. Charcot JM, Joffroy A. Deux cas d'atrophie musculaire progressive avec lésions de la substance grise et des faisceaux antérolatéraux de la moelle épinière. *Arch Physiol Norm Pathol.* (1869) 2:354–744.
2. Strong MJ. The evidence for ALS as a multisystems disorder of limited phenotypic expression. *Can J Neurol Sci.* (2001) 28:283–98. doi: 10.1017/S0317167100001505
3. Strong MJ. Revisiting the concept of amyotrophic lateral sclerosis as a multisystems disorder of limited phenotypic expression. *Curr Opin Neurol.* (2017) 30:599–607. doi: 10.1097/WCO.0000000000000488
4. Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. *Eur J Neurol.* (2020) 27:1918–29. doi: 10.1111/ene.14393
5. Strong MJ. The basic aspects of therapeutics in amyotrophic lateral sclerosis. *Pharmacol Ther.* (2003) 98:379–414. doi: 10.1016/S0163-7258(03)00040-8
6. Strong MJ, Kesavapany S, Pant HC. The pathobiology of amyotrophic lateral sclerosis: a proteinopathy? *J Neuropathol Exp Neurol.* (2005) 64:649–64. doi: 10.1097/01.jnen.0000173889.71434.ea
7. Strong MJ, Abrahams S, Goldstein LH, Woolley S, Mclaughlin P, Snowden J, et al. Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): revised diagnostic criteria. *Amyotr Lateral Scler Frontotemporal Degener.* (2017) 18:153–74. doi: 10.1080/21678421.2016.1267768
8. Elamin M, Phukan J, Bede P, Jordan N, Byrne S, Pender N, et al. Executive dysfunction is a negative prognostic indicator in patients with ALS without dementia. *Neurology.* (2011) 76:1263–9. doi: 10.1212/WNL.0b013e318214359f
9. Phukan J, Elamin M, Bede P, Jordan N, Gallagher L, Byrne S, et al. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J Neurol Neurosurg Psychiatry.* (2012) 83:102–8. doi: 10.1136/jnnp-2011-300188
10. Montuschi A, Iazzolino B, Calvo A, Moglia C, Lopiano L, Restagno G, et al. Cognitive correlates in amyotrophic lateral sclerosis: a population-based study in Italy. *J Neurol Neurosurg Psychiatry.* (2015) 86:168–73. doi: 10.1136/jnnp-2013-307223
11. Oh SI, Park A, Kim HJ, Oh KW, Choi H, Kwon MJ, et al. Spectrum of cognitive impairment in Korean ALS patients without known genetic mutations. *PLoS ONE.* (2014) 9:e87163. doi: 10.1371/journal.pone.0087163
12. Strong MJ. The evidence for altered RNA metabolism in amyotrophic lateral sclerosis (ALS). *J Neurol Sci.* (2010) 288:1–12. doi: 10.1016/j.jns.2009.09.029
13. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun.* (2006) 351:602–11. doi: 10.1016/j.bbrc.2006.10.093
14. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science.* (2006) 314:130–3. doi: 10.1126/science.1134108
15. Moisse K, Volkening K, Leystra-Lantz C, Welch I, Hill T, Strong MJ. Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy. *Brain Res.* (2009) 1249:202–11. doi: 10.1016/j.brainres.2008.10.021
16. Wang IF, Wu LS, Chang HY, Shen CKJ. TDP-43, the signature protein of FTLD-U, is a neuronal activity responsive factor. *J Neurochem.* (2008) 105:797–806. doi: 10.1111/j.1471-4159.2007.05190.x
17. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science.* (2009) 323:1205–8. doi: 10.1126/science.1166066
18. Vance C, Rogelj B, Hortobágyi T, De Vos KJ, Nishimura AL, Sreedharan J, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science.* (2009) 323:1208–11. doi: 10.1126/science.1165942
19. Couthouis J, Hart MP, Shorter J, Dejesus-Hernandez M, Erion R, Oristano R, et al. A yeast functional screen predicts new candidate ALS disease genes. *Proc Natl Acad Sci USA.* (2011) 108:20881–90. doi: 10.1073/pnas.1109434108
20. Collins M, Riascos D, Kovalik T, An J, Krupa K, Krupa K, et al. The RNA-binding motif 45 (RBM45) protein accumulates in inclusion bodies in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) patients. *Acta Neuropathol.* (2012) 124:717–32. doi: 10.1007/s00401-012-1045-x
21. Droppelmann CA, Keller BA, Campos-Melo D, Volkening K, Strong MJ. Rho guanine nucleotide exchange factor is an NFL mRNA destabilizing factor that forms cytoplasmic inclusions in amyotrophic lateral sclerosis. *Neurobiol Aging.* (2013) 34:242–62. doi: 10.1016/j.neurobiolaging.2012.06.021
22. Keller BA, Volkening K, Droppelmann CA, Ang LC, Rademakers R, Strong MJ. Co-aggregation of RNA binding proteins in ALS spinal motor neurons: evidence of a common pathogenic mechanism. *Acta Neuropathol.* (2012) 124:733–47. doi: 10.1007/s00401-012-1035-z
23. Ash PE, Bieniek KF, Gendron TF, Caulfield T, Lin WL, Dejesus-Hernandez M, et al. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron.* (2013) 77:639–46. doi: 10.1016/j.neuron.2013.02.004
24. Mori K, Arzberger T, Grasser FA, Gijselinck I, May S, Rentzsch K, et al. Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. *Acta Neuropathol.* (2013) 126:881–93. doi: 10.1007/s00401-013-1189-3
25. Ge WW, Wen W, Strong WL, Leystra-Lantz C, Strong MJ. Mutant copper/zinc superoxide dismutase binds to and destabilizes human low molecular weight neurofilament mRNA. *J Biol Chem.* (2005) 280:118–24. doi: 10.1074/jbc.M405065200
26. Lagier-Tourenne C, Polymenidou M, Cleveland DW. TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum Mol Genet.* (2010) 19:R46–64. doi: 10.1093/hmg/ddq137
27. Lagier-Tourenne C, Cleveland DW. Rethinking ALS: the FUS about TDP-43. *Cell.* (2009) 136:1001–4. doi: 10.1016/j.cell.2009.03.006
28. Strong MJ. The syndromes of frontotemporal dysfunction in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler.* (2008) 9:323–38. doi: 10.1080/17482960802372371
29. Bak TH, Hodges JR. Motor neurone disease, dementia and aphasia; coincidence, co-occurrence or continuum? *J Neurol.* (2001) 248:260–70. doi: 10.1007/s004150170199
30. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord.* (2000) 1:293–9. doi: 10.1080/146608200300079536
31. de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, et al. The Awaji criteria for diagnosis of ALS. *Muscle Nerve.* (2011) 44:456–7. doi: 10.1002/mus.22175
32. Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain.* (2011) 134:2456–77. doi: 10.1093/brain/awr179
33. Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology.* (2002) 59:1077–9. doi: 10.1212/WNL.59.7.1077
34. Lillo P, Savage S, Mioshi E, Kiernan MC, Hodges JR. Amyotrophic lateral sclerosis and frontotemporal dementia: a behavioural and cognitive continuum. *Amyotroph Lateral Scler.* (2012) 13:102–9. doi: 10.3109/17482968.2011.639376
35. Cykowski MD, Powell SZ, Peterson LE, Appel JW, Rivera AL, Takei H, et al. Clinical significance of TDP-43 neuropathology in amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol.* (2017) 76:402–13. doi: 10.1093/jnen/nlx025
36. Irwin DJ, Cairns NJ, Grossman M, McMillan CT, Lee EB, Van Deerlin VM, et al. Frontotemporal lobar degeneration: defining phenotypic diversity through personalized medicine. *Acta Neuropathol.* (2015) 129:469–91. doi: 10.1007/s00401-014-1380-1
37. Mackenzie IR, Neumann M. Molecular neuropathology of frontotemporal dementia: insights into disease mechanisms from postmortem studies. *J Neurochem.* (2016) 138(Suppl. 1):54–70. doi: 10.1111/jnc.13588
38. Chornenkyy Y, Fardo DW, Nelson PT. Tau and TDP-43 proteinopathies: kindred pathologic cascades and genetic pleiotropy. *Lab Invest.* (2019) 99:993–1007. doi: 10.1038/s41374-019-0196-y

39. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci USA*. (1975) 72:1858–62. doi: 10.1073/pnas.72.5.1858
40. Baas PW, Pienkowski TP, Cimbalnik KA, Toyama K, Bakalis S, Ahmad FJ, et al. Tau confers drug stability but not cold stability to microtubules in living cells. *J Cell Sci.* (1994) 107:135–43.
41. Caceres A, Kosik KS. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature.* (1990) 343:461–3. doi: 10.1038/343461a0
42. Knops J, Kosik KS, Lee G, Pardee JD, Cohen-Gould L, McConlogue L. Overexpression of tau in a nonneuronal cell induces long cellular processes. *J Cell Biol.* (1991) 114:725–33. doi: 10.1083/jcb.114.4.725
43. Jeganathan S, von BM, Brutlach H, Steinhoff HJ, Mandelkow E. Global hairpin folding of tau in solution. *Biochemistry.* (2006) 45:2283–93. doi: 10.1021/bi0521543
44. Arendt T, Stieler JT, Holzer M. Tau and tauopathies. *Brain Res Bull.* (2016) 126:238–92. doi: 10.1016/j.brainresbull.2016.08.018
45. Li D, Scarano S, Lisi S, Palladino P, Minunni M. Real-time tau protein detection by sandwich-based piezoelectric biosensing: exploring tubulin as a mass enhancer. *Sensors.* (2018) 18:946. doi: 10.3390/s18040946
46. Zabik NL, Imhof MM, Martic-Milne S. Structural evaluations of tau protein conformation: methodologies and approaches. *Biochem Cell Biol.* (2017) 95:338–49. doi: 10.1139/bcb-2016-0227
47. Jeganathan S, Hascher A, Chinnathambi S, Biernat J, Mandelkow EM, Mandelkow E. Proline-directed pseudo-phosphorylation at AT8 and PHF1 epitopes induces a compaction of the paperclip folding of Tau and generates a pathological (MC-1) conformation. *J Biol Chem.* (2008) 283:32066–76. doi: 10.1074/jbc.M805300200
48. Fichou Y, Al-Hilaly YK, Devred F, Smet-Nocca C, Tsvetkov PO, Verelst J, et al. The elusive tau molecular structures: can we translate the recent breakthroughs into new targets for intervention? *Acta Neuropathol Commun.* (2019) 7:31. doi: 10.1186/s40478-019-0682-x
49. Zempel H, Mandelkow E. Lost after translation: missorting of Tau protein and consequences for Alzheimer disease. *Trends Neurosci.* (2014) 37:721–32. doi: 10.1016/j.tins.2014.08.004
50. Mukrasch MD, Bibow S, Korukottu J, Jeganathan S, Biernat J, Griesinger C, et al. Structural polymorphism of 441-residue tau at single residue resolution. *PLoS Biol.* (2009) 7:e34. doi: 10.1371/journal.pbio.1000034
51. Martin L, Latypova X, Terro F. Post-translational modifications of tau protein: implications for Alzheimer's disease. *Neurochem Int.* (2011) 58:458–71. doi: 10.1016/j.neuint.2010.12.023
52. Mandell JW, Banker GA. A spatial gradient of tau protein phosphorylation in nascent axons. *J Neurosci.* (1996) 16:5727–40. doi: 10.1523/JNEUROSCI.16-18-05727.1996
53. Hoover BR, Reed MN, Su J, Penrod RD, Kotilinek LA, Grant MK, et al. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron.* (2010) 68:1067–81. doi: 10.1016/j.neuron.2010.11.030
54. 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) 156:1051–63. doi: 10.1083/jcb.200108057
55. Sultan A, Nesslany F, Violet M, Begard S, Loyens A, Talahari S, et al. Nuclear tau, a key player in neuronal DNA protection. *J Biol Chem.* (2011) 286:4566–75. doi: 10.1074/jbc.M110.199976
56. Wei Y, Qu MH, Wang XS, Chen L, Wang DL, Liu Y, et al. Binding to the minor groove of the double-strand, tau protein prevents DNA from damage by peroxidation. *PLoS ONE.* (2008) 3:e2600. doi: 10.1371/journal.pone.0002600
57. Benhelli-Mokrani H, Mansuroglu Z, Chauderlier A, Albaud B, Gentien D, Sommer S, et al. Genome-wide identification of genic and intergenic neuronal DNA regions bound by Tau protein under physiological and stress conditions. *Nucleic Acids Res.* (2018) 46:11405–22. doi: 10.1093/nar/gky929
58. Bukar Maina M, Al-Hilaly YK, Serpell LC. Nuclear Tau and its potential role in Alzheimer's disease. *Biomolecules.* (2016) 6:9. doi: 10.3390/biom6010009
59. Liu C, Gotz J. Profiling murine tau with 0N, 1N and 2N isoform-specific antibodies in brain and peripheral organs reveals distinct subcellular localization, with the 1N isoform being enriched in the nucleus. *PLoS ONE.* (2013) 8:e84849. doi: 10.1371/journal.pone.0084849
60. Maina MB, Bailey LJ, Wagih S, Biasetti L, Pollack SJ, Quinn JP, et al. The involvement of tau in nucleolar transcription and the stress response. *Acta Neuropathol Commun.* (2018) 6:70. doi: 10.1186/s40478-018-0565-6
61. Silva JM, Rodrigues S, Sampaio-Marques B, Gomes P, Neves-Carvalho A, Dioli C, et al. Dysregulation of autophagy and stress granule-related proteins in stress-driven tau pathology. *Cell Death Differ.* (2019) 26:1411–27. doi: 10.1038/s41418-018-0217-1
62. Fernandes N, Eshleman N, Buchan JR. Stress granules and ALS: a case of causation or correlation? *Adv Neurobiol.* (2018) 20:173–212. doi: 10.1007/978-3-319-89689-2_7
63. Li YR, King OD, Shorter J, Gitler AD. Stress granules as crucibles of ALS pathogenesis. *J Cell Biol.* (2013) 201:361–72. doi: 10.1083/jcb.201302044
64. Fan AC, Leung AK. RNA granules and diseases: a case study of stress granules in ALS and FTLD. *Adv Exp Med Biol.* (2016) 907:263–96. doi: 10.1007/978-3-319-29073-7_11
65. Meier S, Bell M, Lyons DN, Ingram A, Chen J, Gensel JC, et al. Identification of novel tau interactions with endoplasmic reticulum proteins in Alzheimer's disease brain. *J Alzheimers Dis.* (2015) 48:687–702. doi: 10.3233/JAD-150298
66. Vanderweyde T, Yu H, Varnum M, Liu-Yesucevitz L, Citro A, Ikezu T, et al. Contrasting pathology of the stress granule proteins TIA-1 and G3BP in tauopathies. *J Neurosci.* (2012) 32:8270–83. doi: 10.1523/JNEUROSCI.1592-12.2012
67. Vanderweyde T, Apicco DJ, Youmans-Kidder K, Ash PEA, Cook C, Lummertz da Rocha E, et al. Interaction of tau with the RNA-binding protein TIA1 regulates tau pathophysiology and toxicity. *Cell Rep.* (2016) 15:1455–66. doi: 10.1016/j.celrep.2016.04.045
68. Babinchak WM, Haider R, Dumm BK, Sarkar P, Surewicz K, Choi JK, et al. The role of liquid-liquid phase separation in aggregation of the TDP-43 low-complexity domain. *J Biol Chem.* (2019) 294:6306–17. doi: 10.1074/jbc.RA118.007222
69. Hernandez-Vega A, Braun M, Scharrel L, Jahnel M, Wegmann S, Hyman BT, et al. Local nucleation of microtubule bundles through tubulin concentration into a condensed tau phase. *Cell Rep.* (2017) 20:2304–12. doi: 10.1016/j.celrep.2017.08.042
70. Wegmann S, Eftekharzadeh B, Tepper K, Zoltowska KM, Bennett RE, Dujardin S, et al. Tau protein liquid-liquid phase separation can initiate tau aggregation. *EMBO J.* (2018) 37:e98049. doi: 10.15252/embj.201798049
71. Ambadipudi S, Biernat J, Riedel D, Mandelkow E, Zweckstetter M. Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. *Nat Commun.* (2017) 8:275. doi: 10.1038/s41467-017-00480-0
72. Kanaan NM, Hamel C, Grabinski T, Combs B. Liquid-liquid phase separation induces pathogenic tau conformations *in vitro*. *Nat Commun.* (2020) 11:2809. doi: 10.1038/s41467-020-16580-3
73. Montalbano M, McAllen S, Puangmalai N, Sengupta U, Bhatt N, Johnson OD, et al. RNA-binding proteins musashi and tau soluble aggregates initiate nuclear dysfunction. *Nat Commun.* (2020) 11:4305. doi: 10.1038/s41467-020-18022-6
74. Kim HJ, Taylor JP. Lost in transportation: nucleocytoplasmic transport defects in ALS and other neurodegenerative diseases. *Neuron.* (2017) 96:285–97. doi: 10.1016/j.neuron.2017.07.029
75. van der Harg JM, Nolle A, Zwart R, Boerema AS, van Haastert ES, Strijkstra AM, et al. The unfolded protein response mediates reversible tau phosphorylation induced by metabolic stress. *Cell Death Dis.* (2014) 5:e1393. doi: 10.1038/cddis.2014.354
76. Garruto RM. Amyotrophic lateral sclerosis and parkinsonism-dementia of guam: clinical, epidemiological and genetic patterns. *Am J Human Biol.* (1989) 1:367–82. doi: 10.1002/ajhb.1310010317
77. Hirano A. Neuropathology of amyotrophic lateral sclerosis and parkinsonism-dementia complex on Guam. In: Luthy L, Bischoff A, editors. *Proceedings of the Fifth International Congress of Neuropathology*. Amsterdam: Excerpta Medica (1966). p. 190–4.
78. Hirano A, Malamud N, Elizan TS, Kurland LT. Amyotrophic lateral sclerosis and parkinsonism-dementia complex on Guam. Further pathologic studies. *Arch Neurol.* (1966) 15:35–51. doi: 10.1001/archneur.1966.00470130039004
79. Moszczynski AJ, Strong W, Xu K, McKee A, Brown A, Strong MJ. Pathological Thr^{175} tau phosphorylationin CTE and CTE with ALS. *Neurology.* (2018) 90:e380–e7. doi: 10.1212/WNL.0000000000004899

80. McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, et al. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol.* (2009) 68:709–35. doi: 10.1097/NEN.0b013e3181a9d503
81. Mez J, Daneshvar DH, Kiernan PT, Abdolmohammadi B, Alvarez VE, Huber BR, et al. Clinicopathological evaluation of chronic traumatic encephalopathy in players of American football. *JAMA.* (2017) 318:360–70. doi: 10.1001/jama.2017.8334
82. Dobson-Stone C, Luty AA, Thompson EM, Blumbergs P, Brooks WS, Short CL, et al. Frontotemporal dementia-amyotrophic lateral sclerosis syndrome locus on chromosome 16p12.1-q12.2: genetic, clinical and neuropathological analysis. *Acta Neuropathol.* (2013) 124:523–33. doi: 10.1007/s00401-013-1078-9
83. Nakamura S, Wate R, Kaneko S, Ito H, Oki M, Tsuge A, et al. An autopsy case of sporadic amyotrophic lateral sclerosis associated with the I113T SOD1 mutation. *Neuropathology.* (2014) 34:58–63. doi: 10.1111/neup.12049
84. Orrell RW, King AW, Hilton DA, Campbell MJ, Lane RJ, de Belleroche JS. Familial amyotrophic lateral sclerosis with a point mutation of SOD-1: intrafamilial heterogeneity of disease duration associated with neurofibrillary tangles. *J Neurol Neurosurg Psychiatry.* (1995) 59:266–70. doi: 10.1136/jnnp.59.3.266
85. Soma K, Fu YJ, Wakabayashi K, Onodera O, Kakita A, Takahashi H. Co-occurrence of argyrophilic grain disease in sporadic amyotrophic lateral sclerosis. *Neuropathol Appl Neurobiol.* (2012) 38:54–60. doi: 10.1111/j.1365-2990.2011.01175.x
86. Takeuchi R, Toyoshima Y, Tada M, Tanaka H, Shimizu H, Shiga A, et al. Globular glial mixed four repeat tau and TDP-43 proteinopathy with motor neuron disease and frontotemporal dementia. *Brain Pathol.* (2016) 26:82–94. doi: 10.1111/bpa.12262
87. Origone P, Geroldi A, Lamp M, Sanguineri F, Caponnetto C, Cabona C, et al. Role of MAPT in pure motor neuron disease: report of a recurrent mutation in Italian patients. *Neurodegener Dis.* (2018) 18:310–4. doi: 10.1159/000497820
88. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron.* (2011) 72:257–68. doi: 10.1016/j.neuron.2011.09.010
89. Dejesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron.* (2011) 72:245–56. doi: 10.1016/j.neuron.2011.09.011
90. Ferrer I, Legati A, Garcia-Monco JC, Gomez-Beldarrain M, Carmona M, Blanco R, et al. Familial behavioral variant frontotemporal dementia associated with astrocyte-predominant tauopathy. *J Neuropathol Exp Neurol.* (2015) 74:370–9. doi: 10.1097/NEN.0000000000000180
91. Vatsavayai SC, Nana AL, Yokoyama JS, Seeley WW. C9orf72-FTD/ALS pathogenesis: evidence from human neuropathological studies. *Acta Neuropathol.* (2019) 137:1–26. doi: 10.1007/s00401-018-1921-0
92. Tacik P, Sanchez-Contreras M, Rademakers R, Dickson DW, Wszolek ZK. Genetic disorders with tau pathology: a review of the literature and report of two patients with tauopathy and positive family histories. *Neurodegener Dis.* (2016) 16:12–21. doi: 10.1159/000440840
93. Bieniek KF, Murray ME, Rutherford NJ, Castanedes-Casey M, Dejesus-Hernandez M, Liesinger AM, et al. Tau pathology in frontotemporal lobar degeneration with C9ORF72 hexanucleotide repeat expansion. *Acta Neuropathol.* (2013) 125:289–302. doi: 10.1007/s00401-012-1048-7
94. King A, Al-Sarraj S, Troakes C, Smith BN, Maekawa S, Iovino M, et al. Mixed tau, TDP-43 and p62 pathology in FTLD associated with a C9ORF72 repeat expansion and p.Ala239Thr MAPT (tau) variant. *Acta Neuropathol.* (2013) 125:303–10. doi: 10.1007/s00401-012-1050-0
95. He H, Huang W, Wang R, Lin Y, Guo Y, Deng J, et al. Amyotrophic lateral sclerosis-associated GGGGCC repeat expansion promotes tau phosphorylation and toxicity. *Neurobiol Dis.* (2019) 130:104493. doi: 10.1016/j.nbd.2019.104493
96. Sproviero W, La Bella V, Mazzei R, Valentino P, Rodolico C, Simone IL, et al. FUS mutations in sporadic amyotrophic lateral sclerosis: clinical and genetic analysis. *Neurobiol Aging.* (2012) 33:837.e1–5. doi: 10.1016/j.neurobiolaging.2011.10.005
97. Lai SL, Abramzon Y, Schymick JC, Stephan DA, Dunckley T, Dillman A, et al. FUS mutations in sporadic amyotrophic lateral sclerosis. *Neurobiol Aging.* (2011) 32:550.e1–4. doi: 10.1016/j.neurobiolaging.2009.12.020
98. Lattante S, Rouleau GA, Kabashi E. TARDBP and FUS mutations associated with amyotrophic lateral sclerosis: summary and update. *Hum Mutat.* (2013) 34:812–26. doi: 10.1002/humu.22319
99. Orozco D, Edbauer D. FUS-mediated alternative splicing in the nervous system: consequences for ALS and FTLD. *J Mol Med.* (2013) 91:1343–54. doi: 10.1007/s00109-013-1077-2
100. Orozco D, Tahirovic S, Rentzsch K, Schwenk BM, Haass C, Edbauer D. Loss of fused in sarcoma (FUS) promotes pathological Tau splicing. *EMBO Rep.* (2012) 13:759–64. doi: 10.1038/embor.2012.90
101. Ishigaki S, Sobue G. Importance of functional loss of FUS in FTLD/ALS. *Front Mol Biosci.* (2018) 5:44. doi: 10.3389/fmolb.2018.00044
102. Ishigaki S, Riku Y, Fujioka Y, Endo K, Iwade N, Kawai K, et al. Aberrant interaction between FUS and SFPQ in neurons in a wide range of FTLD spectrum diseases. *Brain.* (2020) 143:2398–405. doi: 10.1093/brain/awaa196
103. Ishigaki S, Fujioka Y, Okada Y, Riku Y, Udagawa T, Honda D, et al. Altered tau isoform ratio caused by loss of FUS and SFPQ function leads to FTLD-like phenotypes. *Cell Rep.* (2017) 18:1118–31. doi: 10.1016/j.celrep.2017.01.013
104. Wharton SB, Verber NS, Wagner BE, Highley JR, Fillingham DJ, Waller R, et al. Combined fused in sarcoma-positive (FUS+) basophilic inclusion body disease and atypical tauopathy presenting with an amyotrophic lateral sclerosis/motor neurone disease (ALS/MND)-plus phenotype. *Neuropathol Appl Neurobiol.* (2019) 45:586–96. doi: 10.1111/nan.12542
105. Vintilescu CR, Afreen S, Rubino AE, Ferreira A. The neurotoxic TAU45-230 fragment accumulates in upper and lower motor neurons in amyotrophic lateral sclerosis subjects. *Mol Med.* (2016) 22:477–86. doi: 10.2119/molmed.2016.00095
106. Lang AE, Riherd Methner DN, Ferreira A. Neuronal degeneration, synaptic defects, and behavioral abnormalities in tau_{45-230} transgenic mice. *Neuroscience.* (2014) 275:322–39. doi: 10.1016/j.neuroscience.2014.06.017
107. Ferreira A, Bigio EH. Calpain-mediated tau cleavage: a mechanism leading to neurodegeneration shared by multiple tauopathies. *Mol Med.* (2011) 17:676–85. doi: 10.2119/molmed.2010.00220
108. Park SY, Ferreira A. The generation of a 17 kDa neurotoxic fragment: an alternative mechanism by which tau mediates beta-amyloid-induced neurodegeneration. *J Neurosci.* (2005) 25:5365–75. doi: 10.1523/JNEUROSCI.1125-05.2005
109. Yang W, Sopper MM, Leystra-Lantz C, Strong MJ. Microtubule-associated tau protein positive neuronal and glial inclusions in amyotrophic lateral sclerosis. *Neurology.* (2003) 61:1766–73. doi: 10.1212/01.WNL.0000099372.75786.F8
110. Yang W, Strong MJ. Widespread neuronal and glial hyperphosphorylated tau deposition in ALS with cognitive impairment. *Amyotroph Lateral Scler.* (2012) 13:178–93. doi: 10.3109/17482968.2011.622405
111. Yang W, Ang LC, Strong MJ. Tau protein aggregation in the frontal and entorhinal cortices as a function of aging. *Dev Brain Res.* (2005) 156:127–38. doi: 10.1016/j.devbrainres.2005.02.004
112. Strong MJ, Yang W, Strong WL, Leystra-Lantz C, Jaffe H, Pant HC. Tau protein hyperphosphorylation in sporadic ALS with cognitive impairment. *Neurology.* (2006) 66:1770–1. doi: 10.1212/01.wnl.0000218161.15834.db
113. Yang W, Leystra-Lantz C, Strong MJ. Upregulation of GSK3beta expression in frontal and temporal cortex of ALS with cognitive impairment (ALSci). *Brain Res.* (2008) 1196:131–9. doi: 10.1016/j.brainres.2007.12.031
114. Behrouzi R, Liu X, Wu D, Robinson AC, Tanaguchi-Watanabe S, Rollinson S, et al. Pathological tau deposition in motor neurone disease and frontotemporal lobar degeneration associated with TDP-43 proteinopathy. *Acta Neuropathol Commun.* (2016) 4:33. doi: 10.1186/s40478-016-0301-z
115. Stevens CH, Guthrie NJ, van Roijen M, Halliday GM, Ooi L. Increased tau phosphorylation in motor neurons from clinically pure sporadic amyotrophic lateral sclerosis patients. *J Neuropathol Exp Neurol.* (2019) 78:605–14. doi: 10.1093/jnen/nlz041
116. McKee AC, Cairns NJ, Dickson DW, Folkerth RD, Keene CD, Litvan I, et al. The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. *Acta Neuropathol.* (2016) 131:75–86. doi: 10.1007/s00401-015-1515-z

117. Moszczynski AJ, Yang W, Hammond R, Ang LC, Strong MJ. Threonine175, a novel pathological phosphorylation site on tau protein linked to multiple tauopathies. *Acta Neuropathol Commun.* (2017) 5:6. doi: 10.1186/s40478-016-0406-4
118. Kenessey A, Yen SH. The extent of phosphorylation of fetal tau is comparable to that of PHF-tau from Alzheimer paired helical filaments. *Brain Res.* (1993) 629:40–6. doi: 10.1016/0006-8993(93)90478-6
119. Süssmuth S, Tumani H, Ecker D, Ludolph AC. Amyotrophic lateral sclerosis: disease stage related changes of tau protein asn S100 beta in cerebrospinal fluid and creatine kinase in serum. *Neurosci Lett.* (2003) 353:57–60. doi: 10.1016/j.neulet.2003.09.018
120. Bourbouli M, Rentzos M, Bougea A, Zouvelou V, Constantinides VC, Zaganas I, et al. Cerebrospinal fluid TAR DNA-binding protein 43 combined with tau proteins as a candidate biomarker for amyotrophic lateral sclerosis and frontotemporal dementia spectrum disorders. *Dement Geriatr Cogn Disord.* (2017) 44:144–52. doi: 10.1159/000478979
121. Paladino P, Valentino F, Piccoli T, Piccoli F, La Bella V. Cerebrospinal fluid tau protein is not a biological marker in amyotrophic lateral sclerosis. *Eur J Neurol.* (2009) 16:257–61. doi: 10.1111/j.1468-1331.2008.02405.x
122. Sjogren M, Davidsson P, Wallin A, Granerus AK, Grundstrom E, Askmark H, et al. Decreased CSF-beta-amyloid 42 in Alzheimer's disease and amyotrophic lateral sclerosis may reflect mismetabolism of beta-amyloid induced by disparate mechanisms. *Dement Geriatr Cogn Disord.* (2002) 13:112–8. doi: 10.1159/000048642
123. Jiménez-Jiménez FJ, Hernánz A, Medina-Acebrón S, de Bustos F, Zurdo JM, Alonso H, et al. Tau protein concentrations in cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *Acta Neurol Scand.* (2005) 111:114–7. doi: 10.1111/j.1600-0404.2005.00370.x
124. Brettschneider J, Petzold A, Sussmuth SD, Ludolph AC, Tumani H. Axonal damage markers in cerebrospinal fluid are increased in ALS. *Neurology.* (2006) 66:852–6. doi: 10.1212/01.wnl.0000203120.85850.54
125. Gohar M, Yang W, Strong WL, Volkening K, Leystra-Lantz C, Strong MJ. Tau phosphorylation at ^{175}Thr leads to fibril formation. Implications for the tauopathy of amyotrophic lateral sclerosis. *J Neurochem.* (2009) 108:634–43. doi: 10.1111/j.1471-4159.2008.05791.x
126. Moszczynski AJ, Gohar M, Volkening K, Leystra-Lantz C, Strong W, Strong MJ. Thr175-phosphorylated tau induces pathologic fibril formation via GSK3beta-mediated phosphorylation of Thr231 *in vitro*. *Neurobiol Aging.* (2015) 36:1590–9. doi: 10.1016/j.neurobiolaging.2014.12.001
127. Frame S, Cohen P. GSK3 takes centre stage more than 20 years after its discovery. *Biochem J.* (2001) 359:1–16. doi: 10.1042/bj3590001
128. Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgett JR. Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *EMBO.* (1993) 12:803–8. doi: 10.1002/j.1460-2075.1993.tb05715.x
129. Hintermayer MA, Volkening K, Moszczynski AJ, Donison N, Strong MJ. Tau protein phosphorylation at Thr(175) initiates fibril formation via accessibility of the N-terminal phosphatase-activating domain. *J Neurochem.* (2019) 2019:e14942. doi: 10.1111/jnc.14942
130. Kanaan NM, Morfini GA, LaPointe NE, Pigino GF, Patterson KR, Song Y, et al. Pathogenic forms of tau inhibit kinesin-dependent axonal transport through a mechanism involving activation of axonal phosphotransferases. *J Neurosci.* (2011) 31:9858–68. doi: 10.1523/JNEUROSCI.0560-11.2011
131. Kanaan NM, Morfini G, Pigino G, LaPointe NE, Andreadis A, Song Y, et al. Phosphorylation in the amino terminus of tau prevents inhibition of anterograde axonal transport. *Neurobiol Aging.* (2012) 33:826–30. doi: 10.1016/j.neurobiolaging.2011.06.006
132. Avila J, Jimenez JS, Sayas CL, Bolos M, Zabala JC, Rivas G, et al. Tau structures. *Front Aging Neurosci.* (2016) 8:262. doi: 10.3389/fnagi.2016.00262
133. Combs B, Kanaan NM. Exposure of the amino terminus of tau is a pathological event in multiple tauopathies. *Am J Pathol.* (2017) 187:1222–9. doi: 10.1016/j.ajpath.2017.01.019
134. Ward SM, Himmelstein DS, Lancia JK, Binder LI. Tau oligomers and tau toxicity in neurodegenerative disease. *Biochem Soc Trans.* (2012) 40:667–71. doi: 10.1042/BST20120134
135. Kanaan NM, Cox K, Alvarez VE, Stein TD, Poncil S, McKee AC. Characterization of early pathological tau conformations and phosphorylation in chronic traumatic encephalopathy. *J Neuropathol Exp Neurol.* (2016) 75:19–34. doi: 10.1093/jnen/nlv001
136. Nakamura K, Greenwood A, Binder L, Bigio EH, Denial S, Nicholson L, et al. Proline isomer-specific antibodies reveal the early pathogenic tau conformation in Alzheimer's disease. *Cell.* (2012) 149:232–44. doi: 10.1016/j.cell.2012.02.016
137. Lu KP, Hanes SD, Hunter T. A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature.* (1996) 380:544–7. doi: 10.1038/380544a0
138. Lu PJ, Wulf G, Zhou XZ, Davies P, Lu KP. The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature.* (1999) 399:784–8. doi: 10.1038/21650
139. Albayram O, Kondo A, Mannix R, Smith C, Tsai CY, Li C, et al. Cis P-tau is induced in clinical and preclinical brain injury and contributes to post-injury sequelae. *Nat Commun.* (2017) 8:1000. doi: 10.1038/s41467-017-01068-4
140. Liou YC, Sun A, Ryo A, Zhou XZ, Yu ZX, Huang HK, et al. Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. *Nature.* (2003) 424:556–61. doi: 10.1038/nature01832
141. Ma SL, Tang NL, Tam CW, Lui VW, Lam LC, Chiu HF, et al. A PIN1 polymorphism that prevents its suppression by AP4 associates with delayed onset of Alzheimer's disease. *Neurobiol Aging.* (2012) 33:804–13. doi: 10.1016/j.neurobiolaging.2010.05.018
142. Segat L, Pontillo A, Annoni G, Trabattoni D, Vergani C, Clerici M, et al. PIN1 promoter polymorphisms are associated with Alzheimer's disease. *Neurobiol Aging.* (2007) 28:69–74. doi: 10.1016/j.neurobiolaging.2005.11.009
143. Mustroph ML, King MA, Klein RL, Ramirez JJ. Adult-onset focal expression of mutated human tau in the hippocampus impairs spatial working memory of rats. *Behav Brain Res.* (2012) 233:141–8. doi: 10.1016/j.bbr.2012.04.034
144. Moszczynski AJ, Gopaul J, McCunn P, Volkening K, Harvey M, Bartha R, et al. Somatic gene transfer using a recombinant adenoviral vector (rAAV9) encoding pseudophosphorylated human Thr175 tau in adult rat hippocampus induces tau pathology. *J Neuropathol Exp Neurol.* (2018) 77:685–95. doi: 10.1093/jnen/nly044
145. McKee AC, Gavett BE, Stern RA, Nowinski CJ, Cantu RC, Kowall NW, et al. TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. *J Neuropathol Exp Neurol.* (2010) 69:918–29. doi: 10.1097/NEN.0b013e3181ee7d85
146. Josephs KA, Murray ME, Whitwell JL, Parisi JE, Petrucelli L, Jack CR, et al. Staging TDP-43 pathology in Alzheimer's disease. *Acta Neuropathol.* (2014) 127:441–50. doi: 10.1007/s00401-013-1211-9
147. Josephs KA, Whitwell JL, Weigand SD, Murray ME, Tosakulwong N, Liesinger AM, et al. TDP-43 is a key player in the clinical features associated with Alzheimer's disease. *Acta Neuropathol.* (2014) 127:811–24. doi: 10.1007/s00401-014-1269-z
148. Zhou H, Huang C, Chen H, Wang D, Landel CP, Xia PY, et al. Transgenic rat model of neurodegeneration caused by mutation in the TDP gene. *PLoS Genet.* (2010) 6:e1000887. doi: 10.1371/journal.pgen.1000887
149. Moszczynski AJ, Harvey M, Fulcher N, de Oliveira C, McCunn P, Donison N, et al. Synergistic toxicity in an *in vivo* model of neurodegeneration through the co-expression of human TDP-43(M337V) and tau(T175D) protein. *Acta Neuropathol Commun.* (2019) 7:170. doi: 10.1186/s40478-019-0816-1
150. Goedert M, Jakes R, Vanmechelen E. Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205. *Neurosci Lett.* (1995) 189:167–9. doi: 10.1016/0304-3940(95)11484-E
151. Olney RK, Murphy J, Forshew D, Garwood E, Miller BL, Langmore S, et al. The effects of executive and behavioral dysfunction on the course of ALS. *Neurology.* (2005) 65:1774–7. doi: 10.1212/01.wnl.0000188759.87240.8b
152. Liachko NF, McMillan PJ, Strovas TJ, Loomis E, Greenup L, Murrell JR, et al. The tau tubulin kinases TTBK1/2 promote accumulation of pathological TDP-43. *PLoS Genet.* (2014) 10:e1004803. doi: 10.1371/journal.pgen.1004803
153. Sato S, Cerny RL, Buescher JL, Ikezu T. Tau-tubulin kinase 1 (TTBK1), a neuron-specific tau kinase candidate, is involved in tau phosphorylation and aggregation. *J Neurochem.* (2006) 98:1573–84. doi: 10.1111/j.1471-4159.2006.04059.x
154. Tomizawa K, Omori A, Ohtake A, Sato K, Takahashi M. Tau-tubulin kinase phosphorylates tau at Ser-208 and Ser-210, sites found in paired helical filament-tau. *FEBS Lett.* (2001) 492:221–7. doi: 10.1016/S0014-5793(01)02256-6

155. Taylor LM, McMillan PJ, Liachko NF, Strovas TJ, Ghetti B, Bird TD, et al. Pathological phosphorylation of tau and TDP-43 by TTBK1 and TTBK2 drives neurodegeneration. *Mol Neurodegener.* (2018) 13:7. doi: 10.1186/s13024-018-0237-9
156. Taylor LM, McMillan PJ, Kraemer BC, Liachko NF. Tau tubulin kinases in proteinopathy. *FEBS J.* (2019) 286:2434–46. doi: 10.1111/febs.14866
157. Barbier P, Zejneli O, Martinho M, Lasorsa A, Belle V, Smet-Nocca C, et al. Role of tau as a microtubule-associated protein: structural and functional aspects. *Front Aging Neurosci.* (2019) 11:204. doi: 10.3389/fnagi.2019.00204

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 26 November 2020
doi: 10.3389/fneur.2020.602292

Dynamics of Internalization and Intracellular Interaction of Tau Antibodies and Human Pathological Tau Protein in a Human Neuron-Like Model

Dov B. Shamir[1], *Yan Deng*[2], *Qian Wu*[1], *Swananda Modak*[1], *Erin E. Congdon*[1] *and Einar M. Sigurdsson*[1,3]*

[1] Department of Neuroscience and Physiology, Neuroscience Institute, New York University Grossman School of Medicine, New York, NY, United States, [2] Microscopy Core, New York University Grossman School of Medicine, New York, NY, United States, [3] Department of Psychiatry, New York University Grossman School of Medicine, New York, NY, United States

We and others have shown in various *in vivo*, *ex vivo* and cell culture models that several tau antibodies interact with pathological tau within neurons. To further clarify this interaction in a dynamic human model, we differentiated SH-SY5Y cells with retinoic acid and BDNF to create a neuron-like model. Therein, tau antibodies were primarily taken up by receptor-mediated endocytosis, and prevented toxicity of human brain-derived paired helical filament-enriched tau (PHF). Subsequently, we monitored in real-time the interaction of antibodies and PHF within endocytic cellular compartments. Cells were pre-treated with fluorescently-tagged PHF and then incubated with tau antibodies, 4E6, 6B2, or non-specific isotype control IgG1 labeled with a pH sensitive dye. The uptake and binding of the efficacious antibody, 4E6, to PHF occurred mainly within the soma, whereas the ineffective antibody, 6B2, and ineffective control IgG1, were visualized via the processes and showed limited colocalization with PHF within this period. In summary, we have developed a neuron-like model that clarifies the early intracellular dynamics of the interaction of tau antibodies with pathological tau, and identifies features associated with efficacy. Since the model is entirely human, it is suitable to verify the therapeutic potential of humanized antibodies prior to extensive clinical trials.

Keywords: Alzheimer's disease, tau, PHF, antibodies, immunotherapies, live imaging

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Neil Cashman, University of British Columbia, Canada
Abby Olsen, Brigham and Women's Hospital and Harvard Medical School, United States

***Correspondence:**
Einar M. Sigurdsson
einar.sigurdsson@nyumc.org

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *02 September 2020*
Accepted: *02 November 2020*
Published: *26 November 2020*

Citation:
Shamir DB, Deng Y, Wu Q, Modak S, Congdon EE and Sigurdsson EM (2020) Dynamics of Internalization and Intracellular Interaction of Tau Antibodies and Human Pathological Tau Protein in a Human Neuron-Like Model. Front. Neurol. 11:602292. doi: 10.3389/fneur.2020.602292

INTRODUCTION

Immunotherapies targeting pathogenic peptide/protein aggregates are at different stages of development as potential treatment for Alzheimer's disease (AD) and other neurodegenerative diseases (1–4). Our laboratory pioneered targeting pathological tau protein with active and passive immunotherapies for AD and other tauopathies (5, 6), which have been validated and extended by other groups over the last several years and now entered clinical trials (1–4). Antibody-mediated clearance of tau likely involves several mechanisms, which may include: (a) microglia activation and phagocytosis (7–10), (b) neutralization of tau in the extracellular space (11, 12), and (c) intracellular sequestration/degradation of tau within neurons (5, 7, 13–24). Our group and others have focused on elucidating these mechanisms in various cell culture, *ex-vivo*, and *in-vivo* mouse

models. To validate humanized antibodies for clinical trials, models of human origin should ideally be employed, and live imaging at the early stages of treatment may provide valuable insight into the mechanisms involved that cannot be obtained by other means. Not much is known about the interaction and internalization of pathological tau and its antibodies in the first hours of treatment.

We previously showed that colocalization of tau with antibodies is primarily within the soma of neurons using *in vivo*, *ex vivo* and culture models of mouse origin (5, 7, 12, 14, 16, 22, 23). Regarding the specific antibodies examined herein, 4E6 and 6B2, we have previously shown that both are readily taken up into tauopathy mouse neurons in brain slices and primary culture models, where they colocalize with tau primarily in the endosomal lysosomal system, and clear soluble tau, with 4E6 more consistently being effective in clearing pathological tau and preventing its toxicity (7, 12, 14, 23). However, only 4E6 and not 6B2 is effective in clearing tau pathology *in vivo* (12, 22), which is associated with cognitive improvements (12). In addition, we have previously shown extensive internalization of the 6B2 antibody and pathological tau derived from human brain in non-differentiated neuroblastoma model using flow cytometry (19).

In the prior mouse studies, we showed that both the effective 4E6 tau antibody and the relatively ineffective 6B2 tau antibody could localize with somatic intraneuronal tau in mouse brain slices and in mouse primary neurons following an incubation for 24 h or longer. Those findings do not provide insight into why the former is effective and the latter ineffective. Therefore, we set out to determine with live imaging if earlier cellular events, like temporal and spatial differences, might provide better insight into this important issue as both antibodies are clearly taken up into the endosomal-lysosomal system. For early high throughput screening, differentiated neuroblastoma cells are preferred over primary neurons for live imaging as they are less sensitive to the conditions for such analysis. Therefore, we established a neuron-like tauopathy model of human origin that shows the expected neuro- and synaptotoxicity of human-derived paired helical filament (PHF)-enriched pathological tau, and the prevention of its deleterious effect by a tau antibody. Importantly, naïve and differentiated cells utilize different mechanisms of antibody uptake and the tau antibody is only effective in the differentiated more neuron-like cells. Furthermore, live imaging revealed that in differentiated neurons pre-treated with PHF-tau, 4E6 tau antibody, which prevents PHF-tau toxicity, rapidly colocalized with PHF-tau within the soma. In contrast, 6B2 tau antibody and control IgG1, which do not prevent PHF-tau toxicity, were taken up via the neuronal processes and did not colocalize with PHF-tau within this timeframe. This model and experimental design should provide valuable insight into the efficacy and mechanisms of action of tau antibodies that are candidates for clinical trials.

MATERIALS AND METHODS

Paired Helical Filament (PHF) Protein Preparation

Human brain slices from subjects with extensive amyloid-β plaques and neurofibrillary tangles were enriched for PHF for the experiments. Brain slabs were homogenized and prepared as described by us and others (12, 18, 19, 25, 26), and the enriched PHF had similar properties with regard to western profile, ratio of 3R/4R tau and toxicity in culture. Briefly, the brain tissue was homogenized and prepared in buffer [pH 6.5; 0.75 M NaCl, 1 mM EGTA, 0.5 mM $MgSO_4$, and 100 mM 2-(N-morpholino) ethanesulfonic acid] along with protease inhibitor cocktail (Roche) and centrifuged at 11,000 x g for 20 min at 4°C. Supernatant was subsequently centrifuged in an ultracentrifuge at 100,000 x g for 60 min at 4°C. The pellet was then resuspended in paired helical filament (PHF) extraction buffer containing sucrose (10 mM Tris; 10% sucrose; 0.85 M NaCl; and 1 mM EGTA, pH 7.4) and spun at 15,000 x g for 20 min at 4°C. The pellet was extracted again in the sucrose buffer at the same low-speed centrifugation. The supernatants from both sucrose extractions were pooled and subjected to 1% sarkosyl solubilization by briefly heating it and then stirring at ambient temperature, and then centrifuged at 100,000 x g for 60 min at 4°C in a Beckman 60 Ti rotor (Beckman Coulter; Fullerton, CA). The resulting pellet was re-suspended in 50 mM Tris-HCl (pH 7.4), using 0.5 mL of buffer for each milligram of initial weight of brain sample protein. It was then dialyzed in PBS overnight at 4°C, using a 3,500 MW cassette (Thermo Scientific), and designated the PHF enriched preparation.

Antibodies and Fluorescence Labeling

In this study we used tau antibodies, 6B2 and 4E6, that have been generated against the Tau386-408[P-Ser396, 404] region. These IgG1κ mouse antibodies were previously characterized by our laboratory (7, 12, 14, 16, 19, 22, 23, 27). As a control, a non-specific mouse IgG1κ (IgG1, eBioscience) antibody was used. The antibodies were tagged with Cypher5E (GE Healthcare) fluorescent marker where mentioned. Cypher5E is a pH sensitive dye, and only fluoresces within acidic compartments, like endosomes or lysosomes. The PHF-enriched brain fraction was tagged with Alexa Fluor 488 (Invitrogen) where mentioned. All tagging was performed as outlined in the manufacturer's instructions.

Cell Culture and Differentiation

SH-SY5Y human neuroblastoma cells were obtained from ATCC. Cells were cultured in complete media (Dulbecco's Modified Eagle Medium (DMEM) with GlutaMAX (Invitrogen), 10% heat inactivated fetal bovine serum (FBS), 10,000 Units/mL Penicillin, and 10,000 μg/mL streptomycin. Cells were plated at 4×10^2 cells/mm^2, allowed to recover for 3 to 5 days before each experiment, and grown in an incubator with 5% CO_2 at 37°C.

Cells were maintained, prior to differentiation or treatment, in complete media. The cells were double-differentiated as described by other groups (28–30), by adding 10 μM retinoic acid (RA, Sigma Aldrich) and 1% FBS for 5 days. Then after two washes in DMEM media, they were treated with 50 ng/mL brain-derived neurotrophic factor (BDNF, Alomone Labs) for at least 2 days. For all experiments, the differentiated cells (DC) were then treated with the BDNF media, while non-differentiated cells (NDC) were grown and treated in DMEM

media with 1% fetal bovine serum (FBS). For characterization experiments, both cell types were treated with 5 μg/mL 6B2 tau antibody for 24 h, and for clathrin receptor inhibition studies, followed by increasing amounts of inhibitor, Dynasore (0–5 μg/mL). For PHF dose response studies, SH-SY5Y were treated with tagged PHF (0–10 μg/mL) for 24 h, washed, and then incubated for another 24 h. For pre-incubation studies analyzed using Western blot, cells were pre-treated with PHF (1 or 10 μg/mL) for 24 h, then washed with DMEM to remove remaining extracellular PHF, and subsequently incubated with 5 μg/mL tau antibodies (6B2 or 4E6) or IgG1 control for another 24 or 120 h.

Western Blot

All cell lysates analyzed using Western blot were incubated with non-tagged antibodies (6B2, 4E6, and IgG1) and/or PHF. Prior to cell lysis, cells were thoroughly washed with PBS. In preliminary studies, we verified by confocal imaging using fluorescently labeled antibodies and PHF that this method cleared all extracellular antibodies/PHF. Under our conditions, trypsin digestion had the same effect but did damage the cell membranes to a variable degree, which included some membrane rupture and extracellular release of its contents including antibodies and PHF. Hence, we opted for the milder repeated PBS wash. All samples were then homogenized in RIPA buffer and prepared as described previously (7). Samples were boiled and loaded onto 10% SDS-PAGE gels, electrophoresed, and then transferred to nitrocellulose membranes, which were blocked in 5% milk with 0.1% TBS-T. Blots were then probed for total tau (Dako polyclonal antibody), NeuN (Millipore polyclonal antibody), synaptophysin (Sigma Aldrich monoclonal antibody), PSD-95 (UC Davis NeuroMab, monoclonal antibody), or GAPDH (Abcam polyclonal antibody) primary antibodies overnight at 4°C, washed and then probed with anti-horseradish peroxidase (HRP) conjugated rabbit or mouse secondary antibody (Pierce) for 1 h. For antibody uptake detection, membranes were incubated with an anti-mouse IgG1 HRP-conjugated secondary antibody with specificity against the heavy chain (Bethyl Laboratories), and signal was detected with an ECL substrate (Thermo Scientific). Images of immunoreactive bands were then acquired, normalized relative to internal controls and quantified using the Fuji LAS-4000 imaging system. For immunoprecipitation studies, blots were incubated with anti-CD16 (Santa Cruz, RRID: AB_2104020), anti-CD32 (Santa Cruz, RRID: AB_2103599) polyclonal antibodies overnight at 4°C. All blots were washed and probed with a fluorescent anti-rabbit secondary antibody at a 1:10,000 dilution (LI-COR, RRID:AB_621843), and bands were visualized using a LI-COR Odyssey CLX reader.

Immunoprecipitation

Neuroblastoma SH-SY5Y cells were maintained and differentiated as described above. Primary neurons were prepared from a wild type pup at postnatal day 0 as previously described (12, 14, 23). All of the samples were homogenized in RIPA buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM NaF, 1 mM Na_3VO_4, 1 μg/ml complete protease inhibitor cocktail (Roche Applied Science) and assayed for protein concentration. Equal amounts of total protein from each sample was then used for immunoprecipitation.

Immunoprecipitation was carried out using a magnetic Dynabead kit (ThermoFisher) per manufacturer's instructions. Briefly, beads were incubated with an antibody recognizing mouse FcγII/III receptors (eBioscience). Beads were then washed and equal amount of protein from each sample was added. Following incubation, the beads were then washed again, and the target protein eluted. O+ buffer [62.5 mM Tris-HCl (pH 6.8), 5% glycerol, 5% β-mercaptoethanol, 2.3% SDS, 1 mM EDTA, 1 mM ethylene glycol bis(2-aminoethyl) tetraacetic acid (EGTA), 1 mM PMSF, 1 mM NaF, 1 mM Na_3VO_4 and 1 μg/ml complete protease inhibitor cocktail (Roche Applied Science)] was added and samples were boiled before loading them onto an SDS gel for immunoblotting.

RNA Extraction and PCR

Cells were washed thoroughly with PBS and then lysed and total RNA extracted using a kit as described by manufacturer's instructions (Sigma). RNA was then converted to cDNA using reverse transcriptase kit according to manufacturer's instructions (Invitrogen). The cDNA samples were then amplified by PCR using the following primers from Sigma; FcγR2A (NM_001136219), FcγR3A (NM_000569), FcγR3B (NM_001244753), and Actin (NM_001101). All amplified samples were run on a 2% agarose gel and their predicted size confirmed by DNA ladders. DNA bands, representing the extracted RNA, were stained with ethidium bromide, imaged by Protein Simple, Alpha-Imager HP system, and analyzed using ImageJ.

Flow Cytometry

All cells analyzed with flow cytometry were incubated with tagged PHF, and prepared for flow cytometry as previously described (19). Cells were analyzed using FlowJo for Alexa Fluor 647 positive cells. Median fluoresence intensity (MFI) values were obtained for fluorescent signals.

Immunohistochemistry and Cell Staining

Coverslips were coated with Pluripro protein matrix (Cell Guidance Systems) as directed by the manufacturer. Cells were plated onto coverslips at 4×10^2 cells/mm^2, and then allowed to recover for 2 days. For characterization studies, NDC and DC were co-incubated with Dextran (10,000 MW, Alexa Fluor 568, Invitrogen) and 20 μg/mL Alexa Fluor 488 tagged 6B2 tau antibody. Prior to fixation, cells were incubated with Hoechst nuclear staining, as described by the manufacturer (Invitrogen), then fixed with 4% paraformaldehyde, and coverslipped.

Live Cell Imaging

Chamber glasses (Nunc) were coated with Pluripro protein matrix (Cell Guidance Systems) as directed by the manufacturer.

Cells were plated onto these glasses at 2×10^2 cells/mm^2 and allowed to recover for 2 days. Cells were then differentiated as described above. For all pre-incubation studies, cells were then treated with 50 μg/mL fluorescently tagged PHF for 16 h. Prior to live imaging for all experiments, Hoechst nuclear dye was incubated with the live cells as directed by the manufacturer (Invitrogen). Cells were then washed and placed into complete media with no phenol red-DMEM and HEPES (Invitrogen) with BDNF for differentiated cells.

As previously described for time-lapse studies by our group (31), following Hoechst staining, cells were then incubated with tagged 20 μg/mL 4E6, 6B2, IgG1, or 50 μg/mL PHF for 140–150 min, which was added to the chamber glasses within the first 5 min of observation in the live imaging chamber. Cells were imaged every 5 min for up to 140–150 min. Chamber glasses were analyzed using an API DeltaVision PersonalDV system with Olympus PlanApo N 60x/1.42 oil lens, standard fluorescent filterset and full environmental control device. The internal density (IntDen) of either the antibody or PHF signal of the whole image was extracted using ImageJ. In these time-lapse studies, colocalization analysis of the antibody-dextran and time-lapse images were conducted using ImageJ's Intensity Correlation Analysis plugin as previously described by us and others (16, 22, 32–34). Degree of colocalization coefficient (R^2) and product of difference from the mean (PDM) images of corresponding experimental groups were calculated and displayed, where pixels in yellow indicate colocalizing in both channels and blue indicates a negative correlation.

Statistics

All quantified Western blot, flow cytometry, PCR, or live imaging data was analyzed using GraphPad Prism 6. ANOVA or Student's *t*-test were used to compare results of different samples. For three or more groups, one- or two-way ANOVA was used, depending on the number of variables, followed by Bonferroni *post hoc* test. Student's *t*-test was one or two-tailed as specified, depending on the analyzed parameters. The coefficient of determination (r^2) and trend line were generated for degree of colocalization coefficient (R^2) vs. time data in the live time-lapse imaging studies, which was further analyzed using Pearson correlation coefficient.

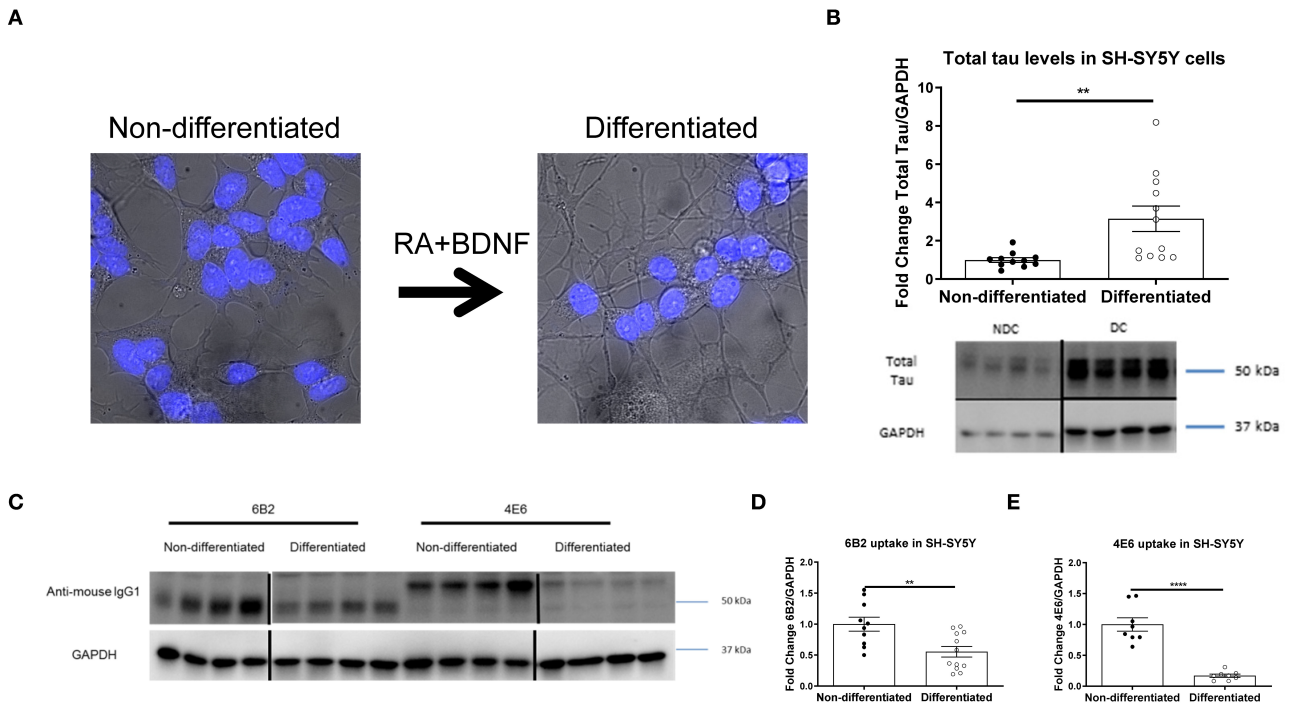


FIGURE 1 | Double differentiation transforms SH-SY5Y cells into a more neuronal-like state, with increase in tau levels and decrease in antibody uptake. Differentiated and non-differentiated SH-SY5Y cells (DC and NDC) were analyzed using live imaging microscopy, or lysed and collected for Western blot analyses. **(A)** Shows live imaging of NDC and DC from the bright-field channel, with nuclei in blue. DC have smaller cell bodies, and longer processes than their NDC counterparts. **(B)** Shows representative total tau and GAPDH Western blots of both cell types with quantification of total tau normalized to GAPDH. DC have significantly more total tau protein than NDC (215% increase, $^{**}p = 0.006$, $n = 11$–12, *t*-test, two-tailed). DC and NDC were also treated with tau antibodies (6B2 and 4E6) for 24 h. Cells were then lysed and collected for Western blot analyses, and probed with an anti-mouse IgG1-HRP to measure antibody uptake. Anti-mouse IgG1 signal was normalized to GAPDH. **(C)** Shows representative Western blots of NDC and DC samples where the IgG1 antibodies (6B2 and 4E6) appear to have slightly different weights, which may be due to their slightly different isoelectric points (~6.8 and ~6.5, respectively), and may explain why they run on the gel differently. **(D,E)** Shows the quantified results of the Western blots. Uptake of the 6B2 tau antibody (45% reduction, $^{**}p = 0.005$, $n = 10$–12, *t*-test, two-tailed) and the 4E6 tau antibody (83% reduction, $^{****}p < 0.0001$, $n = 8$, *t*-test, two-tailed) were less in DC compared to NDC. Note that the blot lanes in **(B,C)** are from the same blots, respectively. The lines show where excess test lanes where sectioned out. All scatter bar graphs are mean ± SEM.

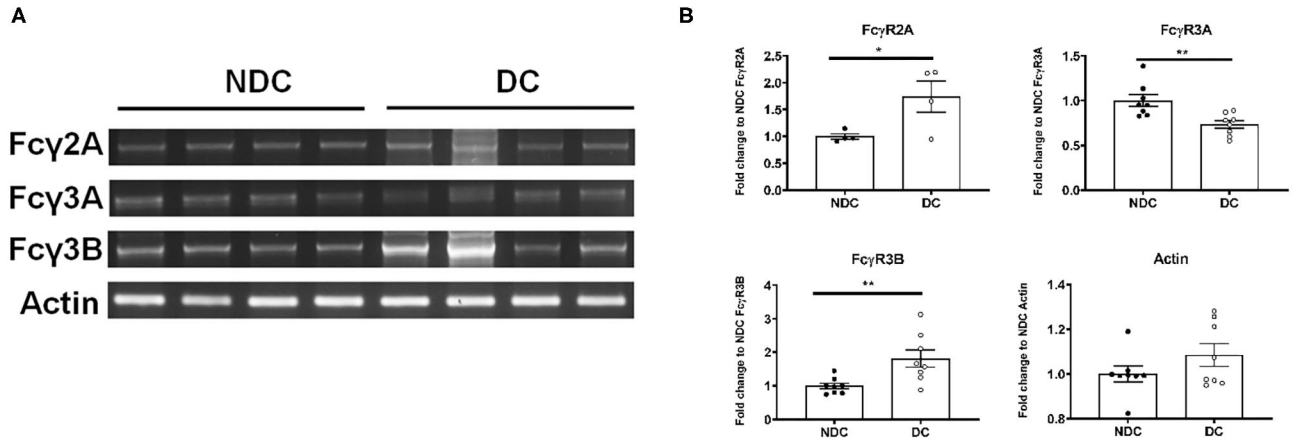


FIGURE 2 | Subtypes of Fcγ receptors are transcribed in NDC and DC. NDC and DC were kept at basal conditions as described in **Figure 1**. Cells were lysed, and RNA was extracted. Total RNA was then subject to reverse transcriptase to generate cDNA, and it subsequently amplified by PCR with FcγR2A, FcγR3A, FcγR3B, and actin primers. All amplified samples (FcγR2A = 3,894 base pairs (bp), FcγR3A = 81 bp, FcγR3B = 1,631 bp, Actin = 189 bp. were run on a 2% agarose gel and imaged for quantification and analysis. **(A)** Shows representative mRNA expression of indicated genes on an agarose gel. **(B)** Shows quantification of those gels. DC expressed more of FcγR2A (74% increase, $^{*}p < 0.05$, $n = 4$, Students *t*-test, two-way) and FcγR3B (82% increase, $^{**}p < 0.01$, $n = 8$, Students *t*-test, two-way) but less of FcγR3A (27% reduction, $^{**}p < 0.01$, $n = 8$, Students *t*-test, two-way), compared to NDC. Actin was unchanged between the cell models. All scatter plots are mean ± SEM.

RESULTS

Differentiation of SH-SY5Y Neuroblastoma Cells Increases Tau Levels and Decreases Antibody Internalization

In prior studies we used several different modeling approaches to examine the efficacy and mechanism of action of tau antibodies. The tau antibodies used herein were generated against Tau386-408[P-Ser396, 404], a prominent pathological epitope in AD and other tauopathies, and have been characterized previously to some extent, showing the efficacy of the 4E6 antibody and relative lack thereof for the 6B2 antibody (7, 12, 14, 16, 19, 22, 23, 27). To better understand the mechanism of action and further clarify the dynamic interaction between tau antibodies and pathological tau, we expanded our research toward alternative culture models, focusing initially on non-differentiated SH-SY5Y cells (NDC), which with the help of flow cytometry provided quantitative insight into uptake and colocalization of the antibodies and their target (19). To improve this model by rendering it more neuron-like, we differentiated these cells with retinoic acid and BDNF as previously described (23, 28–31, 35–37). As expected, differentiated cells (DC) had smaller cell bodies, and elongated processes (**Figure 1A**) compared to NDC, as well as increased total tau levels on Western blots (215% increase, $p < 0.01$, **Figure 1B**). To clarify that DC could take up tau antibodies, both types of cells were treated with the 6B2 or 4E6 tau antibody for 24 h and then analyzed on Western blots, which revealed that DC took up less 6B2 or 4E6 than NDC (6B2; 45% reduction, $p < 0.01$, **Figures 1C,D**; 4E6; 83% reduction, $p < 0.0001$, **Figures 1C,E**). We then showed the efficacy of the 4E6 antibody in preventing toxicity of human brain-derived paired helical filament (PHF)-enriched tau protein in DC, whereas it was ineffective in NDC, presumably because the latter model does not have a fully developed clearance system (**Supplementary Figure 1**). The 6B2 tau antibody was ineffective in both models and neither antibody was toxic on their own (**Supplementary Figure 1**). PHF cytotoxicity is associated with decreases in NeuN levels on Western blots as described previously by us (12, 23) and others (35, 38–42).

Differentiated SH-SY5Y Cells Express a Different Repertoire of Fc Receptors Compared to Non-differentiated Cells

In prior studies in mouse neurons, we concluded that Fcγ receptors (FcγR) were the primary mechanism of uptake of tau antibodies because it could be blocked by Dynasore, an inhibitor of clathrin (receptor)-mediated endocytosis and by an antibody against Fcγ2/3 receptors (mouse BD Fc Block™) (14). We first confirmed that Dynasore prevented antibody uptake in DC, whereas it was ineffective in NDC because its uptake is primarily bulk-mediated as supported by dextran-colocalization studies (**Supplementary Figure 2**). We did not test the Fcγ2/3 blocking antibody because it is made for mouse receptors and therefore unlikely to work in this human culture model. To clarify if these same receptors were expressed in SH-SY5Y cells, we measured their mRNA expression of Fcγ receptors (FcγR) under basal conditions using PCR and quantitative analysis. DC expressed more of FcγR2A (74% increase, $p < 0.05$), and FcγR3B (82% increase, $p < 0.01$), and less of FcγR3A (27% reduction, $p < 0.01$), compared to NDC, with actin levels being comparable in both models (**Figures 2A,B**). Expression of FcγR3A and B was detected consistently in different batches of cells, whereas FcγR2A was not detected in all batches. FcγR2 and FcγR3 expression was further confirmed by immunoprecipitation/western blots

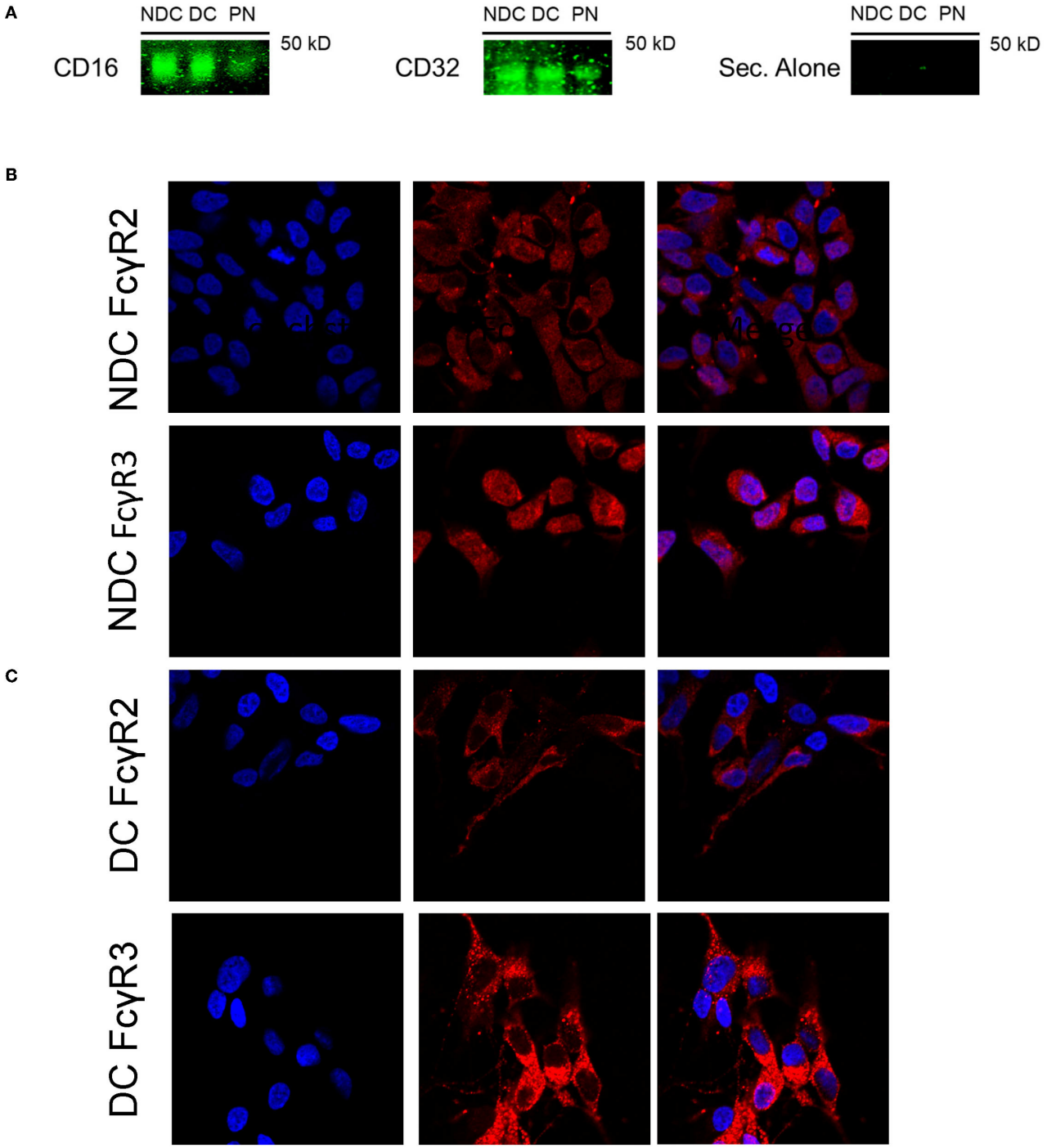


FIGURE 3 | Subtypes of Fcγ receptors are expressed in NDC and DC. Cells were evaluated for presence of FcRs via Western blot and immunohistochemistry. All cells were kept at basal conditions. For Western blot, cells were lysed and equal amounts of protein were added to immunoprecipitation (IP) beads bound to an anti-mouse CD16/32 antibody. Following IP, the samples were subjected to immunoblotting, and the blots probed with antibodies recognizing CD16 and CD32, or fluorescent secondary alone (Sec. Alone). **(A)** Using both antibodies, the signal is similar in the undifferentiated and differentiated neuroblastoma cells. A band is visible in the lane containing primary neuron samples indicating the presence of receptors on these cells. No signal was visible when the blot was probed with only the fluorescent secondary antibody. For immunohistochemistry, cells were fixed, washed, stained for surface bound Fcγ receptors (FcγR); CD16 (FcγR3) and CD32 (FcγR2), and then imaged using confocal microscopy. Cell images were enlarged further 4x from original file size. **(B,C)** Analysis of images revealed diffuse staining of both FcγR in or near the soma for DC and NDC. DC showed staining as well in distal processes and neurites, which are not developed in NDC.

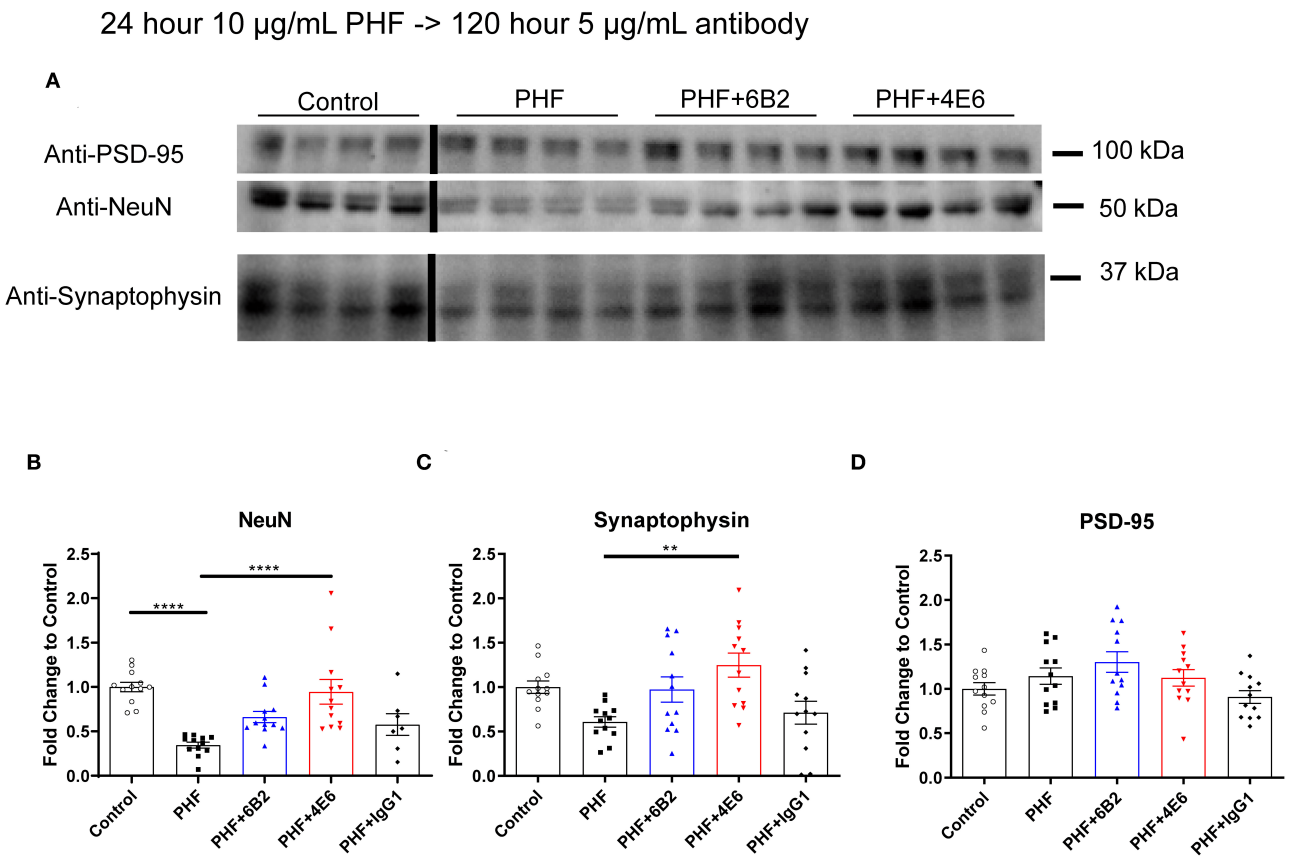


FIGURE 4 | 4E6 tau antibody prevents cytotoxicity and synaptotoxicity in PHF pre-treated DC. To further promote a pathological state and clarify antibody efficacy following long-term treatment, DC were pre-treated with higher PHF concentration (10 µg/mL) for 24 h, washed with DMEM, and subsequently treated with 6B2, 4E6, or IgG1 for 120 h ($n = 7$–12 per group). Cells were then lysed and collected for Western blot analyses, and probed for NeuN, synaptophysin, and PSD-95. **(A)** Shows representative Western blots of DC treated with 10 µg/mL PHF and/or 5 µg/mL antibodies, compared to untreated controls. **(B)** PHF (10 µg/ml) was cytotoxic (65% reduction in NeuN levels, ****$p < 0.0001$, One-way ANOVA, Bonferroni *post hoc* test). Subsequent 4E6 incubation for 120 h significantly increased NeuN levels back to control levels (****$p < 0.0001$), while 6B2 and IgG1 had no effect. **(C)** Likewise, PHF showed a strong trend for synaptotoxicity (39% decrease in the presynaptic marker synaptophysin). Subsequent 4E6 incubation for 120 h significantly increased synaptophysin levels compared to the PHF group (106% increase, **$p < 0.01$, One-way ANOVA, Bonferroni *post hoc* test), while 6B2 and IgG1 had no effect. **(D)** The postsynaptic marker PSD-95 was not altered overall or between the groups. Note that the blot lanes in the individual blots are from the same blot. The lines show where excess test lanes where sectioned out. All scatter bar graphs are mean ± SEM.

and confocal microscopy. FcγR2 (CD32) and FcγR3 (CD16) protein was detected in equal amounts in both cells types via Western blot following immunoprecipitation (**Figure 3A**). All FcγR were localized at the cell body for NDC (**Figure 3B**), as they have no processes, while they could be visualized in the cell body and distal processes for DC (**Figure 3C**). These different expression levels, in addition to other mechanistic differences (**Supplementary Figure 2**), may explain why NDC and DC take up tau antibodies to different degrees.

Long-Term Tau Antibody Treatment Prevents Neuro- and Synaptotoxicity in Differentiated Cells

To further clarify PHF toxicity and antibody efficacy, DC were treated with PHF (10 µg/mL), which creates a more homogenous pathological condition than lower doses (**Supplemental Figure 3**), for 24 h. Cells were subsequently treated with antibodies (5 µg/mL) for 120 h, followed by Western blot analyses (**Figure 4A**). The groups differed overall ($p < 0.0001$). PHF was clearly toxic as assessed by the decreased NeuN levels at 144 h compared to untreated control (65% reduction, $p < 0.0001$, **Figure 4B**). The 4E6 tau antibody prevented PHF toxicity as NeuN levels were similar to untreated control values ($p < 0.0001$), compared to the PHF group. There was a trend for the 6B2 tau antibody to prevent PHF toxicity ($p = 0.101$), whereas IgG1 had no effect on PHF toxicity ($p = 0.988$) (**Figure 4B**). In addition, synaptophysin and PSD-95, which are pre- and post-synaptic markers, were measured to assess synaptic integrity. Synaptophysin levels differed overall between the groups ($p < 0.0016$). They trended downward due to PHF toxicity (39%, **Figure 4C**), and 4E6 prevented this as following its incubation this marker went back to untreated control levels ($p = 0.0018$), compared to the PHF group. There

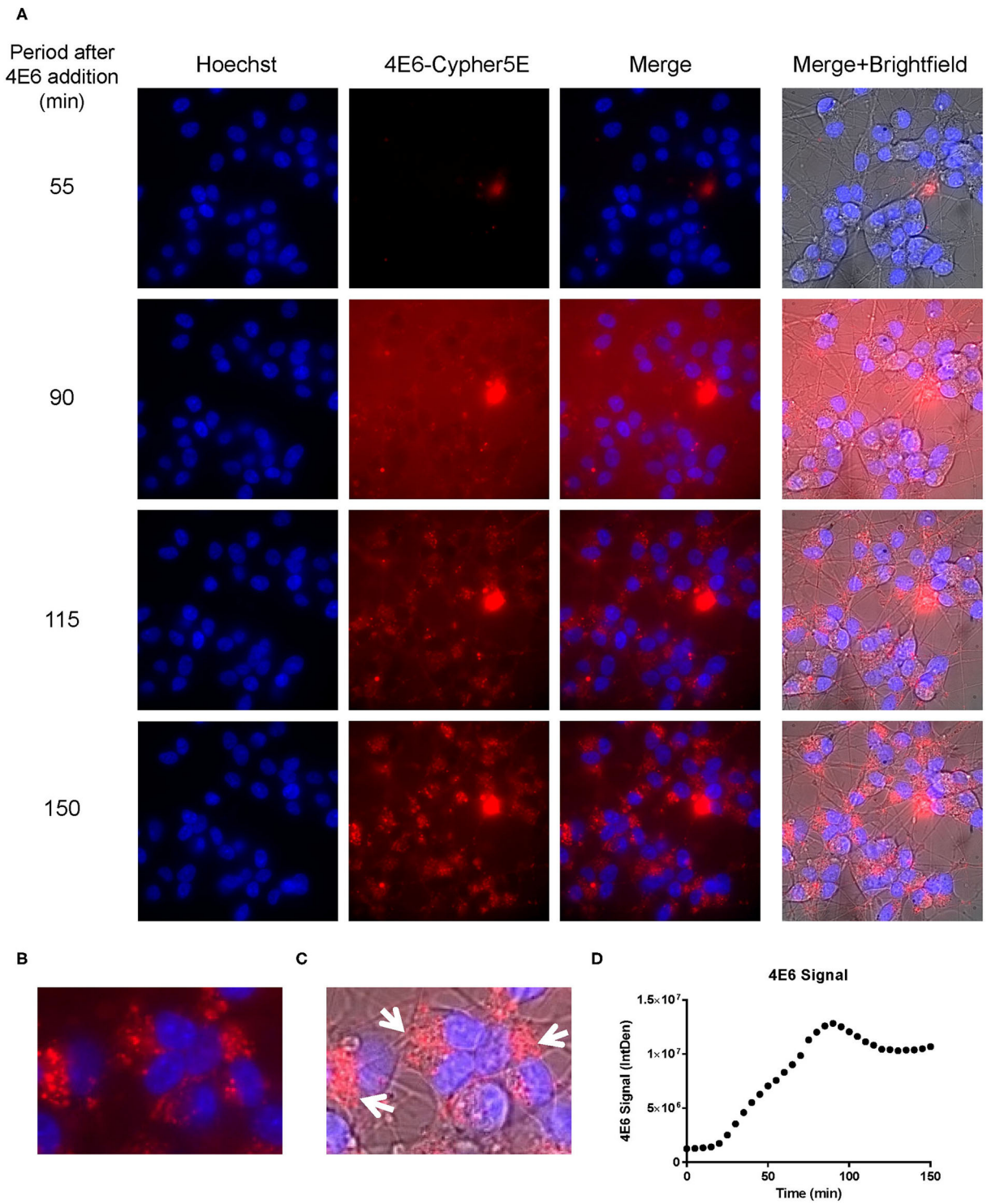


FIGURE 5 | 4E6 tau antibody is time-dependently taken up into the soma of DC in a saturable process. Prior to imaging, DC were incubated with a nuclear stain, Hoechst, washed, and then placed in non-phenol red DMEM with HEPES. Subsequently, DC were treated with 20 μg/mL Cypher5E (a pH sensitive dye)-tagged 4E6 tau antibody for up to 140 min. Cells were analyzed using live time-lapse imaging at 5 min intervals. Time points for analyses were chosen from the first time point with positive antibody signal. **(A)** Shows representative still images from the live imaging from the 55 to 150 min time points, with all analyzed channels. The 4E6-Cypher5E signal increased over time as the antibody enters more acidic compartments within the cell (endosomes to lysosomes). At 90 min there was increased background noise, due to the relatively low antibody signal, but it was gone near 100 min. **(B,C)** The 150 min Merge + Brightfield images were magnified to show more detailed morphology and localization of the 4E6 tau antibody in the cells. Most of the antibody signal was localized in the soma of the cells, and was peri-nuclear as indicated by the white arrows. **(D)** Quantification of the 4E6 tau antibody signal from the Cy5 channel from the Internal Density (IntDen) of the entire frame from 0 to 150 min. The 4E6 signal increased over time, and plateaued near 90–100 min.

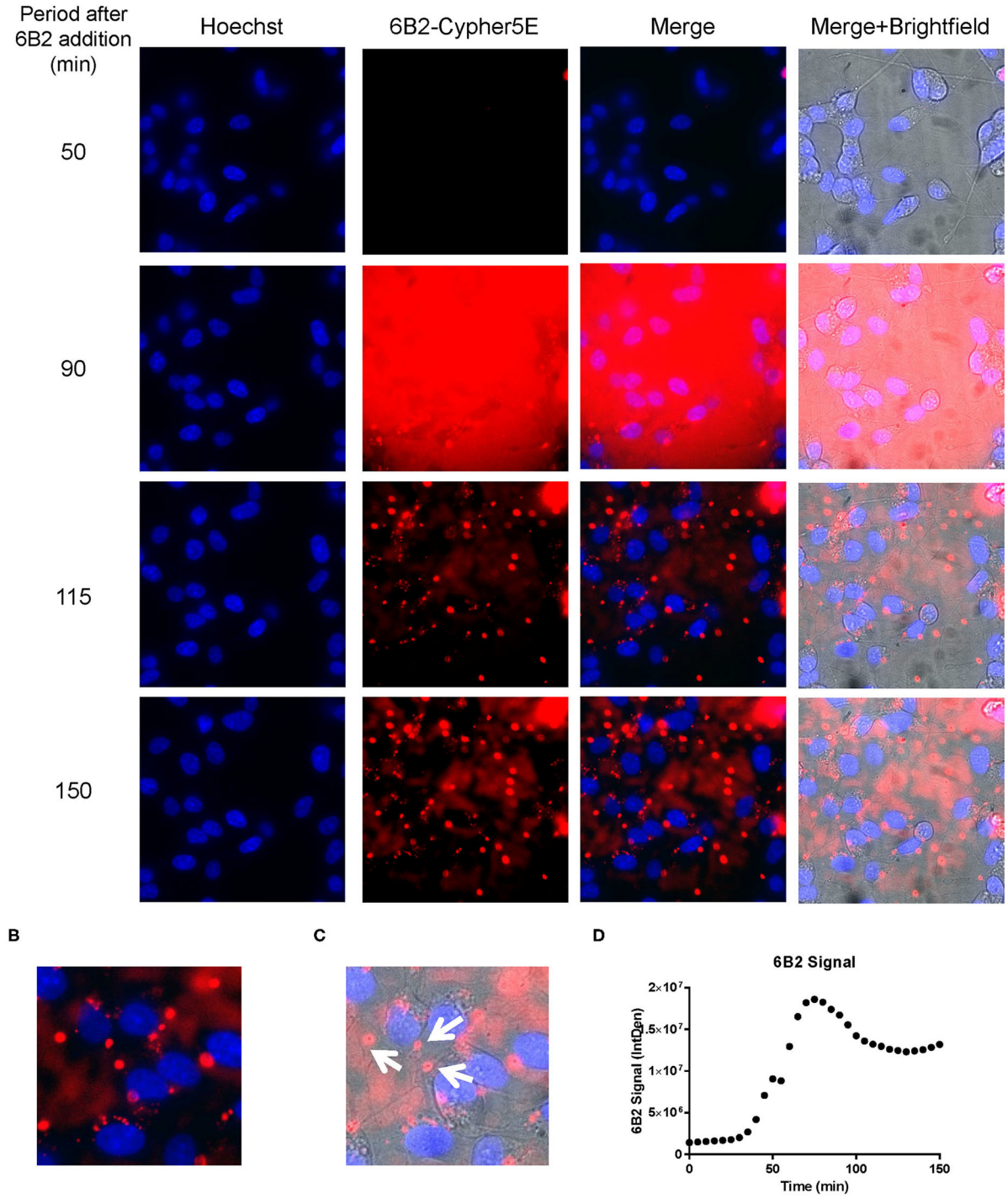


FIGURE 6 | 6B2 tau antibody is time-dependently taken up into the neurites of DC. Prior to imaging, DC were incubated with a nuclear stain, Hoechst, washed, and then placed in non-phenol red DMEM with HEPES. Then, DC were treated with 20 μg/mL Cypher5E-tagged 6B2 tau antibody for up to 150 min. Cells were analyzed using live time-lapse imaging at 5 min intervals. Time points for analyses were chosen from the first time point with positive antibody signal. **(A)** Shows representative still images from the live imaging from the 50 to 150 min time points, with all analyzed channels. The 6B2-Cypher5E signal increased over time. At 90 min there was increased background noise, due to the relatively low antibody signal, but it was gone near 100 min. **(B,C)** The 150 min Merge + Brightfield images were magnified to show more detailed morphology and localization of 6B2 tau antibody in the cells. Most of the antibody signal was localized in the neurites of the cells as indicated by the white arrows. **(D)** Quantification of the 6B2 tau antibody signal from the Cy5 channel from the Internal Density (IntDen) of the entire frame from 0 to 150 min. The 6B2 signal increased over time, and plateaued near 105 min.

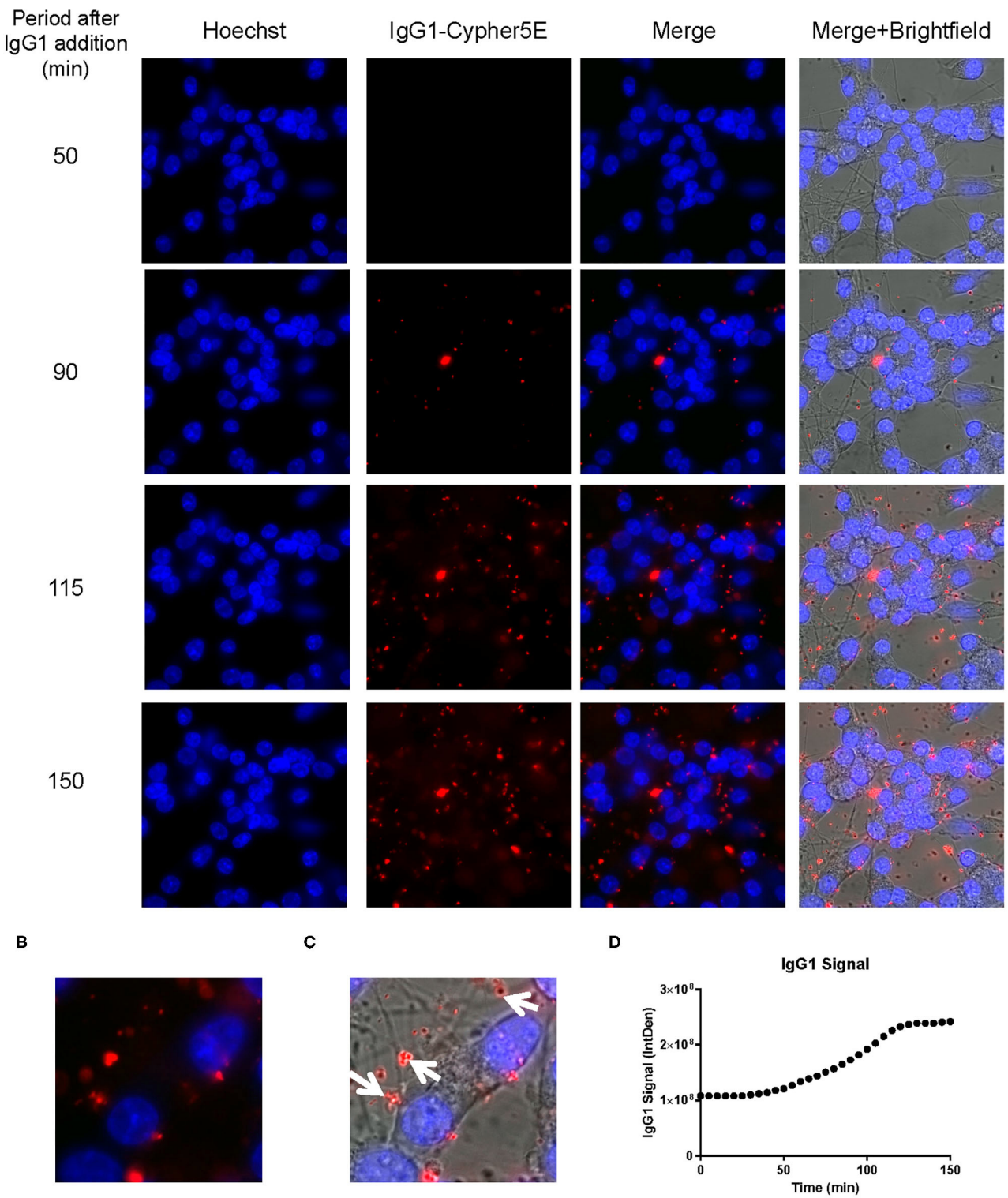


FIGURE 7 | IgG1 antibody is time-dependently taken up into the neurites of DC. Prior to imaging, DC were incubated with a nuclear stain, Hoechst, washed, and then placed in non-phenol red DMEM with HEPES. Subsequently, DC were treated with 20 μg/mL Cypher5E-tagged IgG1 antibody for up to 150 min. Cells were analyzed

(Continued)

FIGURE 7 | using live time-lapse imaging at 5 min intervals. Time points for analyses were chosen from the first time point with positive antibody signal. **(A)** Shows representative still images from the live imaging from the 50 to 150 min time points, with all analyzed channels. The IgG1-Cypher5E signal increases over time. **(B,C)** The 150 min Merge + Brightfield images were magnified to show more detailed morphology and localization of 4E6 tau antibody in the cells. The greater majority of the antibody signal was localized in the neurites of the cells as indicated by the white arrows. **(D)** Quantification of the IgG1 antibody signal from the Cy5 channel from the Internal Density (IntDen) of the entire frame from 0 to 150 min. The IgG1 signal increased over time, and plateaued near 120 min.

was a trend for the 6B2 tau antibody to prevent this type of PHF toxicity ($p = 0.257$), whereas IgG1 had no effect on synaptophysin ($p > 0.999$). Lastly, the post-synaptic marker PSD-95 was not significantly affected by PHF or subsequent antibody treatments (**Figure 4D**). Briefly, these findings are in line with our prior work showing efficacy of 4E6 and lack thereof for 6B2 in preventing tau toxicity in primary mouse neurons and in a transgenic mouse model (12). Hence, these findings validated the use of this DC model for subsequent live imaging studies.

4E6 Tau Antibody Is Taken Up Into the Soma of Differentiated Cells Whereas 6B2 and IgG1 Enter via Neuronal Processes as Assessed by Live Imaging

We had previously shown that both the effective 4E6 tau antibody and the ineffective 6B2 tau antibody could localize with somatic intraneuronal tau in mouse brain slices and in mouse primary neurons following an incubation for 24 h or longer. Those findings do not provide insight into why the former is effective and the latter ineffective. Therefore, we set out to determine with live imaging if earlier cellular events might provide better insight into this important issue. DC are preferred over primary neurons for live imaging as they are less sensitive to the conditions for such analysis. The DC were treated for up to 150 min with 4E6 or 6B2 tau antibodies or control IgG1 (20 μg/mL), which were tagged with a pH sensitive dye (Cypher5E). This is an ideal dye as it is only detectable in acidic compartments within the cell. Its signal therefore confirms intracellular location as opposed to extracellular detection. Larger amounts of antibodies were used than in prior experiments to enhance detection by live imaging under the shorter time period. Antibody internalization was monitored using time-lapse live imaging, which revealed that antibody uptake was time-dependent (**Figure 5A**). 4E6 tau antibody signal was primarily detected in the soma, and was perinuclear (**Figures 5B,C**). In addition, the signal began to plateau near 90 min after incubation began (**Figure 5D**), suggesting a saturable process. Both 6B2 tau antibody and IgG1 were also taken up in a time-dependent manner (**Figures 6A**, **7A**), and distinctly localized in the neurites and more distal processes of the DC, with limited visibility in the soma (**Figures 6B,C**, **7B,C**). 6B2 signal increased over time and plateaued near 100 min, while IgG1 did not have as a distinct plateau over the same period (**Figures 6D**, **7D**), suggesting that its uptake may not have reached capacity. These finding indicate that the dynamics of the uptake of the two tau antibodies and its control antibody are different and needed to be examined further for their interaction with pathological tau protein for a possible insight into their efficacy or lack thereof.

Live Imaging Reveals Dynamics of 4E6 Co-localization With PHF in Differentiated Cells

We have previously shown that added PHF-tau is readily detected intracellularly in NDC and in primary mouse neurons after 24 h incubation, and that it takes at least 48 h for it to result in measurable toxicity (12, 19). Likewise, in these models, treatment with tau antibodies for 24 h results in robust intraneuronal colocalization with the PHF. To clarify the earliest interaction between PHF and tau antibodies under the time-lapse live imaging conditions described above, DC were pre-incubated with 50 μg/mL Alexa Fluor 488 tagged-PHF for 16 h, and were subsequently treated with 20 μg/mL Cypher5E tagged-4E6, 6B2, or IgG1 isotype control antibodies for up to 150 min (**Figures 8**–**10**, See **Supplementary Videos 1**–**3** for detailed view). Larger amounts of PHF and antibodies were used than in prior experiments to enhance detection by live imaging under the shorter time conditions. Antibody and PHF internalization was closely monitored using time-lapse live imaging. These images showed an increase over time of all antibodies' signal (**Figures 8A**, **9A**, **10A**), which began to plateau at 75, 100, and 110 min for 4E6, 6B2, and IgG1, respectively (**Figures 8B**, **9B**, **10B**). Note that the cells had been pretreated with PHF-tau, and the PHF signal stayed relatively constant for up to 150 min for all antibodies (**Figures 8C**, **9C**, **10C**).

Importantly, co-localization with PHF was primarily found within the soma for 4E6, similar to the live imaging results with 4E6 alone (**Figure 5**), and increased over time (**Figures 8A,D,E**). 6B2 and IgG1 did not colocalize with PHF, as the antibody signal was primarily within the neurites, similar to the live imaging with those antibodies alone (**Figures 6**, **7**), whereas the PHF was mainly in the soma (**Figures 9A,D,E**, **10A,D,E**). This was further confirmed by colocalization analysis (R^2), which increased rapidly for 4E6 and showed a strong positive correlation over time ($r^2 = 0.969$, $p = 0.0157$, **Figure 8F**), but poor or non-existent correlation for 6B2 and IgG1 over time (**Figures 9F**, **10F**). These pronounced internalization and localization differences fit nicely with the efficacy divergence between the two tau antibodies, and indicate that this type of live imaging is helpful to identify promising therapeutic tau antibodies.

DISCUSSION

Tau immunotherapies have been shown to be effective in various models (1–4). Despite these advances, mechanistic and dynamic insight into the uptake and interaction of pathological tau protein and tau antibodies has been relatively limited. Here, we show that differentiated human SH-SY5Y neuron-like cells

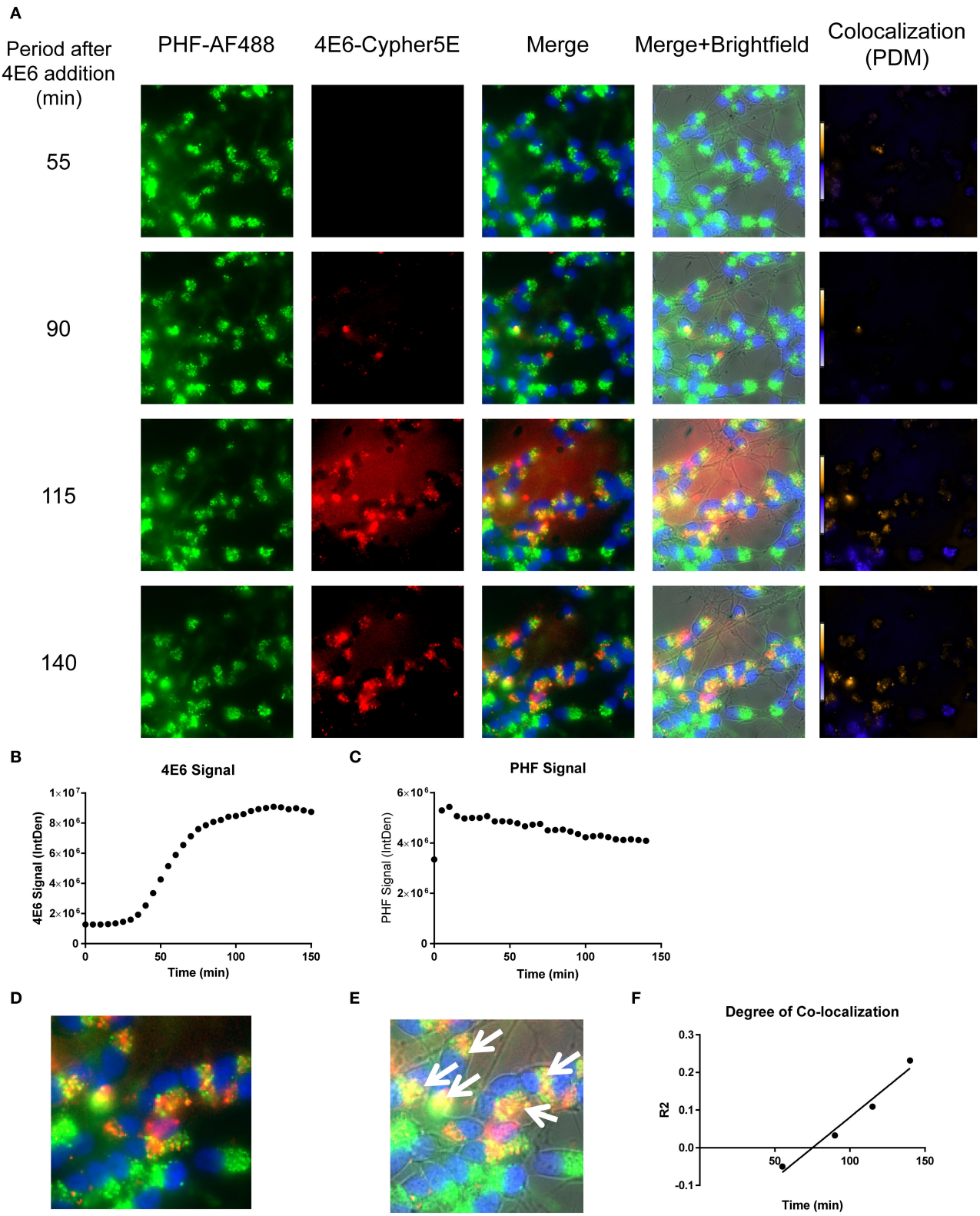


FIGURE 8 | Time-lapse live imaging shows a strong correlation between PHF and 4E6 in pre-treated DC. DC were pre-incubated with 50 μg/mL tagged PHF-AF488 for 16 h, washed, incubated with Hoechst stain, washed again, and then incubated with Cypher5E-tagged 4E6 tau antibody for up to 140 min. Cells were analyzed

(Continued)

FIGURE 8 | using live time-lapse live imaging at 5 min intervals. **(A)** Shows still frames from the live imaging of the 55–140 min time points, with all analyzed channels. The 4E6-Cypher5E signal increased over time, while the PHF signal did not change. In addition, an intensity co-localization analyses was performed between the 4E6 and PHF signals, which generates a co-localization heat map and intensity correlation coefficient (R^2). The colocalization heat map (PDM) showed increasing intensity over time, as indicated by the yellow color, which depicts greater colocalization. Internalization of 4E6 occurred primarily in the soma, and was peri-nuclear, while the PHF resided in the soma at the beginning of the experiment. **(B)** Shows quantification of the 4E6-Cypher5E signal, where the signal increased over time, and began to plateau near 75 min. **(C)** Shows quantification of the PHF-AF488 signal, which showed no change within the 140 min. **(D,E)** The 140 min Merge and Merge + Brightfield images were magnified to show more detailed morphology and localization of 4E6 tau antibody and PHF co-localization in the cells. Most of the antibody signal was localized in the soma of the cells, and was peri-nuclear as indicated by the white arrows. **(F)** Shows the intensity correlation coefficients of PHF and 4E6 ($R^2 = -0.05$ to 0.232), which increased linearly, and correlated well over time ($r^2 = 0.969$, $p = 0.0157$, Pearson, two-tailed).

(DC) treated with human brain-derived pathological tau, is a suitable and expeditious alternative model to assess and clarify tau antibody efficacy, compared to the more time consuming and more expensive to maintain *in-vivo* and induced pluripotent stem cell models. It is well documented that differentiated SH-SY5Y cells (DC) have improved expression of neuronal markers and morphology compared to their non-differentiated (NDC) counterparts (28–30). This DC model is more scalable than both primary mouse neurons and stem cell models, In addition, live time-lapse imaging of this model is more feasible than in those other culture models, and it provides a valuable temporal and spatial insight into the interaction of pathological tau and potential antibody therapies. Specifically, live imaging can be used as a rapid means of analysis of an antibody's: (a) effectiveness to enter neurons; (b) colocalization with its target; (c) subcellular location, and; (d) its association with its target over time.

To create a more physiological cell culture system, a double-differentiated protocol was employed, as described previously by us (23, 31, 36, 37), and other groups (28–30), to differentiate SH-SY5Y neuroblastoma cells with increased neuronal expression and morphology These cells are characteristically different from their NDC counterparts with: (1) improved neuronal morphology; (2) decreased antibody uptake; and (3) increased tau levels (**Figure 1**). In addition, we showed that the neuronal marker NeuN is a sensitive way to measure PHF toxicity in these models, as we had previously shown in primary mouse neurons (12, 23), and others have used as well to assess neurotoxicity (35, 38–42). We have previously discussed that NeuN is expressed throughout the neuron, and that the PHF toxicity likely reflects more retraction of neuronal processes than overt cell death within the analyzed time period (12). PHF (1 μg/mL) was cytotoxic to both human cell lines as measured after 48 h incubation as NeuN levels decreased by 53 and 37%, in NDC and DC, respectively. Importantly, 4E6 tau antibody treatment in PHF pre-incubated DC prevented PHF toxicity, but it was ineffective in NDC (**Supplementary Figure 1**). These DC results are in agreement with findings in primary mouse neuronal cultures under similar conditions (14).

Several human and mouse subtypes of FcγRs exist and are found on various cell types (43). Likewise, several human and mouse IgG isotypes exist with different functions (43). The nomenclature of these receptors and isotypes is not the same for these species, which complicates their comparison. Briefly, regarding tau antibody therapy studies, it is important to keep in mind that most anti-tau mouse monoclonals are of the IgG1 isotype, like the antibodies in this particular study. However, effector function for microglial and macrophage phagocytosis is mainly confined within mouse IgG2a binding to mouse FcγR1 but is limited for mouse IgG1. In contrast, human IgG1 has a strong phagocytic effector function linked to human FcγR1. These differences are though not relevant in the current neuron-like DC model or in mouse primary neuronal cultures because they do not have phagocytic activity. We have previously demonstrated that blocking mouse FcγR1 or phagocytosis has no effect on IgG1 antibody uptake in mouse brain slices or in mouse primary neuronal culture (14). We have also repeatedly shown that the 4E6 IgG1 mouse monoclonal antibody used herein is readily taken up both in mouse neurons (7, 12, 14, 22, 23) and in human NDC (19) and DC in the present article. In contrast, its human IgG1 Fc chimera (c4E6) is poorly taken up in both mouse primary neurons and in human DC because its charge is very different from the mouse IgG1 isotype (23).

Considering that we have previously linked neuronal uptake of antibodies to FcγR2/3 (7), examining their expression in this model was warranted. The mRNA and protein expression levels of FcγR is generally greater in DC than in NDC (**Figures 2**, **3**), which aligns nicely with the main antibody uptake pathway in DC being receptor-mediated, whereas in NDC it is bulk-mediated (**Supplementary Figure 2**). Expression of the FcγR3 subtypes was seen consistently in different cell batches but not always for FcγR2. This phenomenon needs to be further examined, and it may depend on cell passage. Lastly, the proposed mechanism of action in DC is in agreement with our prior work, revealing that antibody uptake in primary mouse neuronal cultures is mainly receptor-mediated – presumably FcγR2/3-mediated based on blocking studies - while bulk-mediated endocytosis was involved to a lesser extent (14). For all these reasons, and their more neuronal properties, DC are a better model than NDC for future studies in this field, and compares favorably to other models used/developed by our laboratory. Other groups have reported involvement of FcγR in uptake of antibodies targeting tau and α-synuclein in different models (17, 44).

Furthermore, to enhance tau pathology in DC, we increased the PHF dosage to 10 μg/mL, which also generates a more homogeneous cellular pathology (**Supplementary Figure 3**). We subsequently treated the cells with antibodies for a longer time period (120 h) to better reflect their efficacy in preventing PHF cytotoxicity. As expected, these experiments showed a more pronounced PHF cytotoxicity as measured by NeuN levels (65%), which was prevented by the 4E6 tau antibody, while 6B2 trended this way, and IgG1 had no effect (**Figure 4**). This

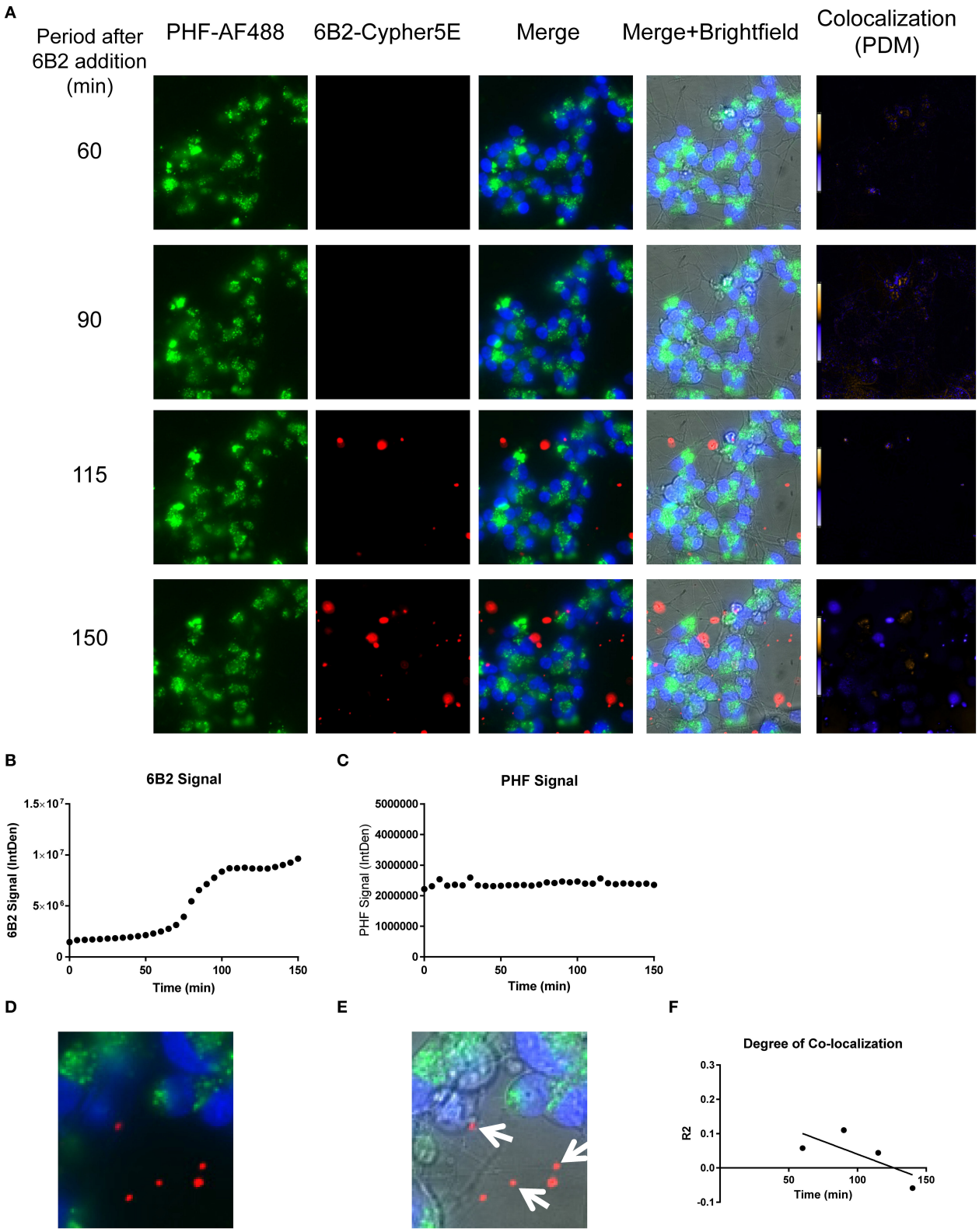


FIGURE 9 | Time-lapse live imaging shows PHF and 6B2 in pre-treated DC, but in separate compartments. DC were pre-incubated with 50 μg/mL tagged PHF-AF488 for 16 h, washed, incubated with Hoechst stain, washed again, and then incubated with Cypher5E-tagged 6B2 tau antibody for up to 150 min. Cells were

(Continued)

FIGURE 9 | analyzed using live time-lapse imaging at 5 min intervals. **(A)** Shows still frames from the live imaging of the 55–150 min time points, with all analyzed channels. The 6B2-Cypher5E signal increased over time, while the PHF signal did not change. In addition, an intensity co-localization analyses was performed between the 6B2 and PHF signals, which generates a co-localization heat map and intensity correlation coefficient (R^2). The colocalization heat map (PDM) showed no change in intensity over time. Internalization of 6B2 occurred primarily in the neurites, while the PHF resided in the soma. **(B)** Shows quantification of the 6B2-Cypher5E signal, where the signal increased over time, and began to plateau near 100 min. **(C)** Shows quantification of the PHF-AF488 signal, which showed no change within the 150 min period. **(D,E)** The 150 min Merge and Merge + Brightfield images were magnified to show more detailed morphology and localization of 6B2 tau antibody and PHF in the cells. Most of the antibody signal was localized in the neurites of the cells, and did not colocalize with PHF, as indicated by the white arrows. **(F)** Shows the intensity correlation coefficients of PHF and 6B2 ($R^2 = 0.058$ to -0.059), which did not correlate over time ($r^2 = 0.5334$).

study confirmed the efficacy differences between 4E6, 6B2, and IgG1 control as we have seen *in vivo* and in primary mouse neuronal cells (12, 22), further supporting the predictive validity of this human cellular model. PHF-induced synaptotoxicity as revealed by a decrease in synaptophysin, a presynaptic marker, mirrored PHF-induced reduction in NeuN. Importantly, as for NeuN, synaptotoxicity was prevented with 4E6 tau antibody treatment, but not with 6B2 or IgG1. Interestingly, PHF was not toxic to the post-synapse as reflected by the lack of change in PSD-95 levels following PHF incubation. This is not particularly surprising because postsynaptic degeneration (loss of PSD-95) is a later phenomenon than presynaptic degeneration (loss of synaptophysin) in AD and related models (45–47). In addition, it has been shown that TrkB, a receptor of BDNF, and PSD-95 complex together within the post-synapse (48–51), and when bound to BDNF a positive feedback loop leads to an increase in PSD-95 (52, 53). The SH-SY5Y neuroblastoma cells are differentiated with an artificially high level of BDNF (50 ng/mL) (28–30), which is much lower in the interstitial space of the brain under physiological or pathological states (54). As a result, the BDNF concentration in our DC model may saturate the PSD-95 levels.

The establishment of DC as a suitable model for PHF toxicity and its prevention by tau antibodies paved the way for the live time-lapse imaging studies. Specifically, to explore internalization and interaction of tau antibodies and their pathological targets in the immediate aftermath of adding these reagents to the cultures. Our findings indicate that their internalization and subsequent interaction occurs in different sub-cellular locations, depending on the antibody used. In cells treated only with the 4E6 tau antibody, the antibody was preferentially taken up directly into the soma. Its signal increased over time and plateaued at about 90 min, suggesting a receptor-mediated process (**Figure 5**). As shown in **Figures 3B,C**, the soma has high density of Fc receptors. For the 6B2 tau antibody and IgG1, uptake into the cells was less prominent and internalization was more extensive in the processes compared to 4E6 (**Figures 6**, **7**). All three antibodies are likely primarily taken up via receptor-mediated process because it is a saturable process, and considering our prior work on elucidating their uptake mechanisms (7, 12, 14, 16, 19, 22, 23, 27). This careful frame-by-frame examination of the video files indicated that the uptake of the 4E6 antibody was predominantly directly into the soma, whereas in contrast 6B2 and IgG1 showed prominent uptake via the neuronal processes. The different primary path of uptake for 6B2 and control IgG1 under these live imaging conditions provides insight into why they are therapeutically ineffective, as further confirmed under the PHF-pre-treated conditions discussed below.

In the PHF pre-treated cells, 4E6 antibody uptake was fast, while the PHF signal stayed constant. The degree of colocalization increased rapidly over time in a highly correlated fashion ($r^2 = 0.969$). This indicates a dynamic antibody being directed to a static target (**Figure 8**, **Supplementary Video 1**). However, the scenario was different for 6B2 or IgG1, as those antibodies showed minimal if any colocalization with PHF during the incubation period (**Figures 9**, **10**, **Supplementary Videos 2**, **3**). Furthermore, the uptake of these antibodies began to plateau later (100–110 min), compared to 4E6 (75 min). In addition, most of the 6B2 and IgG1 antibodies seemed to reside within the neurites, similar to antibody alone controls, and were not detected immediately in the soma, unlike 4E6. A possible explanation for these differences is that 4E6 taken up via somal Fc receptors binds to PHF there in the endosomal/lysosomal system, and is therefore quickly visualized. On the other hand, 6B2 or IgG1 are either not taken up via that somal pathway or do not interact with PHF there and are therefore recycled out of the cell and not seen until they come in through the processes. Transport via that pathway takes longer leading to delayed uptake signal. As we have shown in previous studies (14), 4E6 is taken up into primary mouse neurons mainly through receptor-mediated endocytosis (∼80%), while the remainder is bulk-mediated (∼20%). Together, these findings indicate that 6B2 and IgG1 are internalized through the neurites, while 4E6 is primarily internalized via the soma.

In previous studies, we have shown that tau antibodies can enter tauopathy neurons in a mouse brain within 1 h after intracarotid injection (5). In addition, tau antibodies colocalize strongly with neuronal pathological tau when analyzed at 2 h in *ex vivo* mouse slice culture models (7, 13), and at 24 h in primary mouse neurons (12). Our live time-lapse imaging technique is in agreement with these previous studies, as tau antibodies were internalized into neurons within 50 min after treatment. In addition, we demonstrated the convergence of tau antibody and its pathological PHF target within 90 to 115 min. Interestingly though, under the current live imaging conditions, 4E6 but not 6B2 colocalizes with endosomal/lysosomal tau. This clarifies 4E6's efficacy in clearing tau, preventing its toxicity and lack thereof for 6B2 as shown here and previously *in vivo* in a tauopathy mouse model and in primary mouse neurons (12).

Until now, the dynamics behind the efficacy differences between the two antibodies had been unexplained. It cannot relate to isotype because both antibodies are IgG1. Furthermore, both 4E6 and 6B2 have similar overall charge (∼6.5 and ∼6.8,

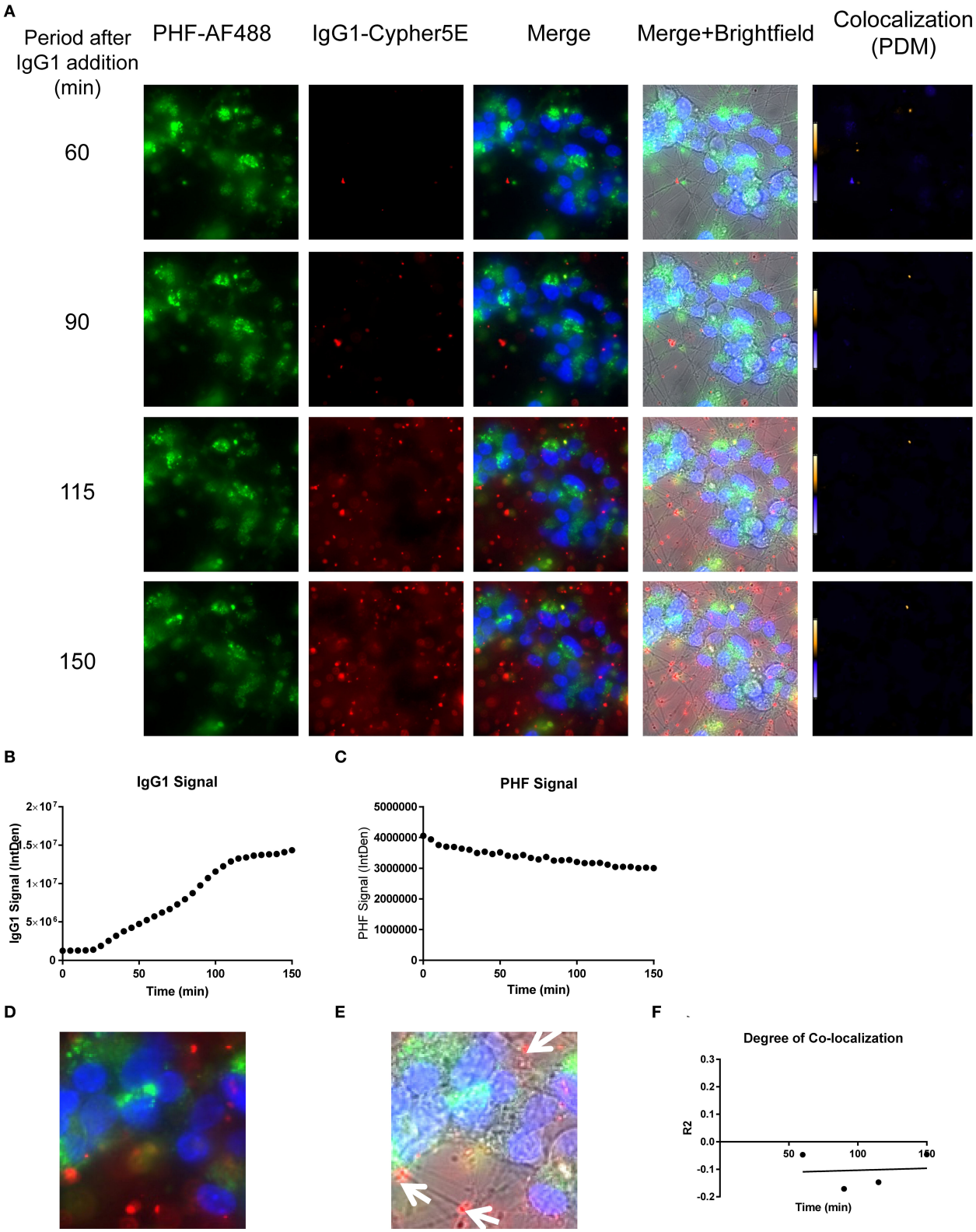


FIGURE 10 | Time-lapse live imaging shows PHF and IgG1 in pre-treated DC, but in separate compartments. DC were pre-incubated with 50 μg/mL tagged PHF-AF488 for 16 h, washed, incubated with Hoechst stain, washed again, and then incubated with Cypher5E-tagged IgG1 isotype control antibody for up to 150 min. Cells were analyzed using live time-lapse imaging at 5 min intervals. **(A)** Shows still frames from the live imaging of the 55–150 min time points, with all

(Continued)

FIGURE 10 | analyzed channels. The IgG1-Cypher5E signal increased over time, while the PHF signal did not change. In addition, an intensity co-localization analyses was performed between the IgG1 and PHF signals, which generates a co-localization heat map (PDM) and intensity correlation coefficient (R^2). The colocalization heat map showed no change in intensity over time. Internalization of IgG1 occurred primarily in the neurites, while the PHF resided in the soma. **(B)** Shows quantification of the IgG1-Cypher5E signal, where the signal increased over time, and began to plateau near 110 min. **(C)** Shows quantification of the PHF-AF488 signal, which showed no change within the 150 min period. **(D,E)** The 150 min Merge and Merge + Brightfield images were magnified to show more detailed morphology and localization of IgG1 and PHF in the cells. Most of the antibody signal was localized in the neurites of the cells, and did not colocalize with PHF, as indicated by the white arrows. **(F)** Shows the intensity correlation coefficients of PHF and IgG1 ($R^2 = -0.047$ to -0.046), which did not correlate over time ($r^2 = 0.0069$).

respectively), reflected in their prominent neuronal uptake as reported previously (7, 12). However, their affinities for various forms of tau differ substantially with 6B2 having much higher affinity for the tau peptide epitope and aggregated tau whereas 4E6 binds primarily to soluble pathological tau (7, 12). Hence, a possible explanation may be that in the PHF pre-treated DC, 6B2 may be binding to extracellular PHF that is present in such small quantities that it does not emit a detectable fluorescent signal. The source of the PHF may be trace amounts that remain after extensive washes following PHF pre-incubation, and/or PHF secreted from the neurons following uptake. This may in effect neutralize the 6B2 and it cannot then interact with more toxic soluble forms of tau within the cell. This 6B2-PHF complex may be preferentially taken up via the neurites, possibly by bulk-endocytosis, as shown previously for recombinant or mouse derived purified tau alone (55–57). As IgG1 does not have an affinity for tau, it is not retained in the soma if it is taken up there, and therefore rapidly recycled out of the cells and not detected. The IgG1 signal within the neurites is likely the result of non-specific uptake as highlighted by the lack of colocalization with PHF throughout the experiment. The possible interaction of 6B2 with trace amounts of extracellular PHF can be seen as an artifact of this model but it may also indicate that extracellular interaction of an antibody with pathological tau neutralizes the antibody so that it cannot prevent intracellular tau toxicity and promote tau clearance.

A more straightforward explanation for the contrasting efficacies of 4E6 and 6B2 relates to the differences in their initial location and uptake within the cell, which is comparable without or with PHF in the system (**Figures 5**, **6** and **8**, **9**, respectively). We previously showed that both antibodies are taken up into tauopathy brain slices, with the key difference being that 4E6 primarily colocalized with tau in the endosomal-lysosomal system, whereas 6B2 showed more diffuse cytoplasmic staining (7). Here, only antibodies in the endosomes/lysosomes are visualized and because 6B2 shows minimal colocalization with tau within this degradation system, it likely explains its lack of efficacy in the different culture models, and *in vivo* in promoting tau clearance and preventing its toxicity and related cognitive impairments (12, 22). The endosomal/lysosomal compartment is acidic, the pH of the cytosol is neutral and thus any 6B2 bound to cytosolic tau would not be seen here using the CypHer5 dye, and further investigation into this compartment may be warranted. It should be noted though that we previously reported colocalization of 6B2 and tau within endosome/lysosomes in NDC after 24 h antibody incubation (18). However, as shown here that primitive model system cannot be used to assess efficacies of tau antibodies, presumably because it does not have efficient lysosomal degradation system and likely relies more on exocytosis for clearance. In addition, the high affinity interaction of 6B2 with tau may make it more difficult to degrade the 6B2-tau complex, whereas the lower affinity interaction of 4E6 with tau may promote tau disassembly and facilitate access of lysosomal enzymes to degrade tau. Overall, live imaging of these early events within a few hours allows for rapid prediction of antibody efficacy prior to lengthier studies in other models.

In summary, we have demonstrated that tau antibody 4E6 prevents PHF toxicity in human neuroblastoma DC, while tau antibody 6B2 or control IgG1 control do not, which mirrors our prior findings in other *ex vivo* and *in vivo* mouse models (12, 22). Furthermore, the live imaging herein provides an important new insight into the mechanisms behind this efficacy difference. Time-lapse live imaging in DC up to 150 min showed that the interaction of PHF and 4E6 tau antibody is dynamic and robust, with rapid colocalization within the soma of the cell, whereas this was not seen for 6B2 or IgG1 control. These qualitative results were supported by our quantitative evaluation of the PHF and antibody signals using colocalization analyses. These findings, and the distinct differences between 4E6 vs. 6B2, or IgG1, support the predictive validity of this assay and method of analysis. In particular, this entirely human model is ideal to examine efficacy of humanized antibodies prior to lengthy clinical trials, for which alternative mouse models are likely not appropriate. In conclusion, this overall approach is very useful to clarify the mechanisms of tau immunotherapies, and to evaluate clinical candidate tau antibodies for their effectiveness at entering cells and finding their pathological target.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The use of human tissue in these studies is exempt from Institutional Review Board (IRB) approval as acknowledged by the IRB Committee of the university. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Primary mouse culture generation was approved

by the Institutional Animal Care and Use Committee (IACUC) of the university and is in accordance with NIH Guidelines, which meet or exceed the ARRIVE guidelines.

AUTHOR CONTRIBUTIONS

DS and ES conceived and designed the experiments, analyzed the data, and wrote the manuscript. DS prepared and performed most of the experiments. YD helped with setup and analysis of data, and wrote relevant method section for live imaging experiments. QW, SM, and EC contributed to the FcR data, determination of the lack of antibody toxicity and relevant text. All authors contributed to the article and approved the submitted version.

FUNDING

ES was supported by NIH R01 grants NS077239, AG032611, and AG020197 and in part by R24OD18340 and R24OD018339. DS was also supported in part with NIH training grants NS 69514-1 and MH 96331-2.

ACKNOWLEDGMENTS

We acknowledge the use of tissues procured by the National Disease Research Interchange (NDRI) with support from NIH grant 2 U42 OD011158. We would like to thank Michael Gregory and Professor Peter Lopez of the NYU School of Medicine Cytometry and Cell Sorting Core (support grant P30CA016087) for flow cytometry advice and training. This facility and the Microscopy core are supported in part by grant UL1 TR00038 from the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.602292/full#supplementary-material

Supplementary Figure 1 | 4E6 tau antibody prevents PHF toxicity in DC, but not in NDC. NDC or DC, maintained as described above, were pre-treated with PHF (1 μg/mL) for 24 h, washed with DMEM, and subsequently treated with tau antibodies (6B2 or 4E6) for 24 h. Cells were then lysed, collected for Western blot analyses, and probed for NeuN. **(A,B)** Shows representative Western blots of NDC and DC treated with PHF and antibodies, compared to untreated controls. **(A)** Shows the quantified NeuN data from the Western blots for NDC samples, where PHF toxicity decreased NeuN levels (53% decrease, $^{**}p < 0.001$, $n = 7$–8, One-way ANOVA, Bonferroni *post hoc* test). Tau antibodies had no effect in NDC. **(B)** Shows quantified NeuN data for DC samples, where PHF toxicity decreased NeuN levels (37% decrease, $^{**}p < 0.01$, $n = 12$, One-way ANOVA, Bonferroni *post hoc* test), which was prevented by 4E6 resulting in NeuN levels comparable to controls. $^{****}p < 0.0001$ compared to PHF alone. Note that the blot lanes in **(A,B)** are from the same blots, respectively. The lines show where excess test lanes where sectioned out. **(C)** Shows representative Western blots from DC treated with 4E6 and 6B2. Quantitation shows no significant difference observed in the NeuN levels for cells treated with 4E6 and 6B2 at 5 and 10 μg/ml each for a duration of 24 h when compared to the untreated control cells demonstrating no neurotoxic effect of antibody treatment alone. All scatter bar graphs are mean ± SEM.

Supplementary Figure 2 | DC take up tau antibodies through receptor-mediated endocytosis. NDC and DC were co-incubated with 300 μg/mL 10,000 MW Alexa 568 tagged Dextran and 20 μg/mL Alexa 488 tagged 6B2 tau antibody for 24 h. Cells were then washed, incubated with Hoechst stain to visualize nuclei, washed, and fixed with paraformaldehyde for microscopy. **(A,B)** Shows confocal microscopy images of NDC and DC, respectively. The merge panel exemplifies the pronounced co-localization of dextran and tau antibody in NDC, while there was significantly less co-localization in DC. Both sets of images were analyzed for intensity correlation coefficient between the dextran and 6B2 signals. The colocalization analyses revealed strong colocalization in NDC as seen by the merged image, and strong intensity correlation between 6B2 and dextran ($R^2 = 0.729$), while limited colocalization was seen in DC as reflected by a weak intensity correlation coefficient ($R^2 = 0.123$). As indicated in the differentiated merged blow-up image, the green dots are much more prominent than the orange/yellow dots that reflect colocalization. Also, since the differentiated cells take up much less dextran because of their limited bulk-endocytosis, the red dots are smaller and not well discerned from the yellow/orange dots to the naked eye. In parallel, 6B2 tau antibody (5 μg/mL) and increasing doses of a clathrin-mediated endocytosis inhibitor, Dynasore (1–5 μg/mL) or vehicle (DMSO) were co-incubated with NDC or DC for 24 h. Cells were then lysed and collected for Western blot analyses. **(C,D)** Shows representative Western blots of NDC and DC treated with Dynasore and probed for anti-mouse IgG1 and GAPDH. **(E,F)** Shows the quantified results of the Western blots where the anti-mouse IgG1 signal was normalized to GAPDH. In NDC, Dynasore did not significantly affect antibody uptake at any dose, whereas it did in DC at the 5 μg/mL dosage (39% decrease, $^{*}p = 0.04$, $n = 7$–8, *t*-test, one-tailed) compared to the vehicle control. All scatter bar graphs are mean ± SEM.

Supplementary Figure 3 | PHF is dose-dependently taken up by NDC. NDC were treated with 0–10 μg/mL Alexa Fluor 647 tagged PHF-tau for 24 h. Cells were then collected and examined with flow cytometry. **(A)** Cells were gated for viable and singlet cells. **(B)** Furthermore, cells were analyzed for Alexa Fluor 647 positive cells, generating histograms for each sample, % of PHF positive cells and Median Fluorescence Intensity (MFI). **(C)** Shows dose-dependent increase in percent PHF positive cells, which plateaued between 1 and 10 μg/mL. **(D)** Quantification of MFI of the Alexa Fluor 647-tagged PHF, normalized to the 0 μg/mL dosage, which shows a dose-dependent increase in the intracellular PHF signal. All plots are mean ± SEM.

Supplementary Video 1 | Time-lapse live imaging shows time-dependent colocalization of PHF and 4E6 in pre-treated DC. DC were pre-incubated with 50 μg/mL tagged PHF-AF488 for 16 h, prepared for live imaging as described above, and subsequently treated with 20 μg/mL Cypher5E (a pH sensitive dye)-tagged 4E6 tau antibody for up to 140 min. Time dependent internalization of 4E6 occurred primarily in the soma, and progressively co-occupied areas where PHF resided in the soma since the beginning of the experiment. Colocalization increased over time, as indicated by the yellow color.

Supplementary Video 2 | Time-lapse live imaging shows 6B2 uptake in pre-treated DC, but it does not colocalize with PHF. DC were pre-incubated with 50 μg/mL tagged PHF-AF488 for 16 h, prepared for live imaging as described above, and subsequently treated with 20 μg/mL Cypher5E (a pH sensitive dye)-tagged 6B2 tau antibody for up to 150 min. Time-dependent internalization of 6B2 occurred primarily in the neurites, while PHF resided in the soma since the beginning of the experiment.

Supplementary Video 3 | Time-lapse live imaging shows IgG1 uptake in pre-treated DC, but it does not colocalize with PHF. DC were pre-incubated with 50 μg/mL tagged PHF-AF488 for 16 h, prepared for live imaging as described above, and subsequently treated with 20 μg/mL Cypher5E (a pH sensitive dye)-tagged IgG1 control antibody for up to 150 min. Time-dependent internalization of IgG1 occurred primarily in the neurites, while PHF resided in the soma since the beginning of the experiment.

REFERENCES

1. Congdon EE, Sigurdsson EM. Tau-targeting therapies for Alzheimer disease. *Nat Rev Neurol*. (2018) 14:399–415. doi: 10.1038/s41582-018-0013-z
2. Bittar A, Bhatt N, Kayed R. Advances and considerations in AD tau-targeted immunotherapy. *Neurobiol Dis*. (2020) 134:104707. doi: 10.1016/j.nbd.2019.104707
3. Colin M, Dujardin S, Schraen-Maschke S, Meno-Tetang G, Duyckaerts C, Courade JP, et al. From the prion-like propagation hypothesis to therapeutic strategies of anti-tau immunotherapy. *Acta Neuropathol*. (2020) 139:3–25. doi: 10.1007/s00401-019-02087-9
4. Sandusky-Beltran LA, Sigurdsson EM. Tau immunotherapies: Lessons learned, current status and future considerations. *Neuropharmacology*. (2020) 175:108104. doi: 10.1016/j.neuropharm.2020.108104
5. Asuni AA, Boutajangout A, Quartermain D, Sigurdsson EM. Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. *J Neurosci*. (2007) 27:9115–29. doi: 10.1523/JNEUROSCI.2361-07.2007
6. Boutajangout A, Ingadottir J, Davies P, Sigurdsson EM. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *J Neurochem*. (2011) 118:658–67. doi: 10.1111/j.1471-4159.2011.07337.x
7. Gu J, Congdon EE, Sigurdsson EM. Two novel Tau antibodies targeting the 396/404 region are primarily taken up by neurons and reduce Tau protein pathology. *J Biol Chem*. (2013) 288:33081–95. doi: 10.1074/jbc.M113.494922
8. Funk KE, Mirbaha H, Jiang H, Holtzman DM, Diamond MI. Distinct therapeutic mechanisms of Tau antibodies: promoting microglial clearance vs. blocking neuronal uptake. *J Biol Chem*. (2015) 290:21652–62. doi: 10.1074/jbc.M115.657924
9. Luo W, Liu W, Hu X, Hanna M, Caravaca A, Paul SM. Microglial internalization and degradation of pathological tau is enhanced by an anti-tau monoclonal antibody. *Sci Rep*. (2015) 5:11161. doi: 10.1038/srep11161
10. Andersson CR, Falsig J, Stavenhagen JB, Christensen S, Kartberg F, Rosenqvist N, et al. Antibody-mediated clearance of tau in primary mouse microglial cultures requires Fcγ-receptor binding and functional lysosomes. *Sci Rep*. (2019) 9:4658. doi: 10.1038/s41598-019-41105-4
11. Holmes BB, Diamond MI. Prion-like properties of Tau protein: the importance of extracellular Tau as a therapeutic target. *J Biol Chem*. (2014) 289:19855–61. doi: 10.1074/jbc.R114.549295
12. Congdon E, Lin Y, Rajamohamedsait H, Shamir D, Rajamohamedsait W, Rasool S, et al. Affinity of tau antibodies for soluble pathological tau species but not their immunogen or insoluble tau aggregates predicts *in vivo* and *ex vivo* efficacy. *Mol Neurodegener*. (2016) 30:62. doi: 10.1186/s13024-016-0126-z
13. Krishnamurthy PK, Deng Y, Sigurdsson EM. Mechanistic studies of antibody-mediated clearance of tau aggregates using an *ex vivo* brain slice model. *Front Psychiatry*. (2011) 2:59. doi: 10.3389/fpsyt.2011.00059
14. Congdon EE, Gu J, Sait HB, Sigurdsson EM. Antibody uptake into neurons occurs primarily via clathrin-dependent Fcgamma receptor endocytosis and is a prerequisite for acute tau protein clearance. *J Biol Chem*. (2013) 288:35452–65. doi: 10.1074/jbc.M113.491001
15. Collin L, Bohrmann B, Gopfert U, Oroszlan-Szovik K, Ozmen L, Gruninger F. Neuronal uptake of tau/pS422 antibody and reduced progression of tau pathology in a mouse model of Alzheimer's disease. *Brain*. (2014) 137(Pt 10):2834–46. doi: 10.1093/brain/awu213
16. Krishnaswamy S, Lin Y, Rajamohamedsait WJ, Rajamohamedsait HB, Krishnamurthy P, Sigurdsson EM. Antibody-derived *in vivo* imaging of tau pathology. *J Neurosci*. (2014) 34:16835–50. doi: 10.1523/JNEUROSCI.2755-14.2014
17. Kondo A, Shahpasand K, Mannix R, Qiu J, Moncaster J, Chen C-H, et al. Antibody against early driver of neurodegeneration cis P-tau blocks brain injury and tauopathy. *Nature*. (2015) 523:431–6. doi: 10.1038/nature14658
18. Shamir DB, Deng Y, Sigurdsson EM. Dynamics of intracellular interaction of a tau antibody and human pathological tau in a human neuron-like model [Abstract]. In: *Society for Neuroscience Annual Meeting Abstract Number 199.10*. San Diego, CA (2016).
19. Shamir DB, Rosenqvist N, Rasool S, Pedersen JT, Sigurdsson EM. Internalization of tau antibody and pathological tau protein detected with a flow cytometry multiplexing approach. *Alzheimer's Dementia*. (2016) 12:1098–107. doi: 10.1016/j.jalz.2016.01.013
20. McEwan WA, Falcon B, Vaysburd M, Clift D, Oblak AL, Ghetti B, et al. Cytosolic Fc receptor TRIM21 inhibits seeded tau aggregation. *Proc Natl Acad Sci USA*. (2017) 114:574–9. doi: 10.1073/pnas.1607215114
21. Nisbet RM, Van der Jeugd A, Leinenga G, Evans HT, Janowicz PW, Götz J. Combined effects of scanning ultrasound and a tau-specific single chain antibody in a tau transgenic mouse model. *Brain*. (2017) 140:1220–30. doi: 10.1093/brain/awx052
22. Wu Q, Lin Y, Gu J, Sigurdsson EM. Dynamic assessment of tau immunotherapies in the brains of live animals by two-photon imaging. *EBioMedicine*. (2018) 35:270–8. doi: 10.1016/j.ebiom.2018.08.041
23. Congdon EE, Chukwu JE, Shamir DB, Deng J, Ujla D, Sait HBR, et al. Tau antibody chimerization alters its charge and binding, thereby reducing its cellular uptake and efficacy. *EBioMedicine*. (2019) 42:157–73. doi: 10.1016/j.ebiom.2019.03.033
24. Krishnaswamy S, Huang HW, Marchal IS, Ryoo HD, Sigurdsson EM. Neuronally expressed anti-tau scFv prevents tauopathy-induced phenotypes in Drosophila models. *Neurobiol Dis*. (2020) 137:104770. doi: 10.1016/j.nbd.2020.104770
25. Lee VMY, Wang J, Trojanowski JQ. [6] Purification of paired helical filament tau and normal tau from human brain tissue. In: Wetzel R, editor. *Methods in Enzymology*. Cambridge, MA: Academic Press (1999). p. 81–9.
26. Rostagno, A., and Ghiso, J. (2009). Isolation and biochemical characterization of amyloid plaques and paired helical filaments. *Curr Protoc Cell Biol*. Chapter 3, Unit 3.33 3.33.1-33. doi: 10.1002/0471143030.cb0333s44
27. Chukwu JE, Congdon EE, Sigurdsson EM, Kong XP. Structural characterization of monoclonal antibodies targeting C-terminal Ser(404) region of phosphorylated tau protein. *MAbs*. (2019) 11:477–88. doi: 10.1080/19420862.2019.1574530
28. Encinas M, Iglesias M, Liu Y, Wang H, Muhaisen A, Ceña V, et al. Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor-dependent, human neuron-like cells. *J Neurochem*. (2000) 75:991–1003. doi: 10.1046/j.1471-4159.2000.0750991.x
29. Jamsa A, Hasslund K, Cowburn RF, Backstrom A, Vasange M. The retinoic acid and brain-derived neurotrophic factor differentiated SH-SY5Y cell line as a model for Alzheimer's disease-like tau phosphorylation. *Biochem Biophys Res Commun*. (2004) 319:993–1000. doi: 10.1016/j.bbrc.2004.05.075
30. Agholme L, Lindstrom T, Kagedal K, Marcusson J, Hallbeck M. An *in vitro* model for neuroscience: differentiation of SH-SY5Y cells into cells with morphological and biochemical characteristics of mature neurons. *J Alzheimers Dis*. (2010) 20:1069–82. doi: 10.3233/JAD-2010-091363
31. Shamir DB, Deng Y, Sigurdsson EM. Live imaging of pathological tau protein and tau antibodies in a neuron-like cellular model. *Methods Mol Biol*. (2018) 1779:371–9. doi: 10.1007/978-1-4939-7816-8_22
32. Li Q, Lau A, Morris TJ, Guo L, Fordyce CB, Stanley EF. A syntaxin 1, Galpha(o), and N-type calcium channel complex at a presynaptic nerve terminal: analysis by quantitative immunocolocalization. *J Neurosci*. (2004) 24:4070–81. doi: 10.1523/JNEUROSCI.0346-04.2004
33. Adler J, Parmryd I. Quantifying colocalization by correlation: the Pearson correlation coefficient is superior to the Mander's overlap coefficient. *Cytometry A*. (2010) 77:733–42. doi: 10.1002/cyto.a.20896
34. Krishnaswamy S, Wu Q, Lin Y, Rajamohamedsait WJ, Rajamohamedsait HB, Sigurdsson EM. *In vivo* imaging of tauopathy in mice. *Methods Mol Biol*. (2018) 1779:513–26. doi: 10.1007/978-1-4939-7816-8_32
35. Karuppagounder SS, Shi Q, Xu H, Gibson GE. Changes in inflammatory processes associated with selective vulnerability following mild impairment of oxidative metabolism. *Neurobiol Dis*. (2007) 26:353–62. doi: 10.1016/j.nbd.2007.01.011
36. Modak SR, Solesio M, Krishnaswamy S, Congdon EE, Sigurdsson EM. Antibodies targeting truncated tau protein reduce tau pathology in primary neuronal and mixed cortical cultures. In: *Society for Neuroscience Annual Meeting #579.14*. Chicago, IL (2015).
37. Shamir DB, Sigurdsson E. Tau antibodies reduce tau levels in differentiated but not in non-differentiated human SH-SY5Y cells. In: *Society for Neuroscience Abstract 579.15*. Chicago, IL (2015).

38. Lesuisse C, Martin LJ. Long-term culture of mouse cortical neurons as a model for neuronal development, aging, and death. *J Neurobiol*. (2002) 51:9–23. doi: 10.1002/neu.10037
39. Park S, Jung Y. Combined actions of Na/K-ATPase, NCX1 and glutamate dependent NMDA receptors in ischemic rat brain penumbra. *Anat Cell Biol*. (2010) 43:201–10. doi: 10.5115/acb.2010.43.3.201
40. McKiernan RC, Jimenez-Mateos EM, Bray I, Engel T, Brennan GP, Sano T, et al. Reduced mature microRNA levels in association with dicer loss in human temporal lobe epilepsy with hippocampal sclerosis. *PLoS ONE*. (2012) 7:e35921. doi: 10.1371/journal.pone.0035921
41. Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, et al. Reduced glucocerebrosidase is associated with increased alpha-synuclein in sporadic Parkinson's disease. *Brain*. (2014) 137(Pt 3):834–48. doi: 10.1093/brain/awt367
42. Ryu HW, Park CW, Ryu KY. Disruption of polyubiquitin gene Ubb causes dysregulation of neural stem cell differentiation with premature gliogenesis. *Sci Rep*. (2014) 4:7026. doi: 10.1038/srep07026
43. Bruhns P, Jönsson F. Mouse and human FcR effector functions. *Immunol Rev*. (2015) 268:25–51. doi: 10.1111/imr.12350
44. Gustafsson G, Eriksson F, Möller C, da Fonseca TL, Outeiro TF, Lannfelt L, et al. Cellular uptake of α-synuclein oligomer-selective antibodies is enhanced by the extracellular presence of α-synuclein and mediated via Fcγ receptors. *Cell Mol Neurobiol*. (2017) 37:121–31. doi: 10.1007/s10571-016-0352-5
45. Proctor DT, Coulson EJ, Dodd PR. Reduction in post-synaptic scaffolding PSD-95 and SAP-102 protein levels in the Alzheimer inferior temporal cortex is correlated with disease pathology. *J Alzheimers Dis*. (2010) 21:795–811. doi: 10.3233/JAD-2010-100090
46. Shao CY, Mirra SS, Sait HB, Sacktor TC, Sigurdsson EM. Postsynaptic degeneration as revealed by PSD-95 reduction occurs after advanced Abeta and tau pathology in transgenic mouse models of Alzheimer's disease. *Acta Neuropathol*. (2011) 122:285–92. doi: 10.1007/s00401-011-0843-x
47. Yuki D, Sugiura Y, Zaima N, Akatsu H, Takei S, Yao I, et al. DHA-PC and PSD-95 decrease after loss of synaptophysin and before neuronal loss in patients with Alzheimer's disease. *Sci Rep*. (2014) 4:7130. doi: 10.1038/srep07130
48. Ji Y, Pang PT, Feng L, Lu B. Cyclic AMP controls BDNF-induced TrkB phosphorylation and dendritic spine formation in mature hippocampal neurons. *Nat Neurosci*. (2005) 8:164–72. doi: 10.1038/nn1381
49. Nagappan G, Lu B. Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. *Trends Neurosci*. (2005) 28:464–71. doi: 10.1016/j.tins.2005.07.003
50. Yoshii A, Constantine-Paton M. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Dev Neurobiol*. (2010) 70:304–22. doi: 10.1002/dneu.20765
51. Cao C, Rioult-Pedotti MS, Migani P, Yu CJ, Tiwari R, Parang K, et al. Impairment of TrkB-PSD-95 signaling in Angelman syndrome. *PLoS Biol*. (2013) 11:e1001478. doi: 10.1371/annotation/f32bc670-c9cf-4bb0-9376-cd8cfd1053c1
52. Yoshii A, Constantine-Paton M. BDNF induces transport of PSD-95 to dendrites through PI3K-AKT signaling after NMDA receptor activation. *Nat Neurosci*. (2007) 10:702–11. doi: 10.1038/nn1903
53. Yoshii A, Murata Y, Kim J, Zhang C, Shokat KM, Constantine-Paton M. TrkB and protein kinase Mzeta regulate synaptic localization of PSD-95 in developing cortex. *J Neurosci*. (2011) 31:11894–904. doi: 10.1523/JNEUROSCI.2190-11.2011
54. Laske C, Stransky E, Leyhe T, Eschweiler GW, Maetzler W, Wittorf A, et al. BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. *J Psychiatr Res*. (2007) 41:387–94. doi: 10.1016/j.jpsychires.2006.01.014
55. Santa-Maria I, Varghese M, Ksiezak-Reding H, Dzhun A, Wang J, Pasinetti GM. Paired helical filaments from Alzheimer disease brain induce intracellular accumulation of Tau protein in aggresomes. *J Biol Chem*. (2012) 287:20522–33. doi: 10.1074/jbc.M111.323279
56. Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, et al. Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem*. (2013) 288:1856–70. doi: 10.1074/jbc.M112.394528
57. Michel CH, Kumar S, Pinotsi D, Tunnacliffe A, St George-Hyslop P, Mandelkow E, et al. Extracellular monomeric tau protein is sufficient to initiate the spread of tau protein pathology. *J Biol Chem*. (2014) 289:956–67. doi: 10.1074/jbc.M113.515445

Conflict of Interest: ES is an inventor on patents on tau immunotherapy and related diagnostics that are assigned to New York University. Some of this technology is licensed to and is being co-developed with H. Lundbeck A/S.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

GENERAL COMMENTARY
published: 23 December 2020
doi: 10.3389/fneur.2020.557509

Commentary: LRP1 Is a Master Regulator of Tau Uptake and Spread

Conor Fearon [1,2] *and Tim Lynch* [1,3]*

[1] Centre for Brain Health, Dublin Neurological Institute at the Mater Misericordiae University Hospital, Dublin, Ireland, [2] Department of Neuropathology, Beaumont Hospital, Dublin, Ireland, [3] Health Affairs, University College Dublin, Dublin, Ireland

Keywords: tau propagation, LRP1, tauopathies, Alzheimer's disease, amyloid (A) 42, corticobasal degeneration (CBD), progressive supranuclear palsy, frontotemporal lobar degeneration

A Commentary on

LRP1 Is a Master Regulator of Tau Uptake and Spread
by Rauch, J. N., Luna, G., Guzmann, E., Audouard, M., Challis, C., Sibih, Y. E., et al. (2020). Nature 580, 381–385. doi: 10.1038/s41586-020-2156-5

Although present as a physiological intracellular protein, pathological aggregation of microtubule-associated tau plays a central role in the pathophysiology of neurodegenerative disease including progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), frontotemporal dementia (FTD), chronic traumatic encephalopathy (CTE), and Alzheimer's disease (AD)—the tauopathies (1). Remarkably, autopsy of patients with tauopathies shows spared gyri often adjacent to devastated gyri (2). Moreover, the topography of neurodegeneration often dictates the clinical syndrome. Why are some brain regions devastated and others spared in the tauopathies? Perhaps abnormal protein spreads within certain pathways but spares others. Like other proteinopathies, animal, and cell models demonstrate spreading of the pathogenic protein from cell to cell in a prion-like fashion. The mechanism of interneuronal spread of tau is poorly understood.

In their recent study, Rauch and colleagues showed that neuronal surface low-density-lipoprotein-receptor-related protein-1 (LRP1), mediates internalization of both physiological tau and pathogenic tau oligomers, and hence, their subsequent neuronal spread (3). The investigators demonstrate that *LRP1* loss reduces internalization of tau in cell culture. This interaction occurs via lysine residues within the tau microtubule-binding region (MTBR). Chemical blockade of these residues prevents tau endocytosis. The authors then used a murine model of tau spread that discriminates between transduced neurons expressing human tau (hTau) and neurons that receive hTau through spread. They demonstrated that downregulation of *LRP1* and subsequent expression of mutant tau limits the spread of tau compared to *LRP1*-wild-type mice.

There are no disease-modifying treatments for the tauopathies, although there are currently 92 clinical trials ongoing (4). Several approaches targeting physiological and pathological tau have been tried, including inhibition of tau aggregation, active and passive immunotherapies and tau silencing with antisense oligonucleotides (5). Many target either monomeric, aggregated, phosphorylated, or conformationally altered forms of tau (5). The discovery that LRP1 is a gatekeeper for transmission of both physiological *and* pathological tau is highly relevant for these trials. There are, however, some caveats: whether pathogenic tau spread is associated with subsequent neurodegeneration was not assessed; effects of fibril conformation, selective vulnerability of specific brain regions, tau mutations and background genomics, other pathogenic proteins, immune processes, and glial pathology may all complicate the neurodegenerative picture in humans compared with the otherwise healthy mouse brains used by Rauch et al. (3).

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Michael L. Shelanski, Columbia University, United States

***Correspondence:**
Tim Lynch
tlynch@dni.ie

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *30 April 2020*
Accepted: *04 December 2020*
Published: *23 December 2020*

Citation:
Fearon C and Lynch T (2020) Commentary: LRP1 Is a Master Regulator of Tau Uptake and Spread. Front. Neurol. 11:557509. doi: 10.3389/fneur.2020.557509

Furthermore, it remains unclear why neurodegeneration occurs in tauopathies associated with an abnormal ratio of 3-repeat (3R) to 4-repeat (4R) tau (either an excess of 3R or an excess of 4R tau). Since the LRP1-interacting tau residues are located within the MTBR, the ratio 3R to 4R tau *in vivo* may be highly relevant to LRP1's pathophysiological role. Although there were no differences in tau uptake between the six main tau isoforms, much of Rauch and colleagues' ligand-binding data refers to 4R tau (3). There is growing evidence, however, of a much wider range of specific tau conformational structures among the tauopathies (6). If LRP1 binds and transmits specific tau conformations differently, these findings may have greater relevance for specific tauopathies where post-translation modifications or other factors impact conformation.

Interestingly, *astrocytic* LRP1 clears $A\beta_{42}$ amyloid across the blood brain barrier (BBB), reducing its cortical deposition (7). Whether LRP1 also clears tau across the BBB was not studied. Although tau deposition probably occurs later than $A\beta_{42}$ amyloid in AD, its distribution correlates more closely with the clinical syndrome. Conversely, $A\beta_{42}$ amyloid deposition is not a prominent feature in the primary tauopathies (such as PSP, CBD and FTD). Rauch and colleagues study was limited to LRP1-mediated *neuronal* spread of tau. Given that many tauopathies have significant glial pathology, the effect of LRP1 on BBB clearance and transmission of tau in other cells types must be investigated.

However, an amyloid precursor protein (APP) mouse model demonstrated that LRP1 enhances production of $A\beta_{42}$ amyloid via APP processing and that this effect outweighs BBB clearance of $A\beta_{42}$ amyloid (8). This dual role of LRP1 in AD pathophysiology is therefore complex and can be modulated by other proteins. The presence of $A\beta_{42}$ amyloid increases ectodomain shedding of the low density lipoprotein receptor and LRP1 in human brain endothelial cells, reducing $A\beta_{42}$ amyloid clearance across the BBB (9). The effect of $A\beta_{42}$ amyloid on LRP1-related APP processing or LRP1-related tau transmission has not been studied, but tau transmission may be similarly reduced by LRP1 shedding. Apolipoprotein E (APOE) also binds LRP1 and Rauch and colleagues demonstrated that tau competes with APOE isoforms for LRP1 binding (3). When $A\beta_{42}$ amyloid is present with different APOE isoforms (APOE ε2, APOE ε3, and APOE ε4), LRP1 shedding is isoform-dependent with greater LRP1 shedding occurring in the presence of APOE ε4 and lesser shedding occurring in the presence of APOE ε2 (9). The possession of the APO ε4 allele is the strongest genetic risk factor for late-onset AD (10). However, the APOE ε2 allele is associated with increased tau pathology in mouse models and in brains of patients with PSP and CBD (11). Thus, APOE ε4 may increase $A\beta_{42}$ amyloid cortical accumulation (subsequent tau spread may be through alternative mechanisms) while APOE ε2 may facilitate clearance of $A\beta_{42}$ amyloid and spreading of tau in the primary tauopathies. The effect of APOE on LRP-1-related APP processing has not been studied. Although APOE competes with tau for LRP1, Rauch and colleagues did not show a difference in neuronal tau transmission between APOE isoforms *in vitro*. The effects of APOE isoforms on tau transmission in astrocytic or endothelial cells and *in vivo*, however, may be a more appropriate comparison. Multiple factors including APOE isoforms, tau conformational strains and the epichaperone system might therefore modulate tau transmission via LRP1, partially explaining the diverse neuropathologies and clinical syndromes found in different tauopathies. The distribution of LRP1 in vulnerable brain regions in specific tauopathies would be of great interest in this respect.

Rauch and colleagues provide new insight into tau physiology and potential therapeutic targets for tauopathies: LRP1; the associated lysine residues within the MTBR; and the yet unstudied processes downstream of this LRP1-tau interaction. Given the complex modulating effect of other proteins on LRP1 transmission, a more direct target may be the MTBR lysine residues. Indeed, antibodies directed against the MTBR (E2814 and DC8E8) show promise in preclinical studies by reducing tau internalization (4). However, *in vitro* and animal data do not always translate to human studies. With numerous failed clinical trials, no community understands this greater than the neurodegenerative fraternity. Success of any future treatment will not rely solely on reduced tau transmission, but on reduced neurodegeneration and motor/cognitive impairment in these relentlessly progressive conditions.

AUTHOR CONTRIBUTIONS

CF: manuscript conception, organization, and writing of first draft on manuscript. TL: manuscript conception and review of manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Höglinger GU, Respondek G, Kovacs GG. New classification of tauopathies. *Rev Neurol.* (2018) 174:664–8. doi: 10.1016/j.neurol.2018.07.001
2. Sima AA, Defendini R, Keohane C, D'Amato C, Foster NL, Parchi P, et al. The neuropathology of chromosome 17-linked dementia. *Ann Neurol.* (1996) 39:734–43. doi: 10.1002/ana.410390609
3. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, et al. LRP1 is a master regulator of tau uptake and spread. *Nature.* (2020) 580:381–385. doi: 10.1038/s41586-020-2156-5
4. US National Library of Medicine (2020). Available online at: Clinicaltrials.gov.
5. Jadhav S, Avila J, Schöll M, Kovacs GG, Kövari E, Skrabana R, et al. A walk through tau therapeutic strategies. *Acta Neuropathol Commun.* (2019) 7:22. doi: 10.1186/s40478-019-0664-z
6. Rösler TW, Tayaranian Marvian A, Brendel M, Nykänen NP, Höllerhage M, Schwarz SC, et al. Four-repeat tauopathies. *Prog Neurobiol.* (2019) 180:101644. doi: 10.1016/j.pneurobio.2019.101644
7. Liu CC, Hu J, Zhao N, Wang J, Wang N, Cirrito JR, et al. Astrocytic LRP1 mediates brain Aβ clearance and impacts amyloid deposition. *J Neurosci.* (2017) 37:4023–31. doi: 10.1523/JNEUROSCI.3442-16.2017
8. Van Gool B, Storck SE, Reekmans SM, Lechat B, Gordts PLSM, Pradier L, et al. LRP1 has a predominant role in production over clearance of Aβ

in a mouse model of Alzheimer's disease. *Mol Neurobiol.* (2019) 56:7234–45. doi: 10.1007/s12035-019-1594-2
9. Bachmeier C, Shackleton B, Ojo J, Paris D, Mullan M, Crawford F. Apolipoprotein E isoform-specific effects on lipoprotein receptor processing. *NeuroMolecular Med.* (2014) 16:686–96. doi: 10.1007/s12017-014-8318-6
10. Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. *Neuron.* (2009) 63:287–303. doi: 10.1016/j.neuron.2009.06.026
11. Zhao N, Liu CC, Van Ingelgom AJ, Linares C, Kurti A, Knight JA, et al. APOE ε2 is associated with increased tau pathology in primary tauopathy. *Nat Commun.* (2018) 9:4388. doi: 10.1038/s41467-018-06783-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 07 January 2021
doi: 10.3389/fneur.2020.595532

Tau Post-translational Modifications: Dynamic Transformers of Tau Function, Degradation, and Aggregation

*Carolina Alquezar†, Shruti Arya† and Aimee W. Kao**

Department of Neurology, Memory and Aging Center, University of California, San Francisco, San Francisco, CA, United States

Post-translational modifications (PTMs) on tau have long been recognized as affecting protein function and contributing to neurodegeneration. The explosion of information on potential and observed PTMs on tau provides an opportunity to better understand these modifications in the context of tau homeostasis, which becomes perturbed with aging and disease. Prevailing views regard tau as a protein that undergoes abnormal phosphorylation prior to its accumulation into the toxic aggregates implicated in Alzheimer's disease (AD) and other tauopathies. However, the phosphorylation of tau may, in fact, represent part of the normal but interrupted function and catabolism of the protein. In addition to phosphorylation, tau undergoes another forms of post-translational modification including (but not limited to), acetylation, ubiquitination, glycation, glycosylation, SUMOylation, methylation, oxidation, and nitration. A holistic appreciation of how these PTMs regulate tau during health and are potentially hijacked in disease remains elusive. Recent studies have reinforced the idea that PTMs play a critical role in tau localization, protein-protein interactions, maintenance of levels, and modifying aggregate structure. These studies also provide tantalizing clues into the possibility that neurons actively choose how tau is post-translationally modified, in potentially competitive and combinatorial ways, to achieve broad, cellular programs commensurate with the distinctive environmental conditions found during development, aging, stress, and disease. Here, we review tau PTMs and describe what is currently known about their functional impacts. In addition, we classify these PTMs from the perspectives of protein localization, electrostatics, and stability, which all contribute to normal tau function and homeostasis. Finally, we assess the potential impact of tau PTMs on tau solubility and aggregation. Tau occupies an undoubtedly important position in the biology of neurodegenerative diseases. This review aims to provide an integrated perspective of how post-translational modifications actively, purposefully, and dynamically remodel tau function, clearance, and aggregation. In doing so, we hope to enable a more comprehensive understanding of tau PTMs that will positively impact future studies.

Keywords: phosphorylation, acetylation, ubiquitination, methylation, sumoylation, glycosylation, glycation, proteolysis

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Chaur-Jong Hu, Taipei Medical University, Taiwan
Isabelle Landrieu, INSERM U1167 Facteurs de risque et déterminants moléculaires des maladies liées au vieillissement, France

***Correspondence:**
Aimee W. Kao
aimee.kao@ucsf.edu

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *16 August 2020*
Accepted: *07 December 2020*
Published: *07 January 2021*

Citation:
Alquezar C, Arya S and Kao AW (2021) Tau Post-translational Modifications: Dynamic Transformers of Tau Function, Degradation, and Aggregation. Front. Neurol. 11:595532. doi: 10.3389/fneur.2020.595532

INTRODUCTION

Post-translational modifications (PTMs) refer to the modifications that occur in a protein either shortly after its translation by ribosomes or after its folding and localization are complete (1). These modifications are usually catalyzed by enzymes and involve the addition of chemical groups, sugars, or proteins to specific residues of the targeted protein. Nearly all cellular processes can be regulated via PTMs. Therefore, PTMs provide a means for the natural increase in the proteome complexity and allow one gene to encode a variety of distinct protein molecules with distinct functional roles, known as proteoforms (2). PTMs alter the charge and hydrophobicity (electrostatics) of a protein, which in turn induces structural changes that influence protein function, protein-protein interactions, and protein aggregation (3, 4). In this way, PTMs also can affect the clearance of proteins by regulating the functionality of degrons, peptide sequences that target a protein for degradation (5). Essentially all proteins in eukaryotes, including the "intrinsically disordered" or "natively unfolded" proteins that tend to aggregate in neurodegenerative diseases, are susceptible to PTMs (3, 6, 7). Unlike folded proteins, natively unfolded proteins do not have a well-defined three-dimensional structure and exist as an ensemble of dynamically fluctuating conformations. The lack of tertiary structure makes natively unfolded proteins more vulnerable to PTMs (8).

Tau is a classic example of a natively unfolded protein (7, 9) that can be modified by a myriad of PTMs. Tau is a microtubule-binding protein found in neurons and glial cells, and is primarily involved in the stabilization of the cytoskeleton (10). Based on its interaction with microtubules and on the basis of its amino acid composition, the primary structure of tau can be divided into an N-terminal projection domain, a proline-rich region, a repeat region, and a C-terminal domain (11) (**Figure 1**). Tau has six different isoforms in human brain that differ from each other in the number of N-terminal inserts and either contain or lack one repeat (R2) of the four repeats (R1-R4) present in the microtubule-binding domain region. Because of its ability to bind to microtubules, the main function of tau is thought to be the modulation of microtubule dynamics, which consequently affects neurite outgrowth, axonal transport, and synapsis (12–14). Recent studies have shown that tau also interacts with several cell membranes, including the plasma membrane, the endoplasmic reticulum, and Golgi (15–17). Tau may also regulate intracellular signaling cascade via interactions with proteins such as 14.3.3, Pin-1, and Fyn (18), suggesting a role for tau in cell signaling (18). Although tau is primarily an axonal protein, it can be found within neuronal nuclei where it binds to either DNA or RNA and regulates gene expression (19–21). Under certain pathological circumstances, tau loses its function as a microtubule-binding protein and accumulates in the cytosol of affected cells, forming first "pre-tangles" and then insoluble cytosolic inclusions or aggregates composed of fibrillar forms of tau known as neurofibrillary tangles (NFTs).

Diseases associated with the presence of tau inclusions in the brain are collectively referred to as tauopathies and include a diverse group of neurodegenerative diseases with distinct pathological characteristics (**Figure 1**). Tau-immunopositive intracellular aggregates are the pathognomonic hallmark of tauopathies (22). However, the forces driving tau, especially non-mutant, wild-type tau, into intracellular aggregates are incompletely understood. The cellular protein homeostasis (or proteostasis) network is fundamental to the proper functioning of tau and prevents the accumulation of tau aggregates. In principle, proteostasis encompasses the entire life cycle of a protein such as tau, including synthesis, proper folding, post-translational processing, and degradation. Thus, PTMs are a key contributor to tau proteostasis and thereby could regulate tau function, levels, and aggregation.

The same PTMs that regulate tau function also have the ability to induce alterations in its clearance, conformation, and aggregation potential (23). However, not all tau PTMs are "pathological". Many PTMs have been identified in tau extracted from healthy brains, suggesting a normal role for PTMs in tau function (24). Among all tau PTMs, phosphorylation is the most studied, and traditionally, it was thought that increased phosphorylation was the trigger for tau intracellular aggregation (25). However, a growing body of evidence suggests that other PTMs also considerably regulate tau function and may even precede phosphorylation in the sequence of events leading to tau inclusions formation.

In this review, we first provide a synopsis of the many tau PTMs that impact its function and potentially contribute to dysfunction (section Effects of PTMs on Tau Function and Dysfunction). We then fold this information into an integrated discussion of how PTMs can regulate tau degradation (section Effect of PTMs on Tau Degradation) and aggregation (section Effect of PTMs on Tau Solubility and Aggregation). A rarely discussed but critically important subject, cross-talk, and competition between PTMs, is considered next (section Cross-Talk and Competition Between PTMs). Finally, we summarize strengths and limitations of current approaches to the study of PTMs (section Approaches and Limitations) and provide concluding remarks (section Concluding Remarks).

EFFECTS OF PTMs ON TAU FUNCTION AND DYSFUNCTION

The main role of PTM addition to any protein, including tau, is to regulate and increase functional diversity usually by altering electrostatics and/or structure. Approximately 35 percent of the amino acid residues in tau are susceptible to modification peri- or post-translationally. These residues are serine (S), threonine (T), tyrosine (Y), lysine (K), arginine (R), asparagine (N), histidine (H), and cysteine (C). Like other proteins, tau undergoes PTMs that involve the addition of small chemical groups or peptides on different side chains of tau: phosphorylation on S, T, or Y; acetylation, ubiquitination, SUMOylation, and glycation on K; methylation on K or R; O-GlcNAcylation on S and T, N-glycosylation on nitrogens, nitration on Y and oxidation on carbons (**Figure 2**). Tau can also undergo protonation on histidine residues and proteolytic cleavage (or truncation), although these modifications are often

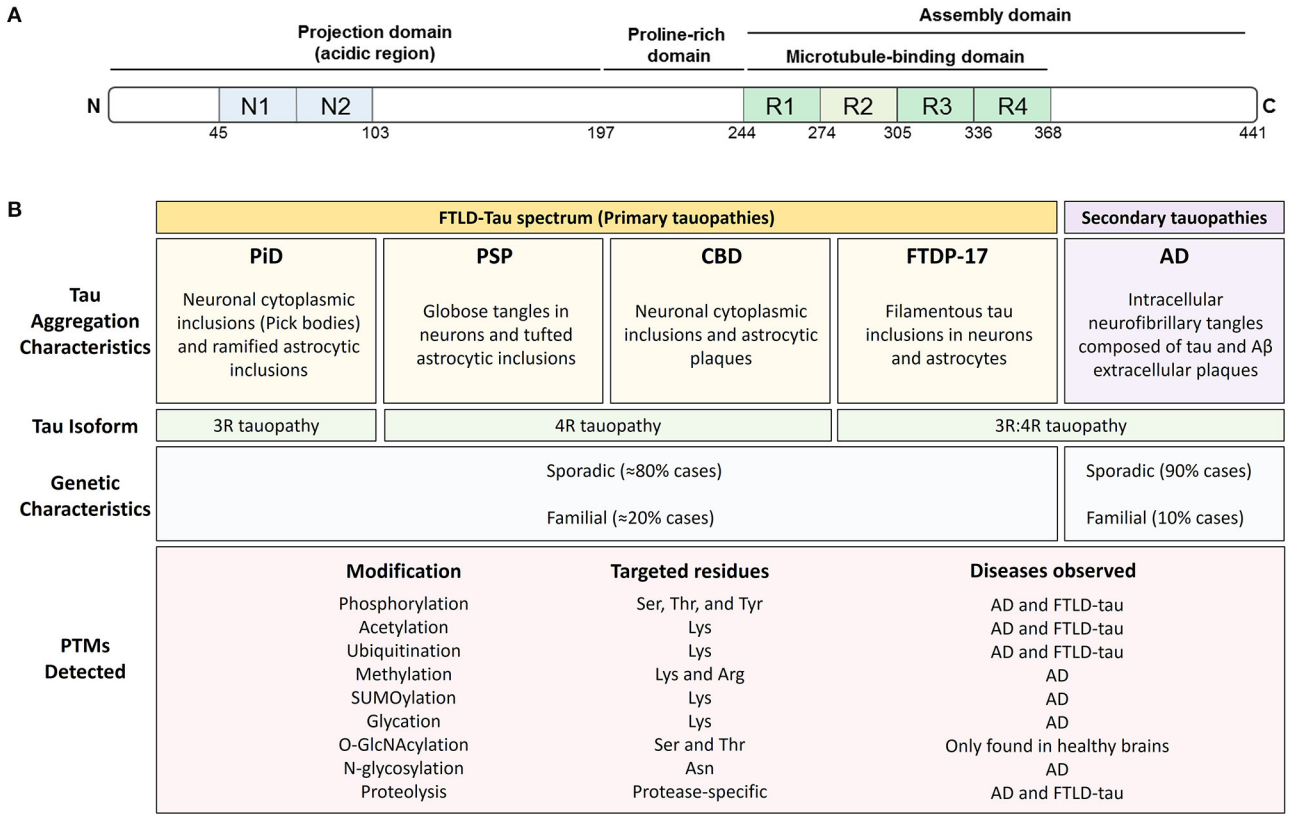


FIGURE 1 | Post-translational modifications are found throughout tau and associate with all tauopathies. **(A)** Schematic representation of the structural features of 2N4R tau protein. Tau exists in 6 isoforms generated by the alternative splicing of exons 2, 3, and 10 of the MAPT gene. The isoforms include 0, 1, or 2 amino-terminal inserts (blue rectangles) and three (3R) or four (4R) microtubule-binding potential repeat domains (green rectangles). **(B)** Pathological and genetic classification of tauopathies as they relate to post-translational modifications (PTMs). Primary tauopathies fall under the umbrella pathological term frontotemporal lobar degeneration with tau inclusions (FTLD-Tau) and include Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and frontotemporal dementia with parkinsonism (FTDP-17). Alzheimer's disease, a secondary or mixed tauopathy, is characterized by the presence of extracellular inclusions containing Amyloid β (Aβ) protein. Tauopathies are also classified depending on tau isoform composing the intracellular inclusions. PiD is a 3R-predominat tauopathy, PSP, and CBD are 4R tauopathies and FTDP-17 and AD are 3R:4R tauopathies. The PTMs observed in tau, the targeted residues and the disease where they have been observed is also shown.

not considered alongside traditional PTMs. The implications of all of these modifications will be discussed in this section.

The clustering of PTMs within short sequence motifs and functional domains suggests that tau function and subcellular localization could be regulated by a complex interplay between different PTMs (**Figure 3**). Since some modifications have been found only in the tau aggregates associated with disease (26, 27), it has been suggested that these modifications may be regulating tau dysfunction. Although a hypothetical sequence of events has been proposed to occur during the formation of tau inclusions (28, 29), what remains elusive is which PTMs actually drive tau self-association vs. those that are simply part of normal tau function and captured or trapped as "bystanders" in aggregates. The transformation from healthy (functional) to aggregated (or pathological) tau is most likely not a consequence of a single PTM, but rather a combination of the intrinsic structural alterations and extrinsic cellular conditions that may ultimately constitute pathogenicity. Below, we describe the PTMs that have been observed in tau and their potential impacts on tau function and dysfunction.

Phosphorylation

Protein phosphorylation is one of the most common PTMs in the proteome (30). Phosphorylation involves the reversible addition of a phosphate (PO_4) group to the polar group of serine, threonine or tyrosine amino acid residues (31) (**Figure 2**). Protein kinases and phosphatases, the enzymes responsible for phosphorylation and dephosphorylation, respectively (32), coordinate the phosphorylation status of proteins. Phosphorylation alters protein electrostatics by introducing a negatively charged, hydrophilic group, resulting in an overall more hydrophilic protein. As a consequence of this increased hydrophilicity, phosphorylation imparts conformational changes, and regulates some important protein functions including protein-protein interactions, signaling cascades, and protein degradation (5, 33). Phosphorylation is involved in essential cellular functions such as metabolism and intracellular signaling, and therefore, is fundamental to several normal cellular processes. However, abnormal phosphorylation has also been described as one of the primary causes for the alteration of a variety

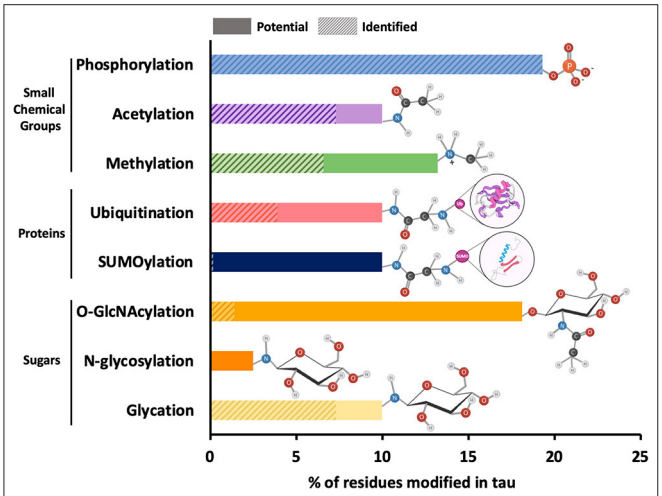


FIGURE 2 | Relative frequency of post-translational modifications on tau. The 2N4R isoform of tau contains 441 amino acid residues, around 35 percent of which can potentially undergo PTMs. These residues include serine (forty-five), threonine (thirty-five), tyrosine (five), asparagine (three), arginine (fourteen), and lysine (forty-four). The most common PTMs in tau are shown on the y-axis of the bar plot. The x-axis of the plot shows the percentage of amino acid residues with solid bars showing the sites that have been identified within total potential sites shown in shaded bars. The functional groups associated with each PTM are also shown next to each bar. Since all potential phosphorylation sites have been identified, the shaded bar is completely masked by the solid bar. Similarly, in case of N-glycosylation, the specific N-glycosylation sites have not been reported and therefore, only potential sites represented by shaded bar have been shown.

of structural, functional, and regulatory proteins under disease conditions.

The longest isoform of tau, known as 2N4R tau, has 85 potential phosphorylation sites (44 S, 35 T, and five Y) (34). Therefore, almost 20 percent of tau has the potential to become phosphorylated (**Figures 2**, **3**). Of these sites, ∼20 residues have been found to undergo phosphorylation in tau extracted from healthy brains (35, 36). In contrast, around 44 residues, some of which overlap with residues found in tau from healthy brains, have been identified as "abnormally" hyperphosphorylated in individuals with a tauopathy (25). Phosphorylation of serine and threonine residues is associated with the completion of degrons (or degradation-signaling motifs) and the subsequent clearance of tau (for a more extensive discussion about phosphodegrons, see section Effect of PTMs on Tau Degradation). If tau degradation is blocked, then these normally transient phosphosites would persist, even though they are a part of the normal metabolism of tau. Thus, the phosphorylation observed in tau extracted from diseased brains could either occur abnormally as part of disease pathogenicity or be a found as a normal event to promote the normal, but interrupted, clearance of tau. Additionally, it is also possible that many other tau phosphorylation sites exist that have not been identified in post-mortem human brain material due to rapid dephosphorylation of tau (37).

As a dynamic and highly regulated process, tau phosphorylation requires a balanced interplay of kinases and phosphatases. Several serine/threonine kinases phosphorylate tau, including glycogen synthase kinase 3 (GSK3β), cyclin dependent kinase 5 (cdk5), casein kinase 1 (CK1), cyclic AMP-dependent protein kinase (PKA), p42/p44 MAPKs (ERKs 1/2), protein kinase C (PKC), calmodulin-dependent protein kinase II (CaMKII), the brain-specific kinases 1 and 2, the tau-tubulin kinases 1 and 2, and some microtubule affinity-regulating kinases (MARKs; also known as PAR1 kinases) (38). Furthermore, tau can be phosphorylated by tyrosine kinases, such as the SRC family members (LCK, SYK, and FYN) and the ABL family members (ARG and ABL1) (39). FYN, SYK, and ABL kinases phosphorylate tau at the Y18, Y197, and Y394 residues (40), which have been associated with tau aggregation and AD pathology (41).

Tau can be dephosphorylated by protein phosphatases (PP) 1, 2A, 2B, 2C, and 5 (25). Among these phosphatases, PP2A accounts for around 70 percent of all tau dephosphorylation in the human brain (42). Interestingly, PP2A also dephosphorylates and modulates the activity of components along the ERK1/2 MAPK cascade that leads to GSK3β activation (43), suggesting potential feedback cycles. As phosphorylation is one of the most studied PTMs in tau, the kinase pathways implicated in tau phosphorylation have been extensively reviewed elsewhere (44). The regulation of the phosphorylation status of tau is essential to maintain the biological function of the protein.

The change in tau electrostatics associated with phosphorylation may have a critical role in the regulation of tau function, localization, and interaction with other molecules. The phosphorylation status of tau is developmentally regulated, implying the significance of phosphorylation in the regulation of specific function of tau in each developmental stage (45, 46). Tau phosphorylation inside the microtubule-binding domain (e.g., S262/S214) and/or the proline-rich (e.g., T231) domain reduces the affinity of tau for negatively charged microtubules (47–50). Binding of tau stabilizes microtubules, therefore tau phosphorylation regulates neuronal functions such as axonogenesis and neurite outgrowth (47, 51–53). Additionally, it has been reported that the specific phosphorylation of tau by FYN kinases induces the relocation of tau from the axon to dendrites (54). However, the effects of tau relocation in neuronal function are not yet well-understood. Additionally, tau interacts with the kinesin-associated protein JIP1 (JUN N-terminal kinase-interacting protein 1) only when phosphorylated, thereby impairing the formation of the kinesin complex that mediates axonal transport (55).

Since phosphorylation regulates the biological functions of tau, inappropriate phosphorylation may be an important contributor to the pathogenesis of tauopathies. The abnormal phosphorylation in several residues inside the KXGS motif (25) and the proline-rich domain (47, 56–58) has been identified in NFTs of tauopathy patients brains. Aberrant tau phosphorylation at these motifs may pose detrimental consequences for neurons, such as the impairment of neurite outgrowth (59) and the deregulation of axonal transport (60, 61), which may lead to synaptic dysfunction and neurodegeneration (62–64). The phosphorylation at residues outside of the microtubules-binding and proline-rich domains does not interfere with the ability of tau

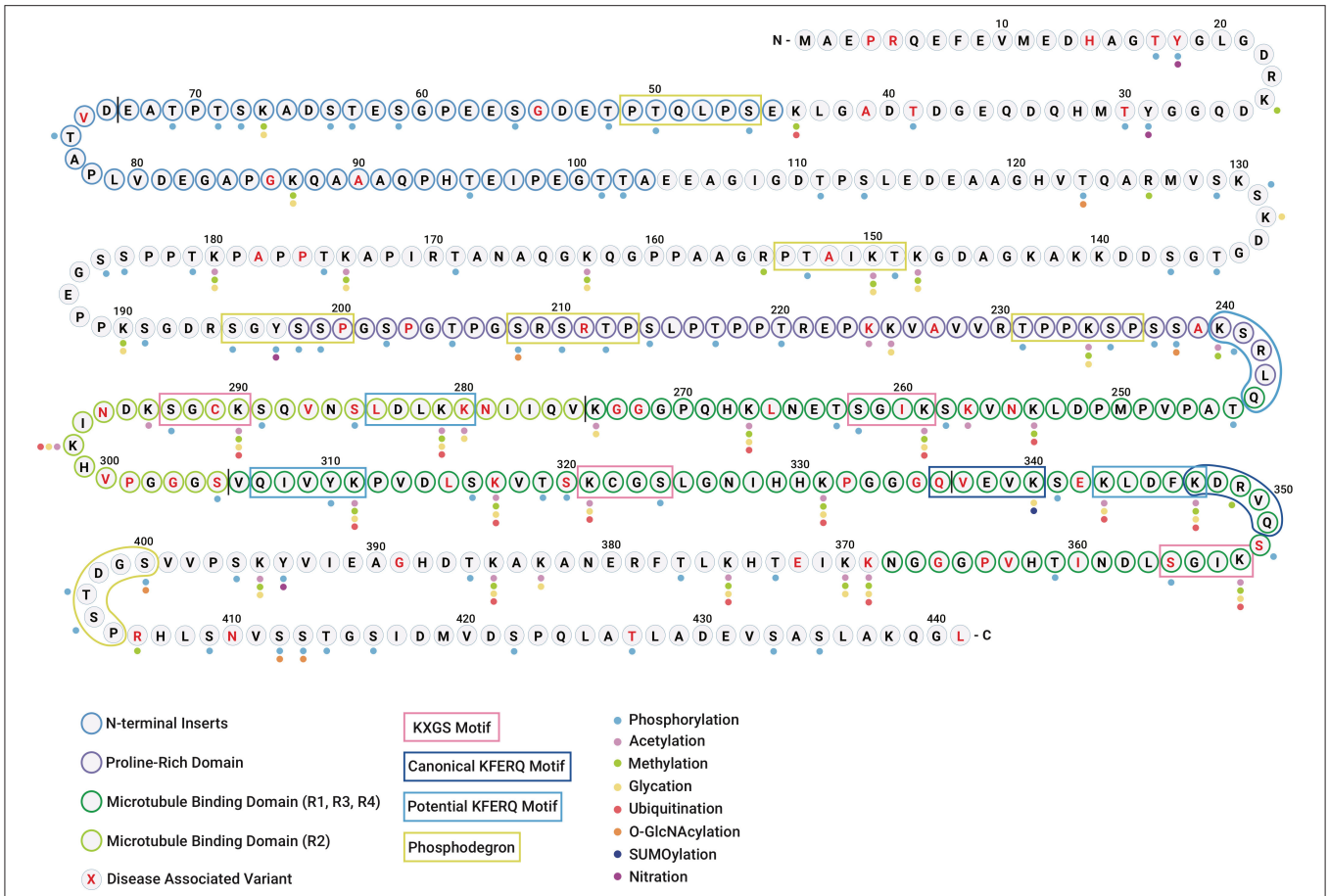


FIGURE 3 | Tau undergoes diverse post-translational modifications that often compete for the same amino acid residues. Diagram showing the polypeptide chain of the longest tau isoform 2N4R tau along with individual amino acid residues highlighting different regions/domains. The N-terminal inserts are represented with blue residue borders, proline-rich domain is represented with purple residue borders, microtubule-binding domains are represented with green residue borders (R1, R3, R4 in dark green, and R2 in light green), and disease-associated amino acid variants are highlighted in red text. The vertical black line between residues is shown for distinction between two N-terminal inserts and four microtubule-binding repeats. The colored dots represent the PTMs identified in tau (WT and mutant) *in vitro* and *in vivo*. The color code for PTMs is as follows: phosphorylation (light blue), acetylation (pink), methylation (green), glycation (yellow), ubiquitination (red), O-GlcNAcylation (orange), SUMOylation (dark blue), and nitration (purple). N-glycosylation is omitted as no specific sites have been identified. The KXGS microtubule-binding motifs are shown in pink boxes. The canonical and potential KFERQ motifs are represented with dark blue and light blue boxes, respectively. The phosphodegron motifs are highlighted with green boxes.

to bind microtubules. Some of these residues such as S396, S404, and S422, have been found abnormally "hyperphosphorylated" in diseased brains (57, 65), and it has been suggested that phosphorylation at these residues may influence tau aggregation (66). While substantial published work suggests that tau phosphorylation may contribute to development of tauopathies, the exact mechanism behind how phosphorylation regulates tau function and aggregation *in vivo* is still unknown. To unravel the role of abnormal tau phosphorylation in tauopathies, it will be necessary to better understand the broader impacts of phosphorylation on normal tau function.

Acetylation

The acetylation of mammalian proteins involves the N-terminal addition of an acetyl group from acetyl coenzyme A (Ac-CoA) to the lysine residue of a polypeptide chain (67) (**Figure 2**). Acetylation was first described on histone proteins, therefore the associated enzymes are known as histone acetyl transferases (HATs) and histone deacetylases (HDACs), although they are less discriminating in their targets than their names would suggest (67). Acetylation is considered an essential modification that plays critical roles in the function of diverse proteins, including p53 and tubulin (68). The addition of an acetyl group neutralizes the positive charge of lysine residues, thereby eliciting its downstream molecular impacts on protein structure and protein-protein recognition. Among its functional roles, acetylation is perhaps best known to regulate gene expression via modification of histone structure and chromatin accessibility (69). However, the acetylation of non-histone proteins regulates a range of cellular processes such as DNA damage repair, cell division, signal transduction, protein folding, autophagy, and metabolism

(70). Incorporation of an acetyl group onto lysine residues prevents other modifications and thus, acetylation also can block the processes regulated by ubiquitination, SUMOylation, methylation, and glycation.

Acetylation has recently arisen to challenge the predominance of phosphorylation as the key PTM on tau. The longest isoform of tau protein (2N4R) contains 44 lysine residues and thus, 10 percent of the protein has the potential to be modified by acetylation (**Figure 3**). Among all HATs implicated in protein acetylation, p300 and its close homolog CREB-binding protein (CBP) appear competent to acetylate tau *in vitro* (71, 72). Moreover, the histone deacetylase 6 (HDAC6) (36, 73) and the NAD^+-dependent sirtuin 1 deacetylase (SIRT1) (72) are the major tau deacetylases. Interestingly, these two HDACs have seemingly opposing effects on tau: while tau deacetylation by SIRT1 has a protective role against tau accumulation, the deacetylation of tau by HDAC6 contributes to the increase of tau phosphorylation and aggregation *in vitro* (74, 75). Tau has also been shown to undergo acetyl-coenzyme A–induced auto-acetylation *in vitro* (76). However, the role of tau as acetyltransferase remains somewhat controversial, given that other groups have not consistently identified tau acetylation in absence of HAT enzymes (26, 76, 77).

Tau acetylation was first recognized in the context of neurodegeneration in mouse models of neurodegenerative disease (72, 78), and subsequently in brain lysates from AD patients at early/moderate Braak stages (79). The dysregulation of the HATs and HDACs involved in tau acetylation was also found in diseased brains (79, 80). However, the underlying abnormalities of HAT and HDAC activity may be both context and disease specific. In this regard, while increased p300/CBP HAT activity was observed in the brains of FTLD-tau patients (80), p300/CBP levels, and activity were found to be lower in the frontal cortex and hippocampus of AD patients (81) and in a mouse model of AD (82). Although these reports failed to show the effect of p300/CBP dysregulation in tau acetylation, they suggest that the role of p300/CBP in tau acetylation may vary between different tauopathies such as FTLD-Tau and AD. Furthermore, the levels and activity of HDACs have also been measured in patients' brains. It has been observed that the expression of SIRT1 is reduced in AD patients (83, 84) while the levels of HDAC6 are increased (73), confirming that these enzymes have opposite effects on tau function and dysfunction. Altogether, this evidence demonstrates that tau acetylation may have a key role in tauopathies.

As with phosphorylation, to fully understand the role of acetylation in tau dysfunction, one must necessarily understand its role in the normal function of tau. While the neutralization of positively charged lysine residues by acetylation appears crucial for regulation of tau, the downstream functional impacts of this regulation seem to be residue specific. For example, acetylation at lysine residue K280 weakens the binding of tau to negatively charged microtubules, potentially destabilizing microtubule networks (26, 27, 85). This contrasts with acetylation at K174, which induces tau aggregation without affecting tau-microtubule binding (79). Finally, HDAC6-regulated tau acetylation at KXGS motif-associated K259, K290, K321, or K353 residues suppresses both tau phosphorylation and aggregation (36). Unlike acetylation of lysine residues that have been identified only in tauopathy brains (26, 79, 86, 87), the acetylation at these four residues occurs in normal tau but is reduced in tauopathy brains (36). Since acetylation has emerged as a key PTM in the regulation of tau function and dysfunction, mapping all the acetylation sites on tau in different biological contexts will help to delineate the ultimate roles and consequences of each modification.

Acetylation on tau is particularly interesting because many lysine residues that are targets for acetylation are also targets for other PTMs, including ubiquitination, SUMOylation, methylation, and glycation (78, 88, 89) (**Figure 3**). Therefore, a potential rivalry between PTMs could exist, where the addition of one chemical group to a given residue blocks the addition of another, suggesting multilayered avenues for the regulation of the biology of the protein (88). While the cross-talk between PTMs will be extensively discussed in section Cross-Talk and Competition Between PTMs, below we review the role of other lysine-associated PTMs in tau function.

Ubiquitination

Covalent modification of proteins with ubiquitin (Ub) (ubiquitination or ubiquitylation) represents one of the most common PTMs in mammalian cells (**Figure 2**). The small, 76 residues, protein ubiquitin is nearly ubiquitously found in eukaryotes (ergo its name) and is used to modify activity, localization, or stability of other proteins (90, 91). Ubiquitination involves a multi-step process carried out sequentially by activating (E1), conjugating (E2), and ligating (E3) enzymes and has been reviewed elsewhere (92). Similar to acetylation, ubiquitination occurs on lysine residues. In case of ubiquitination, the C-terminal carboxyl group of a lysine in the ubiquitin sequence itself is covalently attached to the ε-amino group on a lysine of the "target" protein. Substrates can be modified with a single ubiquitin (monoubiquitination) or with polymeric Ub chains of variable length (polyubiquitination). The ubiquitin protein contains seven lysine residues. Therefore, the nomenclature around ubiquitination is based upon which of these seven lysine residues serves as the initial link to the target protein (i.e., ubiquitinated chains are called K6, K11, K27, K29, K33, K48, or K63) (93). The type of ubiquitin chain determines the biological effects of these modifications. Chains linked through K6, K27, and K33 are often involved in cell proliferation, DNA damage repair, and innate immunity (91). Other linkages are directly related with protein degradation. In this regard, K48 chains generally target substrates for degradation by the 26S proteasome (94), whereas K63-polyubiquitinated proteins are directed to the degradation by the autophagy-lysosomes pathway (93–96).

As a protein rich in lysine residues, tau has a high susceptibility toward ubiquitination. Out of a total of 44 lysine residues, 17 residues of the 2N4R tau isoform have been found ubiquitinated with most of them located in the tau microtubule-binding domain (78, 97). The main role of tau ubiquitination appears to be the regulation of tau clearance by the proteasomal or lysosome-autophagy systems (98). E3

ligases confer ubiquitin residues to target proteins (99). Among ∼600 E3 ligases encoded by the human genome, only three appear competent to ubiquitinate tau: the C-terminus of the Hsc70-interacting protein (CHIP), the TNF receptor-associated factor 6 (TRAF6), and axotrophin/MARCH7 (100–102). Each of these E3 ligases ubiquitinates tau through different linkages and at different residues, suggesting that each ligase modulates tau degradation by different mechanisms. CHIP ubiquitinates tau through K48 or K63 linkages and thus, regulates tau degradation via both proteasomal and autophagy systems (102–104). On the other hand, the E3 ligase TRAF6 ubiquitinates tau via K63 linkages (100), suggesting that ubiquitination mediated by this enzyme may regulate the degradation of tau in the autophagy-lysosomes pathway only (100, 105–107). Additionally, *in vitro* experiments have shown that tau can be monoubiquitinated by axotrophin/MARCH7 (101). However, the effect of monoubiqutination by axotrophin/MARCH7 on tau function and degradation is not yet well-understood.

While E3 ligases attach ubiquitins, deubiquitinases (dUbs) remove them. The cysteine protease Otub1 is the only dUb that has been shown to target tau. Outb1 removes the K48 polyubiquitin chains from endogenous tau, preventing tau degradation in primary neurons derived from a transgenic mouse model (108) and thus implying an important role in the regulation of tau ubiquitination.

Ubiquitin has been found in aggregated tau extracted from the brains of tauopathy patients. However, whether ubiquitin is causal, contributory, or simply a bystander in aggregation remains to be seen. Tau isolated from human AD brains has been shown to be monoubiquitinated at K254, K257, K311, and K317 (109) and polyubiquitinated at K254, K311, and K353 residues (110). Tau was also found to be ubiquitinated at K290 in a mouse model of AD (78). Interestingly, insoluble tau from AD brains is modified predominantly by K48 linkage (110), whereas soluble tau can also be ubiquitinated via K63 polyubiquitin conjugation (102), suggesting that soluble and aggregated tau are degraded by different pathways. While ubiquitin is a component of tau aggregates found in the brains AD patients (109, 111–113), tau "pre-tangles" did not exhibit positive ubiquitin immunostaining (114–116). Moreover, it has been shown that tau phosphorylation precedes ubiquitination in the NFTs of AD patients and that the formation of paired helical filaments precedes tau ubiquitination *in vitro* (109, 114), suggesting that ubiquitin may be linked to tau after the formation of the fibrillar inclusions. This finding potentially supports ubiquitination as a compensatory response to tau accumulation. In contrast, other groups have reported that both mono- and polyubiquitination contribute to the formation of insoluble protein inclusions present in neurodegenerative diseases (96, 117) and that the induction of tau ubiquitination in cell cultures increases aggregation (102). The somewhat contradictory nature of the current literature around ubiquitination suggests that whether this modification is causal, consequential, bystander, or context specific in disease remains to be sorted.

SUMOylation

In SUMOylation, the small ubiquitin-like modifier protein SUMO is transferred to the terminal amino group of lysine side chains of the target protein by an ATP-dependent enzymatic cascade. Analogous to ubiquitination, this SUMOylation cascade involves an E1 activating enzyme, an E2 conjugating enzyme, and an E3-type ligase (**Figure 2**) (118, 119). SUMO groups can be removed by specific proteases known as SUMO-specific proteases/isopeptidases (SENPs). Three main SUMO isoforms are expressed in cells: SUMO1, SUMO2, and SUMO3, of which SUMO2 and SUMO3 are more similar to each other and are different from SUMO1 (120).

SUMO is predominantly found in the nucleus and thus, SUMOylation plays a crucial role in many nuclear processes such as gene expression, genome stability (121), DNA damage response (122), protein trafficking (123), and cell cycle control (124). SUMOylation has emerged as an essential regulator of neuronal function with a growing evidence suggesting that SUMOylation of proteins inside and outside the nucleus plays an important role in neurodegenerative diseases (125). Alterations in protein SUMOylation are observed in a wide range of neurological and neurodegenerative diseases including tauopathies (126–128), and several extranuclear disease-associated proteins including tau have been shown to be directly SUMOylated (125).

In vitro studies have demonstrated that tau can be SUMOylated. Specifically, it has been found that tau is monoSUMOylated *in vitro* at the K340 residue, which is located within the microtubule-binding domain (128, 129). Tau becomes available for SUMOylation only after it is released from microtubules (129), suggesting that SUMOylation may be a secondary PTM that occurs after phosphorylation and/or acetylation. However, little is known about how SUMOylation interferes with the formation of tau inclusions in tauopathies. Tau SUMOylation at K340 inhibits tau ubiquitination and the subsequent proteasome-dependent degradation, suggesting that SUMOylation may block tau degradation and therefore induces tau accumulation and aggregation (128). In contrast, previous evidence indicates that the inhibition of the proteasome pathway stimulates tau ubiquitination and eliminates tau SUMOylation (129), suggesting that SUMOylated tau can be degraded by other pathways such as autophagy. In this context, a more recent study has demonstrated that SUMO1 labels lysosomes in oligodendrocytes from PSP patients containing tau inclusions (127), an indicator that SUMOylation may be regulating autophagy-lysosomes pathway in tauopathies. Additionally, the activation of autophagy-lysosomes pathway appears to reduce tau SUMOylation, tau inclusions and cortical atrophy associated with the rTg4510 mouse model of tauopathy (130). Despite the role of SUMOylation in tau function and dysfunction not being completely understood, substantive evidence support SUMOylation competing with ubiquitin for lysine residues in tau, with consequences on tau clearance in the proteasome and/or autophagy-lysosomes systems.

Methylation

Methylation is a biochemical process that involves the transfer a methyl group (CH_3) to DNA or protein targets (**Figure 2**). DNA methylation was discovered contemporaneously with the discovery of DNA as genetic material (131, 132). On DNA, methylation is regarded as an epigenetic means of regulating gene expression through blocking transcription factor binding or recruiting transcriptional repressors (133). Protein methylation was first described in histones. This was followed by decades of limited interest until the physiological role for protein methylation was documented in the late 1990s and non-histone proteins were also found to be methylated (134). Currently, methylation is fourth on the list of PTMs in terms of overall abundance in the proteome, with more than 18,000 methylation sites described in ∼7,400 proteins (135). The N-methylation of histone and non-histone proteins occurs primarily on lysine and arginine residues. In the process of DNA or protein methylation, methyl groups from an S-adenosyl methionine (SAM) donor are conferred by methyltransferases and removed by demethylases (136). Lysine residues can be methylated up to three times, resulting in a mono-, di-, or trimethyl-lysine, which each give rise to distinctive biological consequences. Indeed, although the methyl group is one of the smallest post-translational modifications, each methylation event removes a proton from the ε-amino group and thereby decreases the hydrogen-bonding potential of lysine. Therefore, methylation also increases the hydrophobicity and bulkiness of lysine side-chains. Based on the electrostatic properties of methylation, this PTM has been shown to regulate different aspects of proteins function such as protein–protein and protein–nucleic acid interactions, protein stability, subcellular localization, and enzyme activity, which in turn affect essential cellular processes including transcription, protein synthesis, signal transduction, and metabolism (137, 138).

Tau methylation is a relatively recent discovery (89). Results from mass spectrometric analyses show that several lysine residues are methylated on tau extracted from either healthy individuals or AD patient brains (89, 139–141). Moreover, in mouse models tau is also methylated at arginine residues R126, R155, and R349 (78). The number of methyl residues in tau protein is relatively low compared to other modifications such as phosphorylation and acetylation (140). Tau from both healthy and AD patient brains can be mono and dimethylated (141, 142). Interestingly the methylation status of tau changes qualitatively with aging and disease progression (89, 140, 143). Inherent in this finding is the possibility that aging alone, rather than disease *per se*, dictates tau methylation, analogous to the "Aging Clock" described for DNA methylation (144).

Until now, the specific enzymes involved in tau methylation have not been identified, and the exact role of methylation in tau function and pathogenesis is unclear. Methylation changes the electrostatics of tau protein. Given that tau is methylated within the KXGS motifs, regions that are essential for interactions with microtubules, lysine methylation may suppress tau's microtubule-binding function (139). Since tau aggregates from AD patients are methylated (89), it has also been suggested that methyl residues can bind to tau after aggregation and perhaps impair ubiquitination and UPS-mediated degradation (89). Future investigation of site-dependent tau methylation *in vivo*, and determination of its relationship to the DNA methylation "Aging Clock", would advance our understanding of the role of this modification in tau function and pathology.

Glycosylation and Glycation

Glycosylation involves the addition of carbohydrate chains to proteins and lipids, of which we will focus on the former. Protein glycosylation is classified into two subtypes depending on the type of reaction involved: enzymatic glycosylation and non-enzymatic glycosylation (aka glycation). Enzymatic glycosylation typically occurs on secreted proteins or those that remain in membrane-bound organelles, hence this process occurs in the endoplasmatic reticulum (ER)/Golgi system. In addition, a specialized enzymatic glycosylation called O-GlcNAcylation occurs almost exclusively on cytoplasmic and nuclear (non-secreted) proteins (145). Given that the addition of sugar groups has steric effects on proteins, both enzymatic and non-enzymatic glycation have been described to play a major role in determining the structure and stability of proteins (146). However, while enzymatic glycosylation is an important PTM that exerts functional effects on glycoproteins, glycation typically results in dysfunctional or defective biomolecules (146, 147). Both enzymatic and non-enzymatic glycosylation of tau function are discussed below.

Enzymatic Glycosylation

Enzymatic glycosylation is a process in which glycosyltransferase enzymes attach activated sugar donor groups (monosaccharides) to proteins via covalent, glycosidic linkages (148–150). Sugar groups are linked to either asparagine (N-glycosylation) or serine/threonine (O-glycosylation) residues (**Figure 2**) (146). N-glycosylation begins as a co-translational event in the ER. There, the oligosaccharyltransferase enzyme adds an immature polysaccharide to a nascent polypeptide chain. After the further maturation of this polysaccharide, the resulting glycoprotein is transferred to the Golgi apparatus, where the sugar groups acquire an even more complex structure during a process called "terminal glycosylation" (149). Conversely, O-glycosylation is achieved post-translationally in the cis-Golgi compartment (151). N- and O-glycosylation also differ in the linker-residue of the targeted protein. N-glycosylation is characterized by the enzymatic addition of N-glycans to asparagine residues in the sequence N-X-S/T (where X is any amino acid except P or D), being X any amino acid except proline (149). O-glycosylation occurs on S/T residues with a β-turn in the proximity of a proline residue, however the O-glycosylation domains are not exactly defined (152). Both N- and O-glycosylation impact the structure, stability, folding, oligomerization, and solubility of glycoproteins (143, 153, 154).

Some proteins can additionally undergo a specialized and highly dynamic form of O-glycosylation called O-GlcNAcylation or *O*-GlcNAc. O-GlcNAcylation differs from other types of glycosylation in that the glycan is not further processed after the addition of a single GlcNAc to S/T residues. It also occurs

almost exclusively on intracellular (nuclear and cytoplasmic) rather than secreted proteins (151). O-GlcNAcylation is highly regulated by two enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) that add and remove sugar groups, respectively (145). Because O-GlcNAcylation affects S/T residues, many known O-GlcNAcylation sites are nearby or overlapping with phosphorylation sites and thus it has been suggested that these two post-translational modifications can regulate each other (145, 155–159).

Enzymatic glycosylation may contribute to tauopathies by modifying proteins other than tau, such as APP and the β-site APP-cleaving enzyme 1 (BACE1). APP and BACE1 have been found to be O-glycosylated (160–162) and N-glycosylated (163, 164) in tauopathy patients' brains. Tau also has the potential to be modified by N-glycosylation and by the specialized form of O-glycosylation, O-GlcNAcylation (165, 166). Interestingly, to date tau has been found to be N-glycosylated only in AD subjects (167–169), while O-GlcNAcylation levels are relatively elevated in brains from normal controls (155, 166, 170). To date, at least five O-GlcNAc sites have been mapped in tau (T123, S208, S238, S400, and one of S409/412/413) (171–173) and all of them are also susceptible to phosphorylation. Therefore, it has been speculated that O-glycosylation may compete with or protect from phosphorylation (155, 174). Since tau O-GlcNAcylation is decreased in diseased brain (155, 170), it has been suggested that this PTMs has a protective role. This hypothesis is potentially supported by the fact that that O-GlcNAcylation increases the interaction of tau with microtubules (175), increases tau degradation (166), and suppresses tau aggregation (171, 175, 176).

Despite tau being N-glycosylated, the exact residues have not been identified. Furthermore, the relevance of N-glycosylation in tau function is a matter of debate since tau is a cytosolic protein, but the N-glycosylation process occurs in the ER-Golgi system (177). Therefore, tau N-glycosylation is thought to be associated with aberrant subcellular localization of tau. However, the mechanisms and consequences behind tau relocation are still elusive. It has been proposed that N-glycosylation precedes and accelerates tau phosphorylation (169), either by suppressing tau dephosphorylation (178) and/or inducing tau phosphorylation by protein kinase A (PKA) (169, 178). While a direct impact of tau N-glycosylation on microtubule polymerization and stability has not been observed (167), the removal of N-glycans and phosphate groups of tau seems to restore its microtubule polymerization activity (164). Although little is known about the role of N-glycosylation in tau biology, this evidence suggests that aberrant N-glycosylation of tau may be involved in neurodegenerative disease pathogenesis.

Glycation or Non-enzymatic Glycosylation

Glycation (or non-enzymatic glycosylation) is a PTM in which sugars or sugar-derived metabolites are covalently attached to the side chain of lysine residues (**Figure 2**). The glycation process involves a set of chemically heterogeneous modifications that lead to the formation of advanced glycation end-products (AGEs) (179). AGEs are produced by the irreversible cross-link between glycated and non-glycated proteins and are considered glycotoxins with significance in aging and age-related diseases (180–182). Both early stage glycation and AGEs have been shown to impair protein activity. Moreover, AGEs deposition has been shown to correlate with age and disease progression in AD (183). Although the study of AGEs is an active area of investigation in the field of aging research (184), it is still unknown how proteins are selected to be glycated and the biological role of glycation in neurodegeneration is not well-understood (185).

Thirty-two lysine residues in 2N4R tau have been identified *in vitro* as potential glycation sites (186, 187) (**Figure 3**). Although most of these sites were detected in both 3R and 4R isoforms, K280 and K281 are absent in the 3R tau, and this difference seems sufficient to slow the glycation of 3R relative to 4R isoforms (186, 187). In AD, glycation has been detected in aggregated tau purified from human AD brains but not in soluble tau (188). The molecular functions for glycation, the sites of these reactions and whether or how they contribute to tau aggregation remain subjects for debate. Since the glycation of tau in microtubule binding domains reduces tau affinity for microtubules *in vitro* (189), it has been suggested that glycation sterically blocks tau-microtubule interactions. Glycation may also induce the accumulation of tau by blocking the ubiquitination of tau at lysine residues and consequently its degradation (189, 190). Furthermore, the late glycosylation product known as AGEs have also been found colocalized with pathological tau aggregates in the brain of sporadic AD cases (191). Similarly, AGEs colocalize with NFTs from PSP, PiD, and ALS patients (180, 191). Although, the role of glycosylation in tau function is not well-understood, evidence presented here implies that this PTM can play an important role in tauopathies.

Proteolysis

Proteolysis refers to the breakdown of proteins into peptides and amino acids through the hydrolysis of peptide bonds via the action of proteases. Most proteases are highly specific and cleave their protein substrates from the N-terminus (aminopeptidases), C-terminus (carboxypeptidases), or more centralized regions (endopeptidases) of a protein. Furthermore, based on their catalytic mechanisms for substrate hydrolysis and the residues associated with their active sites, proteases are categorized into six types: aspartyl, cysteine, glutamic, metallo, serine, and threonine proteases. Proteolysis is a critical modification that can lead to alterations in protein function with important outcomes in many biological processes including signaling pathways and apoptosis. It can regulate the concentration of a protein, transform a protein into an active form, or process a protein to provide the amino acids required to synthesize a different protein. Proteolysis also plays an important function in removing damaged or unnecessary proteins from cells.

Several cytosolic proteases responsible for tau proteolysis have been identified, including caspases, calpains, and thrombin (192). Caspases are a family of evolutionarily conserved cysteine-dependent proteases that cleave proteins after specific aspartic acid residues (193). These proteases play a crucial role in important biological process such as apoptosis and inflammation (194). Thirteen different caspases have been described in humans, and their functional classification is reviewed elsewhere (193).

The role of proteolysis in tau function is incompletely understood. Tau proteolysis could be protective and may promote the removal of abnormal tau or could favor the abnormal accumulation of cleaved tau in the cell (195). It is also possible that tau cleavage is neither protective nor damaging, but is merely a bystander effect of tau accumulation. The proteolytic cleavage of tau has been observed both *in vitro* and *in vivo* (192, 195). Prior research on tau proteolysis has also shown that the proteolytic cleavage of tau precedes its aggregation *in vitro* (196–198). Furthermore, the N-terminal truncation of tau has been shown to alter the cellular localization of the protein from the cytosol to the nucleus (199), indicating a possible link between proteolysis and tau localization. Given that it has been shown that tau fragments induce death in cultured neurons (200), it has been suggested that tau cleavage may induce neurodegeneration. However, the toxic nature of tau fragments has not been demonstrated *in vivo* and therefore, it remains unclear if these fragments are indeed toxic.

The *in vitro* cleavage of tau by caspase-3,−7,−8, and less efficiently by caspases-1, and−6 resulting in the formation of an N-terminal fragment, tau1-421 (Tau-C3) (198, 201, 202) has been demonstrated. Tau-C3 fragment has been observed in AD brains (198) and interestingly, the active forms of both caspase-3 and caspase-6 are elevated in AD brains compared to control brains (203–205). While the exact function of Tau-C3 fragment is unknown, it has been shown that Tau-C3 fragment induce toxicity in neuronal cultures (201, 206, 207), and that the caspase-mediated tau cleavage precedes tangle formation in tau transgenic mice (Tg4510) (208). However, whether caspase-mediated cleavage of tau is toxic or protective for the neurons still remains a matter of debate.

While the proteolytic cleavage of tau by caspases has been extensively studied, the cleavage of tau by calpains and thrombin remains elusive. Calpains are calcium-activated cytosolic cysteine proteases implicated in a variety of calcium associated cellular functions like cell proliferation, migration, invasion, apoptosis, and signal transduction (209). Dysregulation of calcium homeostasis has been proposed to induce abnormal activation of calpains in a number of neurodegenerative diseases (209). The cleavage of tau by calpains, has been demonstrated *in vitro* and in neuronal cultures (210, 211). According to a recent study, calpain-mediated cleavage events are considered a part of normal tau protein processing (212). However, there are studies that have suggested that calpain-mediated proteolysis of tau may induce toxicity, as seen in cell cultures and a *Drosophila* tauopathy model (210, 213). Thrombin is a cytosolic serine protease that is expressed within neurons and astrocytes (195). The cleavage of tau by thrombin has been shown *in vitro* and in cell models of tauopathy (214, 215). Based on cell culture-based studies, thrombin cleavage products of tau are believed to be potentially pathogenic (216, 217). However, due to contradictory reports and lack of *in vivo* evidence for tau proteolysis mediated by these enzymes, the relevance of thrombin, and calpains in tau function remains unclear. Naturally, tau cleavage also occurs in the proteasome and lysosomes, which will be discussed further in section Effect of PTMs on Tau Degradation.

Other PTMs

In addition to the PTMs discussed above, several other post-translational modifications occur on tau, which are less well-recognized. Below, we discuss three different tau PTMs that fall in this category: protonation, oxidation, and nitration.

Protonation

Protonation involves enzyme-independent, rapid, and reversible addition or removal of protons. Protonation adds a positive charge to otherwise uncharged amino acid side chains and therefore can lead to dynamic changes in protein conformation and function (218). The optimum protonation state of a protein is critical to its functioning. Dysregulation in local pH can lead to disruption in the protonation state of a protein and has recently been shown to have implications with neurodegenerative diseases (219, 220). Among several protein sites that can participate in the protonation/deprotonation process, only a few can significantly undergo this modification within the normal cytosolic pH range. Histidine side chains, with a nominal pKa of ∼6.5, are among the most attractive residues for protonation within cytosolic pH range.

Tau contains highly-conserved histidine residues near the C-terminus of each microtubule-binding tau repeat (**Figure 3**) (221). These residues have been proposed to act as pH sensors and influence tau–microtubule interaction within the physiological intracellular pH range (221). The histidines are positively charged at pH <7.5 and show increased tau-microtubule binding (221). However, at pH >7.5, histidine residues in tau are deprotonated and bind microtubules with lower affinity (221). Furthermore, it has been proposed that a highly conserved histidine residue, H299 (**Figure 3**), near the R2 C-terminus seems to contribute to tau–microtubule binding. The labile nature and minimal molecular weight change of protonation renders it difficult to detect by traditional approaches, such as mass spectroscopy or gel electrophoresis, used to detect PTMs. Nonetheless, given its effects on protein structure, solubility and function, protonation should be considered as a post-translational protein modification like the others in this review.

Oxidation

Like protonation, protein conformation is also sensitive to oxidation-reduction (redox) changes. Redox homeostasis is achieved by the regulation of appropriate levels of reactive oxygen (ROS) and nitrogen (RNS) species, that are considered important physiological regulators of intracellular signaling pathways (222). The dysregulation of ROS is a major contributor to oxidative damage and it has been proposed that age-related accumulation of oxidized proteins may contribute to the aging process (223). However, the importance of protein oxidation in the progression of aging still remains poorly understood. Cysteine residues are the prime amino acid that can exist in oxidized or reduced states. The unique properties of cysteine side chain allows it to undergo various oxidative PTMs, which can potentially have diverse regulatory effects. The oxidation of cysteine residues leading to disulfide bond formation is one of the

well-established mechanisms underlying the redox regulation of protein conformation and hence its function.

On tau, there exists a pair of cysteine residues that can undergo oxidation (**Figure 3**). While the effect of oxidation on tau function is unknown, its role in tau aggregation has been studied *in vitro* (224) and is discussed in section Effect of PTMs on Tau Solubility and Aggregation of this review. Tau oxidation has only been described *in vitro*, and therefore the status of oxidized tau *in vivo* remains unclear.

Nitration

Nitration involves the addition of nitrogen dioxide (NO_2) onto tyrosine residues. The mechanism underlying protein nitration is not clear but it most likely involves the presence of ROS/RNS-like peroxynitrite and therefore nitration can also occur during oxidative damage (225). Several lines of evidence suggest a role of nitration in physiological processes like signal transduction (226, 227). Additionally, increased protein nitration has been identified in a large variety of diseases and have also been implicated in the process of aging (227).

The 2N4R tau isoform has five tyrosine residues, and the nitration at four of them has been shown *in vitro*: Y-18, Y-29, Y-197, and Y-394 (**Figure 3**). These studies have shown that residues Y-18 and T-29 are more susceptible toward nitration than residues Y-197 and Y-394. The nitration of tau at Y-197 has been observed in the healthy brain and therefore has been proposed to have important physiological functions (228). However, the nitration of Y-18, Y-29, and Y-394 has only been observed in AD or other tauopathies (229). It is believed that nitration of these tyrosine residues alters tau conformation and reduces its ability to bind to microtubules (230). While there are some interesting insights on tau nitration, most of these studies are based on *in vitro* experiments. Therefore, studies focusing on this PTM will provide additional useful insights into its role in tau pathobiology.

EFFECT OF PTMs ON TAU DEGRADATION

Protein degradation is important for the maintenance of proteostasis and for preventing the accumulation of misfolded or aggregated protein species (231). There are two major protein degradation systems in the cell: the ubiquitin-proteasome system (UPS) and the autophagy-lysosomes pathway (231). Both protein degradation pathways are key effectors of the proteostasis network and are strongly influenced by PTMs. Protein clearance is highly regulated and utilizes ubiquitin as a tag for direct protein degradation in both proteasomal and autophagy systems. However, other PTMs also affect the process of protein degradation indirectly. This is the case for phosphorylation and acetylation, both of which can modify amino acid sequences known as degrons, which are important for protein degradation.

A subset of degrons are regulated by phosphorylation and thus are called phosphodegrons (232, 233). Phosphodegrons are short linear amino acid sequences, D/E-S/T-**G**XX-S/T-P or **LL**-S/T-**P**XX-S/T-P, where the phosphorylation of S/T residues promotes protein clearance through stimulation of subsequent ubiquitination and proteasomal degradation (232, 233). Proteins can also contain other motifs that promote degradation by the autophagy-lysosomes system. These pentapeptide motifs are known as KFERQ motifs as they are composed of one or two positively charged residues (K, R), one or two hydrophobic residues (I, L, V, F), one negatively charged residue (D, E), and one bookending glutamine (Q) (234). "Canonical" KFERQ motifs are complete without modification while "potential" KFERQ motifs require completion via a PTM such as phosphorylation (235) or acetylation (234, 236) (**Figure 3**).

Tau homeostasis is typically maintained via degradation by both UPS and autophagy-lysosome systems (96, 110). Yet at some point in the pathobiology of tauopathies, the local concentration of tau must increase to favor the formation of fibrils, oligomers, and aggregates. Does self-association of potentially abnormally modified tau lead to damage and subsequent impairment of protein degradation systems? In this model, tau accumulation is causal for neurodegenerative disease. Or, alternatively, does tau accumulation result from decreased efficiency or failure of the protein degradation systems? In this case, tau accumulation is a consequence of other pathobiology in neurodegeneration. This type of binary, chicken, or egg approach to the genesis of tauopathies may be over-simplifications of a complex, biological milieu that combines aspects of both tau accumulation as cause and consequence. Nonetheless, the answers to these questions are key, as they dictate certain nodes to target for disease therapy. In this section, we will delineate the pathways for tau clearance and describe how PTMs could play active roles in these processes.

Tau Clearance by the Ubiquitin-Proteasome Pathway

The UPS pathway is responsible for the clearance of soluble, intracellular proteins (237, 238). The proteasome is a highly organized and complex molecular machinery responsible for the selective and efficient degradation of client proteins, whose basic science and function has been reviewed elsewhere (239–242). The 26S proteasome is responsible for the majority of protein clearance in mammalian cells and degrades proteins that are tagged with polyubiquitin chains (195). Therefore, the fact that tau is a target of ubiquitination (as discussed above) (109, 110) suggests that it can be degraded by the proteasome.

While ubiquitination is the proximal signal for protein degradation in the UPS, other PTMs such as phosphorylation are often required for tau ubiquitination to occur (243). For example, tau phosphorylated at proline-directed serine/threonine sites is selectively ubiquitinated by CHIP (103, 243), and thus phosphorylation in these sites increases tau ubiquitination and UPS-mediated degradation. Given that tau has six phosphodegron sequences (**Figure 3**), phosphorylation also regulates the ubiquitination and degradation of tau through this means (233). Phosphorylation of tau degrons is highly regulated by different kinases such as cdk5 and GSK3β (244), which work together in order to phosphorylate tau at specific residues and prepare the molecule for ubiquitination and degradation (245).

However, phosphorylation of tau at alternative sites prevents ubiquitination and clearance. As a case in point, phosphorylation of tau at the KXGS motifs in the C-terminal microtubule binding repeat domains seems to prevent the ubiquitination and degradation of tau (243).

The dysfunction of the UPS and its related PTMs are associated with the development of tauopathies. Insoluble tau aggregates isolated from human AD brains contain proteasome subunits (246). Ubiquitin ligases such as CHIP and TRAF6 also colocalize with tau in AD brains (102, 103). Ubiquitinated tau is a component of NFTs (246). Further, a recent study from our group showed that the PSP-related *A152T* tau variant increases tau phosphorylation at threonine 153, potentially interfering with recognition of the local phosphodegron and thereby UPS degradation (233). Impairment of UPS activity has also been observed in postmortem human AD brains (246–250). While together these data implicate the UPS in tauopathies, they nonetheless represent correlative, rather than causal evidence. To our knowledge, no studies have established UPS failure as necessary and sufficient in promoting tau accumulation, aggregation, neuronal loss, and neurodegeneration.

Tau Clearance by Autophagy

Autophagy is the process by which unnecessary or damaged cellular components are proteolytically degraded in the endosomal-lysosomal pathway. Based on the mechanisms by which the substrate is delivered for degradation, there are three major types of autophagy: chaperone-mediated autophagy (CMA), endosomal microautophagy (e-MI), and macroautophagy. Long-lived proteins or protein aggregates that are too large to be processed through the proteasome are typically degraded via autophagy (242). Tau, being a long-lived protein, is anticipated to be degraded in lysosomes (242) and indeed, it has been shown that both soluble and insoluble tau can be degraded by the three forms of autophagy (251, 252). Furthermore, it has been observed that the use of autophagy inhibitors, such as ammonium chloride (NH_4Cl), chloroquine, and 3-methyladenine (3-MA), as well as the inhibition of lysosomal proteases, delays tau clearance, and enhances tau accumulation (253–255), confirming the importance of autophagy in tau degradation.

Not only is tau cleared by autophagy, but PTMs play distinct roles in modulating these processes. Within the amino acid sequence of the tau protein are found two KFERQ motifs (^{336}QVEVK^{340} and ^{347}KDRVQ^{351}) (**Figure 3**). These consensus sequences direct tau toward either chaperone-mediated autophagy or endosomal-microautophagy (252, 256). In addition to the two canonical KFERQ motifs, tau contains four potential KFERQ motifs that can become canonical in the presence of phosphorylation, acetylation, or a combination of both (**Figure 3**) (*KFERQ finder)* (234), emphasizing that PTMs play an important role in the degradation of tau by these two systems. CMA-mediated protein degradation is a selective form of protein clearance in which the KFERQ motifs are recognized by the Hsc70 chaperone. Upon recognition, the proteins are transported to the lysosomal lumen after binding to the lysosome-associated membrane protein-2 (LAMP-2) (257). Tau protein can be efficiently degraded by CMA as long as the KFERQ motifs are not blocked by PTMs (252).

In addition to initiating CMA, the binding of Hsc70 to KFERQ motifs also targets cytosolic proteins for selective degradation by e-MI. This form of protein degradation occurs in late endosomes/multivesicular bodies instead of lysosomes and involves sequestering substrate proteins into intraluminal vesicles budding in toward the lumen of multivesicular endosomes (234, 256). Unlike CMA, in mammals, the presence of KFERQ motifs is necessary but not sufficient for e-MI (256). In addition to protein cargo delivery by Hsc70, the e-MI pathway relies on the ESCRT (endosomal sorting complexes required for transport) I and III complexes that are required for the formation of vesicles which internalizes the cytosolic cargo (256). A recent study has analyzed the influence of two different PTMs, oxidation and phosphorylation, on the e-MI-dependent degradation of tau (251). A substantial decrease in the association and internalization of tau in late endosomes via e-MI was observed upon oxidation of mutant tau, which suggests that the oxidation of tau at C291/C322 is a prerequisite for completing the internalization of tau through e-MI (251). Moreover, in the same study, the authors found that phosphorylating tau at S262, S293, S324, and S356 inside microtubule-binding domains diminishes its degradation by e-MI (251). In contrast, another study showed e-MI mediated degradation of total tau and of specific phosphorylated species (258). Thus, PTMs regulate the clearance of tau degradation by both CMA and e-MI.

Non-soluble, larger or aggregated species of tau are cleared via macroautophagy, a form of autophagy that involves the sequestration of cytoplasmic cargo into double-membrane vesicles known as autophagosomes, which fuse with the lysosome to degrade its contents (259). Several reports have confirmed that tau aggregates can be degraded by macroautophagy. For example, the aggregates generated by the overexpression of the tau repeat domain with an FTD-17 mutation (TauRDΔK280) (252) as well as the proteolytically cleaved tau isoform D421 (Tau-C3) (260) are degraded by macroautophagy in cell models of tauopathy. Interestingly, tau phosphorylated at KXGS motifs escapes degradation by the UPS but is efficiently degraded by macroautophagy (252), suggesting the that wild type tau can also be degraded by macroautophagy. As PTMs contribute to tau aggregation (see section Effect of PTMs on Tau Solubility and Aggregation), they likely regulate tau clearance via macroautophagy.

Failure of tau degradation by the autophagy-lysosomes pathway has been linked to tauopathies. For example, there are several studies suggesting a defect in macroautophagy in tauopathy patients (261–263). Furthermore, it has been shown that tau in NFTs colocalizes with lysosomes and lysosomal markers in human AD, CBD, and PSP brains (263–267), suggesting that tau is directed toward macroautophagy but fails to be degraded. Moreover, evidence from mouse and *Drosophila* models of FTLD-Tau indicates that the microtubule destabilization associated with the abnormal phosphorylation of tau leads to the disruption of the axonal vesicle transport by impairing the dynein-dynactin complex, vesicle trafficking, and autophagic flux (268, 269). Alterations in CMA may

also contribute to tauopathies, since it has been described that tau carrying the FTD-related mutation P301L reduces tau's susceptibility for degradation by CMA (251). Overall, the regulation of the autophagy-lysosomes pathway seems to be essential for maintaining tau homeostasis in health and disease, and PTMs in tau influence the autophagic degradation of tau in multiple ways.

EFFECT OF PTMs ON TAU SOLUBILITY AND AGGREGATION

The degradation pathways discussed above represent a major component of the proteostasis network and ensure that, under normal conditions, tau is maintained in an optimally functional state (231). However, in tauopathies, there is a breakdown in tau homeostasis that in turn leads to aberrant accumulation, misfolding, and aggregation of tau (45, 242, 270). Recent studies suggest that PTMs contribute in sometimes surprising ways to altering tau solubility and thereby promoting its aggregation.

Various biophysical techniques have demonstrated that under physiological conditions, tau exists in a "natively unfolded" or "intrinsically disordered" state (45, 271, 272). Despite being intrinsically disordered, tau shows a preference for global interactions between its negatively charged N-terminus and positively charged repeat domains, leading to an energetically favored "paperclip" like conformation (273). In contrast to soluble tau, tau aggregates are composed of β-sheet rich structures known as amyloid fibrils (274–277). Amyloid fibrils are highly heterogeneous in nature and are not easily amenable to study via traditional methods used in structural biology. Recent breakthroughs in cryo-electron microscopy (cryo-EM) technology, however, have made it possible to determine high resolution three-dimensional structure of tau amyloid fibrils (278, 279). Determination of the atomic structures of tau aggregates may provide a better understanding of the mechanisms underlying their formation, spreading, and clearance.

To date, cryo-EM has elucidated the structure of tau filaments isolated from brains of patients with several different forms of tauopathies (279–284). According to these studies, each tauopathy is associated with a unique tau filament fold (279). The origin of such structural specificity is not yet known, but could emanate from PTMs (279). For example, tau is phosphorylated at S262 in tau filaments extracted from AD and CBD brains, but not in the filaments of PiD patients (11). Consistent with this phospho-modified residue contributing to aggregate structure, S262 is present in the tau filament core in PiD but not AD (279). Therefore, distinctive patterns of post-translational modification in different tauopathies may underlie not only the structural specificity of amyloids observed in cryo-EM structures, but could even contribute to selective vulnerability to these diseases.

To understand the fundamental role of PTMs in tau aggregation, it is important to account for the influence of PTMs over the charge composition and distribution within a polypeptide chain. The net charge on any protein is dependent on the content of ionizable groups. Net charge is 0 when the pH of the surrounding environment equals that protein's isoelectric point (pI). Similarly, net charge is positive at a pH below pI, and is negative at pH above its pI. Tau contains around 29 percent charged residues, has low hydrophobicity and has a low net charge of +2 at physiological pH. While tau is overall slightly positively charged, the amino-terminal residues (including the two N-terminal inserts) are more negatively charged, the microtubule-binding repeat region is predominantly positive, and the distal carboxy-terminal residues are mostly neutral (285). Such asymmetric distribution of charges has been proposed to play a crucial role in tau interactions with microtubules and other binding partners (45). It may also explain the inability of tau to undergo aggregation *in vitro* without polyanionic aggregation inducers (286–288). The inducers of tau aggregation are not typically present in neurons but their effect may be achieved through the masking of charged residues in tau via PTMs.

PTMs that make tau more negatively charged are phosphorylation, acetylation, and nitration. These PTMs, when present in the microtubule-binding domains, have been proposed to initiate tau aggregation by first weakening its interaction with negatively charged microtubules (26, 45, 289, 290) The reduced affinity of tau toward microtubules upon these modifications would increase the "free" pool of soluble modified tau molecules that might have a higher tendency to self-assemble into aggregates, as has been observed in case of phosphorylated tau (189, 291). The aggregation might be further promoted by electrostatic interactions between negatively charged, modified tau and positively charged, unmodified tau. The effects of phosphorylation on tau aggregation, however, seem to be site-dependent. For example, phosphorylation sites close to the N-terminus tend to prevent tau aggregation but phosphorylation sites in the vicinity of the C-terminus and microtubule-binding region seem to accelerate filament formation *in vitro* (285). A similar site-dependence has been observed in tau nitration, where depending on the residues that are modified, nitration can either promote or inhibit tau aggregation (230, 289). Interestingly, the core of tau filaments isolated from tauopathy patients contains multiple lysine residues that are susceptible to acetylation (280, 281, 283). Since the microtubule binding region constitutes a major part of the core of tau filaments, acetylation of lysine residues in this region likely plays an important role in the assembly of tau filaments.

Unlike phosphorylation, acetylation, and nitration that alter the charge of tau, methylation preserves the positive charge on lysine residues for a neutral effect on net charge of the protein. The role of methylation in tau aggregation is controversial and it is unclear whether tau methylation induces tau aggregation or has a protective role. While some studies have remarked that methylation at lysine residues facilitates abnormal protein aggregation (292, 293), further experimentation using recombinant tau has shown opposite results, demonstrating that methylation reduces the tendency of tau toward aggregation and promotes tubulin assembly (141). Interestingly, the methylation status of tau changes from dimethyl- to monomethyl-lysine with aging and disease (140). Therefore, it is possible that high

stoichiometry (number of methyl groups/protein molecule) of methylation reduces the propensity of tau toward aggregation *in vitro* and promote tubulin assembly (140).

Another important modification in tau that does not affect charge but can affect tau aggregation is oxidation. The oxidation at one of the two cysteine residues C-322 (present in R3 repeat) **(Figure 3)** seems to promote tau aggregation into paired helical filaments (224). However, aggregation is inhibited under reduced conditions, upon mutation of cysteine to alanine and upon the formation of an intramolecular disulfide bond between C-322 and C-291 (224, 294).

While the PTMs described above involve the addition of small chemical groups to tau, other PTMs involve the addition of large sugar groups (glycosylation and glycation) and protein macromolecules (ubiquitination and SUMOylation) **(Figure 2)**. These large molecules can have profound steric effects on the native conformation of tau and therefore can strongly influence misfolding and aggregation. The mechanisms by which glycation and glycosylation regulate tau solubility and aggregation are poorly understood. Glycation appears to promote the polymerization and stabilization of aggregated tau (295). Additionally, the N-glycosylation of tau is thought to be associated with maintaining the structure of NFTs (167). On the other hand, O-GlcNAcylation has been proposed to protect tau against aggregation (175). In fact, a study on tau peptides has shown that O-GlcNAcylation in proline-rich sequences favors a more disordered or extended conformation of tau (296).

Like sugars, the effects of ubiquitin and SUMO addition to tau on aggregation propensity have not been extensively studied. However, both mono- and polyubiquitination can contribute to the formation of insoluble tau inclusions (96, 117). In addition, a recent cryo-electron microscopy (cryo-EM) and mass spectrometry-based study performed on tau filaments isolated from the brains of tauopathy patients, has proposed that incorporation of ubiquitin into tau filaments mediates specific inter-protofilament packing by providing additional contacts between tau molecules in each protofilament via ubiquitin chains (282). SUMOylation is analogous to ubiquitination and has been shown to decrease the solubility of tau (128). However, the mechanisms by which SUMOylation reduces tau solubility and most likely promotes its aggregation, remain unknown.

Overall, PTMs on tau critically influence the assembly of natively unfolded tau into highly ordered β-sheet rich aggregates. It is likely that PTMs drive this process by altering the distinctive charge distribution on tau. It is worth emphasizing that while PTMs undoubtedly affect tau solubility and intermolecular interactions, the effect of PTMs on processes such as protein localization and degradation would also contribute, in a discrete and upstream manner, to when, how and why tau aggregation events occur.

CROSS-TALK AND COMPETITION BETWEEN PTMs

The PTM code hypothesis proposes that the combination of PTMs in proteins generates a dynamic "code" that can be translated into complex biological consequences (297). This hypothesis may apply to tau since it undergoes many modifications, often targeting the same amino acid residue (**Figure 3**). Such heterogeneity and competition between PTMs suggest that tau function and aggregation could be a consequence of the combination of several PTMs and/or the substitution of one PTM by another at the same position. In this regard, and based on the evidence described above, it is likely that while some PTM ensembles are essential for the regulation of tau function and degradation, others are more deterministic for formation of tau aggregates (**Figure 4**).

One level at which competing biological outcomes can be coded is via competition of different PTMs for the same amino acid. For example, S and T residues are modified by phosphorylation and O-GlcNAcylation. O-GlcNAcylation in some S/T residues protects tau from phosphorylation and thus blocks the effects of phosphorylation, which impacts microtubule-binding affinity and aggregation propensity, on these residues (155, 165, 298).

Perhaps even more interesting are lysine residues, which are susceptible to five different PTMs—acetylation, ubiquitination, methylation, SUMOylation, and glycation. Each one of these modifications have potentially different consequences for tau structure, function and aggregation. Since ubiquitination occurs on lysine residues, other lysine-based PTMs may prevent ubiquitination and therefore impair tau degradation (128, 139). In fact, many lysine residues that competent for ubiquitination are also targets for other PTMs (**Figure 3**). For example, K254 residue in tau can undergo ubiquitination and methylation. In fibrillar tau, K254 was found to be primarily methylated and ubiquitinated to a lesser extent, suggesting that methylation may block UPS-mediated tau degradation and lead to an increase in tau levels (89). Like methylation, acetylation could potentially prevent lysine ubiquitination, resulting in the insufficient turnover endogenous tau (72). The veritable log jam of PTMs that can be found on lysine residues marks this amino acid as a particularly interesting and likely important site at which cells orchestrate the biological outcomes they wish to achieve with tau and other proteins.

In addition to competition, crosstalk between PTMs can also be achieved through cooperativity. Some studies suggest that certain PTMs make tau more amenable to other modifications. *In vitro* tau SUMOylation seemingly enhances phosphorylation at residues such as T231 and S262 (128). Other studies have shown that tau methylation at K267 may result in subsequent and possibly abnormal phosphorylation of tau at S262 (89). It has also been proposed that N-glycosylation precedes tau phosphorylation (299). In each of these cases, though, a reasonable alternative interpretation is that SUMOylation, methylation, or N-glycosylation may impair tau clearance, enhancing the half-life of tau in the cytosol, and thus, providing more opportunities for phosphorylation.

On the other hand, phosphorylation can also affect the propensity of tau for other PTMs. For example, in specific experimental conditions the *in vitro* phosphorylation of tau in the microtubule binding domain activates tau autoacetyltransferase activity and therefore promote its

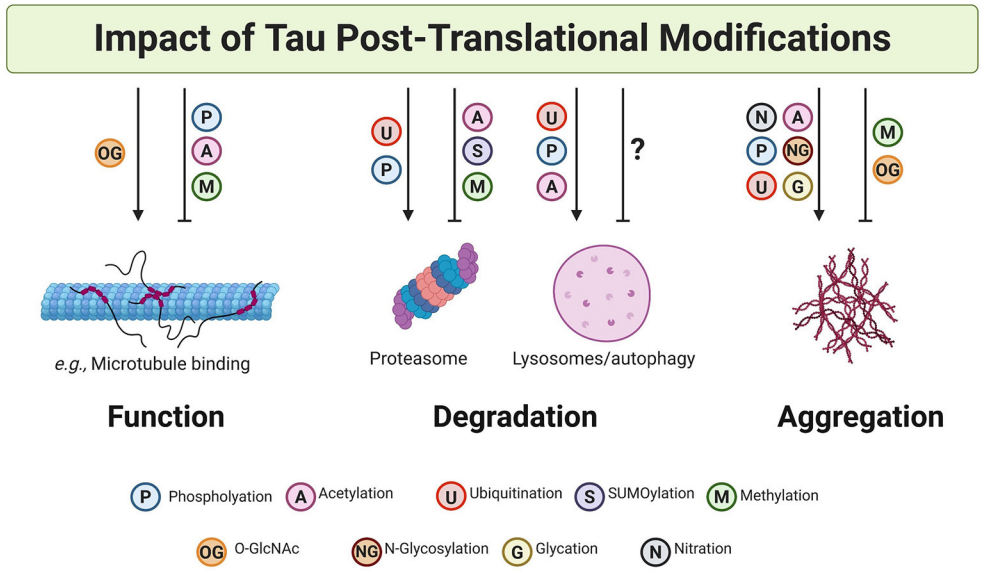


FIGURE 4 | Post-translational modifications on tau impact tau function, degradation, and aggregation. PTMs can both promote and inhibit tau function, clearance, and aggregation. A non-exhaustive list of examples are illustrated here. In many cases, it is likely that cross-talk and competition between PTMs results in the specific and dynamic regulation of tau.

acetylation (76). Furthermore, a protective role of the switch between tau phosphorylation and acetylation has been described. The acetylation of tau at KXGS motifs inhibits phosphorylation and thus prevents the detachment of tau from microtubules and its aggregation (34, 247). Additionally, the phosphorylation of tau at S422 seems to precede and block the cleavage of tau at D421 by caspase-3 (300), suggesting that phosphorylation at S422 could be a protective mechanism for the inhibition of the caspase-mediated cleavage of tau.

Taken together, the current literature suggests that crosstalk, combinations, and competition between PTMs play a critical role in tau function, degradation, and aggregation. An old truism states that "We are the company we keep." If so, then future studies dedicated to documenting and decoding ensembles of tau PTMs will rise in importance in regards to understanding and developing interventions for tauopathies.

APPROACHES AND LIMITATIONS

The complete understanding of the role of PTMs in tau function and dysfunction is obstructed by several limitations in the methodology and models used. Historically, PTMs were identified using immunochemistry-based approaches. Using this method, several phosphorylation sites were identified and thus, a large number of anti-phospho tau antibodies are available (301). While immunochemistry methods are routinely used and are highly effective for the characterization tauopathies, they do not provide a comprehensive and quantitative description of PTMs, nor do they distinguish between single molecules with many PTMs vs. many molecules with single PTMs (302). Some of these problems can be solved by the use of quantitative mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, which provide a more detailed description of PTMs and allow the simultaneous analyses of a variety of PTMs (89, 110, 303–308). However, most of these methods are biased toward the peptides of highest intensity, and prevent the accurate determination of site-specific stoichiometry, associated site-specific dynamics and identification of labile PTMs like protonation (302). The high percentage of PTM sites in tau adds to the complexity, making it incredibly challenging to design and carry out such studies. To overcome some of these challenges, analytical methods including one known as FLEXITau (Full-Length Expressed stable Isotope-labeled tau) have been recently developed (309). This method allows for unbiased analyses of tau PTMs in a more quantitative manner. In this regard, a study using FLEXITau has reported 95 PTMs on tau isolated from postmortem human tissue from AD and has demonstrated that these modifications occur in an ordered manner and lead to tau aggregation (310).

The choice of models to use for studying PTMs also influences the understanding of the effect of PTMs in tau biology. The vast majority of PTMs have been identified *in vitro,* using biochemical assays where purified tau protein was incubated with specific enzymes (71) or using tau/enzymes overexpression systems in cell models (128). Some PTMs have also been confirmed or identified *in vivo* using tauopathy mouse models. However, most of these models are based on the expression of mutant forms human tau, and whether those findings and insights into PTMs extends to wild-type tau and sporadic tauopathies remains to be seen (79).

Tau PTMs have also been studied in post-mortem human brains using immunochemistry techniques and cryo-EM (282). It is important to keep in consideration that some PTMs can be lost or degraded during the process of brain extraction, fixation, storage, and analysis. Therefore, it is possible that in studies performed on human brain extracts, some tau PTMs may not be identified because of the caveats associated with brain preparation processes (37).

The cross-talk between PTMs is very important for the regulation of function, structure, and degradation of the proteins. Many studies on tau PTMs have focused only on one type of modification. Extrapolation of results obtained from single PTM studies lack context in regard to cross-talk and competition. As a result, a major caveat of many studies is the inability to differentiate between causes, consequences and bystander effects of these PTMs. The understanding of and solution to these limitations may enable the discovery of a "PTM code" in tau that will help to define sensitive biomarkers and lead to insights into disease pathobiology. In addition, unravelling the specific effects of PTMs in tau biology will pave the road toward new therapeutic approaches for tau-related neurodegenerative diseases.

CONCLUDING REMARKS

PTMs are essential to the normal function of tau and therefore alterations in the pattern of PTMs has the potential to lead to tau dysfunction, accumulation, and abnormal aggregation. PTMs exert their function either through altering protein electrostatics or conferring steric alterations. Among all tau PTMs, phosphorylation has been studied most extensively and historically, was thought to hold primacy in regulating tau function and pathogenesis of tauopathies. However, increasing evidence suggests that many other PTMs contribute to dynamic regulation of tau. Cross-talk and competition between PTMs, in particular at lysine residues, introduce an intriguing new layer of complexity to orchestration of tau function and dysfunction. At the same time, combinatorial ensembles of PTMs may emerge as a means by which cells achieve differential biological outcomes involving tau. As a robust and fast-moving area of investigation, tau post-translational modification will likely impact the basic, pre-clinical, and clinical-translational aspects of tau biology in interesting ways for years to come.

AUTHOR CONTRIBUTIONS

CA and SA performed the literature review. CA, SA, and AK wrote the manuscript. All authors agreed to the final version of the manuscript.

FUNDING

Tau consortium, P30 P0531421, R01 NS095257, R01 AG059052, Chan Zuckerberg Initiative grant.

ACKNOWLEDGMENTS

We gratefully acknowledge Jacqueline Jia for artistic contributions to figure preparation. We thank Dr. Alma Burlingame and Dr. William Seeley for critical review of the manuscript.

REFERENCES

1. Walsh CT. *Posttranslational Modification of Proteins: Expanding Nature's Inventory - Christopher Walsh - Google Libros*. Greenwood Village, CO: Roberts and Company, Publishers (2006) Available online at: https://books.google.com/books?hl=es&lr=&id=JGBfQXIzdwgC&oi=fnd&pg=PP21&ots=v8ZAw__wOI&sig=owJxdEEdGvj8IHuiAf6pMUkowWM#v=onepage&q&f=false (accessed August 14, 2020).
2. Smith LM, Kelleher NL. Proteoform: a single term describing protein complexity. *Nat Methods.* (2013) 10:186–7. doi: 10.1038/nmeth.2369
3. Darling AL, Uversky VN. Intrinsic disorder and posttranslational modifications: the darker side of the biological dark matter. *Front Genet.* (2018) 9:158. doi: 10.3389/fgene.2018.00158
4. Barber KW, Rinehart J. The ABCs of PTMs. *Nat Chem Biol.* (2018) 14:188–92. doi: 10.1038/nchembio.2572
5. Ravid T, Hochstrasser M. Diversity of degradation signals in the ubiquitin-proteasome system. *Nat Rev Mol Cell Biol.* (2008) 9:679–89. doi: 10.1038/nrm2468
6. Uversky VN. Natively unfolded proteins: a point where biology waits for physics. *Protein Sci.* (2002) 11:739–56. doi: 10.1110/ps.4210102
7. Uversky VN. Intrinsically disordered proteins and their (disordered) proteomes in neurodegenerative disorders. *Front Aging Neurosci.* (2015) 7:18. doi: 10.3389/fnagi.2015.00018
8. Dyson HJ. Expanding the proteome: disordered and alternatively folded proteins. *Q Rev Biophys.* (2011) 44:467–518. doi: 10.1017/S0033583511000060
9. Jeganathan S, Von Bergen M, Mandelkow EM, Mandelkow E. The natively unfolded character of tau and its aggregation to Alzheimer-like paired helical filaments. *Biochemistry.* (2008) 47:10526–39. doi: 10.1021/bi800783d
10. Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev.* (2000) 33:95–130. doi: 10.1016/S0165-0173(00)00019-9
11. Goedert M, Eisenberg DS, Crowther RA. Propagation of tau aggregates and neurodegeneration. *Annu Rev Neurosci.* (2017) 40:189–210. doi: 10.1186/s13041-017-0298-7
12. Dixit R, Ross JL, Goldman YE, Holzbaur ELF. Differential regulation of dynein and kinesin motor proteins by tau. *Science.* (2008) 319:1086–9. doi: 10.1126/science.1152993
13. Zuo YC, Li HL, Xiong NX, Shen JY, Huang YZ, Fu P, et al. Overexpression of Tau rescues nogo-66-induced neurite outgrowth inhibition *in vitro*. *Neurosci Bull.* (2016) 32:577–84. doi: 10.1007/s12264-016-0068-z
14. Merino-Serrais P, Benavides-Piccione R, Blazquez-Llorca L, Kastanauskaite A, Rá A, Avila JS, et al. The influence of phospho-tau on dendritic spines of cortical pyramidal neurons in patients with Alzheimer's disease. *Brain.* (2013) 136:1913–28. doi: 10.1093/brain/awt088
15. Brandt R, Léger J, Lee G. Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. *J Cell Biol.* (1995) 131:1327–40. doi: 10.1083/jcb.131.5.1327
16. Farah CA, Perreault S, Liazoghli D, Desjardins M, Anton A, Lauzon M, et al. Tau interacts with golgi membranes and mediates their association with microtubules. *Cell Motil Cytoskeleton.* (2006) 63:710–24. doi: 10.1002/cm.20157
17. Meier S, Bell M, Lyons DN, Ingram A, Chen J, Gensel JC, et al. Identification of novel tau interactions with endoplasmic reticulum proteins in Alzheimer's disease brain. *J Alzheimer's Dis.* (2015) 48:687–702. doi: 10.3233/JAD-150298
18. Noble W, Hanger DP, Miller CCJ, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. *Front Neurol.* (2013) 4:83. doi: 10.3389/fneur.2013.00083

19. Kampers T, Friedhoff P, Biernat J, Mandelkow EM, Mandelkow E. RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett.* (1996) 399:344–9. doi: 10.1016/S0014-5793(96)01386-5
20. Krylova SM, Musheev M, Nutiu R, Li Y, Lee G, Krylov SN. Tau protein binds single-stranded DNA sequence specifically - the proof obtained *in vitro* with non-equilibrium capillary electrophoresis of equilibrium mixtures. *FEBS Lett.* (2005) 579:1371–5. doi: 10.1016/j.febslet.2005.01.032
21. Sultan A, Nesslany F, Violet M, Bégard S, Loyens A, Talahari S, et al. Nuclear tau, a key player in neuronal DNA protection. *J Biol Chem.* (2011) 286:4566–75. doi: 10.1074/jbc.M110.199976
22. Arendt T, Stieler JT, Holzer M. Tau and tauopathies. *Brain Res Bull.* (2016) 126:238–92. doi: 10.1016/j.brainresbull.2016.08.018
23. Park S, Lee JH, Jeon JH, Lee MJ. Degradation or aggregation: the ramifications of post-translational modifications on tau. *BMB Rep.* (2018) 51:265–23. doi: 10.5483/BMBRep.2018.51.6.077
24. Haj-Yahya M, Lashuel HA. Protein semisynthesis provides access to Tau disease-associated post-translational modifications (PTMs) and paves the way to deciphering the tau PTM code in health and diseased states. *J Am Chem Soc.* (2018) 140:6611–21. doi: 10.1021/jacs.8b02668
25. Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med.* (2009) 15:112–9. doi: 10.1016/j.molmed.2009.01.003
26. Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, et al. The acetylation of tau inhibits its function and promotes pathological tau aggregation. *Nat Commun.* (2011) 2:252. doi: 10.1038/ncomms1255
27. Irwin DJ, Cohen TJ, Grossman M, Arnold SE, Xie SX, Lee VMY, et al. Acetylated tau, a novel pathological signature in Alzheimer's disease and other tauopathies. *Brain.* (2012) 135:807–18. doi: 10.1093/brain/aws013
28. Guillozet-Bongaarts AL, Glajch KE, Libson EG, Cahill ME, Bigio E, Berry RW, et al. Phosphorylation and cleavage of tau in non-AD tauopathies. *Acta Neuropathol.* (2007) 113:513–20. doi: 10.1007/s00401-007-0209-6
29. Guillozet-Bongaarts AL, Garcia-Sierra F, Reynolds MR, Horowitz PM, Fu Y, Wang T, et al. Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. *Neurobiol Aging.* (2005) 26:1015–22. doi: 10.1016/j.neurobiolaging.2004.09.019
30. Sacco F, Perfetto L, Castagnoli L, Cesareni G. The human phosphatase interactome: an intricate family portrait. *FEBS Lett.* (2012) 586:2732–9. doi: 10.1016/j.febslet.2012.05.008
31. Humphrey SJ, James DE, Mann M. Protein phosphorylation: a major switch mechanism for metabolic regulation. *Trends Endocrinol Metab.* (2015) 26:676–87. doi: 10.1016/j.tem.2015.09.013
32. Hunter T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell.* (1995) 80:225–36. doi: 10.1016/0092-8674(95)90405-0
33. Ardito F, Giuliani M, Perrone D, Troiano G, Muzio LL. The crucial role of protein phosphorylation in cell signalingand its use as targeted therapy. *Int J Mol Med.* (2017) 40:271–80. doi: 10.3892/ijmm.2017.3036
34. Reynolds CH, Betts JC, Blackstock WP, Nebreda AR, Anderton BH. Phosphorylation sites on tau identified by nanoelectrospray mass spectrometry: differences *in vitro* between the mitogen-activated protein kinases ERK2, c-Jun N-terminal kinase and P38, and glycogen synthase kinase- 3β. *J Neurochem.* (2000) 74:1587–95. doi: 10.1046/j.1471-4159.2000.0741587.x
35. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida H, Titani K, et al. Proline-directed and non-proline-directed phosphorylation of PHF-tau. *J Biol Chem.* (1995) 270:823–9. doi: 10.1074/jbc.270.2.823
36. Cook C, Stankowski JN, Carlomagno Y, Stetler C, Petrucelli L. Acetylation: a new key to unlock tau's role in neurodegeneration. *Alzheimer's Res Ther.* (2014). 6:29. doi: 10.1186/alzrt259
37. Matsuo ES, Shin RW, Billingsley ML, van deVoorde A, O'Connor M, Trojanowski JQ, et al. 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. doi: 10.1016/0896-6273(94)90264-X
38. Ferrer I, Gomez-Isla T, Puig B, Freixes M, Ribe E, Dalfo E, et al. Current advances on different kinases involved in tau phosphorylation, and implications in Alzheimers disease and tauopathies. *Curr Alzheimer Res.* (2005) 2:3–18. doi: 10.2174/1567205052772713
39. Ji C, Tang M, Zeidler C, Höhfeld J, Johnson GVW. BAG3 and SYNPO (synaptopodin) facilitate phospho-MAPT/Tau degradation via autophagy in neuronal processes. *Autophagy.* (2019) 15:1199–213. doi: 10.1080/15548627.2019.1580096
40. Derkinderen P, Scales TME, Hanger DP, Leung KY, Byers HL, Ward MA, et al. Tyrosine 394 is phosphorylated in Alzheimer's paired helical filament tau and in fetal tau with c-Abl as the candidate tyrosine kinase. *J Neurosci.* (2005) 25:6584–93. doi: 10.1523/JNEUROSCI.1487-05.2005
41. Vega IE, Cui L, Propst JA, Hutton ML, Lee G, Yen SH. Increase in tau tyrosine phosphorylation correlates with the formation of tau aggregates. *Mol Brain Res.* (2005) 138:135–44. doi: 10.1016/j.molbrainres.2005.04.015
42. Gong C -X, Singh TJ, Grundke-Iqbal I, Iqbal K. Phosphoprotein phosphatase activities in Alzheimer disease brain. *J Neurochem.* (1993) 61:921–7. doi: 10.1111/j.1471-4159.1993.tb03603.x
43. Braithwaite SP, Stock JB, Lombroso PJ, Nairn AC. Protein phosphatases and Alzheimer's disease. *Prog Mol Biol Transl Sci.* 106:343–79. doi: 10.1016/B978-0-12-396456-4.00012-2
44. Wadhwa P, Jain P, Jadhav HR. Glycogen synthase kinase 3 (GSK3): its role and inhibitors. *Curr Top Med Chem.* (2020) 20:1522–34. doi: 10.2174/1568026620666200516153136
45. Mandelkow EM, Mandelkow E. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harb Perspect Biol.* (2012) 4:ea006247. doi: 10.1101/cshperspect.a006247
46. Yu Y, Run X, Liang Z, Li Y, Liu F, Liu Y, et al. Developmental regulation of tau phosphorylation, tau kinases, and tau phosphatases. *J Neurochem.* (2009) 108:1480–94. doi: 10.1111/j.1471-4159.2009.05882.x
47. Biernat J, Mandelkow E-M. The development of cell processes induced by tau protein requires phosphorylation of serine 262 and 356 in the repeat domain and is inhibited by phosphorylation in the proline-rich domains. *Mol Biol Cell.* (1999) 10:727–40. doi: 10.1091/mbc.10.3.727
48. Drewes G, Trinczek B, Illenberger S, Biernat J, Schmitt-Ulms G, Meyer HE, et al. Microtubule-associated protein/microtubule affinity-regulating kinase (p110(mark)). a novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer- specific site serine 262. *J Biol Chem.* (1995) 270:7679–88. doi: 10.1074/jbc.270.13.7679
49. Biernat J, Gustke N, Drewes G, Mandelkow E, Mandelkow E. Phosphorylation of Ser262 strongly reduces binding of tau to microtubules: distinction between PHF-like immunoreactivity and microtubule binding. *Neuron.* (1993) 11:153–63. doi: 10.1016/0896-6273(93)90279-Z
50. Wang Y, Mandelkow E. Tau in physiology and pathology. *Nat Rev Neurosci.* (2016) 17:5–21. doi: 10.1038/nrn.2015.1
51. Mandel JE, Banker GA. Microtubule-associated proteins, phosphorylation gradients, and the establishment of neuronal polarity. *Perspect Dev Neurobiol.* (1996) 4:125–35.
52. Mandell JW, Banker GA. A spatial gradient of tau protein phosphorylation in nascent axons. *J Neurosci.* (1996) 16:5727–40. doi: 10.1523/JNEUROSCI.16-18-05727.1996
53. Biernat J, Wu YZ, Timm T, Zheng-Fischhöfer Q, Mandelkow E, Meijer L, et al. Protein kinase MARK/PAR-1 is required for neurite outgrowth and establishment of neuronal polarity. *Mol Biol Cell.* (2002) 13:4013–28. doi: 10.1091/mbc.02-03-0046
54. Li C, Götz J. Somatodendritic accumulation of tau in Alzheimer's disease is promoted by Fyn-mediated local protein translation. *EMBO J.* (2017) 36:3120–38. doi: 10.15252/embj.201797724
55. Ittner LM, Ke YD, Götz J. Phosphorylated tau interacts with c-Jun N-terminal kinase-interacting protein 1 (JIP1) in Alzheimer disease. *J Biol Chem.* (2009) 284:20909–16. doi: 10.1074/jbc.M109.014472
56. Lu PJ, Wulf G, Zhou XZ, Davies P, Lu KP. The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature.* (1999) 399:784–8. doi: 10.1038/21650
57. Cho JH, Johnson GVW. Glycogen synthase kinase 3β phosphorylates tau at both primed and unprimed sites: differential impact on microtubule binding. *J Biol Chem.* (2003) 278:187–93. doi: 10.1074/jbc.M206236200
58. Cho JH, Johnson GVW. Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3β (GSK3β) plays a critical role in regulating tau's

ability to bind and stabilize microtubules. *J Neurochem.* (2004) 88:349–58. doi: 10.1111/j.1471-4159.2004.02155.x
59. Dawson HN, Ferreira A, Ghoshal N, Eyster MV, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from τ deficient mice. *J Cell Sci.* (2001) 114:1179–87.
60. Spittaels K, van Den Haute C, van Dorpe J, Geerts H, Mercken M, Bruynseels K, et al. Glycogen synthase kinase-3β phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice. *J Biol Chem.* (2000) 275:41340–9. doi: 10.1074/jbc.M006219200
61. Tatebayashi Y, Haque N, Tung YC, Iqbal K, Grundke-Iqbal I. Role of tau phosphorylation by glycogen synthase kinase-3β in the regulation of organelle transport. *J Cell Sci.* (2004) 117:1653–63. doi: 10.1242/jcs.01018
62. Jaworski T, Kügler S, van Leuven F. Modeling of tau-mediated synaptic and neuronal degeneration in Alzheimer's disease. *Int J Alzheimer's Dis.* (2010) 2010:573138. doi: 10.4061/2010/573138
63. Hoover BR, Reed MN, Su J, Penrod RD, Kotilinek LA, Grant MK, et al. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron.* (2010) 68:1067–81. doi: 10.1016/j.neuron.2010.11.030
64. Thies E, Mandelkow EM. Missorting of tau in neurons causes degeneration of synapses that can be rescued by the kinase MARK2/Par-1. *J Neurosci.* (2007) 27:2896–907. doi: 10.1523/JNEUROSCI.4674-06.2007
65. Hasegawa M, Jakes R, Crowther RA, Lee VMY, Ihara Y, Goedert M. Characterization of mAb AP422, a novel phosphorylation-dependent monoclonal antibody against tau protein. *FEBS Lett.* (1996) 384:25–30. doi: 10.1016/0014-5793(96)00271-2
66. Haase C, Stieler JT, Arendt T, Holzer M. Pseudophosphorylation of tau protein alters its ability for self-aggregation. *J Neurochem.* (2004) 88:1509–20. doi: 10.1046/j.1471-4159.2003.02287.x
67. Drazic A, Myklebust LM, Ree R, Arnesen T. The world of protein acetylation. *Biochim Biophys Acta - Proteins Proteomics.* (2016) 1864:1372–401. doi: 10.1016/j.bbapap.2016.06.007
68. Ali I, Conrad RJ, Verdin E, Ott M. Lysine acetylation goes global: from epigenetics to metabolism and therapeutics. *Chem Rev.* (2018) 118:1216–52. doi: 10.1021/acs.chemrev.7b00181
69. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature.* (2000) 403:41–5. doi: 10.1038/47412
70. Narita T, Weinert BT, Choudhary C. Functions and mechanisms of non-histone protein acetylation. *Nat Rev Mol Cell Biol.* (2019) 20:156–74. doi: 10.1038/s41580-018-0081-3
71. Kamah A, Huvent I, Cantrelle FX, Qi H, Lippens G, Landrieu I, et al. Nuclear magnetic resonance analysis of the acetylation pattern of the neuronal tau protein. *Biochemistry.* (2014) 53:3020–32. doi: 10.1021/bi500006v
72. Min SW, Cho SH, Zhou Y, Schroeder S, Haroutunian V, Seeley WW, et al. Acetylation of tau inhibits its degradation and contributes to tauopathy. *Neuron.* (2010) 67:953–66. doi: 10.1016/j.neuron.2010.08.044
73. Ding H, Dolan PJ, Johnson GVW. Histone deacetylase 6 interacts with the microtubule-associated protein tau. *J Neurochem.* (2008) 106:2119–30. doi: 10.1111/j.1471-4159.2008.05564.x
74. Min SW, Sohn PD, Li Y, Devidze N, Johnson JR, Krogan NJ, et al. SIRT1 deacetylates tau and reduces pathogenic tau spread in a mouse model of tauopathy. *J Neurosci.* (2018) 38:3680–8. doi: 10.1523/JNEUROSCI.2369-17.2018
75. Carlomagno Y, Chung D eun C, Yue M, Castanedes-Casey M, Madden BJ, Dunmore J, et al. An acetylation–phosphorylation switch that regulates tau aggregation propensity and function. *J Biol Chem.* (2017) 292:15277–86. doi: 10.1074/jbc.M117.794602
76. Cohen TJ, Friedmann D, Hwang AW, Marmorstein R, Lee VMY. The microtubule-associated tau protein has intrinsic acetyltransferase activity. *Nat Struct Mol Biol.* (2013) 20:756–62. doi: 10.1038/nsmb.2555
77. Paik WK, Pearson D, Lee HW, Kim S. Nonenzymatic acetylation of histones with acetyl-CoA. *BBA Sect Nucleic Acids Protein Synth.* (1970) 213:513–22. doi: 10.1016/0005-2787(70)90058-4
78. Morris M, Knudsen GM, Maeda S, Trinidad JC, Ioanoviciu A, Burlingame AL, et al. Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice. *Nat Neurosci.* (2015) 18:1183–9. doi: 10.1038/nn.4067
79. Min SW, Chen X, Tracy TE, Li Y, Zhou Y, Wang C, et al. Critical role of acetylation in tau-mediated neurodegeneration and cognitive deficits. *Nat Med.* (2015) 21:1154–62. doi: 10.1038/nm.3951
80. Chen X, Li Y, Wang C, Tang Y, Mok SA, Tsai RM, et al. Promoting tau secretion and propagation by hyperactive p300/CBP via autophagy-lysosomal pathway in tauopathy. *Mol Neurodegener.* (2020) 15:2. doi: 10.1186/s13024-019-0354-0
81. Schueller E, Paiva I, Blanc F, Wang XL, Cassel JC, Boutillier AL, et al. Dysregulation of histone acetylation pathways in hippocampus and frontal cortex of Alzheimer's disease patients. *Eur Neuropsychopharmacol.* (2020) 33:101–16. doi: 10.1016/j.euroneuro.2020.01.015
82. Chatterjee S, Cassel R, Schneider-Anthony A, Merienne K, Cosquer B, Tzeplaeff L, et al. Reinstating plasticity and memory in a tauopathy mouse model with an acetyltransferase activator. *EMBO Mol Med.* (2018) 1**0**:e8587. doi: 10.15252/emmm.201708587
83. Julien C, Tremblay C, Émond V, Lebbadi M, Salem N, Bennett DA, Calon F. Sirtuin 1 reduction parallels the accumulation of tau in alzheimer disease. *J Neuropathol Exp Neurol.* (2009) 68:48–58. doi: 10.1097/NEN.0b013e3181922348
84. Rizzi L, Roriz-Cruz M. Sirtuin 1 and Alzheimer's disease: an up-to-date review. *Neuropeptides.* (2018) 71:54–60. doi: 10.1016/j.npep.2018.07.001
85. Luo Y, Ma B, Nussinov R, Wei G. Structural insight into tau protein's paradox of intrinsically disordered behavior, self-acetylation activity, and aggregation. *J Phys Chem Lett.* (2014) 5:3026–31. doi: 10.1021/jz501457f
86. Irwin DJ, Cohen TJ, Grossman M, Arnold SE, McCarty-Wood E, van Deerlin VM, et al. Acetylated tau neuropathology in sporadic and hereditary tauopathies. *Am J Pathol.* (2013) 183:344–351. doi: 10.1016/j.ajpath.2013.04.025
87. Grinberg LT, Wang X, Wang C, Sohn PD, Theofilas P, Sidhu M, et al. Argyrophilic grain disease differs from other tauopathies by lacking tau acetylation. *Acta Neuropathol.* (2013) 125:581–93. doi: 10.1007/s00401-013-1080-2
88. Yang XJ, Seto E. Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol Cell.* (2008) 31:449–61. doi: 10.1016/j.molcel.2008.07.002
89. Thomas SN, Funk KE, Wan Y, Liao Z, Davies P, Kuret J, et al. Dual modification of Alzheimer's disease PHF-tau protein by lysine methylation and ubiquitylation: a mass spectrometry approach. *Acta Neuropathol.* (2012) 123:105–17. doi: 10.1007/s00401-011-0893-0
90. Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem.* (1998) 67:425–79. doi: 10.1146/annurev.biochem.67.1.425
91. Behrends C, Harper JW. Constructing and decoding unconventional ubiquitin chains. *Nat Struct Mol Biol.* (2011) 18:520–8. doi: 10.1038/nsmb.2066
92. Cappadocia L, Lima CD. Ubiquitin-like protein conjugation: structures, chemistry, and mechanism. *Chem Rev.* (2018) 118:889–918. doi: 10.1021/acs.chemrev.6b00737
93. Ikeda F, Dikic I. Atypical ubiquitin chains: new molecular signals. "Protein modifications: beyond the usual suspects" Review series. *EMBO Rep.* (2008) 9:536–42. doi: 10.1038/embor.2008.93
94. Nathan JA, Tae Kim H, Ting L, Gygi SP, Goldberg AL. Why do cellular proteins linked to K63-polyubiquitin chains not associate with proteasomes? *EMBO J.* (2013) 32:552–65. doi: 10.1038/emboj.2012.354
95. Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science.* (2007) 315:201–5. doi: 10.1126/science.1127085
96. Tan JMM, Wong ESP, Kirkpatrick DS, Pletnikova O, Ko HS, Tay S-P, et al. Lysine 63-linked ubiquitination promotes the formation and autophagic clearance of protein inclusions associated with neurodegenerative diseases. *Hum Mol Genet.* (2008) 17:431–9. doi: 10.1093/hmg/ddm320
97. Munari F, Barracchia CG, Parolini F, Tira R, Bubacco L, Assfalg M, et al. Semisynthetic modification of tau protein with di-ubiquitin chains for aggregation studies. *Int J Mol Sci.* (2020) 21:4400. doi: 10.3390/ijms21124400
98. Harris LD, Jasem S, Licchesi JDF. The ubiquitin system in Alzheimer's disease. *Adv Exp Med Biol.* (2020) 1233:195–221. doi: 10.1007/978-3-030-38266-7_8

99. Zheng N, Shabek N. Ubiquitin ligases: structure, function, and regulation. *Annu Rev Biochem.* (2017) 86:129–57. doi: 10.1146/annurev-biochem-060815-014922
100. Babu JR, Geetha T, Wooten MW. Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. *J Neurochem.* (2005) 94:192–203. doi: 10.1111/j.1471-4159.2005.03181.x
101. Flach K, Ramminger E, Hilbrich I, Arsalan-Werner A, Albrecht F, Herrmann L, et al. Axotrophin/MARCH7 acts as an E3 ubiquitin ligase and ubiquitinates tau protein *in vitro* impairing microtubule binding. *Biochim Biophys Acta - Mol Basis Dis.* (2014) 1842:1527–38. doi: 10.1016/j.bbadis.2014.05.029
102. Petrucelli L, Dickson D, Kehoe K, Taylor J, Snyder H, Grover A, et al. CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. *Hum Mol Genet.* (2004) 13:703–14. doi: 10.1093/hmg/ddh083
103. Shimura H, Schwartz D, Gygi SP, Kosik KS. CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. *J Biol Chem.* (2004) 279:4869–76. doi: 10.1074/jbc.M305838200
104. Zhang YJ, Xu YF, Liu XH, Li D, Yin J, Liu YH, et al. Carboxyl terminus of heat-shock cognate 70-interacting protein degrades tau regardless its phosphorylation status without affecting the spatial memory of the rats. *J Neural Transm.* (2008) 115:483–91. doi: 10.1007/s00702-007-0857-7
105. Moscat J, Diaz-Meco MT, Wooten MW. Signal integration and diversification through the p62 scaffold protein. *Trends Biochem Sci.* (2007) 32:95–100. doi: 10.1016/j.tibs.2006.12.002
106. Seibenhener ML, Babu JR, Geetha T, Wong HC, Krishna NR, Wooten MW. Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. *Mol Cell Biol.* (2004) 24:8055–68. doi: 10.1128/MCB.24.18.8055-8068.2004
107. Seibenhener ML, Geetha T, Wooten MW. Sequestosome 1/p62 - more than just a scaffold. *FEBS Lett.* (2007) 581:175–9. doi: 10.1016/j.febslet.2006.12.027
108. Wang P, Joberty G, Buist A, Vanoosthuyse A, Stancu IC, Vasconcelos B, et al. Tau interactome mapping based identification of Otub1 as tau deubiquitinase involved in accumulation of pathological tau forms *in vitro* and *in vivo*. *Acta Neuropathol.* (2017) 133:731–49. doi: 10.1007/s00401-016-1663-9
109. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Titani K, Ihara Y. Ubiquitin is conjugated with amino-terminally processed tau in paired helical filaments. *Neuron.* (1993) 10:1151–60. doi: 10.1016/0896-6273(93)90063-W
110. Cripps D, Thomas SN, Jeng Y, Yang F, Davies P, Yang AJ. Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. *J Biol Chem.* (2006) 281:10825–38. doi: 10.1074/jbc.M512786200
111. Mori H, Kondo J, Ihara Y. Ubiquitin is a component of paired helical filaments in Alzheimer's disease. *Science.* (1987) 235:1641–4. doi: 10.1126/science.3029875
112. Perry G, Friedman R, Shaw G, Chau V. Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. *Proc Natl Acad Sci USA.* (1987) 84:3033–6. doi: 10.1073/pnas.84.9.3033
113. Riederer IM, Schiffrin M, Kövari E, Bouras C, Riederer BM. Ubiquitination and cysteine nitrosylation during aging and Alzheimer's disease. *Brain Res Bull.* (2009) 80:233–41. doi: 10.1016/j.brainresbull.2009.04.018
114. Bancher C, Grundke-Iqbal I, Iqbal K, Fried VA, Smith HT, Wisniewski HM. Abnormal phosphorylation of tau precedes ubiquitination in neurofibrillary pathology of Alzheimer disease. *Brain Res.* (1991) 539:11–8. doi: 10.1016/0006-8993(91)90681-K
115. García-Sierra F, Jarero-Basulto JJ, Kristofikova Z, Majer E, Binder LI, Ripova D. Ubiquitin is associated with early truncation of tau protein at aspartic acid 421 during the maturation of neurofibrillary tangles in Alzheimer's disease. *Brain Pathol.* (2012) 22:240–50. doi: 10.1111/j.1750-3639.2011.00525.x
116. Iwatsubo T, Hasegawa M, Esaki Y, Ihara Y. Lack of ubiquitin immunoreactivities at both ends of neuropil threads possible bidirectional growth of neuropil threads. *Am J Pathol.* (1992) 140:277–82.
117. Dickey CA, Yue M, Lin WL, Dickson DW, Dunmore JH, Lee WC, et al. Deletion of the ubiquitin ligase CHIP leads to the accumulation, but not the aggregation, of both endogenous phospho- and caspase-3-cleaved tau species. *J Neurosci.* (2006) 26:6985–96. doi: 10.1523/JNEUROSCI.0746-06.2006
118. Desterro JMP, Thomson J, Hay RT. Ubch9 conjugates SUMO but not ubiquitin. *FEBS Lett.* (1997) 417:297–300. doi: 10.1016/S0014-5793(97)01305-7
119. Gong L, Li B, Millas S, Yeh ETH. Molecular cloning and characterization of human AOS1 and UBA2, components of the sentrin-activating enzyme complex. *FEBS Lett.* (1999) 448:185–9. doi: 10.1016/S0014-5793(99)00367-1
120. Sarge KD, Park-Sarge OK. Sumoylation and human disease pathogenesis. *Trends Biochem Sci.* (2009) 34:200–5. doi: 10.1016/j.tibs.2009.01.004
121. Müller S, Ledl A, Schmidt D. SUMO: a regulator of gene expression and genome integrity. *Oncogene.* (2004) 23:1998–2008. doi: 10.1038/sj.onc.1207415
122. Jackson SP, Durocher D. Regulation of DNA damage responses by ubiquitin and SUMO. *Mol Cell.* (2013) 49:795–807. doi: 10.1016/j.molcel.2013.01.017
123. Melchior F, Schergaut M, Pichler A. SUMO: ligases, isopeptidases and nuclear pores. *Trends Biochem Sci.* (2003) 28:612–8. doi: 10.1016/j.tibs.2003.09.002
124. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. *Nat Rev Mol Cell Biol.* (2012) 13:755–66. doi: 10.1038/nrm3478
125. Henley JM, Carmichael RE, Wilkinson KA. Extranuclear SUMOylation in neurons. *Trends Neurosci.* (2018) 41:198–210. doi: 10.1016/j.tins.2018.02.004
126. Pountney DL, Huang Y, Burns RJ, Haan E, Thompson PD, Blumbergs PC, et al. SUMO-1 marks the nuclear inclusions in familial neuronal intranuclear inclusion disease. *Exp Neurol.* (2003) 184:436–46. doi: 10.1016/j.expneurol.2003.07.004
127. Wong MB, Goodwin J, Norazit A, Meedeniya ACB, Richter-Landsberg C, Gai WP, et al. SUMO-1 is associated with a subset of lysosomes in glial protein aggregate diseases. *Neurotox Res.* (2013) 23:1–21. doi: 10.1007/s12640-012-9358-z
128. Luo H Bin, Xia YY, Shu XJ, Liu ZC, Feng Y, Liu XH, et al. SUMOylation at K340 inhibits tau degradation through deregulating its phosphorylation and ubiquitination. *Proc Natl Acad Sci USA.* (2014) 111:16586–91. doi: 10.1073/pnas.1417548111
129. Dorval V, Fraser PE. Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and α-synuclein. *J Biol Chem.* (2006) 281:9919–24. doi: 10.1074/jbc.M510127200
130. Hernandez I, Luna G, Rauch JN, Reis SA, Giroux M, Karch CM, et al. A farnesyltransferase inhibitor activates lysosomes and reduces tau pathology in mice with tauopathy. *Sci Transl Med.* (2019) 11:eaat3005. doi: 10.1126/scitranslmed.aat3005
131. Avery OT, Macleod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types: induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type iii. *J Exp Med.* (1944) 79:137–58.
132. MacCarty M, AVery OT. Studies on the chemical nature of the substance inducing transformation of pneumococcoal types: effect of desoxyribonuclease on the biological activit of the transforming substance. *J Exp Med.* (1946) 83:89–96.
133. Moore LD, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology.* (2013) 38:23–38. doi: 10.1038/npp.2012.112
134. Murn J, Shi Y. The winding path of protein methylation research: milestones and new frontiers. *Nat Rev Mol Cell Biol.* (2017) 18:517–27. doi: 10.1038/nrm.2017.35
135. Serre NBC, Alban C, Bourguignon J, Ravanel S. An outlook on lysine methylation of non-histone proteins in plants. *J Exp Bot.* (2018) 69:4569–81. doi: 10.1093/jxb/ery231
136. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell.* (2004) 119:941–53. doi: 10.1016/j.cell.2004.12.012
137. Lanouette S, Mongeon V, Figeys D, Couture JF. The functional diversity of protein lysine methylation. *Mol Syst Biol.* (2014) 10:724. doi: 10.1002/msb.134974
138. Falnes P, Jakobsson ME, Davydova E, Ho A, Malecki J. Protein lysine methylation by seven-β-strand methyltransferases. *Biochem J.* (2016) 473:1995–2009. doi: 10.1042/BCJ20160117
139. Kontaxi C, Piccardo P, Gill AC. Lysine-directed post-translational modifications of tau protein in Alzheimer's disease and related tauopathies. *Front Mol Biosci.* (2017) 4:56. doi: 10.3389/fmolb.2017.00056

140. Huseby CJ, Hoffman CN, Cooper GL, Cocuron JC, Alonso AP, Thomas SN, et al. Quantification of tau protein lysine methylation in aging and Alzheimer's disease. *J Alzheimer's Dis.* (2019) 71:979–91. doi: 10.3233/JAD-190604
141. Funk KE, Thomas SN, Schafer KN, Cooper GL, Liao Z, Clark DJ, et al. Lysine methylation is an endogenous post-translational modification of tau protein in human brain and a modulator of aggregation propensity. *Biochem J.* (2014) 462:77–88. doi: 10.1042/BJ20140372
142. Sposito T, Preza E, Mahoney CJ, Setó-Salvia N, Ryan NS, Morris HR, et al. Developmental regulation of tau splicing is disrupted in stem cell-derived neurons from frontotemporal dementia patients with the 10 + 16 splice-site mutation in MAPT. *Hum Mol Genet.* (2015) 24:5260–9. doi: 10.1093/hmg/ddv246
143. Shental-Bechor D, Levy Y. Effect of glycosylation on protein folding: a close look at thermodynamic stabilization. *Proc Natl Acad Sci USA.* (2008) 105:8256–61. doi: 10.1073/pnas.0801340105
144. Bell CG, Lowe R, Adams PD, Baccarelli AA, Beck S, Bell JT, et al. DNA methylation aging clocks: challenges and recommendations. *Genome Biol.* (2019) 20:1–24. doi: 10.1186/s13059-019-1824-y
145. Yang X, Qian K. Protein O-GlcNAcylation: emerging mechanisms and functions. *Nat Rev Mol Cell Biol.* (2017) 18:452–65. doi: 10.1038/nrm.2017.22
146. Reily C, Stewart TJ, Renfrow MB, Novak J. Glycosylation in health and disease. *Nat Rev Nephrol.* (2019) 15:346–66. doi: 10.1038/s41581-019-0129-4
147. Kim C-S, Park S, Kim J. The role of glycation in the pathogenesis of aging and its prevention through herbal products and physical exercise. *J Exerc Nutr Biochem.* (2017) 21:55–61. doi: 10.20463/jenb.2017.0027
148. Liu L, Xu YX, Hirschberg CB. The role of nucleotide sugar transporters in development of eukaryotes. *Semin Cell Dev Biol.* (2010) 21:600–8. doi: 10.1016/j.semcdb.2010.02.002
149. Aebi M. N-linked protein glycosylation in the ER. *Biochim Biophys Acta Mol Cell Res.* (2013) 1833:2430–7. doi: 10.1016/j.bbamcr.2013.04.001
150. Sharma A, Vijayan M. Influence of glycosidic linkage on the nature of carbohydrate binding in β-prism I fold lectins: an X-ray and molecular dynamics investigation on banana lectin-carbohydrate complexes. *Glycobiology.* (2010) 21:23–33. doi: 10.1093/glycob/cwq128
151. Spiro RG. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. *Glycobiology.* (2002) 12:43R—56R. doi: 10.1093/glycob/12.4.43R
152. Haltiwanger RS, Kelly WG, Roquemore EP, Blomberg MA, Dong L-YD, Kreppel L, et al. Glycosylation of nuclear and cytoplasmic proteins is ubiquitous and dynamic. *Biochem Soc Trans.* (1992) 20:264–9. doi: 10.1042/bst0200264
153. Mitra N, Sinha S, Ramya TNC, Surolia A. N-linked oligosaccharides as outfitters for glycoprotein folding, form and function. *Trends Biochem Sci.* (2006) 31:156–63. doi: 10.1016/j.tibs.2006.01.003
154. O'Connor SE, Imperiali B. Modulation of protein structure and function by asparagine-linked glycosylation. *Chem Biol.* (1996) 3:803–12. doi: 10.1016/S1074-5521(96)90064-2
155. Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, Gong CX. O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. *Proc Natl Acad Sci USA.* (2004) 101:10804–9. doi: 10.1073/pnas.0400348101
156. Cheng X, Cole RN, Zaia J, Hart GW. Alternative O-glycosylation/O-phosphorylation of the murine estrogen receptor β. *Biochemistry.* (2000) 39:11609–20. doi: 10.1021/bi000755i
157. Comer FI, Hart GW. Reciprocity between O-GlcNAc and O-phosphate on the carboxyl terminal domain of RNA polymerase II. *Biochemistry.* (2001) 40:7845–52. doi: 10.1021/bi0027480
158. Yang WH, Kim JE, Nam HW, Ju JW, Kim HS, Kim YS, et al. Modification of p53 with O-linked N-acetylglucosamine regulates p53 activity and stability. *Nat Cell Biol.* (2006) 8:1074–83. doi: 10.1038/ncb1470
159. Tarrant MK, Rho HS, Xie Z, Jiang YL, Gross C, Culhane JC, et al. Regulation of CK2 by phosphorylation and O-GlcNAcylation revealed by semisynthesis. *Nat Chem Biol.* (2012) 8:262–9. doi: 10.1038/nchembio.771
160. Cole SL, Vassar R. The Alzheimer's disease β-secretase enzyme, BACE1. *Mol Neurodegener.* (2007) 2:22. doi: 10.1186/1750-1326-2-22
161. Kizuka Y, Kitazume S, Fujinawa R, Saito T, Iwata N, Saido TC, et al. An aberrant sugar modification of BACE 1 blocks its lysosomal targeting in A lzheimer's disease. *EMBO Mol Med.* (2015) 7:175–89. doi: 10.15252/emmm.201404438
162. Chun YS, Kwon OH, Chung S. O-GlcNAcylation of amyloid-β precursor protein at threonine 576 residue regulates trafficking and processing. *Biochem Biophys Res Commun.* (2017) 490:486–91. doi: 10.1016/j.bbrc.2017.06.067
163. Palmigiano A, Barone R, Sturiale L, Sanfilippo C, Bua RO, Romeo DA, et al. CSF N-glycoproteomics for early diagnosis in Alzheimer's disease. *J Proteomics.* (2016) 131:29–37. doi: 10.1016/j.jprot.2015.10.006
164. Kizuka Y, Kitazume S, Taniguchi N. N-glycan and Alzheimer's disease. *Biochim Biophys Acta Gen Subj.* (2017) 1861:2447–54. doi: 10.1016/j.bbagen.2017.04.012
165. Gong C-X, Liu F, Iqbal K. O-GlcNAcylation: a regulator of tau pathology and neurodegeneration. *Alzheimer's Dement.* (2016) 12:1078–89. doi: 10.1016/j.jalz.2016.02.011
166. Shane Arnold C, Johnson GVW, Cole RN, Dong DLY, Lee M, Hart GW. The microtubule-associated protein tau is extensively modified with O- linked N-acetylglucosamine. *J Biol Chem.* (1996) 271:28741–4. doi: 10.1074/jbc.271.46.28741
167. Wang JZ, Grundke-Iqbal I, Iqbal K. Glycosylation of microtubule-associated protein tau: an abnormal posttranslational modification in Alzheimer's disease. *Nat Med.* (1996) 2:871–5. doi: 10.1038/nm0896-871
168. Sato Y, Naito Y, Grundke-Iqbal I, Iqbal K, Endo T. Analysis of N-glycans of pathological tau: Possible occurrence of aberrant processing of tau in Alzheimer's disease. *FEBS Lett.* (2001) 496:152–60. doi: 10.1016/S0014-5793(01)02421-8
169. Liu F, Zaidi T, Iqbal K, Grundke-Iqbal I, Merkle RK, Gong CX. Role of glycosylation in hyperphosphorylation of tau in Alzheimer's disease. *FEBS Lett.* (2002) 512:101–6. doi: 10.1016/S0014-5793(02)02228-7
170. Robertson LA, Moya KL, Breen KC. The potential role of tau protein O-glycosylation in Alzheimer's disease. *J Alzheimer's Dis.* (2004) 6:489–95. doi: 10.3233/JAD-2004-6505
171. Yuzwa SA, Shan X, MacAuley MS, Clark T, Skorobogatko Y, Vosseller K, et al. Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. *Nat Chem Biol.* (2012) 8:393–9. doi: 10.1038/nchembio.797
172. Yuzwa SA, Yadav AK, Skorobogatko Y, Clark T, Vosseller K, Vocadlo DJ. Mapping O-GlcNAc modification sites on tau and generation of a site-specific O-GlcNAc tau antibody. *Amino Acids.* (2011) 40:857–68. doi: 10.1007/s00726-010-0705-1
173. Smet-Nocca C, Broncel M, Wieruszeski JM, Tokarski C, Hanoulle X, Leroy A, et al. Identification of O-GlcNAc sites within peptides of the tau protein and their impact on phosphorylation. *Mol Biosyst.* (2011) 7:1420–9. doi: 10.1039/c0mb00337a
174. Lefebvre T, Ferreira S, Dupont-Wallois L, Bussière T, Dupire MJ, Delacourte A, et al. Evidence of a balance between phosphorylation and O-GlcNAc glycosylation of tau proteins - a role in nuclear localization. *Biochim Biophys Acta Gen Subj.* (2003) 1619:167–76. doi: 10.1016/S0304-4165(02)00477-4
175. Yuzwa SA, Cheung AH, Okon M, McIntosh LP, Vocadlo DJ. O-GlcNAc modification of tau directly inhibits its aggregation without perturbing the conformational properties of tau monomers. *J Mol Biol.* (2014) 426:1736–52. doi: 10.1016/j.jmb.2014.01.004
176. Yu CH, Si T, Wu WH, Hu J, Du JT, Zhao YF, et al. O-GlcNAcylation modulates the self-aggregation ability of the fourth microtubule-binding repeat of tau. *Biochem Biophys Res Commun.* (2008) 375:59–62. doi: 10.1016/j.bbrc.2008.07.101
177. Schedin-Weiss S, Winblad B, Tjernberg LO. The role of protein glycosylation in Alzheimer disease. *FEBS J.* (2014) 281:46–62. doi: 10.1111/febs.12590
178. Liu F, Zaidi T, Iqbal K, Grundke-Iqbal I, Gong CX. Aberrant glycosylation modulates phosphorylation of tau by protein kinase A and dephosphorylation of tau by protein phosphatase 2A and 5. *Neuroscience.* (2002) 115:829–37. doi: 10.1016/S0306-4522(02)00510-9
179. Cho S-J, Roman G, Yeboah F, Konishi Y. The road to advanced glycation end products: a mechanistic perspective. *Curr Med Chem.* (2007) 14:1653–71. doi: 10.2174/092986707780830989

180. Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, et al. Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. *Am J Pathol.* (1998) 153:1149–55. doi: 10.1016/S0002-9440(10)65659-3
181. Yamagishi SI, Nakamura N, Matsui T. Glycation and cardiovascular disease in diabetes: a perspective on the concept of metabolic memory. *J Diabetes.* (2017) 9:141–8. doi: 10.1111/1753-0407.12475
182. Simm A, Müller B, Nass N, Hofmann B, Bushnaq H, Silber RE, et al. Protein glycation - between tissue aging and protection. *Exp Gerontol.* (2015) 68:71–5. doi: 10.1016/j.exger.2014.12.013
183. Luth H-JL, Ogunlade V, Kuhla BB, Kientsch-Engel R, Stahl P, Webster J, et al. Age-and stage-dependent accumulation of advanced glycation end products in intracellular deposits in normal and Alzheimer's disease brains. *Cereb Cortex.* (2005) 15:211–20. doi: 10.1093/cercor/bhh123
184. Chaudhuri J, Bains Y, Guha S, Kahn A, Hall D, Bose N, et al. The role of advanced glycation end products in aging and metabolic diseases: bridging association and causality. *Cell Metab.* (2018) 28:337–52. doi: 10.1016/j.cmet.2018.08.014
185. Ramasamy R, Vannucci SJ, Yan SS Du, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology.* (2005) 15:16R−28R. doi: 10.1093/glycob/cwi053
186. Nacharaju P, Ko L, Yen S-HC. Characterization of *in vitro* glycation sites of tau. *J Neurochem.* (1997) 69:1709–19. doi: 10.1046/j.1471-4159.1997.69041709.x
187. Liu K, Liu Y, Li L, Qin P, Iqbal J, Deng Y, et al. Glycation alter the process of tau phosphorylation to change tau isoforms aggregation property. *Biochim Biophys Acta Mol Basis Dis.* (2016) 1862:192–201. doi: 10.1016/j.bbadis.2015.12.002
188. Ko LW, Ko EC, Nacharaju P, Liu WK, Chang E, Kenessey A, et al. An immunochemical study on tau glycation in paired helical filaments. *Brain Res.* (1999) 830:301–13. doi: 10.1016/S0006-8993(99)01415-8
189. Ledesma MD, Medina M, Avila J. The *in vitro* formation of recombinant τ polymers - effect of phosphorylation and glycation. *Mol Chem Neuropathol.* (1996) 27:249–58. doi: 10.1007/BF02815107
190. Smith MA, Siedlak SL, Richey PL, Nagaraj RH, Elhammer A, Perry G. Quantitative solubilization and analysis of insoluble paired helical filaments from Alzheimer disease. *Brain Res.* (1996) 717:99–108. doi: 10.1016/0006-8993(95)01473-X
191. Yan SD, Chen X, Schmidt AM, Brett J, Godman G, Zou YS, et al. Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. *Proc Natl Acad Sci USA.* (1994) 91:7787–91. doi: 10.1073/pnas.91.16.7787
192. Quinn JP, Corbett NJ, Kellett KAB, Hooper NM. Tau proteolysis in the pathogenesis of tauopathies: neurotoxic fragments and novel biomarkers. *J Alzheimer's Dis.* (2018) 63:13–33. doi: 10.3233/JAD-170959
193. van Opdenbosch N, Lamkanfi M. Caspases in cell death, inflammation, and disease. *Immunity.* (2019) 50:1352–64. doi: 10.1016/j.immuni.2019.05.020
194. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. *Cold Spring Harb Perspect Biol.* (2013) 5:ea008656. doi: 10.1101/cshperspect.a008656
195. Chesser AS, Pritchard SM, Johnson GVW. Tau clearance mechanisms and their possible role in the pathogenesis of Alzheimer disease. *Front Neurol.* (2013) 4:122. doi: 10.3389/fneur.2013.00122
196. Fasulo L, Visintin M, Novak M, Cattaneo A. Tau truncation in Alzheimer's disease: expression of a fragment encompassing PHF core. *Alzheimer's Rep.* (1998) 1:25–32.
197. Novák M. Truncated tau protein as a new marker for Alzheimer's disease. *Acta Virol.* (1994) 38:173–89.
198. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, et al. Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci USA.* (2003) 100:10032–7. doi: 10.1073/pnas.1630428100
199. Paholikova K, Salingova B, Opattova A, Skrabana R, Majerova P, Zilka N, et al. N-terminal truncation of microtubule associated protein tau dysregulates its cellular localization. *J Alzheimer's Dis.* (2015) 43:915–26. doi: 10.3233/JAD-140996
200. Corsetti V, Florenzano F, Atlante A, Bobba A, Ciotti MT, Natale F, et al. NH 2-truncated human tau induces deregulated mitophagy in neurons by aberrant recruitment of Parkin and UCHL-1: implications in Alzheimer's disease. *Hum Mol Genet.* (2015) 24:3058–81. doi: 10.1093/hmg/ddv059
201. Chung CW, Song YH, Kim IK, Yoon WJ, Ryu BR, Jo DG, et al. Proapoptotic effects of tau cleavage product generated by caspase-3. *Neurobiol Dis.* (2001) 8:162–72. doi: 10.1006/nbdi.2000.0335
202. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest.* (2004) 114:121–30. doi: 10.1172/JCI200420640
203. Rohn TT, Rissman RA, Head E, Cotman CW. Caspase activation in the Alzheimer's disease brain: tortuous and torturous. *Drug News Perspect.* (2002) 15:549–57. doi: 10.1358/dnp.2002.15.9.740233
204. Su JH, Zhao M, Anderson AJ, Srinivasan A, Cotman CW. Activated caspase-3 expression in Alzheimer's and aged control brain: correlation with Alzheimer pathology. *Brain Res.* (2001) 898:350–7. doi: 10.1016/S0006-8993(01)02018-2
205. Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. *Am J Pathol.* (2004) 165:523–31. doi: 10.1016/S0002-9440(10)63317-2
206. Matthews-Roberson TA, Quintanilla RA, Ding H, Johnson GVW. Immortalized cortical neurons expressing caspase-cleaved tau are sensitized to endoplasmic reticulum stress induced cell death. *Brain Res.* (2008) 1234:206–12. doi: 10.1016/j.brainres.2008.07.111
207. Quintanilla RA, Matthews-Roberson TA, Dolan PJ, Johnsion GVW. Caspase-cleaved tau expression induces mitochondrial dysfunction in immortalized cortical neurons: implications for the pathogenesis of alzheimer disease. *J Biol Chem.* (2009) 284:18754–66. doi: 10.1074/jbc.M808908200
208. De Calignon A, Fox LM, Pitstick R, Carlson GA, Bacskai BJ, Spires-Jones TL, et al. Caspase activation precedes and leads to tangles. *Nature.* (2010) 464:1201–4. doi: 10.1038/nature08890
209. Suzuki K, Hata S, Kawabata Y, Sorimachi H. Structure, activation, and biology of calpain. *Diabetes.* (2004). 53(Suppl.):S12–8. doi: 10.2337/diabetes.53.2007.S12
210. Park SY, Ferreira A. The generation of a 17 kDa neurotoxic fragment: an alternative mechanism by which tau mediates β-amyloid-induced neurodegeneration. *J Neurosci.* (2005) 25:5365–75. doi: 10.1523/JNEUROSCI.1125-05.2005
211. Liu MC, Kobeissy F, Zheng W, Zhang Z, Hayes RL, Wang KKW. Dual vulnerability of tau to calpains and caspase-3 proteolysis under neurotoxic and neurodegenerative conditions. *ASN Neuro.* (2011) 3:25–36. doi: 10.1042/AN20100012
212. Chen HH, Liu P, Auger P, Lee SH, Adolfsson O, Rey-Bellet L, et al. Calpain-mediated tau fragmentation is altered in Alzheimer's disease progression. *Sci Rep.* (2018) 8:16725. doi: 10.1038/s41598-018-35130-y
213. Reinecke JB, deVos SL, McGrath JP, Shepard AM, Goncharoff DK, Tait DN, et al. Implicating calpain in tau-mediated toxicity *in vivo*. *PLoS ONE.* (2011) 6:e23865. doi: 10.1371/journal.pone.0023865
214. Olesen OF. Proteolytic degradation of microtubule-associated protein τ by thrombin. *Biochem Biophys Res Commun.* (1994) 201:716–21. doi: 10.1006/bbrc.1994.1759
215. Khlistunova I, Biernat J, Wang Y, Pickhardt M, Von Bergen M, Gazova Z, et al. Inducible expression of tau repeat domain in cell models of tauopathy: Aggregation is toxic to cells but can be reversed by inhibitor drugs. *J Biol Chem.* (2006) 281:1205–14. doi: 10.1074/jbc.M507753200
216. Arai T, Guo JP, McGeer PL. Proteolysis of non-phosphorylated and phosphorylated tau by thrombin. *J Biol Chem.* (2005) 280:5145–53. doi: 10.1074/jbc.M409234200
217. Suo Z, Wu M, Citron BA, Palazzo RE, Festoff BW. Rapid tau aggregation and delayed hippocampal neuronal death induced by persistent thrombin signaling. *J Biol Chem.* (2003) 278:37681–9. doi: 10.1074/jbc.M301406200
218. Schönichen A, Webb BA, Jacobson MP, Barber DL. Considering protonation as a posttranslational modification regulating protein structure and function. *Annu Rev Biophys.* (2013) 42:289–314. doi: 10.1146/annurev-biophys-050511-102349
219. Harguindey S, Reshkin S, Orive G, Luis Arranz J, Anitua E. Growth and trophic factors, pH and the Na+/H+ exchanger in Alzheimers

disease, other neurodegenerative diseases and cancer: new therapeutic possibilities and potential dangers. *Curr Alzheimer Res.* (2007) 4:53–65. doi: 10.2174/156720507779939841
220. Syntichaki P, Samara C, Tavernarakis N. The vacuolar H+-ATPase mediates intracellular acidification required for neurodegeneration in *C. elegans*. *Curr Biol.* (2005) 15:1249–54. doi: 10.1016/j.cub.2005.05.057
221. Charafeddine RA, Cortopassi WA, Lak P, Tan R, McKenney RJ, Jacobson MP, et al. Tau repeat regions contain conserved histidine residues that modulate microtubule-binding in response to changes in pH downloaded from. *J Biol Chem.* (2019) 294:8779–90. doi: 10.1074/jbc.RA118.007004
222. Chung HS, Wang SB, Venkatraman V, Murray CI, van Eyk JE. Cysteine oxidative posttranslational modifications: emerging regulation in the cardiovascular system. *Circ Res.* (2013) 112:382–92. doi: 10.1161/CIRCRESAHA.112.268680
223. Reeg S, Grune T. Protein oxidation in aging: does it play a role in aging progression? *Antioxidants Redox Signal.* (2015) 23:239–55. doi: 10.1089/ars.2014.6062
224. Schweers O, Mandelkow EM, Biernat J, Mandelkow E. Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein τ controls the *in vitro* assembly of paired helical filaments. *Proc Natl Acad Sci USA.* (1995) 92:8463–7. doi: 10.1073/pnas.92.18.8463
225. Reynolds MR, Berry RW, Binder LI. Nitration in neurodegeneration: deciphering the "hows" "nYs." *Biochemistry.* (2007) 46:7325–36. doi: 10.1021/bi700430y
226. Tedeschi G, Cappelletti G, Nonnis S, Taverna F, Negri A, Ronchi C, et al. Tyrosine nitration is a novel post-translational modification occurring on the neural intermediate filament protein peripherin. *Neurochem Res.* (2007) 32:433–41. doi: 10.1007/s11064-006-9244-2
227. Radi R. Protein tyrosine nitration: biochemical mechanisms and structural basis of functional effects. *Acc Chem Res.* (2013) 46:550–9. doi: 10.1021/ar300234c
228. Reyes JF, Fu Y, Vana L, Kanaan NM, Binder LI. Tyrosine nitration within the proline-rich region of tau in Alzheimer's disease. *Am J Pathol.* (2011) 178:2275–85. doi: 10.1016/j.ajpath.2011.01.030
229. Reyes JF, Geula C, Vana L, Binder LI. Selective tau tyrosine nitration in non-AD tauopathies. *Acta Neuropathol.* (2012) 123:119–32. doi: 10.1007/s00401-011-0898-8
230. Reyes JF, Reynolds MR, Horowitz PM, Fu Y, Guillozet-Bongaarts AL, Berry R, et al. A possible link between astrocyte activation and tau nitration in Alzheimer's disease. *Neurobiol Dis.* (2008) 31:198–208. doi: 10.1016/j.nbd.2008.04.005
231. Hipp MS, Kasturi P, Hartl FU. The proteostasis network and its decline in ageing. *Nat Rev Mol Cell Biol.* (2019) 20:421–35. doi: 10.1038/s41580-019-0101-y
232. Aoki K, Yoshida K. "Biological Consequences of Priming Phosphorylation in Cancer Development," in *Protein Phosphorylation* (InTech).
233. Butler VJ, Salazar DA, Soriano-Castell D, Alves-Ferreira M, Dennissen FJA, Vohra M, et al. Tau/MAPT disease-associated variant A152T alters tau function and toxicity via impaired retrograde axonal transport. *Hum Mol Genet.* (2019) 28:1498–514. doi: 10.1093/hmg/ddy442
234. Kirchner P, Bourdenx M, Madrigal-Matute J, Tiano S, Diaz A, Bartholdy BA, et al. Proteome-wide analysis of chaperone-mediated autophagy targeting motifs. *PLoS Biol.* (2019) 17:e3000301. doi: 10.1371/journal.pbio.3000301
235. Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. *J Cell Biol.* (2009) 187:1083–1099. doi: 10.1083/jcb.200909067
236. Lv L, Li D, Zhao D, Lin R, Chu Y, Zhang H, et al. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. *Mol Cell.* (2011) 42:719–30. doi: 10.1016/j.molcel.2011.04.025
237. Jung T, Catalgol B, Grune T. The proteasomal system. *Mol Aspects Med.* (2009) 30:191–296. doi: 10.1016/j.mam.2009.04.001
238. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev.* (2002) 82:373–428. doi: 10.1152/physrev.00027.2001
239. Murata S, Yashiroda H, Tanaka K. Molecular mechanisms of proteasome assembly. *Nat Rev Mol Cell Biol.* (2009) 10:104–15. doi: 10.1038/nrm2630
240. Coux O, Tanaka K, Goldberg AL. Structure and Functions of the 20S and 26S proteasomes. *Annu Rev Biochem.* (1996) 65:801–47. doi: 10.1146/annurev.bi.65.070196.004101
241. Jariel-Encontre I, Bossis G, Piechaczyk M. Ubiquitin-independent degradation of proteins by the proteasome. *BBA Rev Cancer.* (2008) 1786:153–77. doi: 10.1016/j.bbcan.2008.05.004
242. Wang Y, Mandelkow E. Degradation of tau protein by autophagy and proteasomal pathways. *Biochem Soc Trans.* (2012) 40:644–52. doi: 10.1042/BST20120071
243. Dickey CA, Kamal A, Lundgren K, Klosak N, Bailey RM, Dunmore J, et al. The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. *J Clin Invest.* (2007) 117:648–58. doi: 10.1172/JCI29715
244. Holt LJ. Regulatory modules: Coupling protein stability to phopshoregulation during cell division. *FEBS Lett.* (2012) 586:2773–7. doi: 10.1016/j.febslet.2012.05.045
245. Engmann O, Giese KP. Crosstalk between Cdk5 and GSK3β: implications for Alzheimer's disease. *Front Mol Neurosci.* (2009) 2:9. doi: 10.3389/neuro.02.002.2009
246. Keck S, Nitsch R, Grune T, Ullrich O. Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. *J Neurochem.* (2003) 85:115–22. doi: 10.1046/j.1471-4159.2003.01642.x
247. Keller JN, Hanni KB, Markesbery WR. Impaired proteasome function in Alzheimer's disease. *J Neurochem.* (2000) 75:436–9. doi: 10.1046/j.1471-4159.2000.0750436.x
248. López Salon M, Morelli L, Castaño EM, Soto EF, Pasquini JM. Defective ubiquitination of cerebral proteins in Alzheimer's disease. *J Neurosci Res.* (2000) 62:302–10. doi: 10.1002/1097-4547(20001015)62:2<302::AID-JNR15>3.0.CO;2-L
249. Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science.* (2001) 292:1552–5. doi: 10.1126/science.292.5521.1552
250. Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. *Nature.* (2003) 426:895–9. doi: 10.1038/nature02263
251. Caballero B, Wang Y, Diaz A, Tasset I, Juste YR, Stiller B, et al. Interplay of pathogenic forms of human tau with different autophagic pathways. *Aging Cell.* (2018) 17:e12692. doi: 10.1111/acel.12692
252. Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, et al. Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. *Hum Mol Genet.* (2009) 18:4153–70. doi: 10.1093/hmg/ddp367
253. Hamano T, Gendron TF, Causevic E, Yen SH, Lin WL, Isidoro C, et al. Autophagic-lysosomal perturbation enhances tau aggregation in transfectants with induced wild-type tau expression. *Eur J Neurosci.* (2008) 27:1119–30. doi: 10.1111/j.1460-9568.2008.06084.x
254. Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, et al. Synergy and antagonism of macroautophagy and chaperone-mediated autophagy in a cell model of pathological tau aggregation. *Autophagy.* (2010) 6:182–3. doi: 10.4161/auto.6.1.10815
255. Kim SI, Lee WK, Kang SS, Lee SY, Jeong MJ, Lee HJ, et al. Suppression of autophagy and activation of glycogen synthase kinase 3beta facilitate the aggregate formation of tau. *Korean J Physiol Pharmacol.* (2011) 15:107–14. doi: 10.4196/kjpp.2011.15.2.107
256. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, et al. Microautophagy of cytosolic proteins by late endosomes. *Dev Cell.* (2011) 20:131–9. doi: 10.1016/j.devcel.2010.12.003
257. Gorantla NV, Chinnathambi S. Autophagic pathways to clear the tau aggregates in Alzheimer's disease. *Cell Mol Neurobiol.* (2020). doi: 10.1007/s10571-020-00897-0. [Epub ahead of print].
258. Vaz-Silva J, Gomes P, Jin Q, Zhu M, Zhuravleva V, Quintremil S, et al. Endolysosomal degradation of tau and its role in glucocorticoid-driven hippocampal malfunction. *EMBO J.* (2018) 37:e99084. doi: 10.15252/embj.201899084
259. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. *Cell Death Differ.* (2005) 12:1542–52. doi: 10.1038/sj.cdd.4401765
260. Dolan PJ, Johnson GVW. A caspase cleaved form of tau is preferentially degraded through the autophagy pathway. *J Biol Chem.* (2010) 285:21978–87. doi: 10.1074/jbc.M110.110940

261. Rohn TT, Wirawan E, Brown RJ, Harris JR, Masliah E, Vandenabeele P. Depletion of Beclin-1 due to proteolytic cleavage by caspases in the Alzheimer's disease brain. *Neurobiol Dis.* (2011) 43:68–78. doi: 10.1016/j.nbd.2010.11.003
262. Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, et al. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J Neuropathol Exp Neurol.* (2005) 64:113–22. doi: 10.1093/jnen/64.2.113
263. Piras A, Collin L, Grüninger F, Graff C, Rönnbäck A. Autophagic and lysosomal defects in human tauopathies: analysis of post-mortem brain from patients with familial Alzheimer disease, corticobasal degeneration and progressive supranuclear palsy. *Acta Neuropathol Commun.* (2016) 4:22. doi: 10.1186/s40478-016-0292-9
264. Caccamo A, Magrì A, Medina DX, Wisely E V, López-Aranda MF, Silva AJ, et al. mTOR regulates tau phosphorylation and degradation: implications for Alzheimer's disease and other tauopathies. *Aging Cell.* (2013) 12:370–80. doi: 10.1111/acel.12057
265. Ikeda K, Akiyama H, Arai T, Kondo H, Haga C, Tsuchiya K, et al. Neurons containing Alz-50-immunoreactive granules around the cerebral infarction: evidence for the lysosomal degradation of altered tau in human brain? *Neurosci Lett.* (2000) 284:187–9. doi: 10.1016/S0304-3940(00)01009-0
266. Ikeda K, Akiyama H, Arai T, Kondo H, Haga C, Iritani S, et al. Alz-50/Gallyas-positive lysosome-like intraneuronal granules in Alzheimer's disease and control brains. *Neurosci Lett.* (1998) 258:113–6. doi: 10.1016/S0304-3940(98)00867-2
267. D'Agostino C, Nogalska A, Cacciottolo M, King Engel W, Askanas V. Abnormalities of NBR1, a novel autophagy-associated protein, in muscle fibers of sporadic inclusion-body myositis. *Acta Neuropathol.* (2011) 122:627–36. doi: 10.1007/s00401-011-0874-3
268. Majid T, Ali YO, Venkitaramani DV, Jang MK, Lu HC, Pautler RG. *in vivo* axonal transport deficits in a mouse model of fronto-temporal dementia. *NeuroImage Clin.* (2014) 4:711–7. doi: 10.1016/j.nicl.2014.02.005
269. Butzlaff M, Hannan SB, Karsten P, Lenz S, Ng J, Voßfeldt H, et al. Impaired retrograde transport by the dynein/dynactin complex contributes to tau-induced toxicity. *Hum Mol Genet.* (2015) 24:3623–37. doi: 10.1093/hmg/ddv107
270. Young ZT, Mok SA, Gestwicki JE. Therapeutic strategies for restoring tau homeostasis. *Cold Spring Harb Perspect Med.* (2018) 8:ea024612. doi: 10.1101/cshperspect.a024612
271. Schweers O, Schonbrunn-Hanebeck E, Man A, Mandelkows E. Structural studies of tau protein and Alzheimer paired helical filaments show no evidence for beta-structure. *J Biol Chem.* (1994) 269:24290–7.
272. Mukrasch MD, Bibow S, Korukottu J, Jeganathan S, Biernat J, Griesinger C, et al. Structural polymorphism of 441-residue tau at single residue resolution. *PLoS Biol.* (2009) 7:e1000034. doi: 10.1371/journal.pbio.1000034
273. Jeganathan S, Von Bergen M, Brutlach H, Steinhoff HJ, Mandelkow E. Global hairpin folding of tau in solution. *Biochemistry.* (2006) 45:2283–93. doi: 10.1021/bi0521543
274. Dobson CM. Protein misfolding, evolution and disease. *Trends Biochem Sci.* (1999) 24:329–32. doi: 10.1016/S0968-0004(99)01445-0
275. Dobson CM. Protein folding and misfolding. *Nature.* (2003) 426:884–90. doi: 10.1038/nature02261
276. Knowles TPJ, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. *Nat Rev Mol Cell Biol.* (2014) 15:384–96. doi: 10.1038/nrm3810
277. Chiti F, Dobson CM. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. *Annu Rev Biochem.* (2017) 86:27–68. doi: 10.1146/annurev-biochem-061516-045115
278. Fitzpatrick AW, Saibil HR. Cryo-EM of amyloid fibrils and cellular aggregates. *Curr Opin Struct Biol.* (2019) 58:34–42. doi: 10.1016/j.sbi.2019.05.003
279. Scheres SH, Zhang W, Falcon B, Goedert M. Cryo-EM structures of tau filaments. *Curr Opin Struct Biol.* (2020) 64:17–25. doi: 10.1016/j.sbi.2020.05.011
280. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature.* (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y
281. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature.* (2017) 547:185–90. doi: 10.1038/nature23002
282. Arakhamia T, Lee CE, Carlomagno Y, Duong DM, Kundinger SR, Wang K, et al. Posttranslational modifications mediate the structural diversity of tauopathy strains. *Cell.* (2020) 180:633–44.e12. doi: 10.1016/j.cell.2020.01.027
283. Falcon B, Zivanov J, Zhang W, Murzin AG, Garringer HJ, Vidal R, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature.* (2019) 568:420–3. doi: 10.1038/s41586-019-1026-5
284. Zhang W, Tarutani A, Newell KL, Murzin AG, Matsubara T, Falcon B, et al. Novel tau filament fold in corticobasal degeneration. *Nature.* (2020) 580:283–7. doi: 10.1038/s41586-020-2043-0
285. Fontaine SN, Sabbagh JJ, Baker J, Martinez-Licha CR, Darling A, Dickey CA. Cellular factors modulating the mechanism of tau protein aggregation. *Cell Mol Life Sci.* (2015) 72:1863–79. doi: 10.1007/s00018-015-1839-9
286. Wille H, Drewes G, Biernat J, Mandelkow EM, Mandelkow E. Alzheimer-like paired helical filaments and antiparallel dimers formed from microtubule-associated protein tau *in vitro*. *J Cell Biol.* (1992) 118:573–84. doi: 10.1083/jcb.118.3.573
287. Crowther RA, Olesen OF, Smith MJ, Jakes R, Goedert M. Assembly of Alzheimer-like filaments from full-length tau protein. *FEMS Lett.* (1994) 337:135–8. doi: 10.1016/0014-5793(94)80260-2
288. Wilson DM, Binder LI. Polymerization of microtubule-associated protein tau under near-physiological conditions. *J Biol Chem.* (1995) 270:24306–14. doi: 10.1074/jbc.270.41.24306
289. Reynolds MR, Reyes JF, Fu Y, Bigio EH, Guillozet-Bongaarts AL, Berry RW, et al. Tau nitration occurs at tyrosine 29 in the fibrillar lesions of Alzheimer's disease and other tauopathies. *J Neurosci.* (2006) 26:10636–45. doi: 10.1523/JNEUROSCI.2143-06.2006
290. Trzeciakiewicz H, Tseng JH, Wander CM, Madden V, Tripathy A, Yuan CX, et al. A dual pathogenic mechanism links tau acetylation to sporadic tauopathy. *Sci Rep.* (2017) 7:44102. doi: 10.1038/srep44102
291. Avila J. Tau aggregation into fibrillar polymers: taupathies. *FEBS Lett.* (2000) 476:89–92. doi: 10.1016/S0014-5793(00)01676-8
292. Sinha S, Lopes DHJ, Du Z, Pang ES, Shanmugam A, Lomakin A, et al. Lysine-specific molecular tweezers are broad-spectrum inhibitors of assembly and toxicity of amyloid proteins. *J Am Chem Soc.* (2011) 133:16958–69. doi: 10.1021/ja206279b
293. West LE, Gozani O. Regulation of p53 function by lysine methylation. *Epigenomics.* (2011) 3:361–9. doi: 10.2217/epi.11.21
294. Bhattacharya K, Rank KB, Evans DB, Sharma SK. Role of cysteine-291 and cysteine-322 in the polymerization of human tau into Alzheimer-like filaments. *Biochem Biophys Res Commun.* (2001) 285:20–6. doi: 10.1006/bbrc.2001.5116
295. Necula M, Kuret J. Pseudophosphorylation and glycation of tau protein enhance but do not trigger fibrillization *in vitro*. *J Biol Chem.* (2004) 279:49694–703. doi: 10.1074/jbc.M405527200
296. Brister MA, Pandey AK, Bielska AA, Zondlo NJ. OGlcNAcylation and phosphorylation have opposing structural effects in tau: phosphothreonine induces particular conformational order. *J Am Chem Soc.* (2014) 136:3803–16. doi: 10.1021/ja407156m
297. Lothrop AP, Torres MP, Fuchs SM. Deciphering post-translational modification codes. *FEBS Lett.* (2013) 587:1247–57. doi: 10.1016/j.febslet.2013.01.047
298. Li X, Lu F, Wang J-Z, Gong C-X. Concurrent alterations of *O* -GlcNAcylation and phosphorylation of tau in mouse brains during fasting. *Eur J Neurosci.* (2006) 23:2078–86. doi: 10.1111/j.1460-9568.2006.04735.x
299. Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. *Acta Neuropathol.* (2017) 133:665–704. doi: 10.1007/s00401-017-1707-9
300. Guillozet-Bongaarts AL, Cahill ME, Cryns VL, Reynolds MR, Berry RW, Binder LI. Pseudophosphorylation of tau at serine 422 inhibits caspase cleavage: *in vitro* evidence and implications for tangle formation *in vivo*. *J Neurochem.* (2006) 97:1005–14. doi: 10.1111/j.1471-4159.2006.03784.x
301. Kimura T, Sharma G, Ishiguro K, Hisanaga S. Phospho-tau bar code: analysis of phosphoisotypes of tau and its application to tauopathy. *Front Neurosci.* (2018) 12:44. doi: 10.3389/fnins.2018.00044

302. Mair W, Muntel J, Tepper K, Tang S, Biernat J, Seeley WW, et al. FLEXITau: quantifying post-translational modifications of tau protein *in vitro* and in human disease. *Anal Chem.* (2016) 88:3704–14. doi: 10.1021/acs.analchem.5b04509
303. Hanger DP, Byers HL, Wray S, Leung KY, Saxton MJ, Seereeram A, et al. Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis. *J Biol Chem.* (2007) 282:23645–54. doi: 10.1074/jbc.M703269200
304. Dammer EB, Lee AK, Duong DM, Gearing M, Lah JJ, Levey AI, et al. Quantitative phosphoproteomics of Alzheimer's disease reveals cross-talk between kinases and small heat shock proteins. *Proteomics.* (2015) 15:508–19. doi: 10.1002/pmic.201400189
305. Kang MJ, Kim C, Jeong H, Cho BK, Ryou AL, Hwang D, et al. Synapsin-1 and tau reciprocal O-GlcNAcylation and phosphorylation sites in mouse brain synaptosomes. *Exp Mol Med.* (2013) 45:e29. doi: 10.1038/emm.2013.56
306. Hanger DP, Betts JC, Loviny TLF, Blackstock WP, Anderton BH. New phosphorylation sites identified in hyperphosphorylated tau (Paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry. *J Neurochem.* (2002) 71:2465–76. doi: 10.1046/j.1471-4159.1998.71062465.x
307. Doll S, Burlingame AL. Mass spectrometry-based detection and assignment of protein posttranslational modifications. *ACS Chem Biol.* (2015) 10:63–71. doi: 10.1021/cb500904b
308. Theillet FX, Smet-Nocca C, Liokatis S, Thongwichian R, Kosten J, Yoon MK, et al. Cell signaling, post-translational protein modifications and NMR spectroscopy. *J Biomol NMR.* (2012) 54:217–36. doi: 10.1007/s10858-012-9674-x
309. Singh S, Springer M, Steen J, Kirschner MW, Steen H. FLEXIQuant: a novel tool for the absolute quantification of proteins, and the simultaneous identification and quantification of potentially modified peptides. *J Proteome Res.* (2009) 8:2201–10. doi: 10.1021/pr800654s
310. Wesseling H, Mair W, Kumar M, Schlaffner CN, Tang S, Beerepoot P, et al. Tau PTM profiles identify patient heterogeneity and stages of Alzheimer's disease. *Cell.* (2020) 183:1–15. doi: 10.1016/j.cell.2020.10.029

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

SYSTEMATIC REVIEW
published: 07 January 2021
doi: 10.3389/fneur.2020.607680

Similarities and Differences in the Pattern of Tau Hyperphosphorylation in Physiological and Pathological Conditions: Impacts on the Elaboration of Therapies to Prevent Tau Pathology

Antoine Duquette[1,2†], *Camille Pernègre*[1,2†], *Ariane Veilleux Carpentier*[1,2] *and Nicole Leclerc*[1,2*]

[1] Research Center of the University of Montreal Hospital (CRCHUM), Montréal, QC, Canada, [2] Département de Neurosciences, Faculty of Medicine, Université de Montréal, Montréal, QC, Canada

OPEN ACCESS

Edited by:
Sonia Do Carmo,
McGill University, Canada

Reviewed by:
Chaur-Jong Hu,
Taipei Medical University, Taiwan
Luc Buee,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

***Correspondence:**
Nicole Leclerc
nicole.leclerc@umontreal.ca

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: 17 September 2020
Accepted: 07 December 2020
Published: 07 January 2021

Citation:
Duquette A, Pernègre C, Veilleux Carpentier A and Leclerc N (2021) Similarities and Differences in the Pattern of Tau Hyperphosphorylation in Physiological and Pathological Conditions: Impacts on the Elaboration of Therapies to Prevent Tau Pathology. Front. Neurol. 11:607680. doi: 10.3389/fneur.2020.607680

Tau protein, a neuronal microtubule-associated protein, becomes hyperphosphorylated in several neurodegenerative diseases called tauopathies. Hyperphosphorylation of tau is correlated to its redistribution from the axon to the somato-dendritic compartment at early stages of tauopathies. Interestingly, tau hyperphosphorylation begins in different regions of the brain in each tauopathy. In some regions, both neurons and glial cells develop tau hyperphosphorylation. Tau hyperphosphorylation is also observed in physiological conditions such as hibernation and brain development. In the first section of present article, we will review the spatiotemporal and cellular distribution of hyperphosphorylated tau in the most frequent tauopathies. In the second section, we will compare the pattern of tau hyperphosphorylation in physiological and pathological conditions and discuss the sites that could play a pivotal role in the conversion of non-toxic to toxic forms of hyperphosphorylated tau. Furthermore, we will discuss the role of hyperphosphorylated tau in physiological and pathological conditions and the fact that tau hyperphosphorylation is reversible in physiological conditions but not in a pathological ones. In the third section, we will speculate how the differences and similarities between hyperphosphorylated tau in physiological and pathological conditions could impact the elaboration of therapies to prevent tau pathology. In the fourth section, the different therapeutic approaches using tau as a direct or indirect therapeutic target will be presented.

Keywords: tau protein, hyperphosphorylation, tauopathies, microtubules, Alzheimer's disease

TAU PROTEIN AND ITS ROLE IN TAUOPATHIES

Tau is mainly a neuronal microtubule-associated protein encoded by the *MAPT* gene on human chromosome 17q21 (1). The *MAPT* gene has 16 exons; three of them are subject to alternative splicing (exons 2, 3, and 10), giving rise to the six isoforms in human adult brain (2, 3). Tau has four functional domains: an amino-terminal projection domain (up to two amino-terminal inserts), a

proline-rich region, the microtubule-binding domain (three or four microtubule-binding repeats), and the carboxy-terminal region (**Figure 1**) (4). Alternative splicing of exon 10 produces isoforms with either three or four microtubule-binding repeats named 3R and 4R isoforms (2). Tau is mainly localized to the axon and plays a role in maintaining neuronal integrity and axonal transport by regulating microtubule assembly and dynamics (5, 6). Its presence in dendrites and nuclei of neurons indicates its involvement in synaptic signaling and genome stability (7). Tau is expressed at very low levels in astrocytes and oligodendrocytes where its role in microtubule assembly and stability remains poorly characterized (7–9).

Tau undergoes several post-translational modifications in physiological conditions; phosphorylation being the predominant modification. Most of these modifications impact tau function and contribute to the heterogeneity of tau forms found in developing and adult brain (7). In neurodegenerative diseases called tauopathies, tau becomes hyperphosphorylated destabilizing its interaction with microtubules, accumulates and self-aggregates in insoluble filaments (7, 10). This aggregation of tau is correlated to neurodegeneration (11, 12). The exact role of other post-translational modifications (glycation, O-GlcNAcylation, nitration, acetylation, methylation, SUMOylation, ubiquitylation, oxidation, and truncation) in aggregation is increasingly investigated (12, 13).

In Alzheimer's disease (AD), both tau and amyloid-beta (Aβ) pathologies are landmarks of the disease whereas in primary tauopathies, tau pathology is the main neuropathological landmark (14–17). Frontotemporal lobar degeneration with predominant tau pathology (FTLD-tau) comprises primary tauopathies affecting the frontal and temporal lobes. This classification encompasses Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), globular glial tauopathies (GGT), and argyrophilic grain disease (AGD). The distribution of cellular tau (neuronal, glial, or mixed), the conformation of tau filaments, and the spatiotemporal pattern distinguish these tauopathies (7, 15). The diagnosis of tauopathies can be challenging. In the aging brain, neurodegenerative diseases frequently coexist and overlapping pathological features are common (14, 18). The clinical phenotype depends on the brain regions affected and not necessarily on the molecular changes occurring at the cellular levels. Thus, while some phenotypes are more frequent in a specific disease, there is a weak correlation between the clinical symptoms and the disease involved.

Tau contains 85 phosphorylation sites. Ten sites are phosphorylated in human control brain whereas in AD brain, 55 sites become phosphorylated (19, 20). Proline directed and non-proline directed kinases were shown to contribute to tau hyperphosphorylation. Phosphatase 2A (PP2A) is the main tau phosphatase and the decrease of its activity in AD brain is believed to contribute to tau hyperphosphorylation (21, 22). Tau hyperphosphorylation is correlated to its redistribution in the somato-dendritic compartment observed at early stages of tau pathology (12). Tau hyperphosphorylation is also observed in physiological conditions. Both in brain development

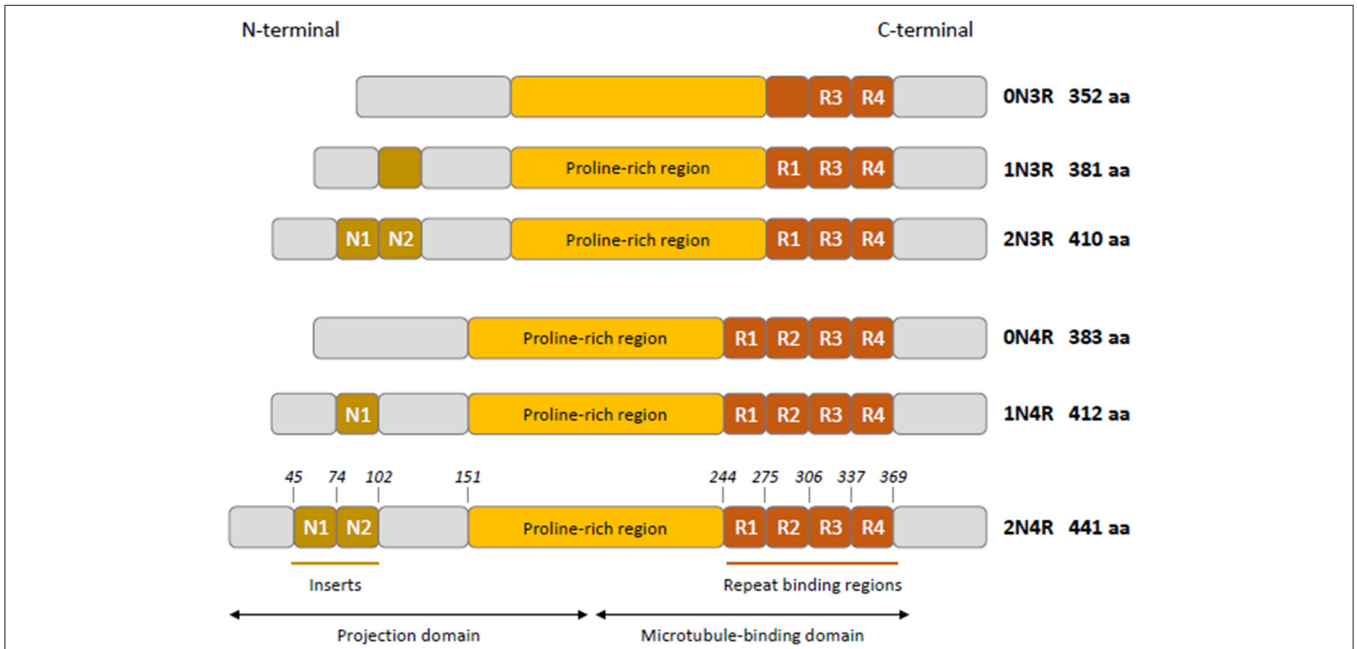


FIGURE 1 | Schematic representation of tau isoforms in human adult brain. Tau isoforms (0N3R, 1N3R, 2N3R, 0N4R, 1N4R, 2N4R) are generated by alternative splicing of exons 2, 3, and 10. Two inserts N1 and N2 (brown boxes) near the N-terminus of tau are produced by exons 2 and 3, respectively. Absence of N1 and N2 gives rise to 0N isoforms, whereas inclusion of N1 produces 1N isoforms and inclusion of both N1 and N2 results in 2N isoforms. The proline-rich region is indicated in yellow. R1–R4 (orange boxes) represent the four microtubule- binding repeats, R2 being encoded by exon 10. The exclusion or inclusion of exon 10 results in isoforms with three or four microtubule-binding repeats named 3R and 4R isoforms.

and hibernation, tau is hyperphosphorylated. In the present article, we will firstly review the spatio-temporal and cellular distribution of hyperphosphorylated tau in tauopathies. Then we will compare the pattern of tau hyperphosphorylation in tauopathies and physiological conditions. Lastly, we will discuss how the similarities and differences of tau hyperphosphorylation in tauopathies and physiological conditions impact the elaboration of therapies to prevent tau hyperphosphorylation and aggregation.

SECTION (1) THE SPATIOTEMPORAL AND CELLULAR DISTRIBUTION OF HYPERPHOSPHORYLATED TAU VARIES IN DIFFERENT TAUOPATHIES

Tau pathology is found in several neurodegenerative diseases that are listed in **Tables 1**, **2**. In the present section, we will review the spatiotemporal pattern of the propagation of tau pathology and the cellular distribution of hyperphosphorylated tau in AD and the three most studied primary tauopathies: PSP, CBD, and PID. Most of the PSP, CBD, and PID cases are sporadic frontotemporal lobar degeneration (FTLD) with tau-immunoreactive inclusions (FTLD-tau). However, some cases affected by one of these diseases present a mutation in the *MAPT* gene. Forrest and al found that 59% of their *MAPT* cases could be pathologically subclassified as other sporadic tauopathies including PSP, CBD, and PiD (23). PSP and CBD are 4R-tauopathies given that tau aggregates are solely composed of 4R isoforms in these diseases whereas PID is a 3R-tauopathy because of its tau aggregates composed of 3R isoforms. Each disease is characterized by unique type of tau filaments (24). In recent studies, the atomic structure of tau filaments in AD, PID, and CBD was analyzed by Cryo-EM (25–27). In each of these diseases, the extent of the ordered cores of the different folds varies.

ALZHEIMER'S DISEASE (AD)

AD is considered a secondary tauopathy because tau pathology is not the sole neuropathological landmark of the disease. Extracellular Aβ plaques are found in AD and are believed to trigger the disease whereas tau pathology would contribute to the progression of the disease by playing a central role in Aβ toxicity (9, 28, 29). In AD, tau pathology is predominant in neurons. Tau filaments are composed of 3R and 4R isoforms and formed neurofibrillary tangles (NFTs). Six stages (Braak stages)

TABLE 1 | Pathological characteristics of tauopathies.

Tauopathy	Neuronal or glial	Tau isoform	Tau filament structure	Main pathologic features of tau inclusions	Other microscopic pathological anomalies
Pick's disease	Neuronal > glial	3R	15–18 nm diameter straight filaments and 22–24 nm twisted	Pick bodies (round neuronal inclusions) Ramified astrocytes	Ballooned neurons (possibly secondary to tau pathology)
Progressive supranuclear palsy	Both	4R	15–18 nm diameter straight filaments	NFT Pretangles Neuropil threads Tufted astrocytes Coiled bodies	
Corticobasal degeneration	Both	4R	24 nm diameter twisted filaments	Neuropil threads Pretangles Astrocytic plaques Coiled bodies	Ballooned neurons (possibly secondary to tau pathology)
Chronic traumatic encephalopathy	Both	3R + 4R	20–25 diameter paired helical filaments	NFT Pretangles Astrocytic tangles	TDP-43 inclusions (unknown if triggered by tau or involved in tau accumulation)
Globular glial tauopathies	Both	4R	Paired helical filaments and straight filaments	Spherical neuronal cytoplasmic inclusions Globular oligodendroglial and astroglial inclusions	
Primary age-related tauopathy	Neuronal > Glial	3R + 4R	Paired helical filaments and straight filaments	Coiled bodies (unknown if related to PART or normal aging)	
Argyrophilic grain disease		4R	9–18 nm diameter straight filaments and 25 nm filaments forming compact bundles (grains)	Argyrophilic grains Pretangles Coiled bodies Diffuse granular astrocytic immunoreactivity	Ballooned neurons (possibly secondary to tau pathology)
Alzheimer's disease	Neuronal	3R + 4R	8–20 nm diameter paired helical filaments 15 nm diameter straight filaments	NFT Neuropil threads	Amyloid-beta plaques (primary neurodegenerative process with intricate relation with tau toxicity)

NFT, neurofibrillary tangles.

TABLE 2 | Spatiotemporal pattern of accumulation of tauopathies.

Tauopathy	Spatiotemporal pattern of neuronal tau accumulation			Main clinical phenotypes
	Early or pre-clinical	Mild stage	Late-stage	
Pick's disease	Limbic system Neocortical frontotemporal cortex	Basal ganglia Brainstem Noradrenergic/serotonergic nuclei Dentate nucleus Sensory tract of the spinal cord	Pre-motor cortex Pre-cerebellar nuclei Primary visual cortex	bvFTD nfaPPA
Progressive supranuclear palsy	Globus pallidus Substantia nigra Subthalamic nucleus	Brainstem, cerebellum (dentate nucleus), posterior frontal lobe	Association cortices	PSP-RS PSP-P PSP-CBS bvFTD nfaPPA
Corticobasal degeneration	Anterior frontal cortex Basal ganglia	Posterior frontal and parietal cortices	Higher tau burden in same regions as early/mild stage Midbrain and pons	CBS bvFTD nfaPPA RS
Chronic traumatic encephalopathy	Depth of sulci in frontal lobe Nucleus basalis Meynert Locus coeruleus	Superficial cortical layers Nucleus basalis Meynert Locus coeruleus	Amygdala Hippocampus Temporal, parietal and insular cortices Diencephalon Brainstem Spinal cord	Cognitive dysfunction Emotional dysregulation Behavior change Motor disturbance
Globular glial tauopathies	(Unknown temporal pattern) Frontal and temporal lobes Motor cortex Corticospinal tracts			bvFTD bvFTD + MND MND PSP-RS CBS
Primary age-related tauopathy	Transentorhinal region	Limbic structures (entorhinal and hippocampus)	Rarely neocortex is affected	Amnestic mild cognitive changes
Argyrophilic grain disease	Ambient gyrus and CA1 of hippocampus	Amygdala Medial temporal lobe	Insular cortex Anterior cingulum Nucleus accumbens Septal nuclei Hypothalamus Gyri recti	Amnestic mild cognitive changes Behavior and psychiatric symptoms bvFTD
Alzheimer's disease	Transentorhinal region	Limbic structures (entorhinal and hippocampus)	Isocortical	From amnestic cognitive changes to dementia

bvFTD, behavioral variant of frontotemporal dementia; nfaPPA, non-fluent-agrammatic variant of primary progressive aphasia; CBS, corticobasal syndrome; RS, Richardson syndrome; PSP, progressive supranuclear palsy; MND, motor neuron disease.

of tau pathology can be observed in AD (30). NFTs are first detected in the transentorhinal region and then extend to the entorhinal and hippocampus at stages I and II. At stages III and IV, NFTs propagate to the neocortical high-order associative areas. In stages V and VI, primary and secondary fields of the neocortex present NFTs. The extent of cognitive decline usually correlates with the accumulation of neocortical tau (31).

PROGRESSIVE SUPRANUCLEAR PALSY (PSP)

PSP is an adult-onset disorder that presents cognitive impairment associated with behavioral changes, akinetic rigid syndrome, and prominent oculomotor dysfunction (32). The most prevalent clinical form is Richardson syndrome. The tau filaments that form NFTs are exclusively composed of 4R isoforms (9). Tau aggregates are also found in glial cells. Tufted astrocytes containing 4R tau aggregates are a specific neuropathological landmark of PSP (9). In oligodendrocytes, coiled bodies composed of 4R tau isoforms are also observed (9, 33). The pattern of tau pathology propagation differs for each cell type (neurons, astrocytes, or oligodendrocytes). Kovacs et al. have divided into six stages the pattern of tau pathology in the Richardson syndrome (34). NFTs firstly begin in the globus pallidus, substantia nigra, and subthalamic nucleus and secondly spread to the brainstem. Thirdly, NFTs are observed in the striatum, dentate nucleus and amygdala. Fourthly, NFTs are noted in the frontal lobe. In the fifth and sixth steps, NFTs develop in the parietal, temporal, and occipital lobes (34, 35). Interestingly, astroglial and oligodendroglial tau pathologies precede neuronal tau pathology in some brain regions. The

oligodendroglial coiled bodies are detected in the globus pallidus at early stages of the disease before NFTs are observed in this region (34). Similarly, the aggregates of tau in astrocytes develop in the striatum before NFTs are noted. In the subsequent steps, astrocytic and oligodendroglial tau pathologies follow a distinct pattern of propagation for finally reaching the neocortex.

CORTICOBASAL DEGENERATION (CBD)

CBD is a tauopathy known for its asymmetric cortical atrophy (36, 37). Corticobasal syndrome (CBS) is the main clinical phenotype and comprise of asymmetric cortical signs (apraxia, hemineglect, alien limb, aphasia) and asymmetric parkinsonism or dystonia (18). In most neurons, tau staining is diffuse or granular resembling a pre-tangle stage. Both neuronal and glial thread-like tau-positive structures in the white and gray matter are observed in CBD. Tau-positive astrocytic plaques are the major neuropathological lesions of CBD. Neuronal loss is observed in focal cortical regions and in the substantia nigra (38). In CBD, the accumulation of tau follows a rostrocaudal gradient (37). It usually starts in the anterior frontal cortex with a higher ratio of astrocytic plaques compared to neuronal tau pathology. It evolves posteriorly and caudally with accumulation in the parietal cortex and brainstem structures. With the progression of the disease, neuronal tau burden increases, and there is an inversion of the ratio, with neuronal tau pathology being more prevalent than astrocytic plaques (37).

PICK'S DISEASE (PID)

PiD is the sole tauopathy presenting neuronal inclusions uniquely composed of 3R isoforms (39). Symptoms and signs are commonly related to the affected frontal lobe and comprise of changes in behavior and personality, apathy, or progressive non-fluent aphasia. Pick bodies are fibrillar intra-neuronal spherical inclusions composed of 3R tau isoforms (9). The propagation of tau pathology occurs in 4 sequential steps. Firstly, tau pathology begins in the dentate gyrus of the hippocampus, the frontotemporal neocortical regions and the angular gyrus. Secondly, tau accumulates in the basal ganglia, the brainstem's noradrenergic and serotonergic nuclei, the dentate nucleus, and the sensory tract of the spinal cord. Thirdly, tau pathology is found in the pre-motor cortex and pre-cerebellar nuclei, and fourthly, in the primary visual cortex (39). Other tau pathologic features include ramified astrocytes and globular oligodendroglial coiled inclusions. However, it remains unclear if tau pathology in glial cells precedes neuronal tau pathology as noted in PSP.

Pattern of Tau Phosphorylation in Tauopathies

In a recent study using mass spectrometry (MS), 55 phosphorylated sites were identified in two cohorts of AD (20). The highest frequency of phosphorylation was noted in the proline-rich region between the amino acids (a.a.) 195–209 and a.a. 212–224 and in the C-terminus in a.a. 396–406. Interestingly, insoluble tau aggregates were found in controls. High frequency phosphorylation sites in control insoluble tau were identified as threonine (T)181 and T231 and lower frequency of phosphorylation at serine (S)198, S199, S202, and T205 were observed in 20–40% of controls indicating that these sites could represent early stages of the disease. These results fit with previous studies where it was reported that T231 and S202/Thr205 were phosphorylated at early stages of AD (40–44). At intermediate stages of AD, 6 additional sites (S199, S202, T212, T217, S237, and S262) were identified in the proline-rich region and one in the C-terminal at S396 (20). The number of phosphorylation sites increased as the disease progresses to reach 55 at late stages indicating that the number of phosphorylation sites is associated with disease stages. Interestingly, no difference of phosphorylation of tau present in the soluble fraction was found between AD and controls as noted by others (20, 45). Tau found in the low molecular weight (monomers) and in the sarkosyl-soluble fractions (monomers) was seed incompetent while tau found in the high molecular weight (oligomers) and in the sarkosyl-insoluble fractions (fibrils) was seed competent.

Sixteen sites phosphorylated in AD were detected in PSP (46). The pattern of tau hyperphosphorylation has not been investigated at different stages of PSP. However, S202/T205 and T231, early sites in AD, are also phosphorylated in PSP (46, 47). As mentioned for PSP, the phosphorylation sites investigated until now for CBD and PiD are also found in AD (38, 39, 48, 49). In contrast to AD and PSP, some studies reported that there was no increase in tau phosphorylation at S262/S356 in PiD (48, 49). This was contradicted in another study (39). However, in a recent study where Cryo-EM was used to analyze the atomic structures of tau filaments in PiD, no phosphorylation at S262 or S356 was found (26).

SECTION (II) COMPARISON OF THE PATTERN OF TAU HYPERPHOSPHORYLATION IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

In both brain development and hibernation, hyperphosphorylated tau is present. Three important points will be compared between these physiological conditions and tauopathies in the present section (1) the pattern of tau hyperphosphorylation; (2) the role of hyperphosphorylated tau; and (3) the reversibility of tau hyperphosphorylation during brain development and hibernation but not in tauopathies.

Pattern of Tau Hyperphosphorylation During Brain Development

Several groups reported that tau is hyperphosphorylated during fetal and post-natal brain development of rodents at sites similar to those observed in AD and primary tauopathies (50–54). This hyperphosphorylation was not correlated to neuronal cell death as noted in tauopathies. Few studies reported that tau is also hyperphosphorylated in human fetal brain (55, 56). However, the most limiting factor of these studies was the small number

of individuals included in their analysis. In a recent study, tau phosphorylation was examined in 20 human fetal brains aged from 14 to 38 post-conceptional weeks and compared to that in tauopathies (AD, PSP, CBD, FTLD-tau), using a combination of immunohistochemistry and immunoblotting analysis (57). The authors showed that tau in human fetal brain is highly phosphorylated but its phosphorylation pattern differs from that of AD and primary tauopathies. Some sites such as S202, S214, and S396/S404 were strongly positive in a high percentage of cases as noted in AD and primary tauopathies. Other sites such as T231 found in high frequency at early stages of AD and S202/T205, less frequently phosphorylated at early stages of AD, were phospshorylated in a very low number of cases (20). All fetal brains were negative to S409, a site highly phosphorylated in AD. Interestingly, in this recent study, phospho-tau positive aggregates were found in fetal human brain. These aggregates were positive to T214 and weakly immunoreactive to the antibodies CP13 (S202) and PHF-1 (S396/S404). These aggregates apparently non-toxic were negative for thioflavin S staining indicating that they were seed-incompetent. Interestingly, in AD brain, S214, S396, and S404 were only found in seed competent tau fractions whereas S202 was found in seed incompent fractions (20). From the above observations, one can speculate that S202 could be one of the pivotal sites in preventing tau seeding activity.

The above studies revealed that in fetal brain, human tau is hyperphosphorylated at multiple epitopes, some of them overlapping with those seen in tauopathies. Human fetal tau appears to be able to form aggregates that are not seed competent. The function of such aggregates remains to be determined. All together, the above observations indicate that it is not hyperphosphorylation *per se* that is toxic but rather the phosphorylation of specific residues and/or the cellular context (development vs. aging) that seem to dictate the toxicity of tau. Further studies are needed to confirm this using more accurate methods such as MS to obtain the full picture of tau phosphorylation sites in fetal brain.

None of the studies mentioned above examined the distinct phosphorylation pattern of each tau isoform. This is an important point to consider since the pattern of tau isoforms is differently regulated in fetal and adult human brains. Only the smallest tau isoform is present in fetal brain whereas all 3R and 4R tau isoforms are found in the adult brain at a ratio of 1:1 (2, 55, 58–60). In rodents, a switch from 3R to 4R is observed during the development resulting in the sole presence of 4R isoforms in adult brain (55, 58, 60, 61). Interestingly, the developmental switch of tau isoforms and the changes of their phosphorylation occur 2–3 weeks postnatally, corresponding to the critical period of neuronal plasticity (62, 63). The 4R tau isoforms present the strongest microtubule-binding, -assembling and -stabilizing abilities and therefore a decrease of their phosphorylation is believed to increase their binding to microtubules, a crucial step for stabilizing the novel established connections in developing brain (64–66).

The difference of phosphorylation between tau isoforms remains poorly characterized. The distinct phosphorylation profile of tau isorforms was recently examined *in vivo* in mouse brain (67). This study, using a combination of the Phostag technology and 3R-/4R-specific antibodies, was the first one to report the differences between 3R and 4R phosphorylation *in vivo* although the phosphorylation sites that differ between 3R and 4R were not identified. During mouse brain development, 3R isoforms that were highly phosphorylated are replaced by the 4R isoforms presenting lower phosphorylation levels than 3R. The phosphorylation levels of both 3R and 4R isoforms decreased during brain development, the dephosphorylation of 4R being less important than that of 3R. A similar pattern of phosphorylation was observed for human 3R and 4R isoforms in knock-in mice indicating that in human brain, 3R and 4R isoforms could also display different profiles of phosphorylation. One has to take into consideration all these observations to compare the phosphorylation state of tau during brain development with that observed in tauopathies for the identification of the sites linked to tau toxicity.

Tau Hyperphosphorylation Associated With Axonal Outgrowth and the Establishment of Synaptic Contacts in Brain Development

During brain development, tau hyperphosphorylation is correlated to the growth of the axon and its suppression results in a reduced axonal length (68, 69). In developing Drosophila, tau was shown to be necessary for formation of synaptic contacts (70). Hyperphosphorylation of tau is believed to occur for decreasing microtubule stability and thereby allowing axonal growth in developing brain (71–73). Interestingly, axonal and dendritic sprouting was observed in AD indicating that neurons might attempt to re-growth their axon and re-establish synaptic contacts (74, 75). Tau hyperphosphorylation at early stages of tauopathies could be part of global reaction intended for re-starting developmental programs. Consistent with this, several studies have reported that markers of cell cycle are increased in AD brain (76). Interestingly, recent studies have shown that tau could be involved in cell cycle (77).

Pattern of Tau Hyperphosphorylation in Hibernating Animals

Hibernation is a biological and adaptive process used by several mammalian species to survive in inhospitable environmental conditions. It is a powerful physiological strategy to restrict energy expenditure and compensate for periodically limited energy supply (78, 79). During hibernation, animals enter a hypometabolic state, called torpor, characterized by extreme changes: a blood-flow reduction, a decrease in brain and body temperature and an immune and metabolically depressed state (79–81). In the torpor state, the metabolic rate can be as low as 5% of that in normal euthermic state (81). Multiple periods of torpor lasting 3–4 days are periodically interrupted by short arousal periods of activity (usually <1 day) in which the animals return to euthermia and normal metabolism, blood flow and body temperature (80, 81). The accumulation of hyperphosphorylated Tau at S/T residues has been reported in several obligate and facultative hibernator species, as Arctic ground squirrels or Syrian hamsters during torpor (82–86). Hyperphosphorylation of tau were also found in neurons of

hibernating European ground squirrels but totally disappeared as tau phosphorylation was fully reversed (82). Although the pattern of hyperphosphorylated tau is similar to the one in AD it appears to be non-toxic as no apparent neuronal damages were observed (79). Five phosphorylation sites (T181, S199, S202, T231, and S404) detected in hibernating animals were also found in tau seed incompetent fractions isolated from AD brain and another five sites (T205, S212, T214, S262, and S396) were found in competent tau seed competent fractions (20). This could indicate that in this physiological context, the phosphorylation sites preventing tau seeding activity override the effects of the sites favoring seeding.

In hibernating animals, hyperphosphorylation of tau is linked to synaptic deafferentation (79). In hibernating golden hamster, a regression of the dendritic spines on apical dendrites but not on basal dendrites of hippocampal neurons was noted (87). Interestingly, these changes were not linked to memory impairment. To explain this, the authors proposed that only unstable spines were removed whereas stable spines were spared.

Tau Hyperphosphorylation Associated With Protective Mechanisms in Hibernating Animals

Torpor in hibernating animals shares several abnormalities observed in AD such as same populations of neurons developing tau hyperphosphorylation, alterations of synaptic connections and cognitive impairment. The hibernation is a process requiring several biological mechanisms, which permit the regulation and adaption of the neural system (88). During torpor, neuronal structural changes have been reported, such as changes in dendritic spines and synaptic connections, in the Golgi apparatus morphology and alterations of microglial cells (89–93). Interestingly, all these changes are reversible. This very fast transition from torpor to arousal requires different remodeling of the brain such as reorganization of membranous organelles and synaptic formation (82, 93–95). In a recent study, the global metabolic changes in Syrian hamster brain during hibernation were analyzed (96). They have identified significant differences in more than 300 metabolites, providing new insights on adaptive and neuroprotective brain processes that are occurring during hibernation and arousal. For example, an increase of brain cryoprotectants such as theitol were increased during torpor whereas an increase of neuroprotective agents such as L-carnitine and acylcarnitine proposed as therapy for AD were elevated during arousal (97, 98). All these changes allow neurons to resist to damages upon cerebral ischemia during torpor and rapid reperfusion during arousal.

Hypometabolism is observed in hibernating animals as well as in AD and primary tauopathies. Some fluorodeoxyglucose positron emission tomography (PET) studies have detected hypometabolic states in isocortical brain areas of patients at preclinical stages of AD and patients with probable AD (subjects at risk) (99, 100). These abnormally low rates of cerebral glucose metabolism occur several decades before the possible onset of dementia and seem to predict cognitive decline (101, 102). Siegfried Hoyer was the first one to propose that hypometabolic states could eventually lead to AD pathology (103–106). In hibernating animals, the hypometabolic state corresponds to a reduction of energy supply and requirements, low neuronal activity and regression of synaptic connections. The neuronal activity is also drastically reduced (107–112). Some groups reported with electroencephalography measurements that almost no brain activity is present in the hibernating brain (110, 113, 114).

It was proposed that tau would play a role in regulating neuronal activity during the hypometabolic state. Indeed, the localization of tau at the synapse and its interaction with post-synaptic proteins modulating the insertion of receptors, in particular AMPA and NMDA receptors, at the plasma membrane make it a plausible player in regulating neuronal activity (115). Hyperexcitability is noted during cooling phase of hibernation as observed at early stages of AD (116, 117). Tau was shown to contribute to hyperexcitability in mouse models of epilepsy (118–120). Its suppression could abolish hyperexcitability in these models (119). The tau mutation V337M linked to frontotemporal dementia was reported to increase neuronal activity by altering the axonal initial segment plasticity (121). Bin1, a protein involved in AD pathogenesis, was shown to regulate tau-dependent hyperexcitability in hippocampal neurons (122). Lastly, two tau mutations, Δ280 and A152T, were reported to induce hypoexcitability and hyperexcitability, respectively, in neuronal cultures (123). Collectively, the above observations strongly support the contribution of tau in the regulation of neuronal activity in physiological and pathological conditions. Hyperphosphorylated tau in AD has recently been suggested to represent some compensatory response to suppress excitatory/inhibitory imbalance at initial stages of the disease. If this imbalance lasts too long, such as under conditions of uncontrolled prolonged hypometabolism, it may become pathological triggering a cascade of events leading to neurodegeneration. All together, the above observations indicate that the increase of tau phosphorylation at early stages of AD could be a physiological reaction to a reduced brain metabolic rate that is required for modulating neuronal activity.

Tau Hyperphosphorylation Is Reversible During Brain Development and Hibernation

In humans, the hyperphosphorylation and aggregation of tau were frequently detected under the age of 30 in a systematic survey of more than 2 300 non-selected autoptic cases aged between 1 and 100 years (124, 125). The authors suggested that this might be the initial step of tauopathies. Moreover, in 2010, the National Institute on Aging and the Alzheimer's Association convened an international workgroup and regrouped some evidence suggesting that the pathological process begins at least years or decades before the onset of clinical impairment (126). These observations indicate that tau hyperphosphorylation is non-reversible in human adult brain leading to neurodegeneration. This fits with the decrease of phosphatase activity in AD brain (21, 127, 128).

During brain development and hibernation, tau hyperphosphorylation is transient and reversible. The highly phosphorylated tau is dephosphorylated around birth and during post-natal brain development to remain less phosphorylated in normal adult brain (51, 56). The role of decreasing tau

phosphorylation during brain development is most likely to increase its binding to microtubules (71–73). In most studies, the hyperphosphorylation of Tau during torpor has been shown to be rapidly and fully reversed after arousal in hibernating animals (79, 82, 85, 86). However, a group reported that in Arctic ground squirrel, some sites were dephosphorylated in arousal animals while others remained phosphorylated, indicating a reversible phosphorylation at selective sites (84). Similarly, tau hyperphosphorylation was shown to be partially reversed in a model inducing a hypothermic torpor-like state (129). Although hypothermia could contribute to tau hyperphosphorylation during hibernation, it was clearly demonstrated that it was not sufficient and that tau phosphorylation is regulated by other mechanisms that remain to be identified.

As mentioned above, during hibernation, multiple periods of torpor lasting few days are periodically interrupted by short arousal periods of activity lasting hours in which the animals return to euthermia and normal metabolism, blood flow and body temperature (80, 81, 130, 131). The reasons for the repeated arousals are still not entirely clear, but it is believed that they allow repair of neuronal damages caused by prolonged hypometabolism and brain inactivity. Interestingly, aged black bears show permanent accumulation of hyperphosphorylated tau at a pretangle-like stage (132). This might be explained by the fact that their hibernation is a continuous torpor period, which is not interrupted by spontaneous arousal. Collectively, the above observations in hibernating animals indicate that tau hyperphosphorylation could be part of neuroprotective reaction but if it is not interrupted by normal periods of tau phosphorylation as noted during arousal in hibernation, it could become permanent making neurons more vulnerable to insults and neurodegeneration as noted in tauopathies.

SECTION (III) IMPLICATIONS OF SIMILARITIES AND DIFFERENCES IN TAU HYPERPHOSPHORYLATION BETWEEN PHYSIOLOGICAL CONDITIONS AND TAUOPATHIES IN THE ELABORATION OF THERAPIES

Three main observations on hyperphosphorylation of tau during brain development and hibernation could impact the elaboration of therapies to prevent tau pathology and/or slow down its propagation in the brain. Firstly, the comparison of tau phosphorylation sites between physiological conditions and tauopathies indicates that the combination of phosphorylation sites is most likely a determinant factor in the toxicity mediated by hyperphosphorylated tau and therefore this should be taking into consideration when designing tools to neutralize phosphorylated forms at specific sites. Secondly, tau hyperphosphorylation is not found in glial cells in physiological conditions as noted in primary tauopathies indicating that they could play important role in the process of neurodegeneration. Thirdly, all tauopathies are linked to aging.

Therapies Targeting Tau Phosphorylation Sites

Examining tau hyperphosphorylation in brain development and hibernation revealed that some sites could be protective preventing tau from forming toxic seed-competent aggregates. However, it is important to mention that a full characterization of tau phosphorylation sites using an approach such as MS is needed to identify the key sites involved in the shift from non-toxic to toxic tau species. Based on what it is known so far, it is clear that the combination of phosphorylation sites is a determinant factor in tau aggregation propensity. For example, in a recent study, it was reported that the amount of phosphorylation on doubly phosphorylated T231 and S235 and on singly phosphorylated S262, positively correlated with seeding capacity of tau whereas some phosphorylation sites negatively correlate with tau seeding such as the single phosphorylation within the clusters of sites contained in peptides S198/S199/S202 or S400/T403/S404 (133). This indicates that phosphorylation at specific sites likely results in conformational changes impacting tau seeding activity. Tau phosphorylation on tyrosine residues can also impact tau aggregation capacity. It was recently reported that tau phosphorylation at tyrosine (Y)18, Y29, Y197 at the N-terminal or at Y310 is sufficient to reduce tau aggregation (134). NMR indicates that there is a local decrease of beta-sheet propensity of tau's PHF6 domain when these sites are phosphorylated. In contrast, Briner et al. showed that the single phosphorylation at Y18 increases tau aggregation *in vitro* in presence of heparin (135). Lastly, tau phosphorylation at T205 was shown to be protective against Aβ in transgenic mice (136). From the above observations, one can conclude that specific pattern of tau phosphorylation can be either beneficial or detrimental to neurons and therefore to merely decrease global tau phosphorylation could result in undesired effects compromising neuronal survival.

Glial Cells: An Unexplored Target

In some tauopathies, both neurons and glial cells are affected (**Figure 2**). It is still unclear if the increase of tau in glial cells is caused by dysfunction of the degradative mechanisms and/or the uptake of tau released by neurons. In PSP, tau pathology develops earlier in glial cells than in neurons in the globus pallidus and striatum (34). Hyperphosphorylated tau is not found in glial cells in developing brain and hibernation indicating that tau accumulation in these cells represents a pathological condition. Consistent with this, tauopathies developing tau pathology in glial cells such as PSP and CBD present a disease progression faster than AD where tau pathology is mainly found in neurons. In a recent study, it was reported that the uptake of extracellular tau oligomers by astrocytes, altered the intracellular Ca2+ signaling and Ca2+-dependent release of gliotransmitters resulting in synaptic dysfunction (137). Glial cells could also contribute the propagation of tau pathology in the brain. It was reported that microglia can facilitate tau pathology propagation between neurons by phagocytosing and exocytosing tau protein (138). Astrocytes were shown to decrease the spreading of tau pathology by taken up extracellular tau and targeting it for

degradation in a mouse model (139). However, in PSP and CBD, the tau taken up by astrocytes might be exocytosed instead of degraded allowing the propagation of tau pathology in the brain.

The fact that glial cells are affected earlier than neurons in some brain regions could indicate that the insults triggering the disease altered the functioning of glial cells more than that of neurons in these regions. The mechanisms leading to tau pathology in glial cells remain poorly characterized. As shown for neurons, tau is believed to be attached to microtubules and to regulate their assembly in glial cells (140). This is supported by studies reporting that the overexpression of human tau in primary rat astrocytes induced selective destruction of detyrosinated microtubules while in oligodendrocytes, alterations of microtubule network were noted (141, 142). From this, one can speculate that microtubules might be a key element in the development of tau pathology in both neurons and glial cells. The most popular hypothesis is that tau becomes hyperphosphorylated and detaches from microtubules decreasing their stability (**Figure 3**) (12). However, the opposite could also occur, meaning microtubules become unstable and this weakens the binding of tau favoring its detachment. Indeed, microtubules are very sensitive to external forces and factors modulating cellular functions. Mutations in the tubulin gene are linked to severe developmental neurological disorders called tubulinopathies (143). Microtubules present in both neurons and glial cells might be involved in the initial phase of the disease. Under normal conditions, alternating cycles of phosphorylation and dephosphorylation regulate tau binding to microtubules. These cycles could be altered in tauopathies leading to tau hyperphosphorylation (144). Another possibility could be that during early stages of tauopathies presenting glial tau pathology, glial cells can proliferate involving the depolymerization of microtubules during this process. This depolymerization would result in tau detachment from microtubules and thereby its access to kinases would be increased as well as its phosphorylation levels. At late stages of AD, it was reported that astrocytes become senescent implying that they stop to divide (145). In such a cellular context, tau binding to microtubules could be blocked making irreversible its detachment from microtubules and its hyperphosphorylation state.

Targeting the Aging Process

The fact that tau hyperphosphorylation does not exert any toxic effects during brain development and hibernation could indicate that the cellular context plays a pivotal role in determining whether hyperphosphorylated tau is part of either a response supporting neuronal survival or a response compromising neuronal survival. Four of the main changes occurring in aging could contribute to tau hyperphosphorylation. Firstly, it is well-documented that the efficacy of the two systems involved in tau degradation, the proteasome and the autophagy, is significantly decreased in aging favoring the accumulation of phosphorylated tau (146). Secondly, the combination of a decrease of energy supply and a decrease of clearance of damaged organelles could contribute to the triggering of tau pathology in aging. For example, the accumulation of dysfunctional mitochondria could restraint the supply of energy in aged cells (147). The inhibitors of the mitochondrial complex I, rotenone and annonacin, were shown to induce tau pathology in rat striatal neurons and glial cells indicating that mitochondrial dysfunction in aging could favor tau pathology (148, 149). Thirdly, changes leading to a progressive increase in vascular resistance and decrease in tissue levels of oxygen and glucose are noted in aging (150). Chronic hypoxia was shown to induce tau hyperphosphorylation in a rat model (151). Fourthly, aging could favor the propagation of tau pathology in the brain. A decrease of glymphatic system, the brain's metabolite clearance system connected to the peripheral lymphatic system is impaired in aging (152). This system was recently shown to contribute to the clearance of extracellular tau from the brain, the pool of tau believed to be involved in the propagation of tau pathology in the brain (153).

SECTION (IV) HYPERPHOSPHORYLATED TAU: DIRECT OR INDIRECT THERAPEUTIC TARGET

Several clinical trials that directly target tau are currently active. The main ones are testing tau immunotherapy for sequestration of pathological tau species (154). Such an approach allows to specifically either target a phosphorylation epitope, a conformational state or a peptidic sequence of tau (155, 156). The epitope S396/S404 was reported to be an efficient therapeutic target. Two vaccines targeting this epitope, one active (ACI-35, ISRCTN13033912) and one passive (Lu AF87908, NCT04149860) are presently in clinical trials (154). An antibody directed against cis-phosphorylated T231 (NCT04096287) is also tested while the vaccine targeting S422 has been discontinued (157). Phosphorylation of tau at certain sites was solely noted in seed competent tau fractions isolated from AD brain (133). More precise identification of the sites involved in the conversion of non-competent to competent seed forms is needed to develop antibodies that could prevent formation of competent seed forms. Epitopes may not be accessible on the majority of tau seeds and therefore an analysis of the atomic structure of such tau species is needed to reveal the epitopes that could be easily accessed by an antibody. Furthermore, one group reported that preventing tau seeding and blocking tau toxicity are not necessarily linked. This was shown using an antibody directed against phosphorylated T212/S214 epitope that could prevent tau seeding but not tau toxicity indicating that seeding could be neuroprotective (158). Another aspect worthy of consideration is that some antibodies only act on extracellular tau to refrain the spreading of tau (159). The characterization of tau phosphorylation sites in CSF by MS has revealed that tau phosphorylation varies depending on the evolution stages of the disease (160). In such a case, different antibodies will have to be used to neutralize these tau species during the progression of the disease.

Phosphorylation epitopes that are shared between the tauopathies could be used to design antibodies that should work for all tauopathies unless these epitopes are rendered inaccessible to antibodies because of tau conformation. Indeed, the analysis of tau atomic structure using Cryo-EM has revealed that tau

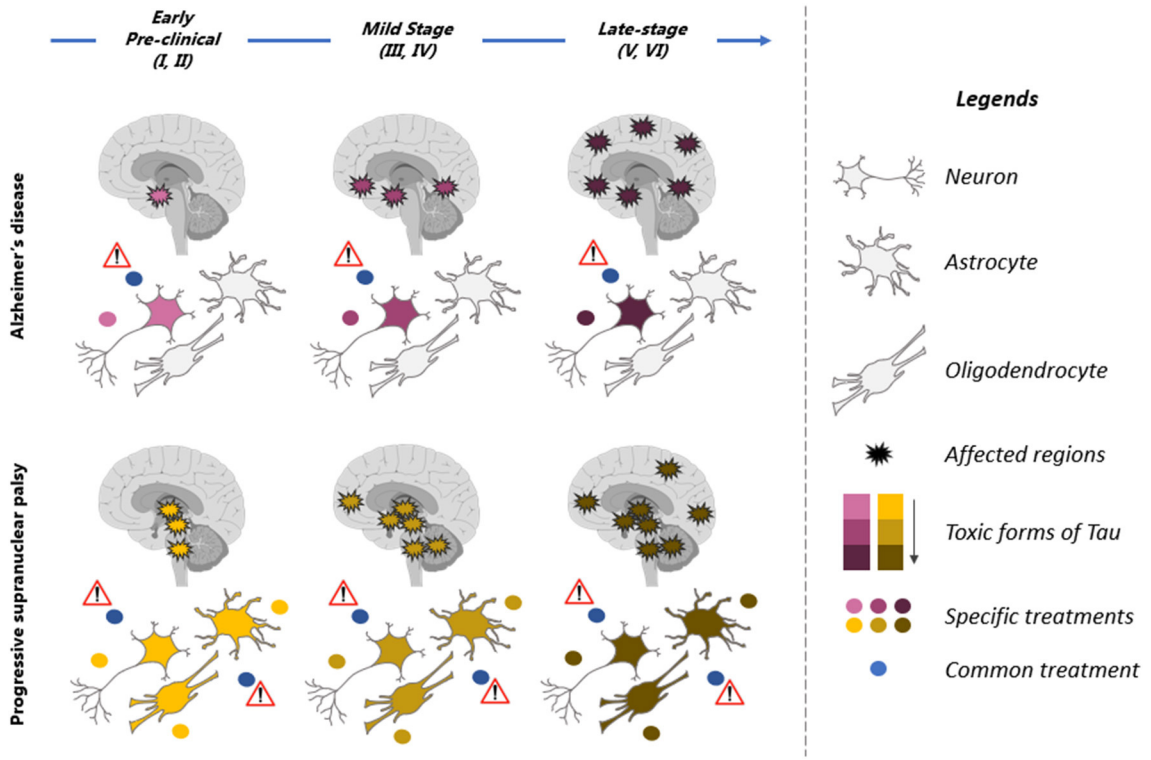


FIGURE 2 | Therapeutic strategies for prevention of tau pathology in tauopathies. Illustration of the spatiotemporal pattern of the propagation of tau pathology in Alzheimer's disease (AD) and Progressive supranuclear palsy (PSP). Six stages (I–VI) of tau pathology can be observed in both diseases (top blue arrow). The affected regions in the brain are indicated by spiky circles. Tau pathology begins in distinct regions in each of these tauopathies and propagates differently. Tau pathology is predominant in neurons in AD brain while neurons and glial cells are affected in PSP. Different toxic forms of tau are observed during the course of the disease. They present a low toxicity (pink in AD and yellow in PSP) at early stages of the disease and high toxicity (dark red in AD and brown in PSP) at late stages of the disease. These forms are distinct in AD (yellow to brown) and PSP (pink to dark red). A therapeutic molecule should be designed for each toxic form of tau (yellow, brown, pink, and dark red dots). The therapeutic strategy should also be adapted to the stages of disease as tau forms vary during the course of the disease. Because the toxic forms of tau differs in AD and PSP, a molecule not specific to these forms (blue dot, common treatment) might be inefficient and even detrimental to patients by affecting forms of tau that are not toxic.

filaments are distinct in AD, PiID and CBD (24). These analyses revealed that some phosphorylation sites could dictate which tauopathie will develop. Falcon et al. (26) reported that Pick body filaments are not phosphorylated at S262/or S356 and proposed that S262 could be protective against PiD (26). A similar conclusion was found for other post-translational modifications. Using Cryo-EM, it was also shown that deamidation of asparagine (N) 279 was present in AD but not in CBD or PSP indicating that as noted for phosphorylation, this modification can dictate the type of disease that will develop (27). This indicates that when targeting either a specific phosphorylation site or any another post-translational modification site, it will be important not to induce a shift from one disease to another. Cryo-EM analysis of hyperphosphorylated tau aggregates in developing brain and in hibernating animals could help to identify the key sites preventing the formation of toxic tau species. Tau conformation might also change throughout the disease evolution, and the affinity of the antibody for a specific conformation as well reducing its affinity for tau (**Figure 2**) (156).

In several clinical trials, hyperphosphorylated tau is an indirect target. In most of these trials, either the inhibition of tau kinases or the activation of PP2A, the main tau phosphatase is tested (161). Tau is phosphorylated by several proline-directed and non-proline-directed kinases (4). One can expect that the inhibition of a sole kinase might not be sufficient to completely prevent tau hyperphosphorylation. Lithium carbonate, a GSK-3 inhibitor, (NCT0318528) and Nilotinb, an inhibitor of Tyrosine kinase Ab1 (NCT02947893) are presently in Phase IV and Phase II, respectively. The phosphatase PP2A can directly and indirectly by regulating the activity of tau kinases modulate tau phosphorylation (162). PP2A activity is decreased in AD brain and therefore several therapeutic strategies have been elaborated to increase its activity (161). PP2A activity can be increased by inhibition of its endogenous inhibitors, I_1^{PP2A} and I_2^{PP2A} (163, 164). PP2A activity can also be increased by preventing its inhibitory demethylation by methyl transferase or phosphotryosinylation at T307 by src kinase (165, 166). Compounds that can increase PP2A activity such as Metformin (NCT01965756), an anti-diabetic drug, and sodium selenite (ACTRN12611001200976), which enhance PP2A activity by promoting the stabilization of its catalytic subunit PP2Ac, are currently in Phase II. So far, the same sites were found

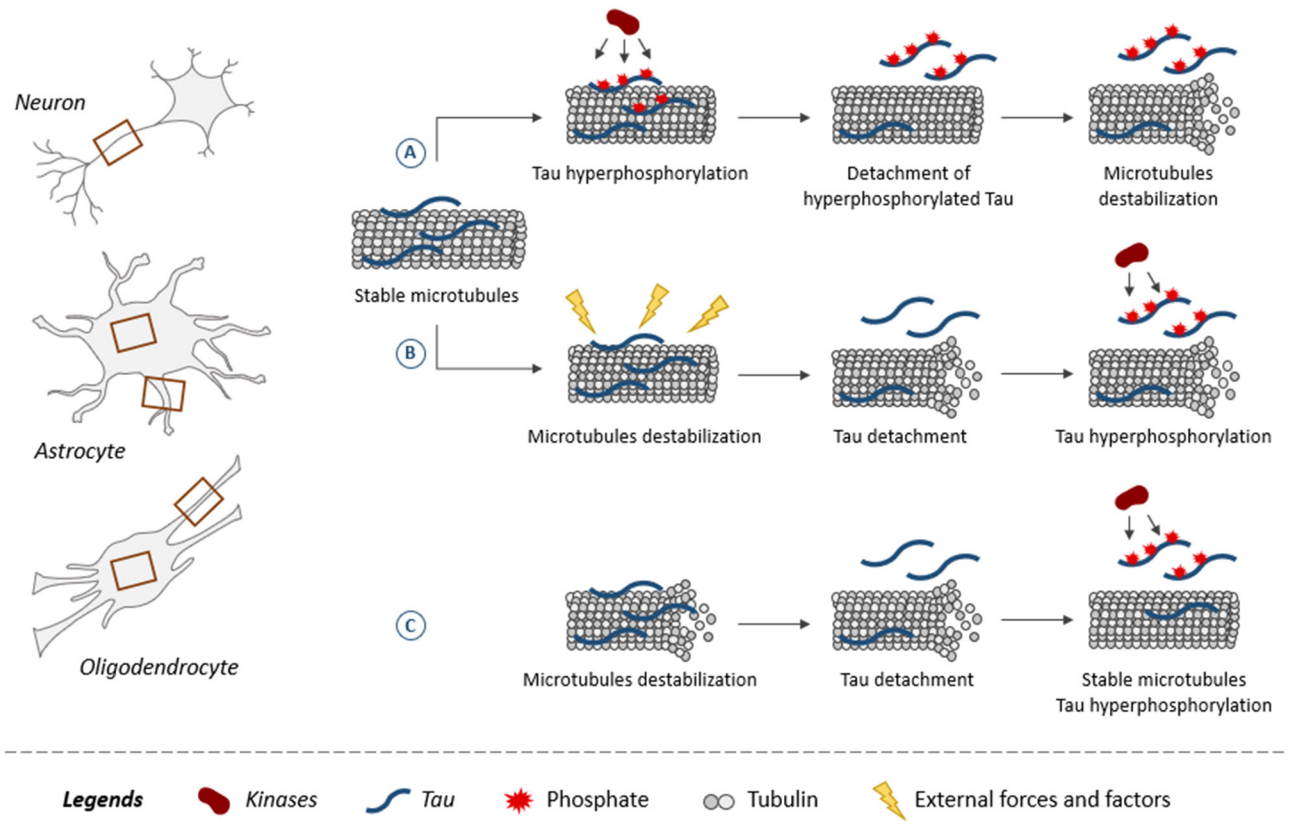


FIGURE 3 | Role of tau and its phosphorylation in microtubule dynamics. Microtubules, composed of tubulin, are found in both neuronal and glial processes (astrocytes and oligodendrocytes) as indicated by brown squares. Illustration of three scenarios on how tau plays a role in microtubule dynamics within neurons and glial cells. Tau promotes assembly and stabilization of microtubules **(A,B)**. **(A)** When tau becomes hyperphosphorylated, it reduces its binding to microtubules resulting in its detachment. Microtubules are destabilized upon the loss of tau at their surface. **(B)** External forces and factors destabilize microtubules, leading to the detachment of tau increasing its access to kinases, which results in its hyperphosphorylation. **(C)** Tau binding does not stabilize microtubules but instead increases their dynamics. The detachment of tau from microtubules and its hyperphosphorylation is associated with an increase of microtubule stabilization.

to be phosphorylated in neurons and glial cells (34). This could signify that the kinases and phosphatases involved in tau hyperphosphorylation are shared by neurons and glial cells and therefore any strategy targeting either tau kinases or phosphatases should work in both neurons and glial cells in tauopathies where these two types of cells are affected.

Tau phosphorylation can be regulated by other post-translational modifications. O-GlcNAcylation was shown to regulate tau phosphorylation (167). In AD brain, the decrease of O-GlcNAcylation of tau was correlated to an increase of tau phosphorylation (168, 169). The inhibition of O-GlcNAcase, the enzyme that removes the O-GlcNAc moieties is tested as an approach to decrease tau phosphorylation. Such inhibitors (AsceNeuron) are presently in Phase I. However, since 100 of proteins undergo O-GlcNAcylation, secondary effects could be triggered by these inhibitors. In a recent study, it was demonstrated that HDAC6 reduce tau acetylation and phosphorylation in the microtubule-binding domain (170). Its suppression in transgenic mice reduces their survival correlated to accelerated tau pathology and cognitive decline revealing HDAC6 as a potential therapeutic target.

At early stages of the disease, tau detachment from microtubules in both neurons and glial cells could be reversible and therefore therapy aiming at increasing tau binding to microtubules could be efficient in stopping the disease. On one hand, if tau first detaches from microtubules because of its hyperphosphorylation, an effective therapy would be to prevent this hyperphosphorylation. On the other hand, if changes in microtubule dynamics trigger the detachment of tau from them, the therapy should act on microtubules instead of tau. This avenue was explored by using drugs that stabilize microtubules. Promising results were obtained in tau mouse models but no beneficial effect was observed in humans (171–173). This could be explained by the fact that a recent study reported that tau does not stabilize microtubules but exerts the opposite effect (**Figure 3**). Indeed, the suppression of tau was associated with an increase of microtubule stabilization in rat cortical neurons (174). The controversy on the stabilizing effects of tau on microtubule needs to be resolved before the elaboration of an efficient therapy.

The interactome of hyperphosphorylated tau was recently characterized (175). In particular, the interactome of

phosphorylated tau found in NFTs microdissected from patients with advanced AD was compared to that of phosphorylated tau immunopurified with the PHF-1 (S396/S404) antibody. Seventy-five proteins of the 125 proteins interacting with immunopurified tau were present in NFTs. These proteins were involved in phagosome maturation, the regulation of synaptic plasticity, DNA binding proteins and members of the 14-3-3 family. From these data, it is reasonable to speculate that these interactions are modulated by tau phosphorylation state and therefore could be modulated during the progression of the disease. The manipulation of tau phosphorylation by any treatment should take this into account for not favoring interactions that are detrimental to neurons.

Lastly, a recent study revealed that excitatory neurons having a selective vulnerability to tau pathology present an elevated expression of tau aggregation-prone proteins and a decreased expression of tau aggregation protector proteins (176). This indicates that a therapy targeting one of these groups of proteins could be beneficial for all tauopathies. Furthermore, these proteins were higher in neurons than in glial cells explaining why neurons are more affected by tau pathology than glial cells in the most frequent tauopathies (176). However, in tauopathies where glial cells are affected before neurons and in tauopathies where only glial cells are affected, the therapy developed for preventing tau pathology in neurons might not be efficient unless it is demonstrated that the same proteins favoring tau aggregation or aggregation protector proteins can play similar roles in glial cells.

From the above observations, one can speculate that therapies targeting mechanisms involved in tau hyperphosphorylation such as the inhibition of tau kinases would be efficient in all tauopathies but a lot more complex to elaborate because of their secondary effects on substrates other than tau. Using tau as a direct target appears to be the most achievable approach although the diversity of pathological tau forms implies the adaptation of a therapy for a each tauopathy. The success of the direct approach relies on a full characterization of tau phosphorylation sites and its other post-translational modifications in both physiological and pathological conditions to identify the pivotal sites in the conversion from non-toxic to toxic species. Furthermore, as revealed in a recent study, tau phosphorylation varies during the progression of AD indicating that the strategy applied at early stages of the disease will be different from that applied at later stages.

AUTHOR CONTRIBUTIONS

AV, CP, AD, and NL wrote the manuscript. AV prepared **Tables 1**, **2**. CP prepared **Figures 1**–**3**. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Canadian Institute of Health Research grants (MOP-114874 and PJT-155993) to NL.

REFERENCES

1. Neve RL, Harris P, Kosik KS, Kurnit DM, Donlon TA. Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2. *Brain Res*. (1986) 387:271–80. doi: 10.1016/0169-328X(86)90033-1
2. 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) 3:519–26. doi: 10.1016/0896-6273(89)90210-9
3. Andreadis A, Brown WM, Kosik KS. Structure and novel exons of the human tau gene. *Biochemistry*. (1992) 31:10626–33. doi: 10.1021/bi00158a027
4. Buée L, Bussiere T, Buee Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev*. (2000) 33:95–130. doi: 10.1016/S0165-0173(00)00019-9
5. Mandell J, Banker G. Microtubule-associated proteins, phosphorylation gradients, and the establishment of neuronal polarity. *Perspect Dev Neurobiol*. (1996) 4:125–35.
6. Baas PW, Rao AN, Matamoros AJ, Leo L. Stability properties of neuronal microtubules. *Cytoskeleton (Hoboken)*. (2016) 73:442–60. doi: 10.1002/cm.21286
7. Arendt T, Stieler JT, Holzer M. Tau and tauopathies. *Brain Res Bull*. (2016) 126:238–92. doi: 10.1016/j.brainresbull.2016.08.018
8. Gorath M, Stahnke T, Mronga T, Goldbaum O, Richter-Landsberg C. Developmental changes of tau protein and mRNA in cultured rat brain oligodendrocytes. *Glia*. (2001) 36:89–101. doi: 10.1002/glia.1098
9. Kovacs GG. Invited review: neuropathology of tauopathies: principles and practice. *Neuropathol Appl Neurobiol*. (2015) 41:3–23. doi: 10.1111/nan.12208
10. Lee V, Goedert M, Trojanowski J. Neurodegenerative tauopathies. *Annu Rev Neurosci*. (2001) 24:1121–59. doi: 10.1146/annurev.neuro.24.1.1121
11. Noble W, Hanger DP, Miller CC, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. *Front Neurol*. (2013) 4:83. doi: 10.3389/fneur.2013.00083
12. Iqbal K, Liu F, Gong CX. Tau and neurodegenerative disease: the story so far. *Nat Rev Neurol*. (2016) 12:15–27. doi: 10.1038/nrneurol.2015.225
13. Grinberg LT, Wang X, Wang C, Sohn PD, Theofilas P, Sidhu M, et al. Argyrophilic grain disease differs from other tauopathies by lacking tau acetylation. *Acta Neuropathol*. (2013) 125:581–93. doi: 10.1007/s00401-013-1080-2
14. Dickson DW, Kouri N, Murray ME, Josephs KA. Neuropathology of frontotemporal lobar degeneration-tau (FTLD-tau). *J Mol Neurosci*. (2011) 45:384–9. doi: 10.1007/s12031-011-9589-0
15. Goedert M, Spillantini MG. Pathogenesis of the tauopathies. *J Mol Neurosci*. (2011) 45:425–31. doi: 10.1007/s12031-011-9593-4
16. Mucke L, Selkoe DJ. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med*. (2012) 2:a006338. doi: 10.1101/cshperspect.a006338
17. Kovacs GG. Molecular pathological classification of neurodegenerative diseases: turning towards precision medicine. *Int J Mol Sci*. (2016) 17:189. doi: 10.3390/ijms17020189
18. Armstrong MJ, Litvan I, Lang AE, Bak TH, Bhatia KP, Borroni B, et al. Criteria for the diagnosis of corticobasal degeneration. *Neurology*. (2013) 80:496–503. doi: 10.1212/WNL.0b013e31827f0fd1
19. Hanger DP, Byers HL, Wray S, Leung KY, Saxton MJ, Seereeram A, et al. Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis. *J Biol Chem*. (2007) 282:23645–54. doi: 10.1074/jbc.M703269200

20. Wesseling H, Mair W, Kumar M, Schlaffner CN, Tang S, Beerepoot P, et al. Tau PTM profiles identify patient heterogeneity and stages of Alzheimer's disease. *Cell*. (2020). 183:1–15 doi: 10.1016/j.cell.2020.10.029
21. Gong CX, Singh TJ, Grundke-Iqbal I, Iqbal K. Phosphoprotein phosphatase activities in Alzheimer disease brain. *J Neurochem*. (1993) 61:921–7. doi: 10.1111/j.1471-4159.1993.tb03603.x
22. Liu F, Grundke-Iqbal I, Iqbal K, Gong CX. Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur J Neurosci*. (2005) 22:1942–50. doi: 10.1111/j.1460-9568.2005.04391.x
23. Forrest SL, Kril JJ, Stevens CH, Kwok JB, Hallupp M, Kim WS, et al. Retiring the term FTDP-17 as MAPT mutations are genetic forms of sporadic frontotemporal tauopathies. *Brain*. (2018) 141:521–34. doi: 10.1093/brain/awx328
24. Scheres SH, Zhang W, Falcon B, Goedert M. Cryo-EM structures of tau filaments. *Curr Opin Struct Biol*. (2020) 64:17–25. doi: 10.1016/j.sbi.2020.05.011
25. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, et al. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature*. (2017) 547:185–90. doi: 10.1038/nature23002
26. Falcon B, Zhang W, Murzin AG, Murshudov G, Garringer HJ, Vidal R, et al. Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature*. (2018) 561:137–40. doi: 10.1038/s41586-018-0454-y
27. Zhang W, Tarutani A, Newell KL, Murzin AG, Matsubara T, Falcon B, et al. Novel tau filament fold in corticobasal degeneration. *Nature*. (2020) 580:283–7. doi: 10.1038/s41586-020-2043-0
28. Ke YD, Suchowerska AK, van der Hoven J, De Silva DM, Wu CW, van Eersel J, et al. Lessons from tau-deficient mice. *Int J Alzheimers Dis*. (2012) 2012:873270. doi: 10.1155/2012/873270
29. Bloom GS. Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurol*. (2014) 71:505–8. doi: 10.1001/jamaneurol.2013.5847
30. Braak H, Braak E. Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol Aging*. (1995) 16:271–8; discussion 278–84. doi: 10.1016/0197-4580(95)00021-6
31. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *J Neuropathol Exp Neurol*. (2012) 71:362–81. doi: 10.1097/NEN.0b013e31825018f7
32. Steele JC, Richardson JC, Olszewski J. Progressive supranuclear palsy. a heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia *Arch Neurol*. (1964) 10:333–59. doi: 10.1001/archneur.1964.00460160003001
33. Williams DR, de Silva R, Paviour DC, Pittman A, Watt HC, Kilford L, et al. Characteristics of two distinct clinical phenotypes in pathologically proven progressive supranuclear palsy: Richardson's syndrome and PSP-parkinsonism. *Brain*. (2005) 128:1247–58. doi: 10.1093/brain/awh488
34. Kovacs GG, Lukic MJ, Irwin DJ, Arzberger T, Respondek G, Lee EB, et al. Distribution patterns of tau pathology in progressive supranuclear palsy. *Acta Neuropathol*. (2020) 140:99–119. doi: 10.1007/s00401-020-02158-2
35. Williams DR, Holton JL, Strand C, Pittman A, de Silva R, Lees AJ, et al. Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. *Brain*. (2007) 130:1566–76. doi: 10.1093/brain/awm104
36. Kouri N, Whitwell JL, Josephs KA, Rademakers R, Dickson DW. Corticobasal degeneration: a pathologically distinct 4R tauopathy. *Nat Rev Neurol*. (2011) 7:263–72. doi: 10.1038/nrneurol.2011.43
37. Ling H, Kovacs GG, Vonsattel JP, Davey K, Mok KY, Hardy J, et al. Astrogliopathy predominates the earliest stage of corticobasal degeneration pathology. *Brain*. (2016) 139:3237–52. doi: 10.1093/brain/aww256
38. Dickson DW, Bergeron C, Chin SS, Duyckaerts C, Horoupian D, Ikeda K, et al. Office of Rare Diseases neuropathologic criteria for corticobasal degeneration. *J Neuropathol Exp Neurol*. (2002) 61:935–46. doi: 10.1093/jnen/61.11.935
39. Irwin DJ, Brettschneider J, McMillan CT, Cooper F, Olm C, Arnold SE, et al. Deep clinical and neuropathological phenotyping of Pick disease. *Ann Neurol*. (2016) 79:272–87. doi: 10.1002/ana.24559
40. Neddens J, Temmel M, Flunkert S, Kerschbaumer B, Hoeller C, Loeffler T, et al. Phosphorylation of different tau sites during progression of Alzheimer's disease. *Acta Neuropathol Commun*. (2018) 6:52. doi: 10.1186/s40478-018-0557-6
41. Su JH, Cummings BJ, Cotman CW. Early phosphorylation of tau in Alzheimer's disease occurs at Ser-202 and is preferentially located within neurites. *Neuroreport*. (1994) 5:2358–62. doi: 10.1097/00001756-199411000-00037
42. Luna-Munoz J, Garcia-Sierra F, Falcon V, Menendez I, Chavez-Macias L, Mena R. Regional conformational change involving phosphorylation of tau protein at the Thr231, precedes the structural change detected by Alz-50 antibody in Alzheimer's disease. *J Alzheimers Dis*. (2005) 8:29–41. doi: 10.3233/JAD-2005-8104
43. Mondragon-Rodriguez S, Perry G, Luna-Munoz J, Acevedo-Aquino MC, Williams S. Phosphorylation of tau protein at sites Ser(396-404) is one of the earliest events in Alzheimer's disease and Down syndrome. *Neuropathol Appl Neurobiol*. (2014) 40:121–35. doi: 10.1111/nan.12084
44. Šimić G, Babić Leko M, Wray S, Harrington C, Delalle I, Jovanov-Milošević N, et al. Tau protein hyperphosphorylation and aggregation in Alzheimer's disease and other tauopathies, and possible neuroprotective strategies. *Biomolecules*. (2016) 6:6. doi: 10.3390/biom6010006
45. Matsuo ES, Shin RW, Billingsley ML, Van deVoorde A, O'Connor M, Trojanowski JQ, et al. 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. doi: 10.1016/0896-6273(94)90264-X
46. Wray S, Saxton M, Anderton BH, Hanger DP. Direct analysis of tau from PSP brain identifies new phosphorylation sites and a major fragment of N-terminally cleaved tau containing four microtubule-binding repeats. *J Neurochem*. (2008) 105:2343–52. doi: 10.1111/j.1471-4159.2008.05321.x
47. Ferrer I, Lopez-Gonzalez I, Carmona M, Arregui L, Dalfo E, Torrejon-Escribano B, et al. Glial and neuronal tau pathology in tauopathies: characterization of disease-specific phenotypes and tau pathology progression. *J Neuropathol Exp Neurol*. (2014) 73:81–97. doi: 10.1097/NEN.0000000000000030
48. Delacourte A, Sergeant N, Wattez A, Gauvreau D, Robitaille Y. Vulnerable neuronal subsets in Alzheimer's and Pick's disease are distinguished by their tau isoform distribution and phosphorylation. *Ann Neurol*. (1998) 43:193–204. doi: 10.1002/ana.410430209
49. Bell K, Cairns NJ, Lantos PL, Rossor MN. Immunohistochemistry distinguishes: between Pick's disease and corticobasal degeneration. *J Neurol Neurosurg Psychiatr*. (2000) 69:835–6. doi: 10.1136/jnnp.69.6.835
50. Bramblett GT, Goedert M, Jakes R, Merrick SE, Trojanowski JQ, Lee VM. Abnormal tau phosphorylation at Ser396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. *Neuron*. (1993) 10:1089–99. doi: 10.1016/0896-6273(93)90057-X
51. Brion JP, Smith C, Couck AM, Gallo JM, Anderton BH. Developmental changes in tau phosphorylation: fetal tau is transiently phosphorylated in a manner similar to paired helical filament-tau characteristic of Alzheimer's disease. *J Neurochem*. (1993) 61:2071–80. doi: 10.1111/j.1471-4159.1993.tb07444.x
52. Goedert M, Jakes R, Crowther RA, Six J, Lubke U, Vandermeeren M, et al. The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. *Proc Natl Acad Sci USA*. (1993) 90:5066–70. doi: 10.1073/pnas.90.11.5066
53. Kenessey A, Yen SH. The extent of phosphorylation of fetal tau is comparable to that of PHF-tau from Alzheimer paired helical filaments. *Brain Res*. (1993) 629:40–6. doi: 10.1016/0006-8993(93)90478-6
54. Seubert P, Mawal-Dewan M, Barbour R, Jakes R, Goedert M, Jonhson G, et al. Detection of phosphorylated Ser262 in fetal tau, adult tau and paired helical filament tau. *J Biol Chem*. (1995) 270:18917–22. doi: 10.1074/jbc.270.32.18917

55. Takuma H, Arawaka S, Mori H. Isoforms changes of tau protein during development in various species. *Brain Res Dev Brain Res*. (2003) 142:121–7. doi: 10.1016/S0165-3806(03)00056-7
56. Yu Y, Run X, Liang Z, Li Y, Liu F, Liu Y, et al. Developmental regulation of tau phosphorylation, tau kinases, and tau phosphatases. *J Neurochem*. (2009) 108:1480–94. doi: 10.1111/j.1471-4159.2009.05882.x
57. Hefti MM, Kim S, Bell AJ, Betters RK, Fiock KL, Iida MA, et al. Tau phosphorylation and aggregation in the developing human brain. *J Neuropathol Exp Neurol*. (2019) 78:930–8. doi: 10.1093/jnen/nlz073
58. Kosik KS, Orecchio LD, Bakalis S, Neve RL. Developmentally regulated expression of specific tau sequences. *Neuron*. (1989) 2:1389–97. doi: 10.1016/0896-6273(89)90077-9
59. Jovanov-Milosevic N, Petrovic D, Sedmak G, Vuksic M, Hof PR, Simic G. Human fetal tau protein isoform: possibilities for Alzheimer's disease treatment. *Int J Biochem Cell Biol*. (2012) 44:1290–4. doi: 10.1016/j.biocel.2012.05.001
60. Trabzuni D, Wray S, Vandrovcova J, Ramasamy A, Walker R, Smith C, et al. MAPT expression and splicing is differentially regulated by brain region: relation to genotype and implication for tauopathies. *Hum Mol Genet*. (2012) 21:4094–103. doi: 10.1093/hmg/dds238
61. Bullmann T, Holzer M, Mori H, Arendt T. Pattern of tau isoforms expression during development in vivo. *Int J Dev Neurosci*. (2009) 27:591–7. doi: 10.1016/j.ijdevneu.2009.06.001
62. Sugiyama S, Prochiantz A, Hensch TK. From brain formation to plasticity: insights on Otx2 homeoprotein. *Dev Growth Differ*. (2009) 51:369–77. doi: 10.1111/j.1440-169X.2009.01093.x
63. Espinosa JS, Stryker MP. Development and plasticity of the primary visual cortex. *Neuron*. (2012) 75:230–49. doi: 10.1016/j.neuron.2012.06.009
64. 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) 9:4225–30. doi: 10.1002/j.1460-2075.1990.tb07870.x
65. Lu M, Kosik K. Competition for microtubule-binding with dual expression of tau missense and splice isoforms. *Mol Biol Cell*. (2001) 12:171–84. doi: 10.1091/mbc.12.1.171
66. 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 USA*. (2003) 100:9548–53. doi: 10.1073/pnas.1633508100
67. Tuerde D, Kimura T, Miyasaka T, Furusawa K, Shimozawa A, Hasegawa M, et al. Isoform-independent and -dependent phosphorylation of microtubule-associated protein tau in mouse brain during postnatal development. *J Biol Chem*. (2018) 293:1781–93. doi: 10.1074/jbc.M117.798918
68. Dawson H, Ferreira A, Eyster M, Ghoshal N, Binder L, Vitek M. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci*. (2001) 114:1179–87.
69. Sennvik K, Boekhoorn K, Lasrado R, Terwel D, Verhaeghe S, Korr H, et al. Tau-4R suppresses proliferation and promotes neuronal differentiation in the hippocampus of tau knockin/knockout mice. *FASEB J*. (2007) 21:2149–61. doi: 10.1096/fj.06-7735com
70. Voelzmann A, Okenve-Ramos P, Qu Y, Chojnowska-Monga M, Del Cano-Espinel M, Prokop A, et al. Tau and spectraplakins promote synapse formation and maintenance through Jun kinase and neuronal trafficking. *Elife*. (2016) 5:38. doi: 10.7554/eLife.14694.038
71. Yoshida H, Ihara Y. Tau in paired helical filaments is functionally distinct from fetal tau: assembly incompetence of paired helical filament-tau. *J Neurochem*. (1993) 61:1183–6. doi: 10.1111/j.1471-4159.1993.tb03642.x
72. Biernat J, Mandelkow EM. The development of cell processes induced by tau protein requires phosphorylation of serine 262 and 356 in the repeat domain and is inhibited by phosphorylation in the proline-rich domains. *Mol Biol Cell*. (1999) 10:727–40. doi: 10.1091/mbc.10.3.727
73. Cho JH, Johnson GV. Glycogen synthase kinase 3beta phosphorylates tau at both primed and unprimed sites. Differential impact on microtubule binding. *J Biol Chem*. (2003) 278:187–93. doi: 10.1074/jbc.M206236200
74. Arendt T. Disturbance of neuronal plasticity is a critical pathogenetic event in Alzheimer's disease. *Int J Dev Neurosci*. (2001) 19:231–45. doi: 10.1016/S0736-5748(01)00007-7
75. Hashimoto M, Masliah E. Cycles of aberrant synaptic sprouting and neurodegeneration in Alzheimer's and dementia with Lewy bodies. *Neurochem Res*. (2003) 28:1743–56. doi: 10.1023/A:1026073324672
76. Tse KH, Herrup K. Re-imagining Alzheimer's disease - the diminishing importance of amyloid and a glimpse of what lies ahead. *J Neurochem*. (2017) 143:432–44. doi: 10.1111/jnc.14079
77. Wang HH, Li Y, Li A, Yan F, Li ZL, Liu ZY, et al. Forskolin induces hyperphosphorylation of tau accompanied by cell cycle reactivation in primary hippocampal neurons. *Mol Neurobiol*. (2018) 55:696–706. doi: 10.1007/s12035-016-0348-7
78. Heldmaier G, Ortmann S, Elvert R. Natural hypometabolism during hibernation and daily torpor in mammals. *Respir Physiol Neurobiol*. (2004) 141:317–29. doi: 10.1016/j.resp.2004.03.014
79. Arendt T, Stieler J, Holzer M. Brain hypometabolism triggers PHF-like phosphorylation of tau, a major hallmark of Alzheimer's disease pathology. *J Neural Transm*. (2015) 122:531–9. doi: 10.1007/s00702-014-1342-8
80. Drew KL, Rice ME, Kuhn TB, Smith MA. Neuroprotective adaptations in hibernation: therapeutic implications for ischemia-reperfusion, traumatic brain injury and neurodegenerative diseases. *Free Radic Biol Med*. (2001) 31:563–73. doi: 10.1016/S0891-5849(01)00628-1
81. Geiser F. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu Rev Physiol*. (2004) 66:239–74. doi: 10.1146/annurev.physiol.66.032102.115105
82. Arendt T, Stieler J, Strijkstra A, Hut R, Rüdiger J, Van der Zee E, et al. Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J Neurosci*. (2003) 23:6972–81. doi: 10.1523/JNEUROSCI.23-18-06972.2003
83. Hartig W, Stieler J, Boerema AS, Wolf J, Schmidt U, Weissfuss J, et al. Hibernation model of tau phosphorylation in hamsters: selective vulnerability of cholinergic basal forebrain neurons - implications for Alzheimer's disease. *Eur J Neurosci*. (2007) 25:69–80. doi: 10.1111/j.1460-9568.2006.05250.x
84. Su B, Wang X, Drew KL, Perry G, Smith MA, Zhu X. Physiological regulation of tau phosphorylation during hibernation. *J Neurochem*. (2008) 105:2098–108. doi: 10.1111/j.1471-4159.2008.05294.x
85. Stieler JT, Bullmann T, Kohl F, Barnes BM, Arendt T. PHF-like tau phosphorylation in mammalian hibernation is not associated with p25-formation. *J Neural Transm*. (2009) 116:345–50. doi: 10.1007/s00702-008-0181-x
86. Stieler JT, Bullmann T, Kohl F, Toien O, Bruckner MK, Hartig W, et al. The physiological link between metabolic rate depression and tau phosphorylation in mammalian hibernation. *PLoS ONE*. (2011) 6:e14530. doi: 10.1371/journal.pone.0014530
87. Bullmann T, Seeger G, Stieler J, Hanics J, Reimann K, Kretzschmann TP, et al. Tau phosphorylation-associated spine regression does not impair hippocampal-dependent memory in hibernating golden hamsters. *Hippocampus*. (2016) 26:301–18. doi: 10.1002/hipo.22522
88. Dave KR, Christian SL, Perez-Pinzon MA, Drew KL. Neuroprotection: lessons from hibernators. *Comp Biochem Physiol B Biochem Mol Biol*. (2012) 162:1–9. doi: 10.1016/j.cbpb.2012.01.008
89. Popov VI, Bocharova LS. Hibernation-induced structural changes in synaptic contacts between mossy fibres and hippocampal pyramidal neurons. *Neuroscience*. (1992) 48:53–62. doi: 10.1016/0306-4522(92)90337-2
90. Popov VI, Bocharova LS, Bragin AG. Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. *Neuroscience*. (1992) 48:45–51. doi: 10.1016/0306-4522(92)90336-Z
91. von der Ohe CG, Darian-Smith C, Garner CC, Heller HC. Ubiquitous and temperature-dependent neural plasticity in hibernators. *J Neurosci*. (2006) 26:10590–8. doi: 10.1523/JNEUROSCI.2874-06.2006
92. Popov VI, Medvedev NI, Patrushev IV, Ignat'ev DA, Morenkov ED, et al. Reversible reduction in dendritic spines in CA1 of rat and ground squirrel subjected to hypothermia-normothermia in vivo: A three-dimensional electron microscope study. *Neuroscience*. (2007) 149:549–60. doi: 10.1016/j.neuroscience.2007.07.059

93. Anton-Fernandez A, Leon-Espinosa G, DeFelipe J, Munoz A. Changes in the golgi apparatus of neocortical and hippocampal neurons in the hibernating hamster. *Front Neuroanat*. (2015) 9:157. doi: 10.3389/fnana.2015.00157
94. Magarinos AM, McEwen BS, Saboureau M, Pevet P. Rapid and reversible changes in intrahippocampal connectivity during the course of hibernation in European hamsters. *Proc Natl Acad Sci USA*. (2006) 103:18775–80. doi: 10.1073/pnas.0608785103
95. Cogut V, Bruintjes JJ, Eggen BJL, van der Zee EA, Henning RH. Brain inflammatory cytokines and microglia morphology changes throughout hibernation phases in Syrian hamster. *Brain Behav Immun*. (2018) 68:17–22. doi: 10.1016/j.bbi.2017.10.009
96. Gonzalez-Riano C, Leon-Espinosa G, Regalado-Reyes M, Garcia A, DeFelipe J, Barbas C. Metabolomic study of hibernating syrian hamster brains: in search of neuroprotective agents. *J Proteome Res*. (2019) 18:1175–90. doi: 10.1021/acs.jproteome.8b00816
97. Virmani A, Binienda Z. Role of carnitine esters in brain neuropathology. *Mol Aspects Med*. (2004) 25:533–49. doi: 10.1016/j.mam.2004.06.003
98. Jeong HS, Park JS, Yang Y, Na SH, Chung YA, Song IU. Cerebral perfusion changes after acetyl-L-carnitine treatment in early Alzheimer's disease using single photon emission computed tomography. *Dement Neurocogn Disord*. (2017) 16:26–31. doi: 10.12779/dnd.2017.16.1.26
99. Reiman EM, Caselli RJ, Yun LS, Chen K, Bandy D, Minoshima S, et al. Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N Engl J Med*. (1996) 334:752–8. doi: 10.1056/NEJM199603213341202
100. Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, et al. Functional brain abnormalities in young adults at genetic risk for late-onset alzheimer's dementia. *Proc Natl Acad Sci USA*. (2004) 101:284–9. doi: 10.1073/pnas.2635903100
101. de Leon MJ, Convit A, Wolf OT, Tarshish CY, DeSanti S, Rusinek H, et al. Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/poitron-emission tomography (FDG/PET). *Proc Natl Acad Sci USA*. (2001) 98:10966–71. doi: 10.1073/pnas.191044198
102. Devous MD, Sr. Functional brain imaging in the dementias: role in early detection, differential diagnosis, and longitudinal studies. *Eur J Nucl Med Mol Imaging*. (2002) 29:1685–96. doi: 10.1007/s00259-002-0967-2
103. Hoyer S, Nitsch R. Cerebral excess release of neurotransmitter amino acids subsequent to reduced cerebral glucose metabolism in early-onset dementia of Alzheimer type. *J Neural Transm*. (1989) 75:227–32. doi: 10.1007/BF01258634
104. Hoyer S, Nitsch R, Oesterreich K. Predominant abnormality in cerebral glucose utilization in late-onset dementia of the Alzheimer type: a cross-sectional comparison against advanced late-onset and incipient early-onset cases. *J Neural Transm Park Dis Dement Sect*. (1991) 3:1–14. doi: 10.1007/BF02251132
105. Hoyer S. Oxidative energy metabolism in Alzheimer brain. Studies in early-onset and late-onset cases. *Mol Chem Neuropathol*. (1992) 16:207–24. doi: 10.1007/BF03159971
106. Hoyer S. Is sporadic Alzheimer disease the brain type of non-insulin dependent diabetes mellitus? A challenging hypothesis. *J Neural Transm (Vienna)*. (1998) 105:415–22. doi: 10.1007/s007020050067
107. Lyman CP, Chatfield PO. Hibernation and cortical electrical activity in the woodchuck (Marmota monax). *Science*. (1953) 117:533–4. doi: 10.1126/science.117.3046.533
108. Chatfield PO, Lyman CP. Subcortical electrical activity in the golden hamster during arousal from hibernation. *Electroencephalogr Clin Neurophysiol*. (1954) 6:403–8. doi: 10.1016/0013-4694(54)90054-1
109. Heller HC. Hibernation: neural aspects. *Annu Rev Physiol*. (1979) 41:305–21. doi: 10.1146/annurev.ph.41.030179.001513
110. Krilowicz BL, Glotzbach SF, Heller HC. Neuronal activity during sleep and complete bouts of hibernation. *Am J Physiol*. (1988) 255:R1008–1019. doi: 10.1152/ajpregu.1988.255.6.R1008
111. Hamill NJ, McGinn MD, Horowitz JM. Auditory brainstem responses in ground squirrels arousing from hibernation. *J Comp Physiol B Biochem Syst Environ Physiol*. (1989) 159:167–72. doi: 10.1007/BF00691737
112. Kilduff TS, Krilowicz B, Milsom WK, Trachsel L, Wang LC. Sleep and mammalian hibernation: homologous adaptations and homologous processes? *Sleep*. (1993) 16:372–86. doi: 10.1093/sleep/16.4.372
113. Walker JM, Glotzbach SF, Berger RJ, Heller HC. Sleep and hibernation in ground squirrels (Citellus spp): electrophysiological observations. *Am J Physiol*. (1977) 233:R213–221. doi: 10.1152/ajpregu.1977.233.5.R213
114. Daan S, Barnes BM, Strijkstra AM. Warming up for sleep? Ground squirrels sleep during arousals from hibernation. *Neurosci Lett*. (1991) 128:265–8. doi: 10.1016/0304-3940(91)90276-Y
115. Hanger DP, Goniotaki D, Noble W. Synaptic localisation of tau. *Adv Exp Med Biol*. (2019) 1184:105–12. doi: 10.1007/978-981-32-9358-8_9
116. Bindman LJ, Lippold OC, Redfearn JW. Comparison of the effects on electrocortical activity of general body cooling of the surface of the brain. *Electroencephalogr Clin Neurophysiol*. (1963) 15:238–45. doi: 10.1016/0013-4694(63)90093-2
117. Volgushev M, Vidyasagar TR, Chistiakova M, Eysel UT. Synaptic transmission in the neocortex during reversible cooling. *Neuroscience*. (2000) 98:9–22. doi: 10.1016/S0306-4522(00)00109-3
118. Garcia-Cabrero AM, Guerrero-Lopez R, Giraldez BG, Llorens-Martin M, Avila J, Serratosa JM, et al. Hyperexcitability and epileptic seizures in a model of frontotemporal dementia. *Neurobiol Dis*. (2013) 58:200–8. doi: 10.1016/j.nbd.2013.06.005
119. Holth JK, Bomben VC, Reed JG, Inoue T, Younkin L, Younkin SG, et al. Tau loss attenuates neuronal network hyperexcitability in mouse and Drosophila genetic models of epilepsy. *J Neurosci*. (2013) 33:1651–9. doi: 10.1523/JNEUROSCI.3191-12.2013
120. Alves M, Kenny A, de Leo G, Beamer EH, Engel T. Tau phosphorylation in a mouse model of temporal lobe epilepsy. *Front Aging Neurosci*. (2019) 11:308. doi: 10.3389/fnagi.2019.00308
121. Sohn PD, Huang CT, Yan R, Fan L, Tracy TE, Camargo CM, et al. Pathogenic tau impairs axon initial segment plasticity and excitability homeostasis. *Neuron*. (2019) 104:458–70. doi: 10.1016/j.neuron.2019.08.008
122. Voskobiynyk Y, Roth JR, Cochran JN, Rush T, Carullo NV, Mesina JS, et al. Alzheimer's disease risk gene BIN1 induces Tau-dependent network hyperexcitability. *Elife*. (2020) 9:e57354. doi: 10.7554/eLife.57354.sa2
123. Decker JM, Mandelkow EM. Presynaptic pathophysiology encoded in different domains of tau - hyper-versus hypoexcitability? *Adv Exp Med Biol*. (2019) 1184:97–103. doi: 10.1007/978-981-32-9358-8_8
124. Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathol*. (2011) 121:171–81. doi: 10.1007/s00401-010-0789-4
125. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol*. (2011) 70:960–9. doi: 10.1097/NEN.0b013e318232a379
126. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. (2011) 7:280–92. doi: 10.1016/j.jalz.2011.03.003
127. Gong CX, Shaikh S, Wang JZ, Zaidi T, Grundke-Iqbal I, Iqbal K. Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain. *J Neurochem*. (1995) 65:732–8. doi: 10.1046/j.1471-4159.1995.65020732.x
128. Liang Z, Liu F, Iqbal K, Grundke-Iqbal I, Wegiel J, Gong CX. Decrease of protein phosphatase 2A and its association with accumulation and hyperphosphorylation of tau in Down syndrome. *J Alzheimers Dis*. (2008) 13:295–302. doi: 10.3233/JAD-2008-13307
129. Luppi M, Hitrec T, Di Cristoforo A, Squarcio F, Stanzani A, Occhinegro A, et al. Phosphorylation and dephosphorylation of tau protein during synthetic torpor. *Front Neuroanat*. (2019) 13:57. doi: 10.3389/fnana.2019.00057
130. Drew KL, Harris MB, LaManna JC, Smith MA, Zhu XW, Ma YL. Hypoxia tolerance in mammalian heterotherms. *J Exp Biol*. (2004) 207:3155–62. doi: 10.1242/jeb.01114
131. Geiser F. Hibernation. *Curr Biol*. (2013) 23:R188–193. doi: 10.1016/j.cub.2013.01.062
132. Hartig W, Klein C, Brauer K, Schuppel KF, Arendt T, Bruckner G, et al. Abnormally phosphorylated protein tau in the cortex of aged individuals

of various mammalian orders. *Acta Neuropathol*. (2000) 100:305–12. doi: 10.1007/s004010000183
133. Dujardin S, Commins C, Lathuiliere A, Beerepoot P, Fernandes AR, Kamath TV, et al. Tau molecular diversity contributes to clinical heterogeneity in Alzheimer's disease. *Nat Med*. (2020) 26:1256–63. doi: 10.1038/s41591-020-0938-9
134. Ait-Bouziad N, Chiki A, Limorenko G, Xiao S, Eliezer D, Lashuel HA. Phosphorylation of the overlooked tyrosine 310 regulates the structure, aggregation, and microtubule- and lipid-binding properties of Tau. *J Biol Chem*. (2020) 295:7905–22. doi: 10.1074/jbc.RA119.012517
135. Briner A, Gotz J, Polanco JC. Fyn kinase controls tau aggregation *in vivo*. *Cell Rep*. (2020) 32:108045. doi: 10.1016/j.celrep.2020.108045
136. Ittner A, Chua SW, Bertz J, Volkerling A, van der Hoven J, Gladbach A, et al. Site-specific phosphorylation of tau inhibits amyloid-beta toxicity in Alzheimer's mice. *Science*. (2016) 354:904–8. doi: 10.1126/science.aah6205
137. Piacentini R, Li Puma DD, Mainardi M, Lazzarino G, Tavazzi B, Arancio O, et al. Reduced gliotransmitter release from astrocytes mediates tau-induced synaptic dysfunction in cultured hippocampal neurons. *Glia*. (2017) 65:1302–16. doi: 10.1002/glia.23163
138. Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, et al. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nat Neurosci*. (2015) 18:1584–93. doi: 10.1038/nn.4132
139. Martini-Stoica H, Cole AL, Swartzlander DB, Chen F, Wan YW, Bajaj L, et al. TFEB enhances astroglial uptake of extracellular tau species and reduces tau spreading. *J Exp Med*. (2018) 215:2355–77. doi: 10.1084/jem.20172158
140. Yi S, Liu Q, Wang X, Qian T, Wang H, Zha G, et al. Tau modulates Schwann cell proliferation, migration and differentiation following peripheral nerve injury. *J Cell Sci*. (2019) 132:jcs222059. doi: 10.1242/jcs.222059
141. Yoshiyama Y, Zhang B, Bruce J, Trojanowski J, Lee V. Reduction of detyrosinated microtubules and Golgi fragmentation are linked to tau-induced degeneration in astrocytes. *J Neurosci*. (2003) 23:10662–71. doi: 10.1523/JNEUROSCI.23-33-10662.2003
142. Richter-Landsberg C. The cytoskeleton in oligodendrocytes. Microtubule dynamics in health and disease. *J Mol Neurosci*. (2008) 35:55–63. doi: 10.1007/s12031-007-9017-7
143. Sferra A, Petrini S, Bellacchio E, Nicita F, Scibelli F, Dentici ML, et al. TUBB variants underlying different phenotypes result in altered vesicle trafficking and microtubule dynamics. *Int J Mol Sci*. (2020) 21:1385. doi: 10.3390/ijms21041385
144. Billingsley ML, Kincaid RL. Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. *Biochem J*. (1997) 323:577–91. doi: 10.1042/bj3230577
145. Han X, Zhang T, Liu H, Mi Y, Gou X. Astrocyte senescence and Alzheimer's disease: a review. *Front Aging Neurosci*. (2020) 12:148. doi: 10.3389/fnagi.2020.00148
146. Tang M, Harrison J, Deaton CA, Johnson GVW. Tau clearance mechanisms. *Adv Exp Med Biol*. (2019) 1184:57–68. doi: 10.1007/978-981-32-9358-8_5
147. Munoz-Carvajal F, Sanhueza M. The mitochondrial unfolded protein response: a hinge between healthy and pathological aging. *Front Aging Neurosci*. (2020) 12:581849. doi: 10.3389/fnagi.2020.581849
148. Hoglinger GU, Lannuzel A, Khondiker ME, Michel PP, Duyckaerts C, Feger J, et al. The mitochondrial complex I inhibitor rotenone triggers a cerebral tauopathy. *J Neurochem*. (2005) 95:930–9. doi: 10.1111/j.1471-4159.2005.03493.x
149. Escobar-Khondiker M, Hollerhage M, Muriel MP, Champy P, Bach A, Depienne C, et al. Annonacin, a natural mitochondrial complex I inhibitor, causes tau pathology in cultured neurons. *J Neurosci*. (2007) 27:7827–37. doi: 10.1523/JNEUROSCI.1644-07.2007
150. Wilson DF, Matschinsky FM. Cerebrovascular blood flow design and regulation; vulnerability in aging brain. *Front Physiol*. (2020) 11:584891. doi: 10.3389/fphys.2020.584891
151. Raz L, Bhaskar K, Weaver J, Marini S, Zhang Q, Thompson JF, et al. Hypoxia promotes tau hyperphosphorylation with associated neuropathology in vascular dysfunction. *Neurobiol Dis*. (2019) 126:124–36. doi: 10.1016/j.nbd.2018.07.009
152. Benveniste H, Liu X, Koundal S, Sanggaard S, Lee H, Wardlaw J. The glymphatic system and waste clearance with brain aging: a review. *Gerontology*. (2019) 65:106–19. doi: 10.1159/000490349
153. Patel TK, Habimana-Griffin L, Gao X, Xu B, Achilefu S, Alitalo K, et al. Dural lymphatics regulate clearance of extracellular tau from the CNS. *Mol Neurodegener*. (2019) 14:11. doi: 10.1186/s13024-019-0312-x
154. Sandusky-Beltran LA, Sigurdsson EM. Tau immunotherapies: lessons learned, current status and future considerations. *Neuropharmacology*. (2020) 175:108104. doi: 10.1016/j.neuropharm.2020.108104
155. Pedersen JT, Sigurdsson EM. Tau immunotherapy for Alzheimer's disease. *Trends Mol Med*. (2015) 21:394–402. doi: 10.1016/j.molmed.2015.03.003
156. Sigurdsson EM. Tau immunotherapies for Alzheimer's disease and related tauopathies: progress and potential pitfalls. *J Alzheimers Dis*. (2018) 66:855–6. doi: 10.3233/JAD-189010
157. Congdon EE, Sigurdsson EM. Tau-targeting therapies for Alzheimer disease. *Nat Rev Neurol*. (2018) 14:399–415. doi: 10.1038/s41582-018-0013-z
158. Congdon EE, Chukwu JE, Shamir DB, Deng J, Ujla D, Sait HBR, et al. Tau antibody chimerization alters its charge and binding, thereby reducing its cellular uptake and efficacy. *EBioMedicine*. (2019) 42:157–73. doi: 10.1016/j.ebiom.2019.03.033
159. Pernegre C, Duquette A, Leclerc N. Tau secretion: good and bad for neurons. *Front Neurosci*. (2019) 13:649. doi: 10.3389/fnins.2019.00649
160. Barthélemy NR, Li Y, Joseph-Mathurin N, Gordon BA, Hassenstab J, Benzinger T, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat Med*. (2020) 26:398–407. doi: 10.1038/s41591-020-0781-z
161. Iqbal K, Liu F, Gong CX. Recent developments with tau-based drug discovery. *Expert Opin Drug Discov*. (2018) 13:399–410. doi: 10.1080/17460441.2018.1445084
162. Iqbal K, Alonso A, del C, Chen S, Chohan MO, El-Akkad E, et al. Tau pathology in Alzheimer disease and other tauopathies. *Biochim et Biophys Acta*. (2005) 1739:198–210. doi: 10.1016/j.bbadis.2004.09.008
163. Tanimukai H, Grundke-Iqbal I, Iqbal K. Up-regulation of inhibitors of protein phosphatase-2A in Alzheimer's disease. *Am J Pathol*. (2005) 166:1761–71. doi: 10.1016/S0002-9440(10)62486-8
164. Basurto-Islas G, Grundke-Iqbal I, Tung YC, Liu F, Iqbal K. Activation of asparaginyl endopeptidase leads to Tau hyperphosphorylation in Alzheimer disease. *J Biol Chem*. (2013) 288:17495–507. doi: 10.1074/jbc.M112.446070
165. Sontag JM, Nunbhakdi-Craig V, Montgomery L, Arning E, Bottiglieri T, Sontag E. Folate deficiency induces in vitro and mouse brain region-specific downregulation of leucine carboxyl methyltransferase-1 and protein phosphatase 2A B(alpha) subunit expression that correlate with enhanced tau phosphorylation. *J Neurosci*. (2008) 28:11477–87. doi: 10.1523/JNEUROSCI.2816-08.2008
166. Arif M, Kazim SF, Grundke-Iqbal I, Garruto RM, Iqbal K. Tau pathology involves protein phosphatase 2A in parkinsonism-dementia of Guam. *Proc Natl Acad Sci USA*. (2014) 111:1144–9. doi: 10.1073/pnas.1322614111
167. Gong CX, Iqbal K. Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Curr Med Chem*. (2008) 15:2321–8. doi: 10.2174/092986708785909111
168. Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, Gong CX. O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. *Proc Natl Acad Sci USA*. (2004) 101:10804–9. doi: 10.1073/pnas.0400348101
169. Liu F, Shi J, Tanimukai H, Gu J, Gu J, Grundke-Iqbal I, et al. Reduced O-GlcNAcylation links lower Brain glucose metabolism and tau pathology in Alzheimer's disease. *Brain*. (2009) 132:1820–32. doi: 10.1093/brain/awp099
170. Trzeciakiewicz H, Ajit D, Tseng JH, Chen Y, Ajit A, Tabassum Z, et al. An HDAC6-dependent surveillance mechanism suppresses tau-mediated neurodegeneration and cognitive decline. *Nat Commun*. (2020) 11:5522. doi: 10.1038/s41467-020-19317-4
171. Barten DM, Fanara P, Andorfer C, Hoque N, Wong PY, Husted KH, et al. Hyperdynamic microtubules, cognitive deficits, and pathology are improved in tau transgenic mice with low doses of the microtubule-stabilizing agent BMS-241027. *J Neurosci*. (2012) 32:7137–45. doi: 10.1523/JNEUROSCI.0188-12.2012
172. Zhang B, Carroll J, Trojanowski JQ, Yao Y, Iba M, Potuzak JS III, 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*. (2012) 32:3601–11. doi: 10.1523/JNEUROSCI.4922-11.2012

173. Tsai RM, Miller Z, Koestler M, Rojas JC, Ljubenkov PA, Rosen HJ, et al. Reactions to multiple ascending doses of the microtubule stabilizer TPI-287 in patients with alzheimer disease, progressive supranuclear palsy, and corticobasal syndrome: a randomized clinical trial. *JAMA Neurol*. (2020) 77:215–24. doi: 10.1001/jamaneurol.2019.3812
174. Qiang L, Sun X, Austin TO, Muralidharan H, Jean DC, Liu M, et al. Tau does not stabilize axonal microtubules but rather enables them to have long labile domains. *Curr Biol*. (2018) 28:2181–9. doi: 10.1016/j.cub.2018.05.045
175. Drummond E, Pires G, MacMurray C, Askenazi M, Nayak S, Bourdon M, et al. Phosphorylated tau interactome in the human Alzheimer's disease Brain. *Brain*. (2020) 143:2803–17. doi: 10.1093/brain/awaa223
176. Fu H, Possenti A, Freer R, Nakano Y, Hernandez Vi, llegas NC, et al. A tau homeostasis signature is linked with the cellular and regional vulnerability of excitatory neurons to tau pathology. *Nat Neurosci*. (2019) 22:47–56. doi: 10.1038/s41593-018-0298-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 07 January 2021
doi: 10.3389/fneur.2020.573324

Impact of Tau on Neurovascular Pathology in Alzheimer's Disease

*Elisa Canepa and Silvia Fossati**

Alzheimer's Center at Temple (ACT), Lewis Katz School of Medicine, Temple University, Philadelphia, PA, United States

Alzheimer's disease (AD) is a chronic neurodegenerative disorder and the most prevalent cause of dementia. The main cerebral histological hallmarks are represented by parenchymal insoluble deposits of amyloid beta (Aβ plaques) and neurofibrillary tangles (NFT), intracellular filamentous inclusions of tau, a microtubule-associated protein. It is well-established that cerebrovascular dysfunction is an early feature of AD pathology, but the detrimental mechanisms leading to blood vessel impairment and the associated neurovascular deregulation are not fully understood. In 90% of AD cases, Aβ deposition around the brain vasculature, known as cerebral amyloid angiopathy (CAA), alters blood brain barrier (BBB) essential functions. While the effects of vascular Aβ accumulation are better documented, the scientific community has only recently started to consider the impact of tau on neurovascular pathology in AD. Emerging compelling evidence points to transmission of neuronal tau to different brain cells, including astrocytes, as well as to the release of tau into brain interstitial fluids, which may lead to perivascular neurofibrillar tau accumulation and toxicity, affecting vessel architecture, cerebral blood flow (CBF), and vascular permeability. BBB integrity and functionality may therefore be impacted by pathological tau, consequentially accelerating the progression of the disease. Tau aggregates have also been shown to induce mitochondrial damage: it is known that tau impairs mitochondrial localization, distribution and dynamics, alters ATP and reactive oxygen species production, and compromises oxidative phosphorylation systems. In light of this previous knowledge, we postulate that tau can initiate neurovascular pathology in AD through mitochondrial dysregulation. In this review, we will explore the literature investigating tau pathology contribution to the malfunction of the brain vasculature and neurovascular unit, and its association with mitochondrial alterations and caspase activation, in cellular, animal, and human studies of AD and tauopathies.

Keywords: tau, neurovascular unit, mitochondria, caspases, Alzheimer's disease, tauopathies, vascular dysfunction

OPEN ACCESS

Edited by:
Sonia Do Carmo,
McGill University, Canada

Reviewed by:
Boon Lead Tee,
Trinity College Dublin, Ireland
Sudeshna Das,
Massachusetts General Hospital and Harvard Medical School, United States

***Correspondence:**
Silvia Fossati
silvia.fossati@temple.edu

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *16 June 2020*
Accepted: *24 November 2020*
Published: *07 January 2021*

Citation:
Canepa E and Fossati S (2021) Impact of Tau on Neurovascular Pathology in Alzheimer's Disease. Front. Neurol. 11:573324. doi: 10.3389/fneur.2020.573324

INTRODUCTION

Alzheimer's Disease, Neurovascular Unit and Blood-Brain Barrier Dysfunction

Alzheimer's disease (AD) is a progressive and deadly neurodegenerative disorder recognized by the World Health Organization as the most prevalent form of dementia (1). AD initial symptoms typically manifest as mild cognitive impairment (MCI). Although MCI does not always convert to AD or dementia, a percentage of MCI cases will worsen over the years, eventually progressing to the severe cognitive decline, emotional and behavioral changes, visuospatial and motor deficits,

characteristics of late-stage AD (2). These devastating cognitive and physical aspects are correlated with neuronal loss and extensive brain atrophy, predominantly in the hippocampus and cortex (3). The most studied and scientifically confirmed neuropathological hallmarks of AD are senile plaques (SP) and neurofibrillary tangles (NFT) in association with neuronal degeneration, loss of synapses, neuroinflammation, and oxidative stress (4). Furthermore, conspicuous studies indicate that brain vascular dysregulation is an early feature of AD, contributing to the progression of the pathology, and suggesting a tight link between cerebrovascular alterations and neurodegeneration (5–8). SP are abnormal parenchymal insoluble deposits of amyloid-beta (Aβ), which derives from amyloid precursor protein (APP) processing. Aβ monomers aggregate into more complex species (oligomers and protofibrils) which represent the toxic species for most brain cells (9, 10) and eventually form fibrils (11). Interestingly, Aβ accumulation also takes place around the brain micro- and macro-vasculature, in a well-described AD pathological feature known as cerebral amyloid angiopathy (CAA) (12), which leads to severe cerebral vascular dysfunction (CVD) (10, 13–16). NFT are intracellular inclusions of paired helical filaments (PHF) of the microtubule-associated protein (MAP) tau. Physiologically, specific phosphorylation and dephosphorylation events regulate tau microtubule (MT)-binding property, thus modulating tau functions, including promoting MT assembly and dynamics, and maintaining neuronal spatial organization and stability (17, 18). In AD brains, tau phosphorylation levels are 3–4-fold higher compared to non-demented adult brains (19, 20), and phosphorylation facilitates tau aggregation, causing cell morphology, functionality, and viability disruption. Besides the documented effects of Aβ deposition around cerebral blood vessels (BVs), recent evidence indicates that tau pathology induces CVD (21–23), and that the presence of hippocampal perivascular tau strictly correlates with blood brain barrier (BBB) permeability and loss of integrity (24), prompting new questions to elucidate the molecular mechanisms through which tau toxicity affects the cerebral vasculature, in both AD and other tauopathies.

The BBB is a highly specialized and selective semipermeable interface between the central nervous system (CNS) and the peripheral circulation, regulating the entrance of blood specific components into the brain and the clearance of potentially neurotoxic substances from the CNS to the blood. The morpho-functional unit, which includes the BBB and is responsible for maintaining its unique properties, is the neurovascular unit (NVU). The NVU is constituted by endothelial cells (ECs) that form the BVs and are strongly attached to each other via a complex network of sealing proteins named tight junctions (TJs) (25), by the surrounding smooth muscle cells (SMC) or pericytes, together with neurons, astrocytes, and microglia cells (8, 26–28). BV-surrounding cells influence and co-operate in maintaining the structural and functional BBB phenotype (26). Astrocytes are the major glial cell type (∼98%) at the parenchymal basal membrane of the microvasculature (29), with microglial cells occupying the remaining surface. In addition to their contribution to BBB maintenance and permeability, astrocytes are considered immunocompetent cells, playing a central role in detecting detrimental signals following which they start secreting cytokines and chemokines, boosting innate and adaptive immune cell activation and trafficking, and thus affecting BBB function (30). Microglia are brain innate resident immune cells, involved in the active surveillance of CNS. When exposed to pathogens, tissue damage or toxic substances, microglial cells become activated, releasing pro-inflammatory cytokines, chemotactic factors, and mediate adaptive immunity, acting as antigen-presenting cells (31, 32). If activated, they can interact with cerebral microvasculature, and increase capillary permeability by producing reactive oxygen species (ROS), and promoting monocyte and lymphocyte migration through the BBB (33, 34). Importantly, the extensive astrogliosis and microgliosis present in AD brains (35–38), may further exacerbate vascular dysfunction. Morphologically, AD brains display BBB changes such as reduced microvascular density, increased capillary tortuosity and fragmentation with fewer intact branches, atrophic string vessels, and changes in vessel diameter (8, 39, 40), that impair vascular fitness causing decreased cerebral blood flow (CBF), hypoxia, dysregulated nutrient and oxygen transport to the CNS, and disrupted cerebral clearance. As a consequence, amplification of cellular stress, accumulation of toxic metabolic waste, uncontrolled detrimental inflammatory response and infiltration of blood-borne molecules and cells occur, eliciting neurodegenerative processes and progressive decrease in cognitive functions (8, 41, 42). All together, these data provide a strong evidence of the connection between BBB malfunction and AD, although the leading molecular mechanisms triggering CVD have not been fully elucidated.

While Aβ aggregation and deposition have long been associated with cerebrovascular alterations in AD, the scientific community has recently started to consider the role of tau in BBB dysfunction, supported by a rapidly growing literature that confirms CVD in other tauopathies.

Here, we will review what is known about tau and neurovascular dysregulation, exploring and providing perspective on some of the possible molecular events through which tau may exert its toxicity on the NVU.

Tau and Its Transmission

The MAPs family includes three classes of polypeptides predominantly expressed in neurons: MAP1, MAP2, and tau. While MAP1 and MAP2 have been largely found in dendrites, tau has been detected mainly in axons (43, 44), where it is involved in primary functions, including neuronal development control (45–47), vesicular and axonal transport (48, 49), and neuronal polarity maintenance (50), amongst others. Structurally, tau presents a basic proline-rich region (aa155–242) which contains serine (S), threonine (T), and tyrosine (Y) potential phosphorylation sites (51). In physiological state, tau is phosphorylated or dephosphorylated based on the balance of kinase (e.g., GSK3β and CDK5) and phosphatase (e.g., PP1, PP2A, B and C) activities (47, 52–55). This equilibrium confers tau the ability to bind and stabilize tubulin polymerization, requisite condition for maintaining axonal and dendritic shape, and thus functionality (56, 57). Due to hyperphosphorylation, tau MT-binding property is lost, inducing its oligomerization and the formation of

PHF which progressively aggregate into NFT (58, 59). As a consequence, alterations of cytoskeleton architecture occur, leading to axonal transport disruption, synaptic dysfunction, and eventually neuronal cell death (47, 60–62). Indeed, a characteristic brain accumulation of highly phosphorylated tau is found in AD, and other tauopathies, such as progressive supranuclear palsy (PSP), Pick's disease (PiD), corticobasal degeneration (CBD), and frontotemporal dementia FTD (63).

Although research in neurodegenerative disorders focuses mainly on tau hyperphosphorylation, it is relevant to mention that tau can also be subject to concurrent or alternative post-translational modifications (PTMs), including N- and C-terminal proteolytic cleavage (truncation), nitration, glycosylation, acetylation, glycation, ubiquitination, or polyamination (64), that exacerbate tau pathology. For instance, in AD human brains, C-terminal tau fragments generated by caspase-3 cleavage at aspartic acid residue 421 (D421) have been detected (65). Considerable evidence shows that caspase-3-truncated tau species are particularly prone to phosphorylation (65), and that caspase-dependent cleavage process increases tau propensity to self-aggregate, boosting the rate of tau polymerization and NFT assembly (45, 47, 59, 66), and fostering dendritic spine loss, synaptic impairment and memory deficits (67, 68). Similarly, caspase-6 activity derived N-terminal truncated tau segments have been found in AD cerebral tissue (69).

Collectively, these data indicate that misfolded conformations of tau facilitate the development of aggregates that appear to be crucial for neuronal demise in AD and other tauopathies.

In the last decade, a wealth of studies has suggested that misfolded tau spreads in a prion-like manner in tauopathy brains (70–72). The hypothesis of the prion-like propagation has been proposed from the observation that the progressive accumulation of tau appeared to spread in a foreseeable pattern, along known anatomically connected neuronal networks (73), analogously to that reported for prion proteins (72).

Albeit tau is an intracellular protein, both tau and P-tau can also be detected in brain interstitial fluid (74), cerebrospinal fluid (CSF) (75), and, as shown more recently, in blood (76–79). In AD patients, CSF and plasma tau and phosphorylated tau (P-tau) concentrations are significantly higher compared to healthy controls. Since tau in biofluids is considered to be for the most part neuron-derived, CSF and plasma tau concentrations typically represent the intensity of neurodegeneration (76, 78, 80, 81). CSF P-tau, on the other hand, is an established AD biomarker also used in clinical trials, reflecting more specifically the progression of tau pathology and correlating with cognitive dysfunction. Recently, plasma P-tau^{181} and P-tau^{217} have also been proposed as novel biomarkers for AD (82–84). Although blood tau and P-tau fragments are of great interest as biomarkers, these peptides are present at very low concentrations in the circulation (in the low pg/ml), and it is still unknown if circulating tau species may be able to induce toxic effects on the vasculature, both in the brain and in the periphery. In particular, more studies are needed to clarify any possible toxic effects of blood P-tau, especially in conditions in which cerebral vessels are already dysfunctional. On the other hand, CSF P-tau, present at higher concentrations in the perivascular spaces, may be a likely contributor to neurovascular pathology, especially in situations in which perivascular clearance is compromised, which may facilitate its accumulation around brain vessels (85–87). Interestingly, it has been reported that, besides prompting intracellular toxicity, tau can be secreted and taken up by healthy neurons (88–90), indicating that extracellular or CSF P-tau may be critical for the progression of tau pathology. Recent studies in the AD field have demonstrated that seed-competent tau spreads along neural connections, is detected in synaptosomes and white matter axons, and that tau seeding precedes the presence of hyperphosphorylated tau in synaptically connected regions (91–94). To further elucidate the propagating properties of tau, many studies have investigated the potential molecular processes involved. Numerous neuronal mechanisms have been identified as non-mutually exclusive sources of extracellular tau, including synaptic secretion (95), direct unconventional translocation across the plasma-membrane (96, 97), release in extracellular vesicles such as exosomes (98–100) and ectosomes (101), or tunneling nanotubes (TNTs) (102, 103). In pathological conditions, neuronal deterioration may also account for extracellular tau which is internalized by neighboring cells via bulk endocytosis (88, 89, 104, 105), clathrin- (106, 107) or low-density lipoprotein receptor related protein 1 (LRP1)-mediated endocytosis (108), micropinocytosis by heparin sulfate proteoglycans (90, 109–111) or TNTs (102, 103) (**Figure 1**). Following cellular entry of tau, neurons can seed physiological monomers, perpetuating the pathological process (112). It has also been demonstrated that neuronal activity can induce tau release (95), enhancing intercellular spread of tau (113).

As mentioned above, AD and tauopathy brains display widespread astrogliosis and microgliosis, which closely correlate with the areas of neurodegeneration (114, 115). Intriguingly, several studies have reported the accumulation of toxic tau in astrocytes and microglia in different tauopathies (116–121). Endogenous glial tau expression has been reported (122, 123), although at much lower levels compared to neurons (124). Therefore, it has been postulated that the intracellular presence of tau in these cell types may be, at least in part, explained by its internalization from the extracellular milieu. Recent evidence has demonstrated that astrocytic tau uptake occurs via micropinocytosis, followed by enhanced lysosomal pathways (125). It is likely that, in pathological conditions, tau engulfed astrocytes exhibit dysregulated clearance mechanisms, further intensifying tau propagation and toxicity. Additionally, it is known that microglia have the competence to phagocytize tau (126–132), and the presence of microglial aggregated or hyperphosphorylated tau has been observed in mice ad humans with tau pathology (119, 120, 126–128, 131, 133). The microglial process of tau internalization seems to be mediated by CX3CR1 receptor (120, 133). More importantly, at the site of tau colocalization with astrocytes and microglia, several pro-inflammatory cytokines have been detected (134), indicating a high neuroinflammatory state. During inflammation, reactive glial cells continuously secrete cytokines and chemokines, which further recruit and activate innate and adaptive immune cells (30–32), initiating a feed-forward detrimental inflammatory response, feature of AD and tauopathies (4, 134), which may

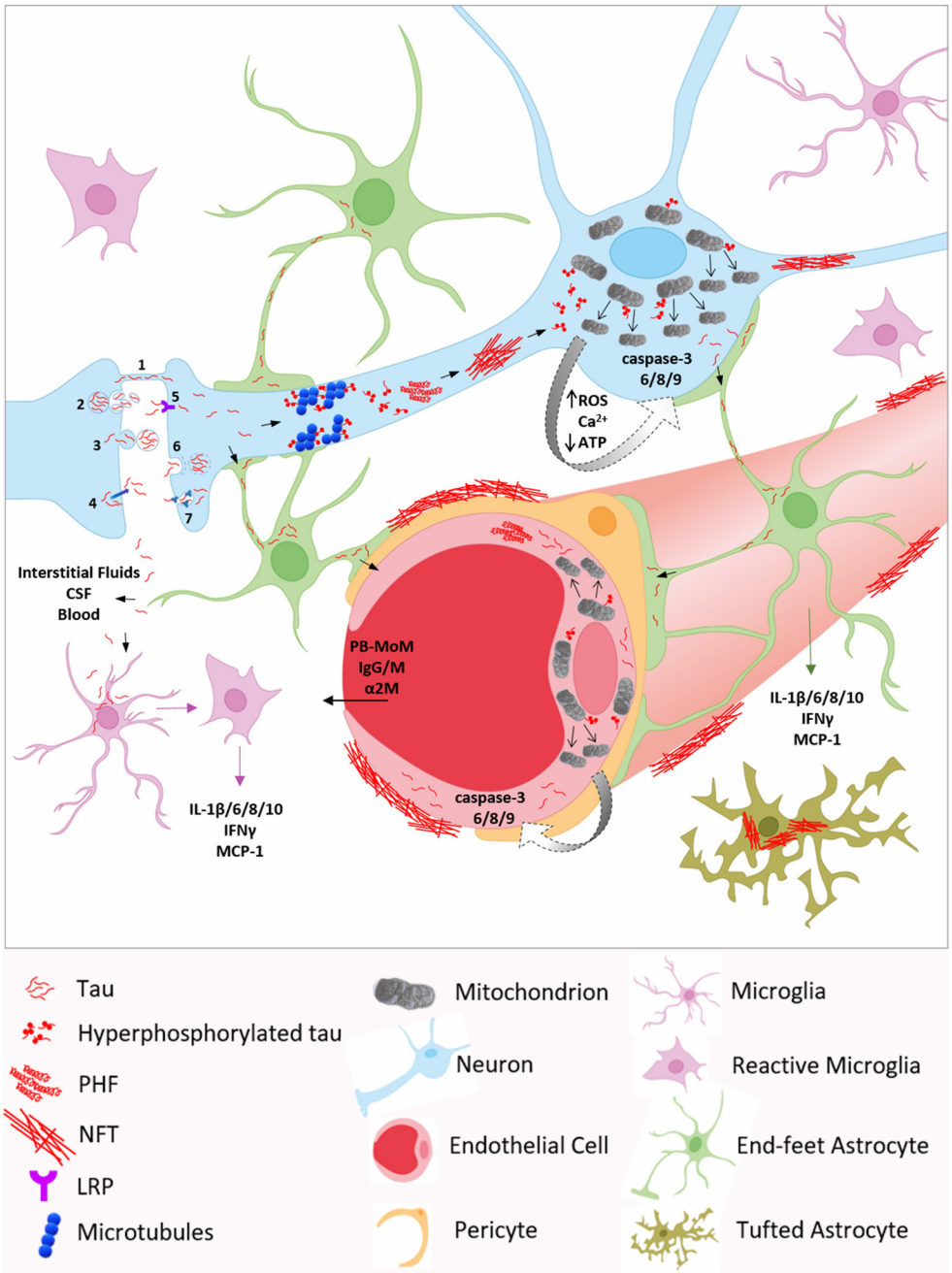


FIGURE 1 | Molecular mechanisms underlying neurovascular tau toxicity in AD and other tauopathies. Neuronal transmission through (1) tunneling nanotubes (TNTs), (2) exosomes, (3) ectosomes, or (4) plasma membrane may account for extracellular tau, which can be internalized by neighboring neurons *via* (5) low-density lipoprotein receptor (LRP), (6) clathrin-mediated endocytosis, (7) micropinocytosis by heparin sulfate proteoglycans, or (1) TNTs. Tau is also detected in brain interstitial fluids, cerebrospinal fluid (CSF) and in blood, reflecting the intensity of neurodegeneration. Inside the neuron, hyperphosphorylated tau promotes microtubules disassembly, aggregates into oligomers and paired helical filaments (PHF) which, in turn, accumulate, leading to neurofibrillary tangles (NFT) deposition. Phosphorylated tau can interact with mitochondrial proteins, such as Drp1, triggering excessive mitochondrial fission and mitochondrial dysfunction, including elevated ROS production, Ca^{2+} homeostasis dysregulation, decreased ATP production, and ultimately caspase activation. Caspase-3 cleaved tau facilitates tau phosphorylation and self-aggregation, further exacerbating tau pathology. Tau can propagate to astrocytes and microglia, and be internalized *via* micropinocytosis and CX3CR1, respectively. In astrocytes, the accumulation of tau fibrils around the nucleus confers the characteristic tufted phenotype. Tau pathological species, through astrocytic end-feet and interstitial fluids, may spread to endothelial cells and pericytes, inducing blood brain barrier (BBB) disruption and permeability to blood-borne components, including peripheral blood monocyte-derived macrophages (PB-MoM), immunoglubulins (IgGs and IgMs), and α2-macroglobulin (α2M). Moreover, the presence of tau within glial cells induces the secretion of several pro-inflammatory cytokines and chemokines, such as IL-1β,−6, - 8,−10, IFNγ, and MCP-1, initiating a high inflammatory state, which contributes to BBB integrity loss.

also affects neurovascular and BBB function. Another hypothesis proposes that tau toxicity might be mediated by the brain vasculature, considering that vascular dysfunction, dendritic deterioration and inflammation take place before neuronal loss in a mouse model of tau-induced neurodegeneration (135) or before tau phosphorylation in a salt-rich diet model (136). In turn, tau toxicity may also be in part responsible for the vascular dysfunction present in AD and related disorders. In confirmation of this, considerable studies have shown that tau pathology triggers alterations of BVs and vascular inflammation (23, 135, 137–139), which may be induced by tau oligomers and fibrils deposition around the microvasculature (23, 24, 140). Moreover, tau-induced neuroinflammation can additionally damage BBB, leading to the infiltration of peripheral immune cells and endothelial signaling molecules expression (141, 142), which keep glial cells active (22, 143), worsening inflammation, neurovascular pathology, and consequentially neuronal activity.

All together, these results suggest how tau toxicity may propagate from neurons to NVU components, and potentially activate harmful mechanisms that interfere with neurovascular function and inflammation, exacerbating neurodegeneration.

TAU AND NEUROVASCULAR DYSFUNCTION

Cell Studies

As previously described, the BBB is a dynamic and extremely specialized barrier which physically separates CNS and systemic circulation. The NVU is a complex functional unit including the BBB and is responsible for its properties and health. ECs, mural cells (including vascular smooth muscle cells and pericytes), astrocytes, microglia and neurons (26, 144) compose the NVU and contribute to its functions. Multiple studies have confirmed NVU abnormalities in AD, describing CVD as an early event in AD pathology, and establishing a direct correlation between brain vasculature dysregulation and neurodegeneration (5–8). The potential molecular pathways linking tau toxicity and neurovascular dysfunction have recently started to be evaluated, both *in vitro* and *in vivo* (**Table 1**). As reported below, a growing body of literature shows that other tauopathies, in addition to AD, also display severe cerebrovascular changes (22, 24, 28, 142, 145–149) (**Table 2**).

A recent study has demonstrated that primary rat brain ECs (RBEC) exposed to human oligomeric tau dramatically increase the expression of genes related to inflammation, endocytosis, angiogenesis, blood coagulation, and vasoconstriction (142). The authors have also observed upregulated expression of genes involved in diapedesis, the process of immune cell migration across the endothelial wall from the blood circulation to brain parenchyma. Using RBEC monolayers as an *in vitro* BBB model, they have analyzed tau-induced transmigration of peripheral blood monocyte-derived macrophages (PB-MoM) isolated from rat. When RBEC are incubated in conditioned media (CM) of astrocytes previously challenged with tau, a 3-fold accelerated transmigration of PB-MoM has been detected. In the same conditions, EC pre-treatment with monoclonal antibodies anti-ICAM-1 and anti-VCAM-1 reduces PB-MoM transmigration, indicating that these specific endothelial adhesion molecules may play a role in tau-induced trafficking of blood cells across BBB (142). The effects of human truncated tau have been evaluated in a similar *in vitro* model of BBB, consisting of a co-culture of primary RBEC and mixed rat primary astrocyte (85–90%)-microglia (10–15%) cultures (22). When tau is added to the abluminal compartment of the co-culture chamber, where astrocytes and microglia are seeded, a significant decrease of trans-endothelial electrical resistance (TEER) has been observed, concomitantly with an augmented mannitol permeability through the BBB, in contrast to controls. The measurement of pro-inflammatory molecules indicates that BBB breakdown is likely mediated by TNF-α and the chemokine MCP-1, which are abundantly released by glial cells, following tau treatment (22). A different *in vitro* study confirmed the harmful effect of tau on NVU components, showing that cultured rat microglia, transfected with human tau40, were significantly activated compared to control. Activation was confirmed by higher ability of migration, enhanced phagocytosis and increased CM levels of nitric oxide (NO), and IL-1β, IL-6, TNF-α, and IL-10 inflammatory cytokines (145), which may act as mediators of tau-induced BBB dysfunction. Indeed, if on one hand IL-1β and TNF-α regulate the expression of endothelial TJ proteins (156, 157), on the other hand, stimulation of ECs with both TNF-α and IFN-γ increases the level of adhesion molecules, such as ICAM-1 and VCAM-1 (158–161), facilitating immune cell paracellular migration to cerebral parenchyma and amplifying tau-triggered toxicity.

Overall, these *in vitro* studies point to deleterious effects of tau on vascular and immune cellular and molecular pathways responsible for NVU function. These effects and the main respective references are summarized in **Table 1**.

Animal Studies

Several *in vivo* studies have contributed to corroborate the impact of tau pathology on the brain vasculature and NVU (**Table 1**). In one of these studies, as a model of tauopathy, wild-type mice have been injected with recombinant adeno-associated virus (AAV) vector to express mutant tau P301L (encoding for familial FTD tau protein), specifically in neurons (135). Compared to control littermates, AAV-tauP301L–injected animals displayed significantly increased capillary thickness as early as 10 days after injection, with many BVs within the CA1 region of the hippocampus surrounded by swollen astrocytes, most likely participating in the substantial constriction of the capillaries. Interestingly, in these animals, neurodegeneration coincided with microgliosis, which occurred 3–6 weeks following injection, demonstrating that tau-mediated vascular defects preceded neuronal loss. In concomitance with the onset of neurodegeneration, BBB integrity was further compromised, as parenchymal IgGs, IgMs, and α2-macroglobulin were detected (135). In aged (12–15-month-old) tetracycline inducible rTg4510 mice, which overexpress tau P301L protein, a variety of vascular abnormalities have been described, mainly in hippocampus and cortex. Progressively with age, rTg4510 animals exhibited

TABLE 1 | Effects of tau on the NVU *in vitro* and *in vivo* models.

In vivo/*in vitro* model	Tau isoform	Effects	References
RBEC (primary rat brain ECs)	Human oligomeric tau	Increased expression of genes related to inflammation, endocytosis, angiogenesis, blood coagulation, vasoconstriction, and diapedesis. Accelerated peripheral blood monocyte-derived macrophages transmigration, when RBEC are exposed to conditioned media of astrocytes pre-treated with tau. Reduced peripheral blood monocyte-derived macrophages transmigration, when RBEC are treated with antibodies anti-ICAM1 and anti-VCAM1.	(142)
co-culture RBEC + primary rat astrocytes and microglia	Human truncated tau	RBEC show decreased TEER and augmented mannitol permeability, when tau is added to the abluminal (glial) side. Glial cells increase TNF-α and MCP-1 release.	(22)
rat microglia	Human tau40	Higher rate of migration, enhanced phagocytosis and release of NO, IL-6, IL-1β, TNF-α, and IL-10.	(145)
WT FVB/NJ mouse	AAV-P310L tau injection (neuronal expression)	10 days after cortical injection, capillary thickness is increased, and hippocampal BVs are surrounded by swollen astrocytes. 3–6 weeks following injection, neuronal loss, microgliosis and IgGs, IgMs, and α2-macroglobulin presence in cerebral parenchyma.	(135)
Tg4510 mouse	P310L tau (neuronal expression)	Perivascular tau accumulation. $CD3^+$ and $CD4^+$ lymphocytes, and RBC infiltration along brain vasculature. Extravasation of IgGs and Evans Blue, mainly in the hippocampus and cortex. Glial activation. BBB functional recovery following doxycycline treatment (which suppresses tau expression).	(24)
Tg SHR-72 rat	Human truncated tau (neuronal expression)	In brainstem, peripheral blood monocyte-derived macrophages transmigration and increased ICAM-1 expression.	(142)
Tg4510 mouse	P310L tau (neuronal expression)	Increased number of capillaries with atypical and spiraling morphologies. Reduced BV diameter and elevated cortical BV density. Altered expression of hypoxia- and angiogenesis-related genes in both ECs and microglia.	(137)
Tg GFAP/tau mouse	T34 human tau (astrocytic expression)	IgGs and albumin presence in brain parenchyma. Tau pathology in astrocytes surrounding BVs.	(146)

tau perivascular accumulation together with BBB breakdown, measured as significant $CD3^+$ and $CD4^+$ lymphocyte, and red blood cell (RBC) infiltration along the vasculature, and extravasation of IgGs and Evans Blue, a dye that binds serum albumin, which does not cross the BBB, unless it is injured (24). In this model, tau pathology triggered glial activation, identified as high expression of glial fibrillary acidic protein (GFAP), astrocytic intermediate filament protein, and heat shock protein 27 (Hsp27), marker of reactive astrocytes (162) and involved in BBB regulation through its role in actin stabilization (163). Noteworthy, in comparison to WT or younger Tg animals, treatment with doxycycline, which suppressed tau expression, led to BBB functional recovery in old rTg4510 mice, with reduced T cell and RBC infiltration, and decreased Hsp27 and GFAP levels (24), indicating tau direct involvement in CVD. Transmigration of blood-borne cells, such as PB-MoM, has also been reported in cerebral areas with increased ICAM-1 expression, associated with neurofibrillary pathology, in Tg SHR-72 rat model which stably expresses human tau protein truncated at amino acids 151–391 (aa 151-391/4R) (142), confirming the role of tau in triggering detrimental changes in BBB. Using the Tg4510 murine model, another study has documented multiple brain vascular changes, including increased number of capillaries characterized by atypical and spiraling morphologies, reduced vessel diameter, and elevated cortical BV density, in 15-month-old Tg animals. RNA analysis has revealed an altered expression of hypoxia- and angiogenesis-related genes specifically in ECs and microglia. The greatest fold change has been found in endothelial *Serpine1,* a gene that encodes for plasminogen activator inhibitor (PAI-1) protein (137), known for stimulating migration of ECs (164), modulating proteolytic activity (165), fundamental for extracellular matrix remodeling during angiogenesis, and for regulating microglia motility and phagocytosis (166). Intriguingly, also Tg mice expressing the T34 human tau isoform specifically in astrocytes, have been shown to develop BBB disruption, verified as IgG and albumin presence in the brain parenchyma, in association with prominent tau pathology in astrocytic foot processes surrounding BVs (146). In summary, tau has been shown to trigger multiple detrimental brain vascular and glial changes in animal models, which are detailed in **Table 1**.

Human Studies

Over the last few decades, the AD cerebral microvasculature has been described as atrophic, thin, fragmented, twisted or tortuous, and with glomerular loop formations (40). Ultrastructural analysis has reported atrophic ECs, and remarkably decreased (167) and compromised morphology (168) of TJs. In addition, AD brains present anomalous focal constrictions and general degeneration of SMC (169–171), swollen astrocytic end-feet

TABLE 2 | Effects of tau on the NVU in AD and other tauopathies.

Disease	Tau isoform	Effects	References
Alzheimer's disease	NFT	Small and medium size artery SMC loss occurs between early onset tau toxicity-Braak stage I and II–III, along with PHF perivascular deposition.	(23)
Pick's disease	Pick bodies	Microvasculature thinning, increased BV tortuosity, fragmented or twisted capillaries in association with decreased number of long microvessels and their branches.	(149)
Progressive supranuclear palsy	NFT	Tau immunoreactivity in brainstem vasculature.	(147)
Parkinsonism dementia complex of Guam	NFT	Reduced cerebral BV density and ramification, increased vascular fragmentation, and thin capillaries. String and coiling BVs, restricted to the areas affected by NFT.	(40, 150)
Chronic traumatic encephalopathy	NFT	Perivascular NFT in frontal, temporal, and parietal cortices. Astrocytic tangles detected around small cortical BVs.	(148)
Progressive supranuclear palsy	NFT	Cortical tau-positive dense-packed fibrils in astrocytes.	(151–153)
Corticobasal degeneration	NFT	Tau astrocytic plaques in gray and white matter of the cortex, basal ganglia, diencephalon, and rostral brainstem.	(154)
Pick's disease	Pick bodies	Reactive astrocytes containing hyperphosphorylated tau.	(155)

(172, 173), and atrophic pericytes (169). As a consequence, AD subjects display disrupted CBF and brain hypoperfusion (28, 174–176). Interestingly, a 2016 study has demonstrated that vascular changes are correlated with Braak stages, which classify the degree of tau pathology, showing that small and medium size artery SMC loss occurs between early onset tau toxicity-Braak stage I and II–III, along with PHF perivascular deposition (23).

These results bolster a direct connection of tau pathology with vascular abnormalities. As a confirmation, in PiD, where cerebral aggregates of hyperphosphorylated tau (Pick bodies) are present (149), hallmarks like microvasculature thinning, increased BV tortuosity, fragmented or twisted capillaries in association with decreased number of long microvessels and their branches, are as severe as in AD (40). Moreover, atrophic brain areas display a considerable disorganization of the vascular basal lamina distribution (150). A recent paper has described oligomeric tau immunoreactivity in brain vasculature of other tauopathies, such as PSP (147). It is also known that Parkinsonism dementia complex of Guam, characterized by abundant tau neurofibrillary pathology, shows reduced cerebral BV density and ramification, increased vascular fragmentation, and thin capillaries (40). Furthermore, string and coiling vessels are typical, and restricted to the areas affected by NFT (150). Repetitive mild or moderate traumatic brain injury (TBI) seems to be associated with higher risk of AD development (177), and may lead to chronic traumatic encephalopathy (CTE) (148, 178, 179). Strikingly, perivascular NFT of hyperphosphorylated tau are one of the most common pathological hallmarks of CTE, starting at very early stages of the disease, mainly in frontal, temporal, and parietal cortices (particularly in the depth of the sulci) (180). With the progression of the disorder, also fibrillar astrocytic tangles are detected around small cortical BVs (148), plausibly exacerbating vascular dysfunction. Similarly, other tauopathies display high phosphorylated tau accumulation in astrocytes. For instance, in PSP, astroglial phenotype is often referred to as tufted (151–153), showing tau-positive dense-packed fibrils forming tufts around single or double nuclei, mainly in the frontal cortex, striatum, and thalamus (181, 182). Additionally, CBD is characterized by tau astrocytic plaques (154), defined as punctate or spindle-shaped aggregates, forming irregular rounded structures (181, 182). Reactive astrocytes containing hyperphosphorylated tau have been found in PiD as well (155).

Associations between tau pathology and white matter hyperintensities (WMH) have also been reported, showing that increasing cortical P-tau burden independently predicted the severity of WMH, indicating a potentially important role of tau in the pathogenesis of WM damage (183, 184).

Collectively, these human studies provide strong evidence for an association between tau toxicity and vascular dysregulation in multiple tauopathies (summarized in **Table 2**).

To better dissect how tau pathology may affect cerebrovascular function, we propose below some of the molecular and cellular mechanisms which have been shown to be affected by tau, and may also mediate its effects on the vessel walls.

TAU AND MITOCHONDRIAL DYSREGULATION

Tau and Mitochondrial Dynamics

Mitochondria are essential organelles for cell survival and death, playing a primary role in energy metabolism and apoptotic processes (185). Balanced mitochondrial fission and fusion dynamics are pivotal events in regulating their shape, size, and number, enabling a correct morphology and distribution, and thus their capacity to meet high energy cellular demands, such as those of brain cells (186–188). A cytosolic guanosine triphosphatase (GTPase), named dynamin-like protein 1 (Drp1), assembling into spiral filaments around mitochondria, interacts with outer membrane proteins, including mitochondrial fission

factor (Mff) and fission protein-1 (Fis1), to regulate the division process (189). On the other hand, the interaction of mitochondrial outer membrane proteins, such as the GTPases mitofusin 1 and 2 (Mfn1 and Mfn2), with optic dominant atrophy 1 (Opa1) inner mitochondrial membrane protein, mediates fusion (190). It has been reported that mitochondrial activity and phenotype are directly and tightly modulated by cellular and environmental stimuli (191). Therefore, it is not surprising that mitochondrial dysfunction and aberrant morphology are predominant pathological early features of AD brains (192–194).

Although with some discrepancies, most likely due to different tau isoforms and cell types used, multiple *in vitro* studies have pointed to direct effects of tau on mitochondrial dynamics (**Table 3**). Both human WT full-length (hTau) and P301L mutated tau isoforms have been shown to promote mitochondrial perinuclear accumulation, a feature of AD brains (203), in animal and cellular models (195, 203). Moreover, in HEK293 cells, hTau enhanced Mfn1, Mfn2, and Opa1 protein expression. Specifically, the decrease of hTau-mediated Mfn2 polyubiquitination underlaid Mfn2 accumulation, and the resulting increased mitochondrial fusion (195). Conversely, primary cortical neurons from tau KO mice, transfected with caspase-3 truncated tau presented mitochondrial fragmentation, together with a significant reduction of Opa1 levels, compared to control neurons (196). Another study has reported that, following treatment with okadaic acid, a well-known PP2A inhibitor, rat brain ECs (RBE4) displayed elevated tau phosphorylation and increased mitochondrial fission (Drp1 and Fis1) and fusion (Mfn1, Mfn2, and Opa1) protein levels, suggesting tau-dependent mitochondrial dynamics alterations, and confirming the expression of tau in ECs (197). Mfn1, Mfn2, and Opa1 accumulation have also been detected in brains of 6-month-old hTau Tg mice, in comparison with age-matched WT littermates (195). Additionally, in tau P301L Tg mice, another *in vivo* model of tauopathy, the partial genetic ablation of Drp1 reduced mitochondrial dysfunction and rescued mitochondrial dynamics (198). Excessive mitochondrial fission triggered by an atypical interaction between hyperphosphorylated tau and Drp1 has been described in multiple AD mouse models (199). Importantly, similar findings have been reported in AD human brains, where phosphorylated tau has been found to physically interact with Drp1, with little or no physical interaction occurring in control subjects (199). Moreover, a disrupted balance of fission and fusion, both at mRNA and protein levels (200, 201), has been found in AD patients, with most studies reporting a shift toward fission (200, 202). Overall, these data point to direct effects of tau on mitochondrial dynamics in multiple cellular (including ECs), animal models of tauopathies, and in human AD brains. These effects and their specific references are summarized in **Table 3**.

Tau and Mitochondrial Dysfunction

Mitochondria are important for multiple cell functions in healthy and diseased brains, including energy production, intracellular Ca^{2+} homeostasis control, cell cycle regulation, ROS generation, apoptosis, and, in neurons, synaptic plasticity maintenance (10, 15, 185, 204–209). Amongst these, a pivotal role of the mitochondria is to provide energy to the cell from nutrient sources, through adenosine trisphosphate (ATP) production, accomplished *via* tricarboxylic acid cycle (TCA or Krebs cycle) and oxidative phosphorylation (OxPhos). Mitochondrial bioenergetics dysfunction and elevated mitochondrial ROS production have been reported to cause neuronal degeneration, and eventually cell death, in AD and other neuropathological conditions (194, 205, 206, 210). Mitochondrial dysfunction is also considered one of the earliest and probable causative steps in the AD pathogenesis (211–215). Tau has been recognized as a mediator of mitochondrial dysfunction, in both *in vitro* and *in vivo* models (216–219), as well as in human tauopathies, including AD (**Table 4**).

Cell experiments showed that rat brain ECs challenged with okadaic acid, prompted tau hyperphosphorylation in concomitance with ROS production, mitochondrial Ca^{2+} overload, and activation of caspase-3 and caspase-9, markers of apoptosis (197). The treatment of rat primary neuronal-astrocytic co-culture with the repeat domain of tau (K18) blocked mitochondrial Ca^{2+} efflux *via* impairment of NCLX, mitochondrial Na^{+}/Ca^{2+} exchanger, in both neurons and astrocytes. Mitochondrial Ca^{2+} overload occurred also in human iPSC-derived cortical neurons expressing 10 + 16 MAPT mutation (linked to FTD), along with mitochondrial depolarization and caspase-3 activation (220). Rat primary hippocampal neurons stably expressing hTau displayed a significant decrease in ATP levels, ATP/ADP ratio, complex I activity, and cell viability (195). Similarly, overexpression of P301L tau in neuronal SHSY5Y cells led to ATP depletion and pronounced complex I activity deficit, along with depolarization of mitochondrial membrane potential (MMP) (219), which is physiologically generated by complexes I, III, and IV. In cortical neurons, the inducible expression of D421-cleaved caspase-3 truncated tau triggered mitochondrial fragmentation, increased ROS production, and a significant reduction of Ca^{2+}-buffering capacity, mitochondrial membrane integrity and MMP. Interestingly, the treatment with cyclosporin A, which inhibits mitochondrial permeability transition pore (mPTP), partially prevented tau-induced mitochondrial impairment (221). mPTP is a mitochondrial channel that opens in pathological circumstances, enhancing mitochondrial permeability to ions and small molecules (230–232), therefore inducing MMP failure, decrease of ATP production, release of mitochondrial content, and cell death (233–237). One of the components of mPTP is voltage-dependent anion channel (VDAC) protein (238). Remarkably, VDAC protein levels have been described to progressively increase in correlation with Braak stages in AD brains, where phosphorylated tau has been found to interact with VDAC (239). WT mice subjected to subcortical injection of tau oligomers exhibited decrease of NADH-ubiquinone oxidoreductase (complex I) protein expression and a considerable activation of caspase-9, when the hemispheres were compared with the ones injected with fibrillar or monomeric tau. Validating the tau propagation hypothesis, these changes have been found in the hippocampus, where IHC analysis revealed the co-localization between tau oligomers and mitochondria, specifically in CA1 cells (218). In another study, P301L mice exhibited increased amounts of hydrogen

TABLE 3 | Tau and mitochondrial dynamics.

Model/disease	Tau isoform	Mitochondrial dynamics	References
HEK293 cells and rat primary hippocampal neurons	Human WT full length tau	Mitochondrial perinuclear accumulation. In HEK293 cells, hTau enhances expression of mitochondrial fusion proteins (Mfn1, Mfn2, and Opa1). In HEK293 cells, Mfn2 accumulation is due to hTau-mediated Mfn2 decreased polyubiquitination.	(195)
Primary cortical neurons (from tau KO mice)	Caspase-3 truncated tau (D421)	Mitochondrial fragmentation. Reduction of Opa1 levels.	(196)
RBE4 (rat brain ECs)	WT	Following treatment with okadaic acid (PP2A inhibitor), increased mitochondrial fission (Drp1 and Fis1) and fusion (Mfn1, Mfn2, and Opa1) protein levels.	(197)
Tg hTau mouse	Human WT full length tau	Hippocampal accumulation of Mfn1, Mfn2, and Opa1.	(195)
Tg4510 × $Drp1^{+/--}$ mouse	P310L tau (neuronal expression)	Drp1 partial genetic ablation decreases Drp1 and Fis1 mRNA and protein levels, in cortical and hippocampal tissues. Drp1 partial genetic ablation increases Mfn1, Mfn2, and Opa1 mRNA and protein levels, in cortical and hippocampal tissues.	(198)
3xTg mouse and Alzheimer's disease	NFT	Excessive mitochondrial fission due to Drp1 and hyperphosphorylated tau interaction.	(199)
Alzheimer's disease	NFT	Disrupted balance of fission and fusion (mRNA and protein), shifted toward fission.	(200–202)

peroxide (H_2O_2) and 4-hydroxy-2-nonenol (HNE), indicator of lipid peroxidation, when compared to WT animals. Moreover, Tg animals showed markedly diminished levels of ATP and cytochrome C oxidase (complex IV) (198), further corroborating the role of tau toxicity in mitochondrial bioenergetics failure. Using the same murine model of tauopathy, it has been demonstrated that brains of 12-month-old Tg mice displayed significantly reduced activities of complex I and V. In these animals, tau-induced mitochondrial dysregulation worsened with aging, since 24-month-old mice showed increasingly reduced electron transport capacity and ATP levels, together with incremented ROS levels (H_2O_2 and superoxide anion radicals) (217). Of note, complex V level reduction has been also found in human familial FTD brains (217). In AD, several studies have shown significant decrease of mitochondrial proteins and activity (222–224). Additional studies have observed decreased ATP production and increased oxidative stress markers, such as free radicals, lipid peroxidation, and DNA and protein oxidation (225–228). Strikingly, mitochondrial structural abnormalities have been reported to occur also in the vascular wall of AD subjects, compared to age-matched controls (229). The literature summarized above points to a contribution of tau to mitochondrial dysfunction. Although more studies specifically targeted to understand the mitochondrial effects of tau in ECs or the NVU are still needed, the available data (197) suggest that mitochondrial dysfunction may be one of the mechanisms by which tau impairs cerebrovascular health.

TAU AND CASPASES

Caspases are cysteine-dependent proteases which cleave multiple intracellular substrate proteins after an aspartic acid residue, playing a primary role in apoptosis (240–244). Based on their structure and their hierarchical position in the apoptotic signaling cascade, caspases are categorized into upstream initiators (caspase-2,−8,−9, and−10) and downstream effectors (caspase-3,−6, and−7) (245–247). In brain cells, including neurons and glial cells, caspase activation does not always result in apoptosis (248, 249).

In neurodegenerative disorders, including AD, several stressors, such as deficits of oxygen and growth factors, excitotoxicity, inflammation, dysregulation of Ca^{2+} homeostasis and oxidative stress, may induce caspase activation, contributing to the etiopathogenesis of the disease (250–252). The associations between tau toxicity and caspase activation, and the relative references are listed in **Table 5**.

Multiple studies have provided evidence that human AD brains display significant caspase-3,−6,−7,−8, and−9 upregulation, compared to controls (69, 255–258). A large body of literature has focused on caspase-3, recognized as the main effector of the apoptotic process (262, 263). The presence of active caspase-3 within NFT is considered one of the earliest biomarkers of AD (259), with a high degree of colocalization in neurons, astrocytes and BVs, in subjects with overt pathology (256). This underpins a tight correlation between tau toxicity and neurovascular dysfunction, which is possibly caspase-mediated. To further corroborate this hypothesis, another study has demonstrated the activation of caspase-3 in $CD68^{+}$ cells, identified by the authors as reactive microglia, in the frontal cortex of AD brains, relative to age- and gender-matched healthy controls (248). Moreover, neurons and a subset of degenerating astrocytes positive for active caspase-3 have been described in FTD cerebral samples (260). The activation of caspase-3 has been described to precede and to lead NFT formation in 7-month-old rTg4510 mice. Remarkably, when animals have been treated

TABLE 4 | Tau and mitochondrial dysfunction.

Model/disease	Tau isoform	Mitochondrial dysfunction	References
RBE4 (rat brain ECs)	WT	Following treatment with okadaic acid (PP2A inhibitor), increased ROS production, mitochondrial Ca^{2+} overload, and activation of caspase-3 and caspase-9.	(197)
Co-culture rat primary neurons + rat primary astrocytes	h4R tau (K18 fragment)	Mitochondrial Ca^{2+} efflux blocked in both neurons and astrocytes.	(220)
Human iPSC-derived cortical neurons	10 + 16 MAPT	Mitochondrial Ca^{2+} overload. Mitochondrial depolarization. Caspase-3 activation.	(220)
Rat primary hippocampal neurons	hTau	Decrease in ATP levels, ATP/ADP ratio and complex I activity.	(195)
SHSY5Y	P301L tau	ATP depletion. Complex I activity deficit and depolarization of MMP.	(219)
Cortical neurons	Caspase-3 truncated tau (D421)	Increased ROS production. Reduction of Ca^{2+}-buffering capacity, mitochondrial membrane integrity and MMP. Cyclosporin A (mPTP inhibitor) treatment partially prevents mitochondrial impairment. Mitochondrial fragmentation.	(221)
WT C57BL/6 mouse	Oligomeric tau injection	Following subcortical tau injection, decrease of complex I protein expression, and activation of caspase-9, in the hippocampus.	(218)
Tg4510 mouse	P310L tau (neuronal expression)	Increased amounts of H_2O_2 and HNE. Diminished levels of ATP and complex IV, in cortical tissues.	(198)
Tg4510 mouse	P310L tau (neuronal expression)	12-month-old mice display reduced activities of complex I and V. 24-month-old mice show reduced electron transport capacity and ATP levels. Increased H_2O_2 and superoxide anion radical levels.	(217)
Frontotemporal dementia	NFT	Complex V level reduction, in temporal cortices.	(217)
Alzheimer's disease	NFT	Cortical complex IV activity is reduced. Reduction of complex I (24- and 75-kDa subunits) and complex V protein levels.	(222–224)
Alzheimer's Disease	NFT	Decreased ATP production. Increased oxidative stress markers (free radicals, lipid peroxidation, and DNA and protein oxidation), in frontal, parietal, and temporal lobes.	(225–228)
Alzheimer's disease	NFT	Mitochondrial structural abnormalities in the vascular wall.	(229)

with doxycycline to suppress tau expression, the levels of cleaved caspase-3 in tangle-bearing neurons were diminished, while tangles remained, suggesting that soluble tau is the upstream initiator of caspase-3 activation, which in turn leads to the fibrillary progression (253).

Interestingly, among more than 400 proteins that can be cleaved by caspases (264–266), tau has been detected as substrate of caspase-2 (267),−3 (65), and−6 (69). Tau fragments derived from caspase-3 and−6 cleavage have been found in AD subjects (65, 69). Specifically, D421-cleaved caspase-3 truncated tau is accounted as an early feature of AD (268), and it has also been observed in other tauopathies, including PiD, PSP and CBD (269). Considering that caspase-3 cleaved tau shows an enhanced propensity to phosphorylation (65), and therefore to self-aggregation, besides the canonical role in apoptosis, caspase-3 activation may also function as a trigger for tau polymerization and NFT assembly (45, 47, 59, 66), ultimately causing dendritic spine loss and synaptic dysfunction (67, 68), as well as possible toxic effects for neurovascular cells. Accordingly, the injection of human tau-4R in WT mice led to the neuronal activation of caspase-3, tau truncation and aggregation. Moreover, when animals were injected with truncated tau, $D421^+$ neurons showed colocalization of cleaved tau with the endogenous WT isoform, together with an accumulation of misfolded tau, further confirming that D421 tau fragment precedes and is sufficient to induce tau conformational and phosphorylation changes, and that the process is likely caspase-3-mediated (and initiated by soluble tau) (253). Active caspase-3 and D421-cleaved caspase-3 truncated tau have also been reported to occur within $GFAP^+$, $CD68^+$, and EBA^+ (endothelial barrier antigen) cells in the white matter of corpus callosum, in a rat model of TBI, corroborating the involvement of caspase-3 activation in tau-mediated neurovascular damage (254). In addition, caspase-3 derived tau fragments have been found in NFT in the hippocampus, DG and frontal cortex, in human vascular dementia brains. Importantly, active caspase-3 has been detected in BVs and pre-tangle (but not NFT) neurons that colocalized with cleaved tau, supporting that caspase-3 activation is a required step for the cleavage of tau and the consequential NFT development at the NVU level (261).

TABLE 5 | Tau and caspases.

Model/disease	Tau isoform	Caspase activation	References
Tg4510 mouse	P310L tau (neuronal expression)	Activation of caspase-3 precedes NFT. Following doxycycline treatment (to suppress tau expression), active caspase-3 levels in tangle-bearing neurons are diminished.	(253)
WT mouse	h4R and caspase-3 truncated tau (D421)	h4R-tau injection triggers neuronal caspase-3 activation associated with tau truncation and aggregation. In truncated tau-injected animals, $D421^+$ neurons showed colocalization of cleaved tau with the endogenous tau, together with an accumulation of misfolded tau.	(253)
Traumatic brain injury (rat)		Active caspase-3 and D421-cleaved caspase-3 truncated tau presence within $GFAP^+$, $CD68^+$, and EBA^+ cells, in the corpus callosum.	(254)
Alzheimer's disease	NFT	Caspase-3,−6,−7,−8, and−9 upregulation, in hippocampus and cortex.	(69, 255–258)
Alzheimer's disease	NFT	Active caspase-3 within NFT in the limbic cortex considered one of the earliest biomarkers of AD.	(259)
Alzheimer's disease	NFT	Active caspase-3 with a high degree of colocalization in neurons, astrocytes and BVs, mainly in hippocampus and entorhinal cortex.	(256)
Alzheimer's disease	NFT	Activation of caspase-3 in $CD68^+$ cells (microglia) in the frontal cortex.	(248)
Frontotemporal dementia	NFT	Neurons and degenerating astrocytes positive for active caspase-3, in the temporal lobe.	(260)
Vascular dementia	NFT	Caspase-3 derived tau fragments found in NFT in the hippocampus, DG and frontal cortex. Active caspase-3 (colocalizing with cleaved tau) detected in BVs and pre-tangle neurons.	(261)
Alzheimer's disease	NFT	2–3-fold increase of active caspase-6 in the temporal and frontal cortex. Caspase-6 cleaved tau found into intracellular, extracellular, and even immature tangles.	(257)

Since Aβ oligomeric aggregates have also been shown to induce caspase-3 activation in neurovascular cells, including endothelial, glial, and neuronal cells (10, 13–15, 206–208), the presence of Aβ deposits surrounding cerebral vessels in AD and CAA, promoting caspase-3 activation, may also prompt an increase in the levels of caspase-3 truncated tau, which is more prone to phosphorylation and aggregation, and may therefore enhance tau toxicity at the NVU (270). In addition, AD subjects display a 2–3-fold increase of active caspase-6 in the temporal and frontal cortex, where caspase-6 cleaved tau is found into intracellular, extracellular, and even immature tangles (257).

Overall, this evidence supports the hypothesis that both tau and Aβ can initiate caspase activation, which may lead to apoptosis and, concomitantly, promote tau aggregation and NFT formation, further exacerbating tau-induced neurovascular pathology. In particular, the presence of active caspases in the brain vasculature (254, 256, 261), accompanied by the discussed evidence of tau transmission within neurovascular cells, supports the hypothesis that caspase activation may induce accumulation of toxic caspase-3 cleaved tau in cerebrovascular and glial cells, thus precipitating neurovascular pathology in AD, CAA, and tauopathies.

DISCUSSION

The impact of tau on neurovascular pathology, although previously understudied, has recently become an active topic of research for the AD and dementia scientific community. Although more specific mechanistic studies in vessel wall cells are needed, here we postulate that tau may propagate from neurons to NVU cellular components such as ECs, astrocytes and microglia, and that inflammatory and mitochondrial alterations induced by tau in these cells may underlie its toxicity at the NVU (**Figure 1**). We further hypothesize that caspase activation, and in particular active caspase-3 tau cleavage, may also play a primary role in pathological tau-induced vascular dysfunction. Whether oligomeric Aβ aggregates, possibly through caspase-mediated mechanisms, contribute to perivascular tau oligomerization and NFT deposition is an intriguing hypothesis in need of additional exploration. Contextually, the lack of knowledge of the cell-specific detrimental molecular mechanisms initiated by tau arises new questions. Further studies will be necessary to elucidate tau-driven endothelial, pericyte, astrocytic, and microglial harmful cellular events. Amongst these, it will be critical to dissect the possible link between tau dysregulated clearance, altered inflammatory response and development of tau aggregates around cerebral BVs, in neurodegenerative diseases, including AD and other tauopathies, such as PiD, PSP, and CTE. Although tau toxicity seems to be responsible for neurovascular compromise, other studies suggest that early CVD can also cause tau pathological accumulation (136). Both these mechanisms may induce a vicious cycle between CVD and tau pathology, which still remains largely understudied. Establishing causality of these effects when studying a disease like AD, presenting with different pathologies, such as amyloidosis, tauopathy, cerebrovascular dysfunction, and neuroinflammation, often overlapping at the same time in the same brains, will require multiple and properly-designed studies.

Moreover, developing strategies to promote perivascular clearance, such as immunotherapy (271) and to target

neurovascular cell-specific mechanisms, including mitochondrial dysfunction (207, 208, 272), to counteract toxicity of both amyloid and tau, will be essential research efforts to ameliorate CVD in AD and other tauopathies.

AUTHOR CONTRIBUTIONS

EC and SF designed and conceptualized the review. EC wrote the review draft and did the literature search. SF critically revised and edited the manuscript, provided relevant insights, additional literature search, and acquired funding. Both authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by NIH R01NS104127 and R01AG062572 grants and the Edward N. and Della L. Thome Memorial Foundation, Awards Program in Alzheimer's Disease Drug Discovery Research, awarded to SF.

ACKNOWLEDGMENTS

We thank Nicole Lemon for editing the manuscript and Thaira Torielli for drafting the figure.

REFERENCES

1. World Health Organization. *Dementia Fact Sheet*. (2019). Available online at: https://www.who.int/news-room/fact-sheets/detail/dementia (accessed September 19, 2019).
2. Cummings JL. Alzheimer's disease. *N Engl J Med.* (2004) 351:56–67. doi: 10.1056/NEJMra040223
3. Caselli RJ, Beach TG, Yaari R, Reiman EM. Alzheimer's disease a century later. *J Clin Psychiatry.* (2006) 67:1784–800. doi: 10.4088/JCP.v67n1118
4. Querfurth HW, LaFerla FM. Alzheimer's disease. *N Engl J Med.* (2010) 362:329–44. doi: 10.1056/NEJMra0909142
5. Kalaria RN. The role of cerebral ischemia in Alzheimer's disease. *Neurobiol Aging.* (2000) 21:321–30. doi: 10.1016/S0197-4580(00)00125-1
6. Farkas E, Luiten PG. Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol.* (2001) 64:575–611. doi: 10.1016/S0301-0082(00)00068-X
7. de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *Lancet Neurol.* (2004) 3:184–90. doi: 10.1016/S1474-4422(04)00683-0
8. Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* (2005) 28:202–8. doi: 10.1016/j.tins.2005.02.001
9. Finder VH, Glockshuber R. Amyloid-beta aggregation. *Neurodegener Dis.* (2007) 4:13–27. doi: 10.1159/000100355
10. Fossati S, Cam J, Meyerson J, Mezhericher E, Romero IA, Couraud PO, et al. Differential activation of mitochondrial apoptotic pathways by vasculotropic amyloid-beta variants in cells composing the cerebral vessel walls. *FASEB J.* (2010) 24:229–41. doi: 10.1096/fj.09-139584
11. Muller-Hill B, Beyreuther K. Molecular biology of Alzheimer's disease. *Annu Rev Biochem.* (1989) 58:287–307. doi: 10.1146/annurev.bi.58.070189.001443
12. Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D, et al. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, part XV. *Neurology.* (1996) 46:1592–6. doi: 10.1212/WNL.46.6.1592
13. Fossati S, Ghiso J, Rostagno A. TRAIL death receptors DR4 and DR5 mediate cerebral microvascular endothelial cell apoptosis induced by oligomeric Alzheimer's abeta. *Cell Death Dis.* (2012) 3:e321. doi: 10.1038/cddis.2012.55
14. Fossati S, Ghiso J, Rostagno A. Insights into caspase-mediated apoptotic pathways induced by amyloid-beta in cerebral microvascular endothelial cells. *Neurodegener Dis.* (2012) 10:324–8. doi: 10.1159/000332821
15. Ghiso J, Fossati S, Rostagno A. Amyloidosis associated with cerebral amyloid angiopathy: cell signaling pathways elicited in cerebral endothelial cells. *J Alzheimers Dis.* (2014) 42(Suppl. 3):S167–76. doi: 10.3233/JAD-140027
16. Fossati S, Todd K, Sotolongo K, Ghiso J, Rostagno A. Differential contribution of isoaspartate post-translational modifications to the fibrillization and toxic properties of amyloid beta and the Asn23 Iowa mutation. *Biochem J.* (2013) 456:347–60. doi: 10.1042/BJ20130652
17. Drubin DG, Kirschner MW. Tau protein function in living cells. *J Cell Biol.* (1986) 103(6 Pt 2):2739–46. doi: 10.1083/jcb.103.6.2739
18. 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) 3:1141–54. doi: 10.1091/mbc.3.10.1141
19. Khatoon S, Grundke-Iqbal I, Iqbal K. Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein. *J Neurochem.* (1992) 59:750–3. doi: 10.1111/j.1471-4159.1992.tb09432.x
20. Khatoon S, Grundke-Iqbal I, Iqbal K. Levels of normal and abnormally phosphorylated tau in different cellular and regional compartments of Alzheimer disease and control brains. *FEBS Lett.* (1994) 351:80–4. doi: 10.1016/0014-5793(94)00829-9
21. Vidal R, Calero M, Piccardo P, Farlow MR, Unverzagt FW, Mendez E, et al. Senile dementia associated with amyloid beta protein angiopathy and tau perivascular pathology but not neuritic plaques in patients homozygous for the APOE-epsilon4 allele. *Acta Neuropathol.* (2000) 100:1–12. doi: 10.1007/s004010051186
22. Kovac A, Zilkova M, Deli MA, Zilka N, Novak M. Human truncated tau is using a different mechanism from amyloid-beta to damage the blood-brain barrier. *J Alzheimers Dis.* (2009) 18:897–906. doi: 10.3233/JAD-2009-1197
23. Merlini M, Wanner D, Nitsch RM. Tau pathology-dependent remodelling of cerebral arteries precedes Alzheimer's disease-related microvascular cerebral amyloid angiopathy. *Acta Neuropathol.* (2016) 131:737–52. doi: 10.1007/s00401-016-1560-2
24. Blair LJ, Frauen HD, Zhang B, Nordhues BA, Bijan S, Lin YC, et al. Tau depletion prevents progressive blood-brain barrier damage in a mouse model of tauopathy. *Acta Neuropathol Commun.* (2015) 3:8. doi: 10.1186/s40478-015-0186-2
25. Farquhar MG, Palade GE. Junctional complexes in various epithelia. *J Cell Biol.* (1963) 17:375–412. doi: 10.1083/jcb.17.2.375
26. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis.* (2010) 37:13–25. doi: 10.1016/j.nbd.2009.07.030
27. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev.* (2005) 57:173–85. doi: 10.1124/pr.57.2.4
28. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci.* (2004) 5:347–60. doi: 10.1038/nrn1387
29. Oksanen M, Lehtonen S, Jaronen M, Goldsteins G, Hamalainen RH, Koistinaho J. Astrocyte alterations in neurodegenerative pathologies and their modeling in human induced pluripotent stem cell platforms. *Cell Mol Life Sci.* (2019) 76:2739–60. doi: 10.1007/s00018-019-03111-7
30. Farina C, Aloisi F, Meinl E. Astrocytes are active players in cerebral innate immunity. *Trends Immunol.* (2007) 28:138–45. doi: 10.1016/j.it.2007.01.005
31. Streit WJ, Conde JR, Fendrick SE, Flanary BE, Mariani CL. Role of microglia in the central nervous system's immune response. *Neurol Res.* (2005) 27:685–91. doi: 10.1179/016164105X49463
32. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci.* (2007) 10:1538–43. doi: 10.1038/nn2014
33. Persidsky Y, Ghorpade A, Rasmussen J, Limoges J, Liu XJ, Stins M, et al. Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis.

Am J Pathol. (1999) 155:1599–611. doi: 10.1016/S0002-9440(10)65476-4
34. Hudson LC, Bragg DC, Tompkins MB, Meeker RB. Astrocytes and microglia differentially regulate trafficking of lymphocyte subsets across brain endothelial cells. *Brain Res.* (2005) 1058:148–60. doi: 10.1016/j.brainres.2005.07.071
35. Rogers J, Luber-Narod J, Styren SD, Civin WH. Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. *Neurobiol Aging.* (1988) 9:339–49. doi: 10.1016/S0197-4580(88)80079-4
36. McGeer PL, Akiyama H, Itagaki S, McGeer EG. Activation of the classical complement pathway in brain tissue of Alzheimer patients. *Neurosci Lett.* (1989) 107:341–6. doi: 10.1016/0304-3940(89)90843-4
37. Sheng JG, Jones RA, Zhou XQ, McGinness JM, Van Eldik LJ, Mrak RE, et al. Interleukin-1 promotion of MAPK-p38 overexpression in experimental animals and in Alzheimer's disease: potential significance for tau protein phosphorylation. *Neurochem Int.* (2001) 39:341–8. doi: 10.1016/S0197-0186(01)00041-9
38. Hayes A, Thaker U, Iwatsubo T, Pickering-Brown SM, Mann DM. Pathological relationships between microglial cell activity and tau and amyloid beta protein in patients with Alzheimer's disease. *Neurosci Lett.* (2002) 331:171–4. doi: 10.1016/S0304-3940(02)00888-1
39. Ostergaard L, Aamand R, Gutierrez-Jimenez E, Ho YC, Blicher JU, Madsen SM, et al. The capillary dysfunction hypothesis of Alzheimer's disease. *Neurobiol Aging.* (2013) 34:1018–31. doi: 10.1016/j.neurobiolaging.2012.09.011
40. Buee L, Hof PR, Delacourte A. Brain microvascular changes in Alzheimer's disease and other dementias. *Ann N Y Acad Sci.* (1997) 826:7–24. doi: 10.1111/j.1749-6632.1997.tb48457.x
41. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci.* (2011) 12:723–38. doi: 10.1038/nrn3114
42. Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. *Acta Neuropathol.* (2009) 118:103–13. doi: 10.1007/s00401-009-0522-3
43. Dehmelt L, Halpain S. The MAP2/Tau family of microtubule-associated proteins. *Genome Biol.* (2005) 6:204. doi: 10.1186/gb-2004-6-1-204
44. Halpain S, Dehmelt L. The MAP1 family of microtubule-associated proteins. *Genome Biol.* (2006) 7:224. doi: 10.1186/gb-2006-7-6-224
45. Ding H, Matthews TA, Johnson GV. Site-specific phosphorylation and caspase cleavage differentially impact tau-microtubule interactions and tau aggregation. *J Biol Chem.* (2006) 281:19107–14. doi: 10.1074/jbc.M511697200
46. McMillan PJ, Kraemer BC, Robinson L, Leverenz JB, Raskind M, Schellenberg G. Truncation of tau at E391 promotes early pathologic changes in transgenic mice. *J Neuropathol Exp Neurol.* (2011) 70:1006–19. doi: 10.1097/NEN.0b013e31823557fb
47. Kolarova M, Garcia-Sierra F, Bartos A, Ricny J, Ripova D. Structure and pathology of tau protein in Alzheimer disease. *Int J Alzheimers Dis.* (2012) 2012:731526. doi: 10.1155/2012/731526
48. Dolan PJ, Johnson GV. The role of tau kinases in Alzheimer's disease. *Curr Opin Drug Discov Devel.* (2010) 13:595–603.
49. Rodriguez-Martin T, Cuchillo-Ibanez I, Noble W, Nyenya F, Anderton BH, Hanger DP. Tau phosphorylation affects its axonal transport and degradation. *Neurobiol Aging.* (2013) 34:2146–57. doi: 10.1016/j.neurobiolaging.2013.03.015
50. Avila J, Jimenez JS, Sayas CL, Bolos M, Zabala JC, Rivas G, et al. Tau structures. *Front Aging Neurosci.* (2016) 8:262. doi: 10.3389/fnagi.2016.00262
51. Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol.* (1985) 101:1371–8. doi: 10.1083/jcb.101.4.1371
52. Arrasate M, Perez M, Avila J. Tau dephosphorylation at tau-1 site correlates with its association to cell membrane. *Neurochem Res.* (2000) 25:43–50. doi: 10.1023/A:1007583214722
53. Maas T, Eidenmuller J, Brandt R. Interaction of tau with the neural membrane cortex is regulated by phosphorylation at sites that are modified in paired helical filaments. *J Biol Chem.* (2000) 275:15733–40. doi: 10.1074/jbc.M000389200
54. Maccioni RB, Otth C, Concha, II, Munoz JP. The protein kinase Cdk5. Structural aspects, roles in neurogenesis and involvement in Alzheimer's pathology. *Eur J Biochem.* (2001) 268:1518–27. doi: 10.1046/j.1432-1327.2001.02024.x
55. Hernandez F, Lucas JJ, Avila J. GSK3 and tau: two convergence points in Alzheimer's disease. *J Alzheimers Dis.* (2013) 33(Suppl. 1):S141–4. doi: 10.3233/JAD-2012-129025
56. Wolfe MS. The role of tau in neurodegenerative diseases and its potential as a therapeutic target. *Scientifica.* (2012) 2012:796024. doi: 10.6064/2012/796024
57. Liu SL, Wang C, Jiang T, Tan L, Xing A, Yu JT. The role of Cdk5 in Alzheimer's disease. *Mol Neurobiol.* (2016) 53:4328–42. doi: 10.1007/s12035-015-9369-x
58. Crespo-Biel N, Theunis C, Van Leuven F. Protein tau: prime cause of synaptic and neuronal degeneration in Alzheimer's disease. *Int J Alzheimers Dis.* (2012) 2012:251426. doi: 10.1155/2012/251426
59. de Calignon A, Spires-Jones TL, Hyman BT. Caspase activation precedes and leads to neurodegeneration in a murine model of Alzheimer's disease. *Med Sci.* (2010) 26:787–9. doi: 10.1051/medsci/20102610787
60. Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci USA.* (2001) 98:6923–8. doi: 10.1073/pnas.121119298
61. Dorostkar MM, Zou C, Blazquez-Llorca L, Herms J. Analyzing dendritic spine pathology in Alzheimer's disease: problems and opportunities. *Acta Neuropathol.* (2015) 130:1–19. doi: 10.1007/s00401-015-1449-5
62. Liu F, Li B, Tung EJ, Grundke-Iqbal I, Iqbal K, Gong CX. Site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation. *Eur J Neurosci.* (2007) 26:3429–36. doi: 10.1111/j.1460-9568.2007.05955.x
63. Liu F, Gong CX. Tau exon 10 alternative splicing and tauopathies. *Mol Neurodegener.* (2008) 3:8. doi: 10.1186/1750-1326-3-8
64. Beharry C, Cohen LS, Di J, Ibrahim K, Briffa-Mirabella S, Alonso Adel C. Tau-induced neurodegeneration: mechanisms and targets. *Neurosci Bull.* (2014) 30:346–58. doi: 10.1007/s12264-013-1414-z
65. Park SY, Ferreira A. The generation of a 17 kDa neurotoxic fragment: an alternative mechanism by which tau mediates beta-amyloid-induced neurodegeneration. *J Neurosci.* (2005) 25:5365–75. doi: 10.1523/JNEUROSCI.1125-05.2005
66. Hanger DP, Wray S. Tau cleavage and tau aggregation in neurodegenerative disease. *Biochem Soc Trans.* (2010) 38:1016–20. doi: 10.1042/BST0381016
67. D'Amelio M, Cavallucci V, Middei S, Marchetti C, Pacioni S, Ferri A, et al. Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. *Nat Neurosci.* (2011) 14:69–76. doi: 10.1038/nn.2709
68. Zhao Y, Tseng IC, Heyser CJ, Rockenstein E, Mante M, Adame A, et al. Appoptosin-mediated caspase cleavage of tau contributes to progressive supranuclear palsy pathogenesis. *Neuron.* (2015) 87:963–75. doi: 10.1016/j.neuron.2015.08.020
69. Rohn TT, Rissman RA, Davis MC, Kim YE, Cotman CW, Head E. Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. *Neurobiol Dis.* (2002) 11:341–54. doi: 10.1006/nbdi.2002.0549
70. Goedert M, Clavaguera F, Tolnay M. The propagation of prion-like protein inclusions in neurodegenerative diseases. *Trends Neurosci.* (2010) 33:317–25. doi: 10.1016/j.tins.2010.04.003
71. Brundin P, Melki R, Kopito R. Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat Rev Mol Cell Biol.* (2010) 11:301–7. doi: 10.1038/nrm2873
72. Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature.* (2013) 501:45–51. doi: 10.1038/nature12481
73. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* (1991) 82:239–59. doi: 10.1007/BF00308809
74. Yamada K, Cirrito JR, Stewart FR, Jiang H, Finn MB, Holmes BB, et al. *In vivo* microdialysis reveals age-dependent decrease of brain interstitial fluid tau levels in P301S human tau transgenic mice. *J Neurosci.* (2011) 31:13110–7. doi: 10.1523/JNEUROSCI.2569-11.2011
75. Johnson GV, Seubert P, Cox TM, Motter R, Brown JP, Galasko D. The tau protein in human cerebrospinal fluid in Alzheimer's disease

consists of proteolytically derived fragments. *J Neurochem.* (1997) 68:430–3. doi: 10.1046/j.1471-4159.1997.68010430.x
76. Dage JL, Wennberg AMV, Airey DC, Hagen CE, Knopman DS, Machulda MM, et al. Levels of tau protein in plasma are associated with neurodegeneration and cognitive function in a population-based elderly cohort. *Alzheimers Dement.* (2016) 12:1226–34. doi: 10.1016/j.jalz.2016.06.001
77. Deters KD, Risacher SL, Kim S, Nho K, West JD, Blennow K, et al. Plasma tau association with brain atrophy in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis.* (2017) 58:1245–54. doi: 10.3233/JAD-161114
78. Fossati S, Ramos Cejudo J, Debure L, Pirraglia E, Sone JY, Li Y, et al. Plasma tau complements CSF tau and P-tau in the diagnosis of Alzheimer's disease. *Alzheimers Dement.* (2019) 11:483–92. doi: 10.1016/j.dadm.2019.05.001
79. Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther.* (2013) 5:9. doi: 10.1186/alzrt163
80. Blennow K, Zetterberg H. The past and the future of Alzheimer's disease fluid biomarkers. *J Alzheimers Dis.* (2018) 62:1125–40. doi: 10.3233/JAD-170773
81. Mielke MM, Hagen CE, Wennberg AMV, Airey DC, Savica R, Knopman DS, et al. Association of plasma total tau level with cognitive decline and risk of mild cognitive impairment or dementia in the mayo clinic study on aging. *JAMA Neurol.* (2017) 74:1073–80. doi: 10.1001/jamaneurol.2017.1359
82. Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol.* (2020) 19:422–33. doi: 10.1016/S1474-4422(20)30071-5
83. Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med.* (2020) 26:379–86. doi: 10.1038/s41591-020-0755-1
84. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA.* (2020) 324:772–81. doi: 10.1001/jama.2020.12134
85. de Leon MJ, Li Y, Okamura N, Tsui WH, Saint-Louis LA, Glodzik L, et al. Cerebrospinal fluid clearance in Alzheimer disease measured with dynamic PET. *J Nucl Med.* (2017) 58:1471–6. doi: 10.2967/jnumed.116.187211
86. Tarasoff-Conway JM, Carare RO, Osorio RS, Glodzik L, Butler T, Fieremans E, et al. Clearance systems in the brain-implications for Alzheimer disease. *Nat Rev Neurol.* (2015) 11:457–70. doi: 10.1038/nrneurol.2015.119
87. Carare RO, Aldea R, Agarwal N, Bacskai BJ, Bechman I, Boche D, et al. Clearance of interstitial fluid (ISF) and CSF (CLIC) group-part of vascular professional interest area (PIA): cerebrovascular disease and the failure of elimination of amyloid-beta from the brain and retina with age and Alzheimer's disease-opportunities for therapy. *Alzheimers Dement.* (2020) 12:e12053. doi: 10.1002/dad2.12053
88. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem.* (2009) 284:12845–52. doi: 10.1074/jbc.M808759200
89. Guo JL, Lee VM. Seeding of normal tau by pathological tau conformers drives pathogenesis of Alzheimer-like tangles. *J Biol Chem.* (2011) 286:15317–31. doi: 10.1074/jbc.M110.209296
90. Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Trans-cellular propagation of tau aggregation by fibrillar species. *J Biol Chem.* (2012) 287:19440–51. doi: 10.1074/jbc.M112.346072
91. DeVos SL, Corjuc BT, Oakley DH, Nobuhara CK, Bannon RN, Chase A, et al. Synaptic tau seeding precedes tau pathology in human Alzheimer's disease brain. *Front Neurosci.* (2018) 12:267. doi: 10.3389/fnins.2018.00267
92. Furman JL, Vaquer-Alicea J, White CL 3rd, Cairns NJ, Nelson PT, Diamond MI. Widespread tau seeding activity at early braak stages. *Acta Neuropathol.* (2017) 133:91–100. doi: 10.1007/s00401-016-1644-z
93. Kaufman SK, Del Tredici K, Thomas TL, Braak H, Diamond MI. Tau seeding activity begins in the transentorhinal/entorhinal regions and anticipates phospho-tau pathology in Alzheimer's disease and PART. *Acta Neuropathol.* (2018) 136:57–67. doi: 10.1007/s00401-018-1855-6
94. Kaufman SK, Thomas TL, Del Tredici K, Braak H, Diamond MI. Characterization of tau prion seeding activity and strains from formaldehyde-fixed tissue. *Acta Neuropathol Commun.* (2017) 5:41. doi: 10.1186/s40478-017-0442-8
95. Pooler AM, Phillips EC, Lau DH, Noble W, Hanger DP. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep.* (2013) 14:389–94. doi: 10.1038/embor.2013.15
96. Katsinelos T, Zeitler M, Dimou E, Karakatsani A, Muller HM, Nachman E, et al. Unconventional secretion mediates the trans-cellular spreading of tau. *Cell Rep.* (2018) 23:2039–55. doi: 10.1016/j.celrep.2018.04.056
97. Merezhko M, Brunello CA, Yan X, Vihinen H, Jokitalo E, Uronen RL, et al. Secretion of tau via an unconventional non-vesicular mechanism. *Cell Rep.* (2018) 25:2027–35.e4. doi: 10.1016/j.celrep.2018.10.078
98. Polanco JC, Scicluna BJ, Hill AF, Gotz J. Extracellular vesicles isolated from the brains of rTg4510 mice seed tau protein aggregation in a threshold-dependent manner. *J Biol Chem.* (2016) 291:12445–66. doi: 10.1074/jbc.M115.709485
99. Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem.* (2012) 287:3842–9. doi: 10.1074/jbc.M111.277061
100. Wang Y, Balaji V, Kaniyappan S, Kruger L, Irsen S, Tepper K, et al. The release and trans-synaptic transmission of tau via exosomes. *Mol Neurodegener.* (2017) 12:5. doi: 10.1186/s13024-016-0143-y
101. Dujardin S, Begard S, Caillierez R, Lachaud C, Delattre L, Carrier S, et al. Ectosomes: a new mechanism for non-exosomal secretion of tau protein. *PLoS ONE.* (2014) 9:e100760. doi: 10.1371/journal.pone.0100760
102. Abounit S, Wu JW, Duff K, Victoria GS, Zurzolo C. Tunneling nanotubes: a possible highway in the spreading of tau and other prion-like proteins in neurodegenerative diseases. *Prion.* (2016) 10:344–51. doi: 10.1080/19336896.2016.1223003
103. Tardivel M, Begard S, Bousset L, Dujardin S, Coens A, Melki R, et al. Tunneling nanotube (TNT)-mediated neuron-to neuron transfer of pathological tau protein assemblies. *Acta Neuropathol Commun.* (2016) 4:117. doi: 10.1186/s40478-016-0386-4
104. Santa-Maria I, Varghese M, Ksiezak-Reding H, Dzhun A, Wang J, Pasinetti GM. Paired helical filaments from Alzheimer disease brain induce intracellular accumulation of tau protein in aggresomes. *J Biol Chem.* (2012) 287:20522–33. doi: 10.1074/jbc.M111.323279
105. Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, et al. Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem.* (2013) 288:1856–70. doi: 10.1074/jbc.M112.394528
106. Calafate S, Flavin W, Verstreken P, Moechars D. Loss of bin1 promotes the propagation of tau pathology. *Cell Rep.* (2016) 17:931–40. doi: 10.1016/j.celrep.2016.09.063
107. Evans LD, Wassmer T, Fraser G, Smith J, Perkinton M, Billinton A, et al. Extracellular monomeric and aggregated tau efficiently enter human neurons through overlapping but distinct pathways. *Cell Rep.* (2018) 22:3612–24. doi: 10.1016/j.celrep.2018.03.021
108. Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, et al. LRP1 is a master regulator of tau uptake and spread. *Nature.* (2020) 580:381–5. doi: 10.1038/s41586-020-2156-5
109. Falcon B, Cavallini A, Angers R, Glover S, Murray TK, Barnham L, et al. Conformation determines the seeding potencies of native and recombinant Tau aggregates. *J Biol Chem.* (2015) 290:1049–65. doi: 10.1074/jbc.M114.589309
110. Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, et al. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci USA.* (2013) 110:E3138–47. doi: 10.1073/pnas.1301440110
111. Rauch JN, Chen JJ, Sorum AW, Miller GM, Sharf T, See SK, et al. Tau internalization is regulated by 6-O sulfation on heparan sulfate proteoglycans (HSPGs). *Sci Rep.* (2018) 8:6382. doi: 10.1038/s41598-018-24904-z
112. Falcon B, Noad J, McMahon H, Randow F, Goedert M. Galectin-8-mediated selective autophagy protects against seeded tau aggregation. *J Biol Chem.* (2018) 293:2438–51. doi: 10.1074/jbc.M117.809293

113. Wu JW, Hussaini SA, Bastille IM, Rodriguez GA, Mrejeru A, Rilett K, et al. Neuronal activity enhances tau propagation and tau pathology *in vivo*. *Nat Neurosci.* (2016) 19:1085–92. doi: 10.1038/nn.4328
114. Bierer LM, Hof PR, Purohit DP, Carlin L, Schmeidler J, Davis KL, et al. Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol.* (1995) 52:81–8. doi: 10.1001/archneur.1995.00540250089017
115. Togo T, Dickson DW. Tau accumulation in astrocytes in progressive supranuclear palsy is a degenerative rather than a reactive process. *Acta Neuropathol.* (2002) 104:398–402. doi: 10.1007/s00401-002-0569-x
116. Buee L, Delacourte A. Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and pick's disease. *Brain Pathol.* (1999) 9:681–93. doi: 10.1111/j.1750-3639.1999.tb00550.x
117. Kovacs GG, Ferrer I, Grinberg LT, Alafuzoff I, Attems J, Budka H, et al. Aging-related tau astrogliopathy (ARTAG): harmonized evaluation strategy. *Acta Neuropathol.* (2016) 131:87–102. doi: 10.1007/s00401-015-1509-x
118. Ferrer I, Garcia MA, Gonzalez IL, Lucena DD, Villalonga AR, Tech MC, et al. Aging-related tau astrogliopathy (ARTAG): not only tau phosphorylation in astrocytes. *Brain Pathol.* (2018) 28:965–85. doi: 10.1111/bpa.12593
119. Odawara T, Iseki E, Kosaka K, Akiyama H, Ikeda K, Yamamoto T. Investigation of tau-2 positive microglia-like cells in the subcortical nuclei of human neurodegenerative disorders. *Neurosci Lett.* (1995) 192:145–8. doi: 10.1016/0304-3940(95)11595-N
120. Bolos M, Llorens-Martin M, Jurado-Arjona J, Hernandez F, Rabano A, Avila J. Direct evidence of internalization of tau by microglia *in vitro* and *in vivo*. *J Alzheimers Dis.* (2016) 50:77–87. doi: 10.3233/JAD-150704
121. Leyns CEG, Holtzman DM. Glial contributions to neurodegeneration in tauopathies. *Mol Neurodegener.* (2017) 12:50. doi: 10.1186/s13024-017-0192-x
122. Hallmann AL, Arauzo-Bravo MJ, Mavrommatis L, Ehrlich M, Ropke A, Brockhaus J, et al. Astrocyte pathology in a human neural stem cell model of frontotemporal dementia caused by mutant TAU protein. *Sci Rep.* (2017) 7:42991. doi: 10.1038/srep42991
123. Walker LC. Glial tauopathy: neurons optional? *J Exp Med.* (2020) 217:e20191915. doi: 10.1084/jem.20191915
124. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci.* (2014) 34:11929–47. doi: 10.1523/JNEUROSCI.1860-14.2014
125. Martini-Stoica H, Cole AL, Swartzlander DB, Chen F, Wan YW, Bajaj L, et al. TFEB enhances astroglial uptake of extracellular tau species and reduces tau spreading. *J Exp Med.* (2018) 215:2355–77. doi: 10.1084/jem.20172158
126. Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, et al. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nat Neurosci.* (2015) 18:1584–93. doi: 10.1038/nn.4132
127. Brelstaff J, Tolkovsky AM, Ghetti B, Goedert M, Spillantini MG. Living neurons with tau filaments aberrantly expose phosphatidylserine and are phagocytosed by microglia. *Cell Rep.* (2018) 24:1939–48.e4. doi: 10.1016/j.celrep.2018.07.072
128. de Calignon A, Polydoro M, Suarez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron.* (2012) 73:685–97. doi: 10.1016/j.neuron.2011.11.033
129. De Marco D, Taggenbrock R, Crespo R, Koudstaal W, Ramsburg E, Apetri A. Cell-based assay to study antibody-mediated tau clearance by microglia. *J Vis Exp*. 2018:58576. doi: 10.3791/58576
130. Funk KE, Mirbaha H, Jiang H, Holtzman DM, Diamond MI. Distinct therapeutic mechanisms of tau antibodies: promoting microglial clearance versus blocking neuronal uptake. *J Biol Chem.* (2015) 290:21652–62. doi: 10.1074/jbc.M115.657924
131. Hopp SC, Lin Y, Oakley D, Roe AD, DeVos SL, Hanlon D, et al. The role of microglia in processing and spreading of bioactive tau seeds in Alzheimer's disease. *J Neuroinflammation.* (2018) 15:269. doi: 10.1186/s12974-018-1309-z
132. Luo W, Liu W, Hu X, Hanna M, Caravaca A, Paul SM. Microglial internalization and degradation of pathological tau is enhanced by an anti-tau monoclonal antibody. *Sci Rep.* (2015) 5:11161. doi: 10.1038/srep11161
133. Bolos M, Llorens-Martin M, Perea JR, Jurado-Arjona J, Rabano A, Hernandez F, et al. Absence of CX3CR1 impairs the internalization of Tau by microglia. *Mol Neurodegener.* (2017) 12:59. doi: 10.1186/s13024-017-0200-1
134. Nilson AN, English KC, Gerson JE, Barton Whittle T, Nicolas Crain C, Xue J, et al. Tau oligomers associate with inflammation in the brain and retina of tauopathy mice and in neurodegenerative diseases. *J Alzheimers Dis.* (2017) 55:1083–99. doi: 10.3233/JAD-160912
135. Jaworski T, Lechat B, Demedts D, Gielis L, Devijver H, Borghgraef P, et al. Dendritic degeneration, neurovascular defects, and inflammation precede neuronal loss in a mouse model for tau-mediated neurodegeneration. *Am J Pathol.* (2011) 179:2001–15. doi: 10.1016/j.ajpath.2011.06.025
136. Faraco G, Hochrainer K, Segarra SG, Schaeffer S, Santisteban MM, Menon A, et al. Dietary salt promotes cognitive impairment through tau phosphorylation. *Nature.* (2019) 574:686–90. doi: 10.1038/s41586-019-1688-z
137. Bennett RE, Robbins AB, Hu M, Cao X, Betensky RA, Clark T, et al. Tau induces blood vessel abnormalities and angiogenesis-related gene expression in P301L transgenic mice and human Alzheimer's disease. *Proc Natl Acad Sci USA.* (2018) 115:E1289–E98. doi: 10.1073/pnas.1710329115
138. Kim HJ, Park S, Cho H, Jang YK, San Lee J, Jang H, et al. Assessment of extent and role of tau in subcortical vascular cognitive impairment using 18F-AV1451 positron emission tomography imaging. *JAMA Neurol.* (2018) 75:999–1007. doi: 10.1001/jamaneurol.2018.0975
139. Majerova P, Garruto RM, Kovac A. Cerebrovascular inflammation is associated with tau pathology in guam parkinsonism dementia. *J Neural Transm.* (2018) 125:1013–25. doi: 10.1007/s00702-018-1883-3
140. Castillo-Carranza DL, Guerrero-Munoz MJ, Sengupta U, Hernandez C, Barrett AD, Dineley K, et al. Tau immunotherapy modulates both pathological tau and upstream amyloid pathology in an Alzheimer's disease mouse model. *J Neurosci.* (2015) 35:4857–68. doi: 10.1523/JNEUROSCI.4989-14.2015
141. Laurent C, Dorothee G, Hunot S, Martin E, Monnet Y, Duchamp M, et al. Hippocampal T cell infiltration promotes neuroinflammation and cognitive decline in a mouse model of tauopathy. *Brain.* (2017) 140:184–200. doi: 10.1093/brain/aww270
142. Majerova P, Michalicova A, Cente M, Hanes J, Vegh J, Kittel A, et al. Trafficking of immune cells across the blood-brain barrier is modulated by neurofibrillary pathology in tauopathies. *PLoS ONE.* (2019) 14:e0217216. doi: 10.1371/journal.pone.0217216
143. Davalos D, Ryu JK, Merlini M, Baeten KM, Le Moan N, Petersen MA, et al. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. *Nat Commun.* (2012) 3:1227. doi: 10.1038/ncomms2230
144. Lecrux C, Hamel E. The neurovascular unit in brain function and disease. *Acta Physiol.* (2011) 203:47–59. doi: 10.1111/j.1748-1716.2011.02256.x
145. Wang L, Jiang Q, Chu J, Lin L, Li XG, Chai GS, et al. Expression of Tau40 induces activation of cultured rat microglial cells. *PLoS ONE.* (2013) 8:e76057. doi: 10.1371/journal.pone.0076057
146. Forman MS, Lal D, Zhang B, Dabir DV, Swanson E, Lee VM, et al. Transgenic mouse model of tau pathology in astrocytes leading to nervous system degeneration. *J Neurosci.* (2005) 25:3539–50. doi: 10.1523/JNEUROSCI.0081-05.2005
147. Castillo-Carranza DL, Nilson AN, Van Skike CE, Jahrling JB, Patel K, Garach P, et al. Cerebral microvascular accumulation of tau oligomers in Alzheimer's disease and related tauopathies. *Aging Dis.* (2017) 8:257–66. doi: 10.14336/AD.2017.0112
148. McKee AC, Stein TD, Kiernan PT, Alvarez VE. The neuropathology of chronic traumatic encephalopathy. *Brain Pathol.* (2015) 25:350–64. doi: 10.1111/bpa.12248
149. Rohn TT, Day RJ, Catlin LW, Brown RJ, Rajic AJ, Poon WW. Immunolocalization of an amino-terminal fragment of apolipoprotein E in the pick's disease brain. *PLoS ONE.* (2013) 8:e80180. doi: 10.1371/journal.pone.0080180
150. Buee L, Hof PR, Bouras C, Delacourte A, Perl DP, Morrison JH, et al. Pathological alterations of the cerebral microvasculature in Alzheimer's disease and related dementing disorders. *Acta Neuropathol.* (1994) 87:469–80. doi: 10.1007/BF00294173

151. Arima K. Ultrastructural characteristics of tau filaments in tauopathies: immuno-electron microscopic demonstration of tau filaments in tauopathies. *Neuropathology.* (2006) 26:475–83. doi: 10.1111/j.1440-1789.2006.00669.x
152. Arima K, Nakamura M, Sunohara N, Ogawa M, Anno M, Izumiyama Y, et al. Ultrastructural characterization of the tau-immunoreactive tubules in the oligodendroglial perikarya and their inner loop processes in progressive supranuclear palsy. *Acta Neuropathol.* (1997) 93:558–66. doi: 10.1007/s004010050652
153. Dickson DW, Ahmed Z, Algom AA, Tsuboi Y, Josephs KA. Neuropathology of variants of progressive supranuclear palsy. *Curr Opin Neurol.* (2010) 23:394–400. doi: 10.1097/WCO.0b013e32833be924
154. Kouri N, Whitwell JL, Josephs KA, Rademakers R, Dickson DW. Corticobasal degeneration: a pathologically distinct 4R tauopathy. *Nat Rev Neurol.* (2011) 7:263–72. doi: 10.1038/nrneurol.2011.43
155. Takeda N, Kishimoto Y, Yokota O. Pick's disease. *Adv Exp Med Biol.* (2012) 724:300–16. doi: 10.1007/978-1-4614-0653-2_23
156. Trickler WJ, Mayhan WG, Miller DW. Brain microvessel endothelial cell responses to tumor necrosis factor-alpha involve a nuclear factor kappa B (NF-kappaB) signal transduction pathway. *Brain Res.* (2005) 1048:24–31. doi: 10.1016/j.brainres.2005.04.028
157. Wu B, Ma Q, Khatibi N, Chen W, Sozen T, Cheng O, et al. Ac-YVAD-CMK decreases blood-brain barrier degradation by inhibiting caspase-1 activation of interleukin-1beta in intracerebral hemorrhage mouse model. *Transl Stroke Res.* (2010) 1:57–64. doi: 10.1007/s12975-009-0002-z
158. Ohara Y, McCarron RM, Hoffman TT, Sugano H, Bembry J, Lenz FA, et al. Adrenergic mediation of TNF alpha-stimulated ICAM-1 expression on human brain microvascular endothelial cells. *Acta Neurochir Suppl.* (2000) 76:117–20. doi: 10.1007/978-3-7091-6346-7_24
159. Larochelle C, Alvarez JI, Prat A. How do immune cells overcome the blood-brain barrier in multiple sclerosis? *FEBS Lett.* (2011) 585:3770–80. doi: 10.1016/j.febslet.2011.04.066
160. Ruprecht K, Kuhlmann T, Seif F, Hummel V, Kruse N, Bruck W, et al. Effects of oncostatin M on human cerebral endothelial cells and expression in inflammatory brain lesions. *J Neuropathol Exp Neurol.* (2001) 60:1087–98. doi: 10.1093/jnen/60.11.1087
161. Sonar SA, Lal G. Differentiation and transmigration of CD4 T cells in neuroinflammation and autoimmunity. *Front Immunol.* (2017) 8:1695. doi: 10.3389/fimmu.2017.01695
162. Koren J 3rd, Jinwal UK, Lee DC, Jones JR, Shults CL, Johnson AG, et al. Chaperone signalling complexes in Alzheimer's disease. *J Cell Mol Med.* (2009) 13:619–30. doi: 10.1111/j.1582-4934.2008.00557.x
163. Leszczynski D, Joenvaara S, Reivinen J, Kuokka R. Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects. *Differentiation.* (2002) 70:120–9. doi: 10.1046/j.1432-0436.2002.700207.x
164. Isogai C, Laug WE, Shimada H, Declerck PJ, Stins MF, Durden DL, et al. Plasminogen activator inhibitor-1 promotes angiogenesis by stimulating endothelial cell migration toward fibronectin. *Cancer Res.* (2001) 61:5587–94.
165. Czekay RP, Wilkins-Port CE, Higgins SP, Freytag J, Overstreet JM, Klein RM, et al. PAI-1: an integrator of cell signaling and migration. *Int J Cell Biol.* (2011) 2011:562481. doi: 10.1155/2011/562481
166. Jeon H, Kim JH, Kim JH, Lee WH, Lee MS, Suk K. Plasminogen activator inhibitor type 1 regulates microglial motility and phagocytic activity. *J Neuroinflammation.* (2012) 9:149. doi: 10.1186/1742-2094-9-149
167. Stewart PA, Hayakawa K, Akers MA, Vinters HV. A morphometric study of the blood-brain barrier in Alzheimer's disease. *Lab Invest.* (1992) 67:734–42.
168. Claudio L. Ultrastructural features of the blood-brain barrier in biopsy tissue from Alzheimer's disease patients. *Acta Neuropathol.* (1996) 91:6–14. doi: 10.1007/s004010050386
169. Miyakawa T, Uehara Y, Desaki J, Kimura T, Kuramoto R. Morphological changes of microvessels in the brain with Alzheimer's disease. *Jpn J Psychiatry Neurol.* (1988) 42:819–24. doi: 10.1111/j.1440-1819.1988.tb01171.x
170. Hashimura T, Kimura T, Miyakawa T. Morphological changes of blood vessels in the brain with Alzheimer's disease. *Jpn J Psychiatry Neurol.* (1991) 45:661–5. doi: 10.1111/j.1440-1819.1991.tb01187.x
171. Kimura T, Hashimura T, Miyakawa T. Observations of microvessels in the brain with Alzheimer's disease by the scanning electron microscopy. *Jpn J Psychiatry Neurol.* (1991) 45:671–6. doi: 10.1111/j.1440-1819.1991.tb01189.x
172. Higuchi Y, Miyakawa T, Shimoji A, Katsuragi S. Ultrastructural changes of blood vessels in the cerebral cortex in Alzheimer's disease. *Jpn J Psychiatry Neurol.* (1987) 41:283–90. doi: 10.1111/j.1440-1819.1987.tb00414.x
173. Yamashita K, Miyakawa T, Katsuragi S. Vascular changes in the brains with Alzheimer's disease. *Jpn J Psychiatry Neurol.* (1991) 45:79–84. doi: 10.1111/j.1440-1819.1991.tb00509.x
174. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, et al. Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med.* (2000) 343:450–6. doi: 10.1056/NEJM200008173430701
175. Greenberg SM, Gurol ME, Rosand J, Smith EE. Amyloid angiopathy-related vascular cognitive impairment. *Stroke.* (2004) 35(11 Suppl. 1):2616–9. doi: 10.1161/01.STR.0000143224.36527.44
176. Vinters HV, Farag ES. Amyloidosis of cerebral arteries. *Adv Neurol.* (2003) 92:105–12.
177. Fann JR, Ribe AR, Pedersen HS, Fenger-Gron M, Christensen J, Benros ME, et al. Long-term risk of dementia among people with traumatic brain injury in Denmark: a population-based observational cohort study. *Lancet Psychiatry.* (2018) 5:424–31. doi: 10.1016/S2215-0366(18)30065-8
178. Katsumoto A, Takeuchi H, Tanaka F. Tau pathology in chronic traumatic encephalopathy and Alzheimer's disease: similarities and differences. *Front Neurol.* (2019) 10:980. doi: 10.3389/fneur.2019.00980
179. McKee AC. The neuropathology of chronic traumatic encephalopathy: the status of the literature. *Semin Neurol.* (2020) 40:359–69. doi: 10.1055/s-0040-1713632
180. Stein TD, Alvarez VE, McKee AC. Chronic traumatic encephalopathy: a spectrum of neuropathological changes following repetitive brain trauma in athletes and military personnel. *Alzheimers Res Ther.* (2014) 6:4. doi: 10.1186/alzrt234
181. Ikeda K, Akiyama H, Kondo H, Haga C, Tanno E, Tokuda T, et al. Thorn-shaped astrocytes: possibly secondarily induced tau-positive glial fibrillary tangles. *Acta Neuropathol.* (1995) 90:620–5. doi: 10.1007/BF00318575
182. Schultz C, Ghebremedhin E, Del Tredici K, Rub U, Braak H. High prevalence of thorn-shaped astrocytes in the aged human medial temporal lobe. *Neurobiol Aging.* (2004) 25:397–405. doi: 10.1016/S0197-4580(03)00113-1
183. McAleese KE, Firbank M, Dey M, Colloby SJ, Walker L, Johnson M, et al. Cortical tau load is associated with white matter hyperintensities. *Acta Neuropathol Commun.* (2015) 3:60. doi: 10.1186/s40478-015-0240-0
184. Strain JF, Smith RX, Beaumont H, Roe CM, Gordon BA, Mishra S, et al. Loss of white matter integrity reflects tau accumulation in Alzheimer disease defined regions. *Neurology.* (2018) 91:e313–e8. doi: 10.1212/WNL.0000000000005864
185. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. *Cell.* (2012) 148:1145–59. doi: 10.1016/j.cell.2012.02.035
186. Chen H, Chan DC. Mitochondrial dynamics–fusion, fission, movement, and mitophagy–in neurodegenerative diseases. *Hum Mol Genet.* (2009) 18:R169–76. doi: 10.1093/hmg/ddp326
187. Santos RX, Correia SC, Wang X, Perry G, Smith MA, Moreira PI, et al. A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease. *J Alzheimers Dis.* (2010) 20(Suppl. 2):S401–12. doi: 10.3233/JAD-2010-100666
188. Itoh K, Nakamura K, Iijima M, Sesaki H. Mitochondrial dynamics in neurodegeneration. *Trends Cell Biol.* (2013) 23:64–71. doi: 10.1016/j.tcb.2012.10.006
189. Kageyama Y, Zhang Z, Sesaki H. Mitochondrial division: molecular machinery and physiological functions. *Curr Opin Cell Biol.* (2011) 23:427–34. doi: 10.1016/j.ceb.2011.04.009
190. Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC. Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. *Mol Biol Cell.* (2009) 20:3525–32. doi: 10.1091/mbc.e09-03-0252
191. Picard M, Shirihai OS, Gentil BJ, Burelle Y. Mitochondrial morphology transitions and functions: implications for retrograde signaling? *Am J Physiol Regul Integr Comp Physiol.* (2013) 304:R393–406. doi: 10.1152/ajpregu.00584.2012

192. Wang X, Wang W, Li L, Perry G, Lee HG, Zhu X. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochim Biophys Acta.* (2014) 1842:1240–7. doi: 10.1016/j.bbadis.2013.10.015
193. Gao J, Wang L, Liu J, Xie F, Su B, Wang X. Abnormalities of mitochondrial dynamics in neurodegenerative diseases. *Antioxidants.* (2017) 6:25. doi: 10.3390/antiox6020025
194. Guo L, Tian J, Du H. Mitochondrial dysfunction and synaptic transmission failure in Alzheimer's disease. *J Alzheimers Dis.* (2017) 57:1071–86. doi: 10.3233/JAD-160702
195. Li XC, Hu Y, Wang ZH, Luo Y, Zhang Y, Liu XP, et al. Human wild-type full-length tau accumulation disrupts mitochondrial dynamics and the functions via increasing mitofusins. *Sci Rep.* (2016) 6:24756. doi: 10.1038/srep 24756
196. Perez MJ, Vergara-Pulgar K, Jara C, Cabezas-Opazo F, Quintanilla RA. Caspase-cleaved tau impairs mitochondrial dynamics in Alzheimer's disease. *Mol Neurobiol.* (2018) 55:1004–18. doi: 10.1007/s12035-017-0385-x
197. Placido AI, Pereira CMF, Correira SC, Carvalho C, Oliveira CR, Moreira PI. Phosphatase 2A inhibition affects endoplasmic reticulum and mitochondria homeostasis via cytoskeletal alterations in brain endothelial cells. *Mol Neurobiol.* (2017) 54:154–68. doi: 10.1007/s12035-015-9640-1
198. Kandimalla R, Manczak M, Fry D, Suneetha Y, Sesaki H, Reddy PH. Reduced dynamin-related protein 1 protects against phosphorylated Tau-induced mitochondrial dysfunction and synaptic damage in Alzheimer's disease. *Hum Mol Genet.* (2016) 25:4881–97. doi: 10.1093/hmg/ddw312
199. Manczak M, Reddy PH. Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer's disease neurons: implications for mitochondrial dysfunction and neuronal damage. *Hum Mol Genet.* (2012) 21:2538–47. doi: 10.1093/hmg/dds072
200. Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, et al. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci.* (2009) 29:9090–103. doi: 10.1523/JNEUROSCI.1357-09.2009
201. Manczak M, Calkins MJ, Reddy PH. Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. *Hum Mol Genet.* (2011) 20:2495–509. doi: 10.1093/hmg/ddr139
202. Bonda DJ, Wang X, Perry G, Smith MA, Zhu X. Mitochondrial dynamics in Alzheimer's disease: opportunities for future treatment strategies. *Drugs Aging.* (2010) 27:181–92. doi: 10.2165/11532140-000000000-00000
203. Kopeikina KJ, Carlson GA, Pitstick R, Ludvigson AE, Peters A, Luebke JI, et al. Tau accumulation causes mitochondrial distribution deficits in neurons in a mouse model of tauopathy and in human Alzheimer's disease brain. *Am J Pathol.* (2011) 179:2071–82. doi: 10.1016/j.ajpath.2011.07.004
204. Jacobson J, Duchen MR. Interplay between mitochondria and cellular calcium signalling. *Mol Cell Biochem.* (2004) 256–7:209–18. doi: 10.1023/B:MCBI.0000009869.29827.df
205. Mattson MP, Gleichmann M, Cheng A. Mitochondria in neuroplasticity and neurological disorders. *Neuron.* (2008) 60:748–66. doi: 10.1016/j.neuron.2008.10.010
206. Parodi-Rullan R, Sone JY, Fossati S. Endothelial mitochondrial dysfunction in cerebral amyloid angiopathy and Alzheimer's disease. *J Alzheimers Dis.* (2019) 72:1019–39. doi: 10.3233/JAD-190357
207. Fossati S, Giannoni P, Solesio ME, Cocklin SL, Cabrera E, Ghiso J, et al. The carbonic anhydrase inhibitor methazolamide prevents amyloid beta-induced mitochondrial dysfunction and caspase activation protecting neuronal and glial cells *in vitro* and in the mouse brain. *Neurobiol Dis.* (2016) 86:29–40. doi: 10.1016/j.nbd.2015.11.006
208. Solesio ME, Peixoto PM, Debure L, Madamba SM, de Leon MJ, Wisniewski T, et al. Carbonic anhydrase inhibition selectively prevents amyloid beta neurovascular mitochondrial toxicity. *Aging Cell.* (2018) 17:e12787. doi: 10.1111/acel.12787
209. Swerdlow RH, Burns JM, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis. *J Alzheimers Dis.* (2010) 20(Suppl. 2):S265–79. doi: 10.3233/JAD-2010-100339
210. Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Hum Mol Genet.* (2011) 20:4515–29. doi: 10.1093/hmg/ddr381
211. Balietti M, Giorgetti B, Casoli T, Solazzi M, Tamagnini F, Burattini C, et al. Early selective vulnerability of synapses and synaptic mitochondria in the hippocampal CA1 region of the Tg2576 mouse model of Alzheimer's disease. *J Alzheimers Dis.* (2013) 34:887–96. doi: 10.3233/JAD-121711
212. Du H, Guo L, Yan S, Sosunov AA, McKhann GM, Yan SS. Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. *Proc Natl Acad Sci USA.* (2010) 107:18670–5. doi: 10.1073/pnas.1006586107
213. Gibson GE, Shi Q. A mitocentric view of Alzheimer's disease suggests multi-faceted treatments. *J Alzheimers Dis.* (2010) 20(Suppl. 2):S591–607. doi: 10.3233/JAD-2010-100336
214. Xie H, Guan J, Borrelli LA, Xu J, Serrano-Pozo A, Bacskai BJ. Mitochondrial alterations near amyloid plaques in an Alzheimer's disease mouse model. *J Neurosci.* (2013) 33:17042–51. doi: 10.1523/JNEUROSCI.1836-13.2013
215. Varghese M, Zhao W, Wang J, Cheng A, Qian X, Chaudhry A, et al. Mitochondrial bioenergetics is defective in presymptomatic Tg2576 AD mice. *Transl Neurosci.* (2011) 2:1–5. doi: 10.2478/s13380-011-0011-8
216. Amadoro G, Corsetti V, Stringaro A, Colone M, D'Aguanno S, Meli G, et al. A NH2 tau fragment targets neuronal mitochondria at AD synapses: possible implications for neurodegeneration. *J Alzheimers Dis.* (2010) 21:445–70. doi: 10.3233/JAD-2010-100120
217. David DC, Hauptmann S, Scherping I, Schuessel K, Keil U, Rizzu P, et al. Proteomic and functional analyses reveal a mitochondrial dysfunction in P301L tau transgenic mice. *J Biol Chem.* (2005) 280:23802–14. doi: 10.1074/jbc.M500356200
218. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener.* (2011) 6:39. doi: 10.1186/1750-1326-6-39
219. Schulz KL, Eckert A, Rhein V, Mai S, Haase W, Reichert AS, et al. A new link to mitochondrial impairment in tauopathies. *Mol Neurobiol.* (2012) 46:205–16. doi: 10.1007/s12035-012-8308-3
220. Britti E, Ros J, Esteras N, Abramov AY. Tau inhibits mitochondrial calcium efflux and makes neurons vulnerable to calcium-induced cell death. *Cell Calcium.* (2020) 86:102150. doi: 10.1016/j.ceca.2019.102150
221. Quintanilla RA, Matthews-Roberson TA, Dolan PJ, Johnson GV. Caspase-cleaved tau expression induces mitochondrial dysfunction in immortalized cortical neurons: implications for the pathogenesis of Alzheimer disease. *J Biol Chem.* (2009) 284:18754–66. doi: 10.1074/jbc.M808908200
222. Mutisya EM, Bowling AC, Beal MF. Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. *J Neurochem.* (1994) 63:2179–84. doi: 10.1046/j.1471-4159.1994.63062179.x
223. Schagger H, Ohm TG. Human diseases with defects in oxidative phosphorylation. 2. F1F0 ATP-synthase defects in Alzheimer disease revealed by blue native polyacrylamide gel electrophoresis. *Eur J Biochem.* (1995) 227:916–21. doi: 10.1111/j.1432-1033.1995.tb20219.x
224. Kim SH, Vlkolinsky R, Cairns N, Fountoulakis M, Lubec G. The reduction of NADH ubiquinone oxidoreductase 24- and 75-kDa subunits in brains of patients with Down syndrome and Alzheimer's disease. *Life Sci.* (2001) 68:2741–50. doi: 10.1016/S0024-3205(01)01074-8
225. Gibson GE, Sheu KF, Blass JP. Abnormalities of mitochondrial enzymes in Alzheimer disease. *J Neural Transm.* (1998) 105:855–70. doi: 10.1007/s007020050099
226. Parker WD Jr, Filley CM, Parks JK. Cytochrome oxidase deficiency in Alzheimer's disease. *Neurology.* (1990) 40:1302–3. doi: 10.1212/WNL.40.8.1302
227. Maurer I, Zierz S, Moller HJ. A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. *Neurobiol Aging.* (2000) 21:455–62. doi: 10.1016/S0197-4580(00)00112-3

228. Wang J, Xiong S, Xie C, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J Neurochem.* (2005) 93:953–62. doi: 10.1111/j.1471-4159.2005.03053.x
229. Aliyev A, Chen SG, Seyidova D, Smith MA, Perry G, de la Torre J, et al. Mitochondria DNA deletions in atherosclerotic hypoperfused brain microvessels as a primary target for the development of Alzheimer's disease. *J Neurol Sci.* (2005) 229–30:285–92. doi: 10.1016/j.jns.2004.11.040
230. Haworth RA, Hunter DR. The Ca^{2+}-induced membrane transition in mitochondria. II. Nature of the Ca^{2+} trigger site. *Arch Biochem Biophys.* (1979) 195:460–7. doi: 10.1016/0003-9861(79)90372-2
231. Hunter DR, Haworth RA. The Ca^{2+}-induced membrane transition in mitochondria. I. The protective mechanisms. *Arch Biochem Biophys.* (1979) 195:453–9. doi: 10.1016/0003-9861(79)90371-0
232. Hunter DR, Haworth RA. The Ca^{2+}-induced membrane transition in mitochondria. III. Transitional Ca^{2+} release. *Arch Biochem Biophys.* (1979) 195:468–77. doi: 10.1016/0003-9861(79)90373-4
233. Kroemer G, Blomgren K. Mitochondrial cell death control in familial parkinson disease. *PLoS Biol.* (2007) 5:e206. doi: 10.1371/journal.pbio.0050206
234. Bonora M, Bononi A, De Marchi E, Giorgi C, Lebiedzinska M, Marchi S, et al. Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition. *Cell Cycle.* (2013) 12:674–83. doi: 10.4161/cc.23599
235. Bernardi P, Di Lisa F. The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. *J Mol Cell Cardiol.* (2015) 78:100–6. doi: 10.1016/j.yjmcc.2014.09.023
236. Bernardi P, Di Lisa F, Fogolari F, Lippe G. From ATP to PTP and back: a dual function for the mitochondrial ATP synthase. *Circ Res.* (2015) 116:1850–62. doi: 10.1161/CIRCRESAHA.115.306557
237. Jonas EA, Porter GA Jr, Beutner G, Mnatsakanyan N, Alavian KN. Cell death disguised: the mitochondrial permeability transition pore as the c-subunit of the F(1)F(O) ATP synthase. *Pharmacol Res.* (2015) 99:382–92. doi: 10.1016/j.phrs.2015.04.013
238. Rao VK, Carlson EA, Yan SS. Mitochondrial permeability transition pore is a potential drug target for neurodegeneration. *Biochim Biophys Acta.* (2014) 1842:1267–72. doi: 10.1016/j.bbadis.2013.09.003
239. Manczak M, Reddy PH. Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease. *Hum Mol Genet.* (2012) 21:5131–46. doi: 10.1093/hmg/dds360
240. Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet TA, et al. Molecular cloning of the interleukin-1 beta converting enzyme. *Science.* (1992) 256:97–100. doi: 10.1126/science.1373520
241. Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, et al. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature.* (1995) 376:37–43. doi: 10.1038/376037a0
242. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, et al. Human ICE/CED-3 protease nomenclature. *Cell.* (1996) 87:171. doi: 10.1016/S0092-8674(00)81334-3
243. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell.* (1996) 86:147–57. doi: 10.1016/S0092-8674(00)80085-9
244. Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science.* (1998) 281:1312–6. doi: 10.1126/science.281.5381.1312
245. Hengartner MO. The biochemistry of apoptosis. *Nature.* (2000) 407:770–6. doi: 10.1038/35037710
246. Shi Y. Mechanisms of caspase activation and inhibition during apoptosis. *Mol Cell.* (2002) 9:459–70. doi: 10.1016/S1097-2765(02)00482-3
247. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. *Nat Rev Mol Cell Biol.* (2004) 5:897–907. doi: 10.1038/nrm1496
248. Burguillos MA, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, et al. Caspase signalling controls microglia activation and neurotoxicity. *Nature.* (2011) 472:319–24. doi: 10.1038/nature09788
249. Hyman BT, Yuan J. Apoptotic and non-apoptotic roles of caspases in neuronal physiology and pathophysiology. *Nat Rev Neurosci.* (2012) 13:395–406. doi: 10.1038/nrn3228
250. Bamberger ME, Landreth GE. Inflammation, apoptosis, and Alzheimer's disease. *Neuroscientist.* (2002) 8:276–83. doi: 10.1177/1073858402008003013
251. Supnet C, Bezprozvanny I. The dysregulation of intracellular calcium in Alzheimer disease. *Cell Calcium.* (2010) 47:183–9. doi: 10.1016/j.ceca.2009.12.014
252. Chen X, Guo C, Kong J. Oxidative stress in neurodegenerative diseases. *Neural Regen Res.* (2012) 7:376–85. doi: 10.3969/j.issn.1673-5374.2012.05.009
253. de Calignon A, Fox LM, Pitstick R, Carlson GA, Bacskai BJ, Spires-Jones TL, et al. Caspase activation precedes and leads to tangles. *Nature.* (2010) 464:1201–4. doi: 10.1038/nature08890
254. Glushakova OY, Glushakov AO, Borlongan CV, Valadka AB, Hayes RL, Glushakov AV. Role of caspase-3-mediated apoptosis in chronic caspase-3-cleaved tau accumulation and blood-brain barrier damage in the corpus callosum after traumatic brain injury in rats. *J Neurotrauma.* (2018) 35:157–73. doi: 10.1089/neu.2017.4999
255. Selznick LA, Holtzman DM, Han BH, Gokden M, Srinivasan AN, Johnson EM, et al. *In situ* immunodetection of neuronal caspase-3 activation in Alzheimer disease. *J Neuropathol Exp Neurol.* (1999) 58:1020–6. doi: 10.1097/00005072-199909000-00012
256. Su JH, Zhao M, Anderson AJ, Srinivasan A, Cotman CW. Activated caspase-3 expression in Alzheimer's and aged control brain: correlation with Alzheimer pathology. *Brain Res.* (2001) 898:350–7. doi: 10.1016/S0006-8993(01)02018-2
257. Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. *Am J Pathol.* (2004) 165:523–31. doi: 10.1016/S0002-9440(10)63317-2
258. Matsui T, Ramasamy K, Ingelsson M, Fukumoto H, Conrad C, Frosch MP, et al. Coordinated expression of caspase 8, 3 and 7 mRNA in temporal cortex of Alzheimer disease: relationship to formic acid extractable abeta42 levels. *J Neuropathol Exp Neurol.* (2006) 65:508–15. doi: 10.1097/01.jnen.0000229238.05748.12
259. Gastard MC, Troncoso JC, Koliatsos VE. Caspase activation in the limbic cortex of subjects with early Alzheimer's disease. *Ann Neurol.* (2003) 54:393–8. doi: 10.1002/ana.10680
260. Su JH, Nichol KE, Sitch T, Sheu P, Chubb C, Miller BL, et al. DNA damage and activated caspase-3 expression in neurons and astrocytes: evidence for apoptosis in frontotemporal dementia. *Exp Neurol.* (2000) 163:9–19. doi: 10.1006/exnr.2000.7340
261. Day RJ, Mason MJ, Thomas C, Poon WW, Rohn TT. Caspase-cleaved tau co-localizes with early tangle markers in the human vascular dementia brain. *PLoS ONE.* (2015) 10:e0132637. doi: 10.1371/journal.pone.0132637
262. Yuan J, Yankner BA. Apoptosis in the nervous system. *Nature.* (2000) 407:802–9. doi: 10.1038/35037739
263. Shimohama S. Apoptosis in Alzheimer's disease–an update. *Apoptosis.* (2000) 5:9–16. doi: 10.1023/A:1009625323388
264. Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem.* (1999) 68:383–424. doi: 10.1146/annurev.biochem.68.1.383
265. Fischer U, Janicke RU, Schulze-Osthoff K. Many cuts to ruin: a comprehensive update of caspase substrates. *Cell Death Differ.* (2003) 10:76–100. doi: 10.1038/sj.cdd.4401160
266. Luthi AU, Martin SJ. The CASBAH: a searchable database of caspase substrates. *Cell Death Differ.* (2007) 14:641–50. doi: 10.1038/sj.cdd.4402103
267. Zhao X, Kotilinek LA, Smith B, Hlynialuk C, Zahs K, Ramsden M, et al. Caspase-2 cleavage of tau reversibly impairs memory. *Nat Med.* (2016) 22:1268–76. doi: 10.1038/nm.4199
268. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest.* (2004) 114:121–30. doi: 10.1172/JCI2004 20640
269. Newman J, Rissman RA, Sarsoza F, Kim RC, Dick M, Bennett DA, et al. Caspase-cleaved tau accumulation in neurodegenerative diseases associated with tau and alpha-synuclein pathology. *Acta Neuropathol.* (2005) 110:135–44. doi: 10.1007/s00401-005-1027-3

270. You Y, Perkins A, Cisternas P, Munoz B, Taylor X, You Y, et al. Tau as a mediator of neurotoxicity associated to cerebral amyloid angiopathy. *Acta Neuropathol Commun.* (2019) 7:26. doi: 10.1186/s40478-019-0680-z
271. Pedersen JT, Sigurdsson EM. Tau immunotherapy for Alzheimer's disease. *Trends Mol Med.* (2015) 21:394–402. doi: 10.1016/j.molmed.2015.03.003
272. Provensi G, Carta F, Nocentini A, Supuran CT, Casamenti F, Passani MB, et al. A New kid on the block? Carbonic anhydrases as possible new targets in Alzheimer's disease. *Int J Mol Sci.* (2019) 20:4724. doi: 10.3390/ijms20194724

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 15 January 2021
doi: 10.3389/fneur.2020.589471

Tau Isoform-Driven CBD Pathology Transmission in Oligodendrocytes in Humanized Tau Mice

*Justyna Zareba-Paslawska[1], Kalicharan Patra[1], Luca Kluzer[1], Tamas Revesz[2] and Per Svenningsson[1]**

[1] Laboratory of Translational Neuropharmacology, Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden, [2] Queen Square Brain Bank, Department of Clinical and Movement Neurosciences, Queen Square Institute of Neurology, University College London, London, United Kingdom

The aggregation of abnormally phosphorylated tau protein in neurons and glia is a neuropathological hallmark of several neurodegenerative disorders, collectively known as tauopathies. They are further subclassified based on the preferential pathological aggregation of three carboxyl-terminal repeat domains (3R) and/or 4R tau. Corticobasal degeneration (CBD) is a rare neurodegenerative disorder classified as a 4R tauopathy. In the present study, we extend analysis of CBD-tau cell-type specific pathology transmission with 3R and 4R tau isoform distinguishable changes. We use a humanized tau (hTau) mouse line, which overexpress all six human tau isoforms in a murine tau knockout background and perform intrastriatal inoculation of control and CBD-tau enriched human brain homogenate. We show that CBD-tau causes hyperphosphorylation of tau at Ser202 predominantly in oligodendrocytes. Next, we demonstrate the spread of tau pathology from striatum to the overlaying corpus callosum and further to the contralateral side. Finally, we demonstrate that the almost exclusive oligodendrocyte-based transmission of hyperphosphorylated tau is reflected in the endogenous 4R tau isoform expression and corresponds to subclassification of CBD as a 4R tauopathy. Additionally, we identify functional changes in oligodendrocytes reflected by myelin basic protein abnormalities upon CBD-tau inoculation. These changes are not observed in murine tau knockout mice lacking both human and murine tau. Our study presents not only *in vivo* tau isoform–driven region- and cell-specific tau pathology, but also underlines that tau pathology seeding and transmission might be oligodendrocyte-based. These results, which need to be extended to more cases, give new insights into why tauopathies might vary greatly in both histopathological and neuroanatomical patterns.

Keywords: tau, CBD, oligodendrocytes, myelin basic protein, hTau mice

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Isidre Ferrer, University of Barcelona, Spain
Eliana Cristina De Brito Toscano, Federal University of Minas Gerais, Brazil

***Correspondence:**
Per Svenningsson
per.svenningsson@ki.se

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *30 July 2020*
Accepted: *07 December 2020*
Published: *15 January 2021*

Citation:
Zareba-Paslawska J, Patra K, Kluzer L, Revesz T and Svenningsson P (2021) Tau Isoform-Driven CBD Pathology Transmission in Oligodendrocytes in Humanized Tau Mice. Front. Neurol. 11:589471. doi: 10.3389/fneur.2020.589471

INTRODUCTION

Corticobasal degeneration (CBD) is a rare neurodegenerative disorder with 0.6–0.9 cases per 100,000 individuals per year (1, 2), and in a more recent study of a Russian population, it was estimated at 0.02 cases per 100,000 individuals per year (3). The cardinal CBD features are progressive asymmetric rigidity and apraxia, accompanied by aphasia and dystonia. The

neuropathological hallmark of CBD is astrocytic plaque formation and accumulation of abnormally phosphorylated tau in neurons and glia (oligodendroglia and astrocytes) predominantly in forebrain structures (4). Although the CBD tau pathology is mainly astrocytic and neuronal, the oligodendroglia tau aggregates are also present in a form of numerous and widespread cytoplasmic process inclusions (argyrophilic threads) (5) and oligodendroglia cell body inclusions (coiled bodies) (6). Although astrogliopathy predominates in the earliest stage of CBD pathology, the oligodendroglia involvement in the early preclinical stages is also reported (7). Furthermore, in connection to the oligodendroglia pathology, the white matter volume loss in subcortical structure is observed in CBD (8, 9). Although CBD is a predominantly sporadic disorder, familial cases with tau protein mutation (N296N) have been reported (10).

Abnormalities in tau protein, resulting in its aggregation in neurons and glia, are a neuropathological hallmark of more than 20 neurodegenerative disorders collectively named tauopathies (11). Human tau protein is encoded by the microtubule associated protein tau (MAPT) gene containing 16 exons. The alternative splicing of exon 2 (E2), E3, and E10 result in expression of 6 tau isoforms in the adult human brain. The E2 and E3 determine the presence of 0, 1, or 2 near-amino-terminal inserts (0N, 1N, or 2N, respectively), and the existence of 3 or 4 carboxy-terminal repeat domains (3R or 4R, respectively) is dictated by alternative splicing of E10, in which only 4R variants include E10. The carboxy-terminal repeat domains are part of the microtubule assembly domain, and the presence of an extra repeat in 4R tau accelerates the microtubule assembly process 2.5–3.0 times compared to 3R (12). The presence or absence of E10 is particularly interesting in tauopathies. The preferential accumulation of 3R and/or 4R tau provides a subclassification of tauopathies (13). The most frequent neurodegenerative disorder, Alzheimer disease (AD), is a 3R + 4R tauopathy; progressive supranuclear palsy (PSP), CBD, and argyrophilic grain disease are 4R tauopathies; and Pick's disease (PiD) represents a 3R tauopathy (13). The tau protein also undergoes many post-translational modifications, among which phosphorylation is of particular interest due to insoluble hyperphosphorylated tau deposits found in postmortem brains from patients with tauopathy (14). Since the first classification of tau as a phospho-protein (15), at least 85 phosphorylation sites have been identified (16). Under normal physiological conditions, phosphorylation/dephosphorylation of tau modulates its microtubule binding properties. Tau affinity to the microtubule decreases upon phosphorylation (14), which increases the amount of more aggregation-prone cytosolic tau fraction (17). In addition, a recently published study challenges the current hypothesis that tau protein is a microtubule-stabilizing protein in axon and favor a new theory in which tau rather enables axonal microtubules to have the long labile domains (18).

Over the years, much attention has been placed on the neuronal connectome from the injection side and specific tau strains to elucidate the intraneuronal spread of tau aggregates in tauopathies. Recently, more focus is placed on explaining the role of astrocytes, oligodendrocytes, and microglia in tau-driven neurodegenerative disorders. Moreover, the striatum and prefrontal cortex are indicated as the earliest sites of tau pathology in CBD (7). Therefore, we utilize humanized tau (hTau) animals, overexpressing all six human tau isoforms in a murine knockout background and perform intrastriatal CBD-tau enriched brain homogenate inoculation to study a CBD-tau cell-type-specific pathology transmission. We show an oligodendrocyte predominant disease transmission, which we further identify to be 4R tau dependent.

MATERIALS AND METHODS

Subjects and Sample Collections

Subjects' consent was obtained according to the Declaration of Helsinki. All experiments involving human subjects were approved by the regional ethical committees at Karolinska University Hospital (2016/19–31/1). Formalin-fixed, paraffin-embedded 5-μm human brain sections and fresh frozen human brain blocks from subjects without neurological disorders ($n = 1$) or pathologically typical CBD ($n = 1$) were obtained from the Queens Square Brain Bank, London. The control case was an 88-year-old male with pathological aging-related amyloid beta deposition, limbic TDB-43, and mild small vessel disease. The CBD case was a 78-year-old male with characteristic CBD pathology, including typical AT8 positive astrocytic plaques, neuropil threads, and scattered oligodendroglial coiled bodies in the subcortical white matter. He also exhibited pathological aging (CERAD sparse amyloid beta plaques), Braak & Braak stage II. A detailed neuropathological description of the cases is presented in the **Supplementary Data**.

Purification of Insoluble Tau From CBD and Control Brains

The fresh frozen blocks of human frontal cortex from the CBD and control cases were homogenized at 1:5 (w/v) in sterile phosphate-buffered saline (PBS) with complete protease inhibitors (1836153, Roche, Basel, Switzerland), sonicated 5 × 1 s and centrifuged at 3,000 *g* at 4°C for 5 min. Next, the supernatant was centrifuged at 100,000 *g* at 4°C for 20 min. The supernatant, containing soluble tau, was aliquoted and stored at −70°C, and the pellet fraction containing insoluble tau was resuspended in the original volume of homogenization buffer, sonicated 5 × 1 s, aliquoted, and stored at −70°C (19).

Animals

hTau transgenic mice B6.Cg-Mapt$^{tm1(EGFP)Klt}$ Tg(MAPT)8cPdav/J (stock #004808 005491, The Jackson laboratory, Bar Harbor, ME, USA) and tau knockout (KO) mice B6.Cg-Mapt$^{tm1(EGFP)Klt}$ (stock #004779, The Jackson laboratory, Bar Harbor, ME, USA) were bred in the laboratory according to supplier instructions.

Stereotaxic Surgery

The 3-month-old mice were randomly assigned (4–8 per group) to the experimental groups. All experiments were

performed in agreement with the European Communities Council (86/609/EEC) and approved by the Stockholm North Ethical Committee (Ethical permit # N13014). Stereotaxic surgery was performed under anesthesia in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). The right striatum was injected with 8 μg of insoluble tau fractions from brain lysates of CBD or control cases at the coordinates 0.8 mm posterior, 1.95 mm lateral to bregma, and 3.0 mm ventral according to bregma. The infusion of 2 μl was conducted with a 10-μl Hamilton syringe (Hamilton, Bonaduz, Switzerland) at a rate of 0.2 μl/30 s. After infusion completion, the needle was left at the position for an additional 5 min and then slowly retracted. The skin was sutured and postoperative pain relief, Temgesic (s.c.; Indivior UK Limited, Slough, UK), was given directly after the surgery and additionally two times within 48 h. The animals were placed back in the cage with unlimited access to water and food until the experimental endpoint.

Immunocytochemistry

At 1 and 12 months postsurgery, mice were deeply anesthetized and perfused transcardially with saline followed by buffered 4% paraformaldehyde. Both hemispheres were collected, dehydrated, and embedded in paraffin and then sectioned into 4-μm-thick sagittal sections using a microtome. Next, slides were deparaffinized, rehydrated, and subjected to the antigen retrieval step in sodium citrate pH 8.5 for 30 min at 80°C. All washes were made with 1X PBS (7.2 mM Na_2HPO_4, 2.8 mM NaH_2PO_4, 140 mM NaCl, pH 7.4). Blocking of endogenous IgG with a Mouse on Mouse (M.O.M.™) Basic kit (BMK-2202, Vector Laboratories, Burlingame, CA, USA) was performed according to manufacturer protocol for mouse primary antibodies. The overnight incubation was performed at 4°C with the following primary antibodies: CP13 antibody (gift from P. Davis, dilution 1:1,000) for detection of phosphorylated tau at Ser202, Olig2 (ab109186, Abcam, Cambridge, UK; dilution 1:300), Sox9 (ab185966, Abcam, Cambridge, UK; dilution 1:300), NeuN (ab177487, Abcam, Cambridge, UK; dilution 1:300), MBP (sc-271524, Santa Cruz Biotechnology, Dallas, TX, USA; dilution 1:300), and RD4 (05-804, Merck Millipore, Burlington, MA, USA; dilution 1:300). Next, sections were washed and quenched of endogenous peroxidase in 3% hydrogen peroxide and 10% methanol in PBS. Thereafter, sections were washed, and secondary antibody incubation was performed with M.O.M.™ Biotinylated Anti-Mouse IgG Reagent (BMK-2202, Vector Laboratories, Burlingame, CA, USA) or ImmPRESS™-AP REAGENT Anti-Rabbit IgG (MP-5401, Vector Laboratories, Burlingame, CA, USA) according to manufacturer protocol. The subsequent washes were performed, and avidin-biotin complex horseradish peroxidase kit (ABC Elite) (PK-6100, Vector Laboratories, Burlingame, CA, USA) with 3,3-diaminobenzidine (DAB, D5637, Sigma-Aldrich, St. Louis, MO, USA) based detection, yielding brown product, was applied for CP13, MBP, and RD4 antibody detection. The Vector® Red AP substrate (SK-5100, Vector Laboratories, Burlingame, CA, USA), yielding red/pink reaction product, was applied for Olig2, Sox9, and NeuN antibodies. The multiple antigen labeling was performed for detection of CP13 or RD4 antibody with DAB chromogen and followed by subsequent Olig2, Sox9, or NeuN overnight incubation and detection with Vector® Red AP substrate. Negative controls, omitting primary antibodies, were always conducted in parallel for immunohistochemical staining. The human paraffin sections were treated as described above for mice sections with the following alterations: no M.O.M. kit incubation was applied, and horseradish peroxidase directly conjugated secondary antibody (P044701-2, Dako, Denmark; dilution 1:100) was applied to detect CP13 mouse antibody.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE was performed as previously described (20). The sample was mixed in a 3:1 ratio with denaturing loading buffer (106 mM Tris-HCl, 141 mM Tris, 2% lithium dodecyl sulfate (LDS), 10% glycerol, 6% β-mercaptoethanol, 0.51 mM EDTA, 0.22 mM SERVA Blue G-250, 0.175 mM Phenol Red, pH 8.5) and boiled at 95°C for 5 min. Next, samples were separated using a bis-tris acrylamide gel using MES running buffer (50 mM Tris, 50 mM 2-(N-morpholino) ethanesulfonic acid (MES), 0.1% SDS, 1 mM EDTA, pH 7.3).

Western Blotting

After SDS/PAGE, gels were assembled with 0.45 μm pore size polyvinylidene difluoride membranes, and semidry transfer was performed. Membranes were blocked during 1 h incubation in 5% skim milk at RT. Next, membranes were incubated overnight at 4°C with a primary antibody diluted 1:1,000 (vol/vol) in 1% skim milk. Membranes were thereafter incubated for 2 h with appropriate horseradish peroxidase (HRP)-conjugated secondary antibody (Dako, Denamrk) diluted 1:10,000 (vol/vol) in 1% skim milk at RT. The signal was developed by a Clarity Western ECL Substrate (BioRad, USA). A colorimetric scan was used to visualize the protein ladder. The intensity of protein bands was analyzed using ImageJ (21). Primary antibodies used were RD3 (05-803, Merck Millipore, USA), RD4 (05-804, Merck Millipore, USA), CP13 (gift from P. Davis, 1:5,000 dilution), AT8 (MN1020, Thermo Fisher Scientific, USA), AT100 (MN1060, Thermo Fisher Scientific, USA), pThr181 (12855, Cell Signaling, USA) and DA9 (gift from P. Davis).

Dot Blotting and Ponceau S Staining

One μl of the insoluble tau fractions from brain lysates of CBD and control cases was placed on a nitrocellulose membrane and air dried. The Ponceau S staining (P7170-1L, Sigma-Aldrich, St. Louis, MO, USA) was performed according to manufacture protocol. A colorimetric scan was used to visualize the signal. The intensity of the protein signal was analyzed using ImageJ (21).

Negative Stain TEM

Three μl of the sample was applied on glow-discharged, carbon-coated, and formvar-stabilized 400 mesh copper grids (Ted Pella) and incubated for approximately 30 s. Excess sample was blotted off, and the grid was washed with MilliQ water prior to negative staining using 2% uranyl acetate. TEM imaging was done using Hitachi HT7700 (Hitachi High-Technologies) transmission

electron microscope operated at 100 kV equipped with a 2k x 2k Veleta CCD camera (Olympus Soft Imaging System).

Cell Counting

Cell counting was performed on representative light microscopy pictures. The digital images of the striatum and corpus callosum were obtained by a microscopic objective lens (20×, Plan Fluor; Nikon, Tokyo, Japan) and analyzed using ImageJ. For each animal, three sections were analyzed. From each section, three locations of the structures were selected for microphotography. For the striatum, the two dorsal (one anterior and one posterior) and one ventral images were analyzed. The dorsal anterior striatum analysis was performed, omitting the internal capsule. For the corpus callosum, pictures corresponding to the anterior, medial, and posterior parts of the corpus callosum overlaying the striatum were analyzed. The manual exhausted counting of single and double positive cells was performed blindly. The data are presented as a fraction of double positive ($CP13^+/Olig2^+$) cells of all single positive ($Olig2^+$) cells and displayed as a percentage of control injected animals.

Densitometry

The optical density of MBP immunoreactivity was performed on representative light microscopy pictures. The digital images of the corpus callosum were obtained by a microscopic objective lens (10×, Plan Fluor; Nikon, Tokyo, Japan) during one microscopic session and analyzed in gray scale using ImageJ. The same microscope light intensity was applied to all acquired pictures. For each animal, three sections were analyzed. From each section, three locations from the anterior, medial, and posterior parts of the corpus callosum overlaying the striatum were selected, omitting backgrounds gaps. The picture collections and data analysis were performed blindly. The optical density for each location was corrected for non-specific background density. The data are presented as a percentage of control injected animals.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (GraphPad Inc). A Mann–Whitney *U* test was applied for nonparametric comparison between two groups. The Student *t* test was applied for parametric comparison between two groups after assessing normality with the Shapiro–Wilk normality test. A Spearman's rank correlation coefficient was used to analyze dependence between two sets of data. All statistical tests were 2-tailed, and a P-value < 0.05 was considered statistically significant.

RESULTS

To investigate cell-specific propagation of CBD-tau, we prepared an insoluble tau fraction from a neuropathologically confirmed CBD case and a control non-neuropathological case. The detailed neuropathological case description is presented in the **Supplementary Data**. The Western blot analysis of the tau insoluble fraction of control and the CBD brain homogenate used for inoculation is presented in **Supplementary Figure 1A**. The 3R tau isoforms are presented in both CBD and control cases, and no high molecular weight aggregates were detected in either of them. Importantly, the 4R tau isoforms were also detected in both cases, but only the CBD case possessed a high molecular aggregated form of 4R tau. Further analysis reveals that only the CBD case possessed CP13 (pSer202), AT8 (pSer202, pThr205), AT100 (pThr212, pSer214), and pThr181 positive tau in the tau insoluble brain homogenate fraction. All of them were also detected as a high molecular weight species. In addition, the CBD inoculated material had 3-fold greater signal intensity for total tau than the control case (DA9, pan-tau antibody). Importantly, Ponceau S staining showed that the protein load was 2-fold lower in the CBD case than in the control. Therefore, the observed greater total tau load in the CBD case was due to tau pathology, not difference in total protein load. Further, we performed TEM analysis of injected insoluble material from both the CBD and control cases. Only a few short tangles were observed in the control case (**Supplementary Figure 1B**). In material isolated from the CBD case, numerous short tangles can be seen (**Supplementary Figure 1C**). We have not observed any long tangles in any of the samples, which is connected with performing a sonication step of the material prior to injection into mice brains. In addition, we performed immunohistochemistry analysis of frontal cortices from the control and CBD cases using CP13 antibody (**Supplementary Figures 1D,F**) specific for tau phosphorylated at Ser202 (pSer202), commonly used to detect tau pathology in both early (pretangle) and more advanced stages of neurofibrillary tangle accumulation (22–24). The CP13 positive ($CP13^+$) astrocytic plaques were observed only in the CBD case. The neuronal CP13 staining was regularly detected in the CBD case, and only few neurons were observed in the control case. The aging-related tau astrogliopathy was present in both cases in the form of $CP13^+$ thorn-shaped astrocytes. No fuzzy $CP13^+$ astrocytes were detected in any of the cases. The negative microphotography representation of CP13 staining highlights abundant pSer202 involvement in CBD pathology compared to the control case (**Supplementary Figures 1E,G**).

The insoluble tau fraction of CBD or control case brain homogenate was stereotaxically injected into the right striatum of 3-month-old humanized tau (hTau) animals and tau knockout mice (KO) based on neuropathological findings highlighting the striatum in preclinical and early stages of CBD (7). The hTau mice overexpress all six human tau isoforms under the tau promoter in the murine tau KO background. Next, we examined tau pathology at 1 and 12 months post-surgery using CP13 antibody (pSer202, pretangles, and more advanced stages of neurofibrillary tangle accumulation) (22–24).

We started analysis of oligodendrocytes using the Olig2 nuclear marker combined with CP13 immunoreactivity. Interestingly, 1 month after inoculation, the vast majority of CP13 positive ($CP13^+$) oligodendrocytes in the striatum were located within or in close proximity to white matter tracts, and CP13 negative ($CP13^-$) were equally distributed (**Figure 1A**). No significant differences were observed in Ser202 phosporylated tau positive oligodendrocytes ($CP13^+/Olig2^+$) between control-tau and CBD-tau injected hTau animals in either contra- or ipsilateral striatum (**Figures 1B,C**, respectively). However, 12-month inoculation of CBD-tau resulted in a significantly higher presence of $CP13^+$ oligodendrocytes in the striatum

(**Figures 1A,D,E**). Interestingly, the location of the $CP13^+$ oligodendrocytes was no longer mainly restricted to the white matter tracts and their near proximity, but $CP13^+$ oligodendrocytes were also regularly observed in striatal gray matter (**Figure 1A**). Importantly, the significantly increased number of $CP13^+$ oligodendrocytes was not restricted to the ipsilateral hemisphere but was also present in the contralateral striatum (**Figures 1A,D,E**). This suggests that CBD-tau can induce transmittable changes in oligodendrocyte population across hemispheres.

Due to anatomical connections of striatal white matter tracts to the corpus callosum, we also analyzed $CP13^+$ oligodendrocytes in the corpus callosum. After 1 month of the control-tau homogenate inoculation, approximately one third of oligodendrocytes were $CP13^+$ (**Figure 1A**). At that time point, no significant differences in $CP13^+$ oligodendrocytes were observed between control-tau and CBD-tau injected hTau animals in either the contra- or ipsilateral sides (**Figures 1F,G**, respectively). Although in line with striatal results, 12 months after homogenate inoculation, $CP13^+$ oligodendrocytes were significantly more frequently observed in CBD-tau than in control-tau injected hTau animals (**Figure 1A**) in both the contra- and ipsilateral sides (**Figures 1H,I**, respectively). This confirms that tau-enriched brain homogenate from a CBD case induced transferrable changes in the oligodendrocyte population also in the corpus callosum. Furthermore, the significant positive correlation in the fraction of $CP13^+$ oligodendrocytes was observed between the striatum and corpus callosum (**Figure 1J**), suggesting the presence of the oligodendrocytes' transmissible changes in tau phosphorylation between these structures. Interestingly, when comparing the spread of Ser202 phosporylated tau pathology across hemispheres, the significant positive correlation between the number of ipsi- and contralateral $CP13^+$ oligodendrocytes was present only in CBD-tau (**Figure 1K**) and not control-tau (**Figure 1L**) injected animals. This supports that only the CBD-tau, not control-tau, is able to trigger and transmit pathology across hemispheres.

Subsequently, we studied if an increased fraction of $CP13^+$ oligodendrocytes could be reflected in their functional changes. To assess this possibility, we measured myelin basic protein (MBP) immunoreactivity in the corpus callosum of 12-month post-injected hTau and KO animals. The densitometric analysis revealed a significant decrease in MBP immunoreactivity in CBD-tau compared with control-tau injected hTau animals (**Figures 2A–C**). On the contrary, analysis of tau KO animals did not show any significant differences in MBP density between CBD-tau and control-tau injected animals (**Figures 2A,D,E**), highlighting the importance of human endogenous tau presence in triggering CBD-tau pathology. In addition, the significant negative correlation was detected between MBP density and the number of $CP13^+$ oligodendrocytes in the corpus callosum of hTau mice (**Figure 2F**), indicating the myelin disruption as a functional consequence of increased Ser202 hyperphosphorylated tau in oligodendrocytes.

Because CBD is primarily an astrogliopathy, we analyzed tau pathology in astrocytes. We applied Sox9, a nuclear marker specific for astrocytes, to identify astrocytes in the adult mouse brain outside the neurogenic regions. Examination of striatal astrocytes revealed that $CP13^+$ astrocytes are a very rare event in hTau animals, and the vast majority of astrocytes are $CP13^-$. Similar results were observed in control-tau and CBD-tau injected animals at both experimental time points (**Figure 3**). Likewise, the astrocyte population in the corpus callosum also remained mainly $CP13^-$ in all experimental groups (**Figure 3**).

Furthermore, we also analyzed Ser202-specific tau phosphorylation in the neurons of the striatum, and no significant alterations were observed in any of the experimental groups at any of the time points (**Figures 1A**, **3**, based on neuronal morphological discrimination). The corpus callosum was not examined due to a lack of neurons in this structure. The clear neuronal immunoreactivity of CP13 was present in the hippocampus (**Supplementary Figure 2**) and other brain regions in hTau animals indicating that CP13 antibody recognized pSer202 tau not only in oligodendrocytes, but also neurons (22–24).

Finally, to determine the possible cause of preferential oligodendrocyte susceptibility to CBD-tau induced changes over other cell types in the striatum and corpus callosum, we performed 4R tau-specific immunorectivity to follow subclassification of CBD as a 4R tauopathies (**Figure 4**). Interestingly, we identified that the only 4R positive cells in the striatum and corpus callosum were oligodendrocytes, and no 4R tau-positive astrocytes or neurons were observed. Furthermore, none or weak expression of 4R tau were detected in oligodendrocytes in the cortex or hippocampus. Only a few 4R tau-positive neurons were observed in the cortex and some in the hippocampus. In none of the analyzed structures were 4R tau positive astrocytes identified. Interestingly, this suggests that 4R tau expression in hTau animals is both cell and site specific.

DISCUSSION

Numerous studies have been performed with intracerebral injection of human brain homogenates from a range of tauopathies in mice expressing human tau (19, 25–29) or WT animals (29–33). In the present study, by applying a humanized tau (hTau) mouse line (22), we generated a new model in which CBD-tau transmission could be examined not only in the presence of all six human tau isoforms, but also excluding any interaction resulting from endogenous murine tau. The important advantage of the applied model is a non-predominant 4R tau environment, which allows the 3R and 4R tau isoforms distinguishable study. Additionally, tau overexpression in this line is driven by the endogenous tau promoter. Furthermore, performed striatal inoculation relates to the brain region mostly affected by astrogliopathy in the early stages of CBD (7). In our research, the manifestation of pathological tau was analyzed by monitoring the presence of phosphoepitope Ser202 on tau protein by CP13 antibody immunoreactivity, commonly used to detect tau pathology in both early (pretangle) and more advanced stages of neurofibrillary tangle accumulation (22–24).

We show that CBD-tau brain homogenate inoculation in striatum of hTau animals caused hyperphosphorylation of

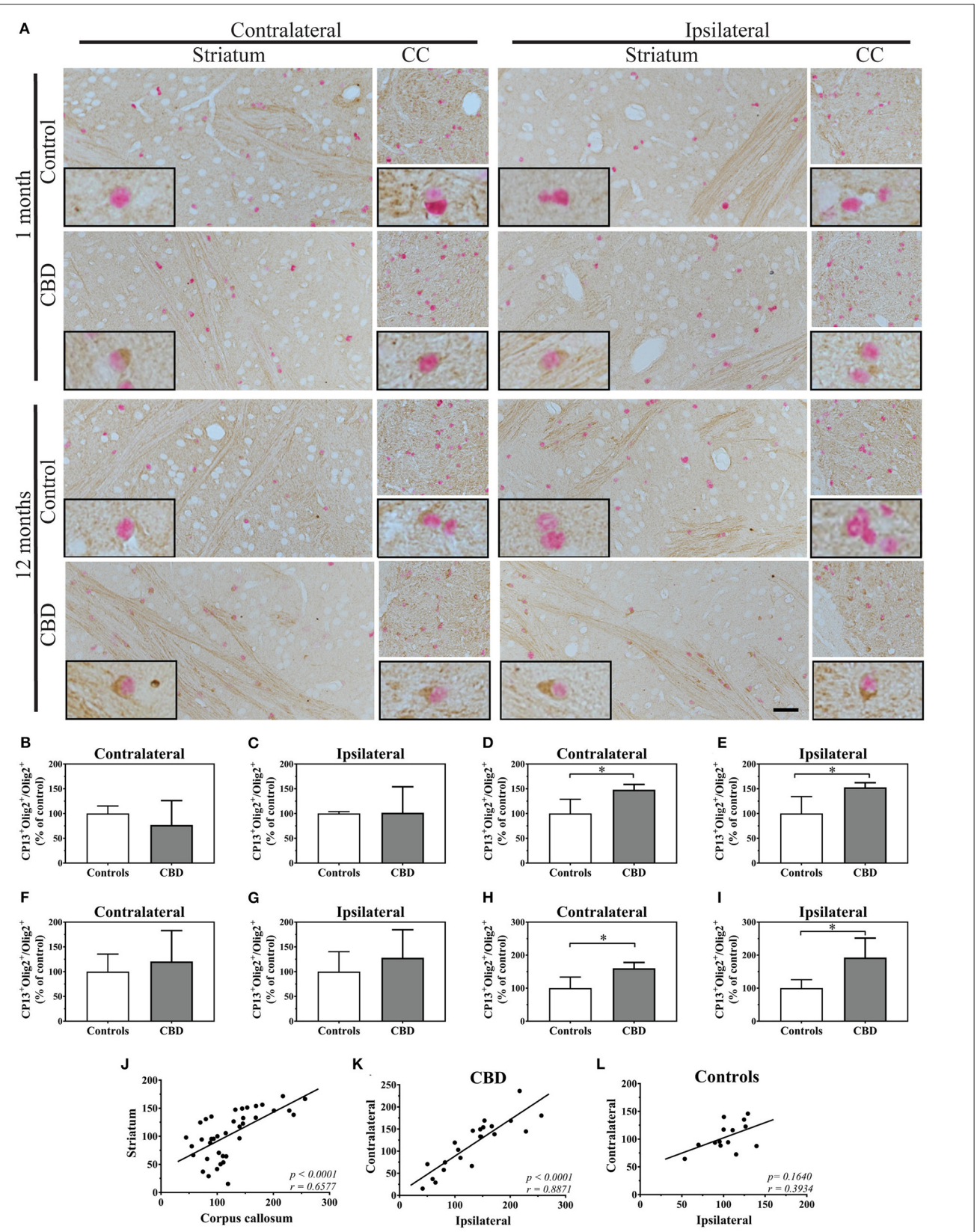


FIGURE 1 | The tau pathology transmission in oligodendrocytes. **(A)** The microphotography pictures of double immunohistochemistry staining of tau phosphorylated at Ser202 (CP13 antibody, brown chromogen) in oligodendrocytes (Olig2, nuclear marker, red/pink chromogen). The cell distribution in the contralateral (left panel) and *(Continued)*

FIGURE 1 | ipsilateral side (right panel) of the striatum (left subpanel) and corpus callosum (right subpanel) after 1 month (upper panel) and 12 months (lower panel) of control-tau (upper subpanel) or CBD-tau (lower subpanel) inoculation. The representative pictures of cells in each group are presented as the black bordered inserts. The presence of pathological tau in oligodendrocytes increases upon CBD-tau inoculation at 12 months postsurgery. No changes for control-tau were observed. The scale bar represents 50 μm and applies to all pictures; the black-bordered inserts represent 4× digital enlargement. **(B–I)** the quantitative analysis of double positive oligodendrocytes ($CP13^+/Olig2^+$): 1 month postsurgery in the contralateral (**B,** controls $n = 3$, CBD $n = 8$) and ipsilateral (**C,** controls $n = 4$, CBD $n = 8$) striatum; 12 month postsurgery in the contralateral (**D,** controls $n = 6$, CBD $n = 4$) and ipsilateral (**E,** controls $n = 4$, CBD $n = 4$) striatum; 11 months postsurgery in the contralateral (**F,** controls $n = 3$, CBD $n = 7$) and ipsilateral (**G,** controls $n = 4$, CBD $n = 7$) corpus callosum; and 12 month postsurgery in the contralateral (**H,** controls $n = 5$, CBD $n = 4$) and ipsilateral (**I,** controls $n = 4$, CBD $n = 4$) corpus callosum. The significant increase of tau phosphorylated at Ser202 positive oligodendrocytes in CBD-tau injected animals was observed in striatum and corpus callosum 12 months postsurgery compared to control-tau animals. **(J–L)** the correlation of double-positive oligodendrocytes ($CP13^+/Olig2^+$) as a fraction of all oligodendrocytes between the corpus callosum and striatum **(J)**, between the ips- and contralateral side of CBD-tau **(K)** and control-tau **(L)** injected animals. The striatum and corpus callosum transmissible changes in oligodendrcytic tau pathology were detected by the presence of the significant positive correlation between them **(J)**. The significant positive correlation between ipsi-and contralateral hemispheres of oligodendrcytic tau pathology was observed only in CBD-tau **(K)**, and not control-tau **(L)** injected animals strengthening that only pathological tau is able to trigger transmissible changes in oligodendrocytes across hemispheres. Comparison between 2 groups was performed using the Mann–Whitney *U* test. Spearman's rank correlation coefficient was used to analyze dependence between 2 sets of data. *P* value < 0.05 was considered statistically significant. $^*P < 0.05$.

tau at Ser202 predominantly in oligodendrocytes. Next, the pathology of tau hyperphosphorylated at Ser202 was transmitted from the striatum to the overlaying corpus callosum in the ipsilateral side and 12 months postinjection was also detected in oligodendrocytes in the contralateral corpus callosum and striatum. These results are in line with well-documented oligodendrocytes and white matter pathology in CBD patients (4–6, 8, 9). Although astrogliopathy predominates at the earliest stage of CBD pathology, the oligodendroglia involvement in the early preclinical stages is also reported in patients (7). Interestingly, the striatum (caudate and putamen) was the most affected by astrocytic plaque in the earliest stages, and astrogliopathy was accompanied by oligodendroglia pathology present in form of coiled bodies in the putamen (7).

Furthermore, the results showing oligodendrocyte involvement in CBD-tau propagation in our model are in line with previously reported studies in WT (31) and other transgenic mice (26, 27, 29, 34). These studies report oligodendrocytic next to neuronal and astrocytic involvement in CBD-tau propagation although with discrepancy in the cell types involved and timing in both WT and transgenic animals. Interestingly, intracerebral inoculation of the same tauopathies' brain homogenates in the same mouse brain region, presented the diverse magnitude of the cell type involvement in tau spreading depending on whether the animals were WT or transgenic (PS19) (26). In the PS19 transgenic mouse line, expression of the T34 isoform of tau (4R1N) containing the P301S tau mutation is driven by the murine prion protein promoter (35). It is, therefore, not surprising to observe disease pathology in all reported cell types (neurons, astrocytes, and oligodendrocytes) because they have been preconditioned and unified toward it not only by mutation *per se* but also 4R tau isoform presence (both human and endogenous murine). However, even in this preconditioned environment, predominantly oligodendrocytes and white matter pathology was observed, over infrequent intraneuronal tau aggregates upon CBD-tau inoculation for 1 month. Astrocytic pathology was scarcely detected later in time, 6 months postinjection (26). This agrees with our current report, in which exclusive oligodendrocyte involvement over neuronal and astrocytic is observed after striatal CBD-tau inoculation in hTau mice. A non-significant increase in $CP13^+$ oligodendrocytes was also detected as early as after 1 month CBD-tau inoculation, and it became significant at 12 months. We did not observe any significant neuronal or astrocytic involvement. However, it should be noted that robust acceleration of pathological changes caused by P301S mutation reported by Lee, Trojanowki, and colleagues (35) and other studies (23, 36–38), together with the tau isoform expression pattern, need to be taken into consideration. On the contrary, in the other study reported by Lee, Trojanowki, and colleagues, CBD-tau injection into WT mice triggered tau aggregates in oligodendrocytes and astrocytes simultaneously 1 month post-injection with accompanying systematic neuronal inclusions (31). In addition, similarly to our study, propagation of oligodendrocyte aggregates was shown over time from the ipsi- to contralateral side of the white matter tracks (fimbria and corpus callosum). Importantly, with our quantitative data, we reported a significant positive correlation between the number of ipsi- and contralateral $CP13^+$ oligodendrocytes only in CBD-tau and not control-tau injected animals, reinforcing that only the CBD-tau was able to trigger and transmit pathology across hemispheres.

Likewise, CBD-tau inoculation in ALZ17 animals (27), expressing the longest human tau isoform (4R2N) under the neuron-specific promotor (39) also caused oligodendrocytic tau pathology. The presence of small neurofibrillary tangles and abundant neuropil threads in the hippocampus were observed 6 months after post-hippocampal CBD-tau injection, and were accompanied by tau inclusions in oligodendrocytes in the form of coiled bodies. In line with the aforementioned studies (31, 35) the astrocytic plaques were identified later in time (after 12 months inoculation).

The observed changes in diverse magnitude of the cell type involvement and timing in tau spreading, reported in our and previous studies (26, 27, 31, 34), might be explained by heterologous promoters controlling overexpression of different tau variants (normal or mutated), use of transgenic or WT animals, as well as differences in tau isoform expression. The ratio of 3R and 4R tau isoforms in the human adult brain is 1:1, and adult mice express exclusively 4R isoforms (12, 22, 23, 40–42). The radical changes in tau isoform expression were reported in the literature upon introduction of all six human tau isoforms in a mouse null background (with endogenous tau knockout) animal lines (22, 41), and the predominant

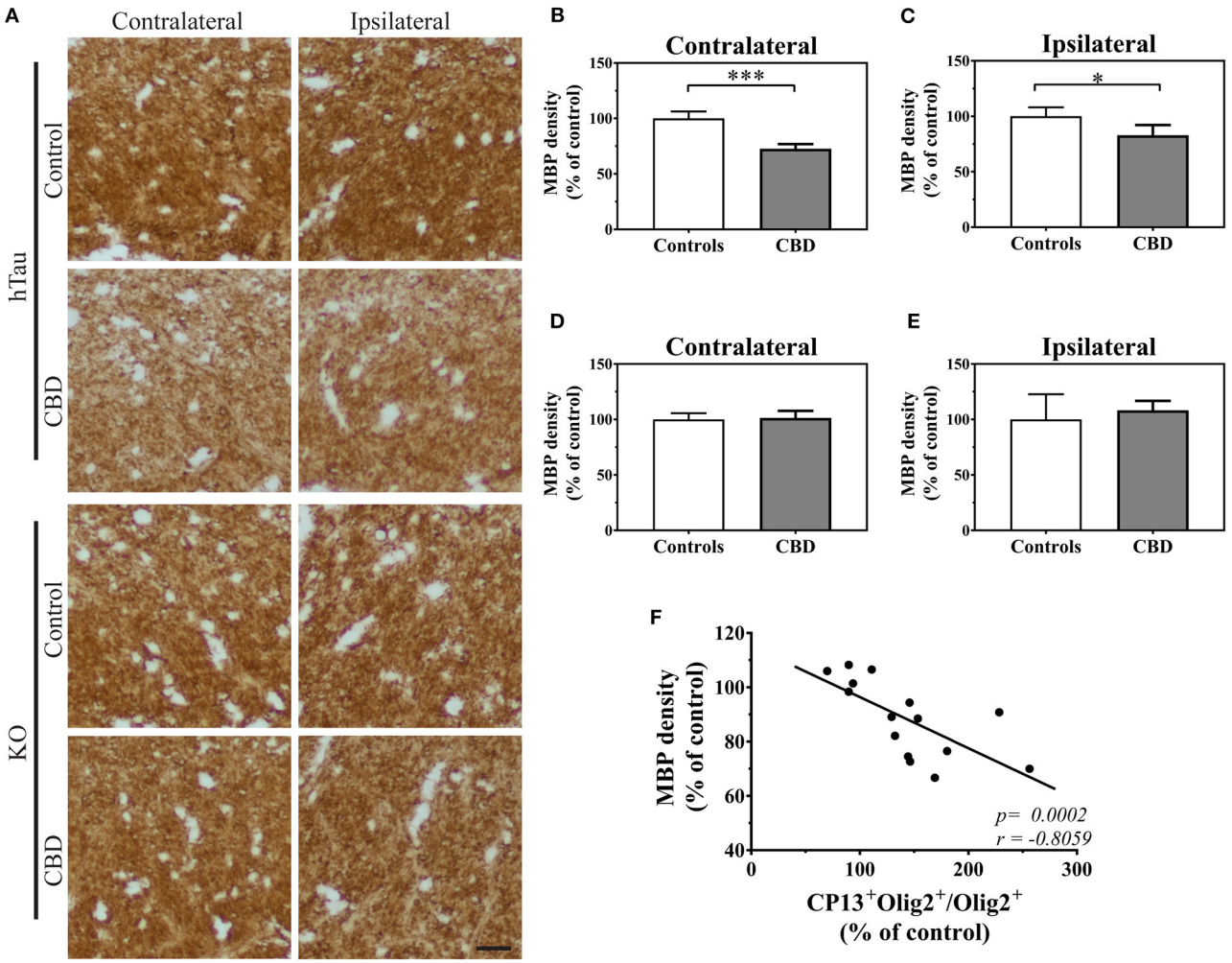


FIGURE 2 | The functional changes in oligodendrocytes triggered by CBD-tau inoculation. **(A)**, the corpus callosum microphotography pictures of myelin basic protein (MBP) immunoreactivity in the contralateral (left panel) and ipsilateral (right panel) sides in hTau (upper panel) or KO (lower panel) animals 12 months after control-tau (upper subpanel) or CBD-tau (lower sub panel) injection. The scale bar represents 25 μm and applies to all pictures. **(B–E)**, The quantitative analysis of densitometric data of MBP immunoreactivity in the corpus callosum of hTau animals contralateral (**B,** controls $n = 4$, CBD $n = 4$) and ipsilateral (**C,** controls $n = 4$, CBD $n = 4$) hemispheres; and KO animals contralateral (**D,** controls $n = 3$, CBD $n = 4$) and ipsilateral (**E,** controls $n = 3$, CBD $n = 4$) hemispheres. The significant decrease in MBP densitometry was observed bilaterally only in hTau animals, reflecting presence of tau-dependent changes. **(F)**, correlation of MBP density and double-positive oligodendrocytes in the corpus callosum. The presence of significant positive correlation indicates functional changes in oligodendrocytes upon tau pathology presence. Comparison between two groups was performed using the Student *t* test. Spearman's rank correlation coefficient was used to analyze dependence between two sets of data. *P*-value < 0.05 was considered statistically significant. $^{*}P < 0.05$, $^{***}P < 0.0001$.

expression of 3R over 4R human tau isoforms is observed. Furthermore, as in other mouse lines with null background expressing all six human tau isoforms (hT-PAC-N, Mapt$^{-/-}$), we observed indications of the region- and cell-specific tau isoform expression in hTau animals. We found that 4R tau isoforms are only expressed in oligodendrocytes in the striatum and corpus callosum. Furthermore, none or weak expression of 4R tau isoforms were observed in oligodendrocytes in the cortex or hippocampus. No astrocytes in any of the analyzed structures were 4R immunoreactive, and only a few 4R tau positive neurons were detected in cortex, some in the hippocampus, and none were identified in the striatum. This supports our observation that CBD, as a predominantly 4R tau disorder, triggered tau pathology mainly in oligodendrocytes, the only striatal cells in hTau animals expressing 4R tau. In addition, we show that disease transmission is triggered in anatomically connected regions only in the cells expressing 4R tau isoforms. This greatly correlates with a previously published *in vitro* study in which tau fibrils from different tauopathies recruited tau isoforms corresponding to the original human cases (4R recruits 4R, 3R+4R recruits 3R and 4R) (31). Likewise, a recent study using a newly generated 6hTau transgenic line (expressing an equal ratio of 3R and 4R human tau isoforms in null-background mice) states that distinct tauopathy

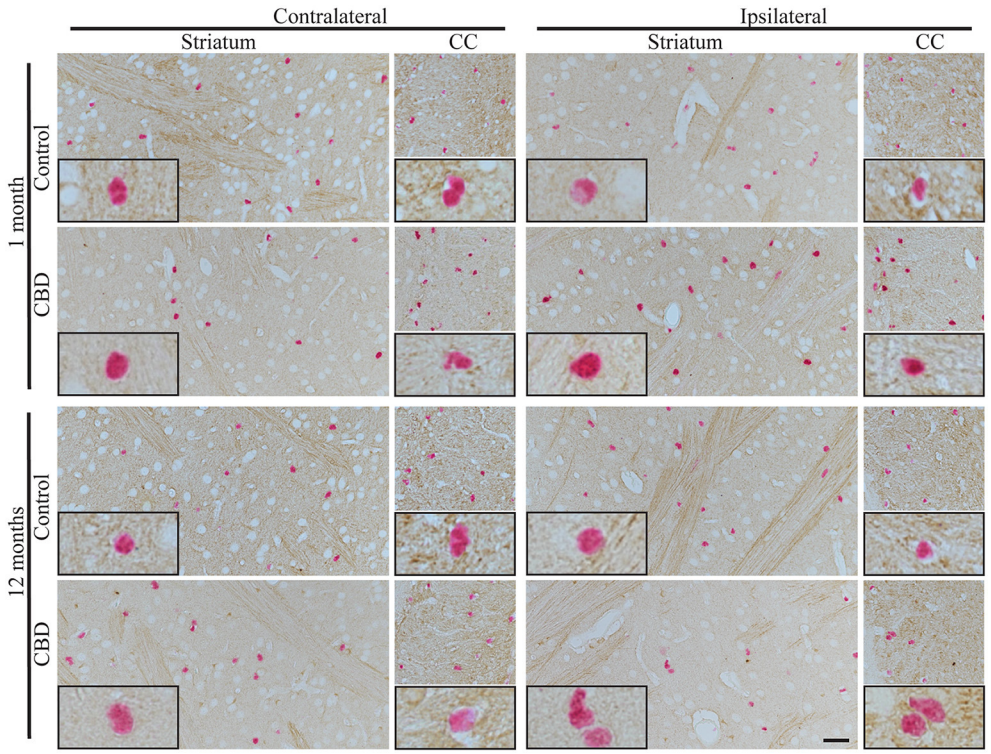


FIGURE 3 | The absence of tau pathology transmission in astrocytes. The microphotography pictures of double immunohistochemistry staining of tau phosphorylated at Ser202 (CP13 antibody, brown chromogen) in astrocytes (Sox9, nuclear marker, red/pink chromogen). The cell distribution in the contralateral (left panel) and ipsilateral side (right panel) of striatum (left subpanel) and corpus callous (right subpanel) after 1 month (upper panel) and 12 months (lower panel) of control-tau (upper subpanel) or CBD-tau (lower sub panel) inoculation. The representative pictures of cells in each group are presented as black-bordered inserts and demonstrate the absence of pathological tau in astrocytes in any of the experimental group at any time points, and similarly, the absence of pathological tau in neurons (morphological cell distinction) in any of the experimental group at any time points. The scale bar represents 50 μm and applies to all pictures; the black-bordered inserts represent 4× digital enlargement.

strains recruit the corresponding tau isoforms present in AD, PSP, CBD, and PiD into tau aggregates in 6hTau mice (34). Importantly, in line with our report, the cross-seeding of non-corresponding tau isoforms was inefficient in PSP and CBD in contrary to AD. Moreover, oligodendrocytes together with neuronal and astrocyte tau pathologies was observed upon CBD-tau inoculation.

Furthermore, a recent study on human tau pathology transition by glia in the absence of neuronal tau supports our observation (29). In the absence of neuronal tau pathology (no neuronal tau expression), the propagation of CBD-tau pathology across the mouse brain was observed only in oligodendroglia in the fimbria and corpus callosum, which agrees with our report. In addition, astrocytic tau aggregates did not spread in CBD-tau injected animals in the absence of neuronal tau, and neuronal tau aggregate release and local uptake by astrocytes was suggested as a basis of astrogliopathy. Furthermore, in this study reported by Lee, Trojanowki, and colleagues (29) the oligodendrocyte loss is reported, and "oligodendroglial connectome" is suggested as a tau propagation road. Our data also suggest oligodendrocyte connectome-based tau transmission. The oligodendrocyte connectome is based on the gap junctions (GJ) formed by connexin and pannexin transmebrane proteins (43–45). The GJ allows exchange of small molecules, ions, and metabolites between coupled cells. The connexin-43 (Cx42), as reported by the Lee and Trojanowki team, is highlighted together with aquaporin-4 as markers of aging-related tau astrogliopathy (46). Although this study is connecting tau pathology with connexin, highly expressed in astrocytes and generating homotypic channels (Cx42/Cx42) in astrocyte–astrocyte coupling, Cx42 is also highly involved in generating heterotypic channels in astrocyte–oligodendrocyte coupling with connexin-32 (Cx32). The Cx32 is highly expressed by oligodendrocytes and recently reported to be pivotal in α-synuclein oligomer uptake and transfer in neurons and oligodendrocytes (47). Furthermore, a number of small compounds and ions that alter tau aggregation have been reported (48, 49). For example, the zinc ion (Zn^{2+}) is one of the most potent tau aggregation stimulators. Importantly, in mature oligodendrocytes, the myelin sheath integrity was indicated to be greatly dependent on MBP and PLP complexes with Zn^{2+} (50–53). In addition, a Zn^{2+} involvement in pathologies of Alzheimer's, Parkinson's, and other neurodegenerative diseases is reported (51).

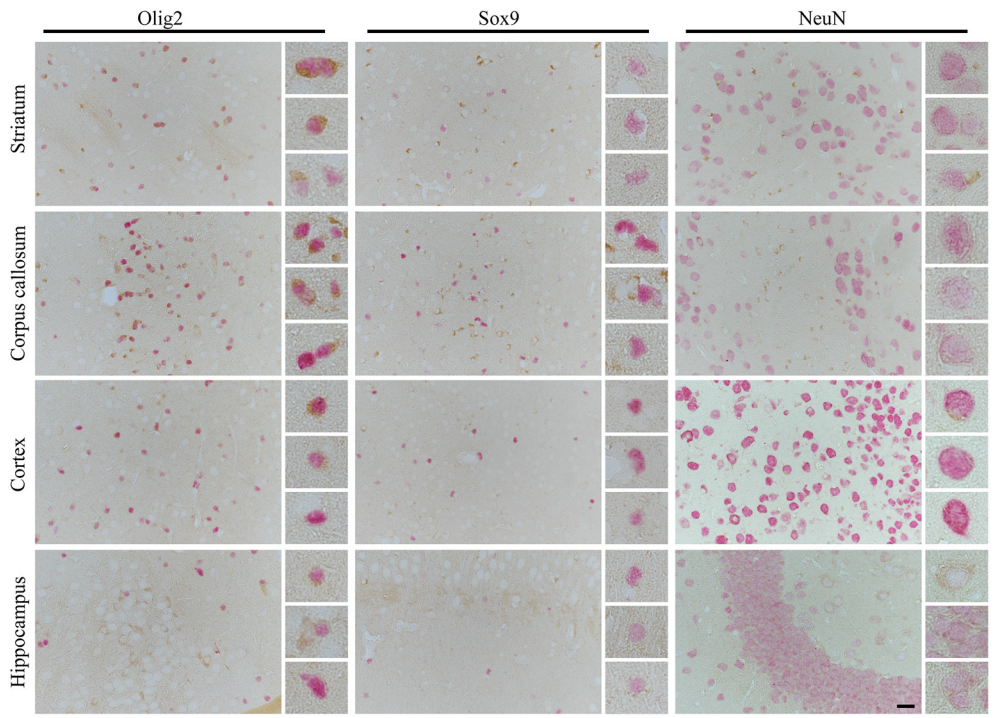


FIGURE 4 | The cell- and side-specific expression of four repeats of tau (4R) isoform in hTau animals brain. The high-power microphotography of double immunohistochemistry staining of 4R tau isoform (RD4, brown chromogen) combined with oligodendrocytes (Olig2, left panel, red/pink chromogen), astrocytic (Sox9, middle panel, red/pink chromogen) or neuronal (NeuN, right panel, red/pink chromogen) markers. The representative pictures of four brain regions: striatum (upper panel), corpus callosum (second upper panel), cortex (third upper panel), and hippocampus (lower panel) with higher magnification of representative cells on the right side of each image. In striatum and corpus callosum only oligodendrocytes were RD4 positive, and no RD4 immunoreactive astrocytes or neurons were detected. In cortex only, some oligodendrocytes and rear neurons were RD4 positive, and no RD4 immunoreactive astrocytes were identified. Similarly, in the hippocampus, no astrocytes were RD4 positive, and only some oligodendrocytes and neurons were weakly RD4 immunoreactive. The scale bar represents 25 μm and applies to all pictures; inserts represent 4× digital enlargement.

Although it did not include CBD, a previous study describing oligodendrocytic involvement in tau seeding and spreading from a variety of tauopathies by white matter injection is in line with our present data (33). The inoculation of sarkosyl-insoluble homogenates into the corpus callosum of WT animals triggered tau pathology in oligodendrocytes without any neuronal and astrocytic pathology. Furthermore, both tau seeding and spreading in oligodendrocytes were active processes with phospho-kinase involvement and, comparable to our data, extending from the ipsi- to contralateral hemisphere. Also, several reports using transgenic mice expressing tau mutations display inclusions in oligodendrocytes in addition to neuronal and astrocytes (54–58). Besides this, the pattern of glial tau pathology spreading in PS19 animals upon CBD-tau inoculation differed dramatically from intra-axonal spreading and the occurrence of the axonal afferent- and efferent-independent pattern has been proposed, which is consistent with our report (26).

Importantly, in the current study, we also report the functional consequences of increased oligodendrocytes with tau hyperphosphorylated at Ser202. We show the significant decrease in MBP density in the corpus callosum, both ipsi- and contralaterally, in 12 months CBD-tau inoculated hTau animals in comparison to controls. Furthermore, reduction in MBP density was not observed in tau KO mice injected either with CBD-tau or control-tau. This highlights the importance of an endogenous tau presence in CBD-tau pathology transmission and triggering of the subsequent functional changes. In addition, the significant negative correlation was detected between MBP density and number of $CP13^{+}$ oligodendrocytes in corpus callosum, further strengthening the myelin disruption as a functional consequence of increased Ser202 hyperphosphorylated tau in oligodendrocytes. This is in line with the aforementioned well-documented oligodendrocytic and white matter pathology in CBD patients (8, 9). It is also interesting to note that large-scale genetic studies have reported that gene variants in myelin-associated oligodendrocyte basic protein confers risk to develop CBD and PSP (59, 60).

Our research supports the emerging hypothesis that the accumulation of tau in oligodendrocytes might cause neurodegeneration by disrupting axonal transport (36). In this context, it is important to mention that oligodendrocytes are the most numerous glia cells in the brain (61). Moreover, their classical role, myelin-based electric insulation to axons, in optimizing axon potential conduction has been extended to providing a trophic support to long axons (62, 63), white matter

angiogenesis (64), and increasing tightness of the blood–brain barrier (65). To our knowledge, we are the first reporting that non-mutated human tau could contribute to MBP dysfunction upon CBD-tau inoculation. The previous study presents structural disruption and progressive loss of myelin in T34 P301S mutant mice, but not in T34 tau overexpressing oligodendrocytes in CNP promotor-driven (oligodendrocyte-specific) transgenic animals (36). In this study, impairment in axonal transport preceded axonal degeneration and correlates with early stages of tau aggregate accumulation in oligodendrocytes. In another report, the signs of slightly disrupted myelin are reported in corpus callosum–injected WT animals by a variety of tauopathies in diverse magnitudes by presenting proteolipid protein 1 immunoreactive balls and dots (although this study did not include any CBD cases) (33).

In conclusion, we demonstrate new insights into cell and regional selectivity of tau spreading in sporadic CBD tauopathy. We show that CBD-tau brain homogenate inocculation in the striatum of hTau animals caused hyperphosphorylation of tau at Ser202, predominantly in oligodendrocytes. Next, the pathology was transmitted from striatum to overlaying corpus callosum in the ipsilateral side and 12 months postinjection also in oligodendrocytes in the contralateral corpus callosum and striatum. Moreover, we report that the almost exclusive oligodendrocyte-based transmission of hyperphosphorylated tau is reflected by the endogenous 4R tau isoforms immunoreactivity. Furthermore, we demonstrate that non-mutated human tau could contribute to myelin dysfunction, reflected by decreased MBP immunoreactivity, upon CBD-tau inoculation as the functional consequence of increased oligodendrocytes with tau hyperphosphorylated at Ser202.

The question of why tauopathies vary greatly not only in histopathological but also neuroanatomical patterns could be partially answered by region- and cell-specific tau isoform presence in the human brain; however, additional larger studies with more CBD cases are required to elucidate this issue.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Stockholm North Ethical Committee.

AUTHOR CONTRIBUTIONS

KP and PS designed research. JZ-P, KP, and LK performed research. JZ-P, TR, and PS analyzed data and JZ-P and PS wrote the paper. All authors contributed to the article and approved the submitted version.

FUNDING

We thank Karin and Sten Mörtstedts CBD solution for funding the project.

ACKNOWLEDGMENTS

We would like to thank Dr. Therese Fagerqvist and Dr. Marcela Pereira for the scientific and technical support in the presented work. The Queens Square Brain Bank, UCL, to provide postmortem human brain tissue.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.589471/full#supplementary-material

REFERENCES

1. Togasaki DM, Tanner CM. Epidemiologic aspects. *Adv Neurol.* (2000) 82:53–9.
2. Schrag A, Ben-Shlomo Y, Quinn NP. Prevalence of progressive supranuclear palsy and multiple system atrophy: a cross-sectional study. *Lancet.* (1999) 354:1771–5. doi: 10.1016/S0140-6736(99)04137-9
3. Winter Y, Bezdolnyy Y, Katunina E, Avakjan G, Reese JP, Klotsche J, et al. Incidence of Parkinson's disease and atypical parkinsonism: Russian population-based study. *Mov Disord.* (2010) 25:349–56. doi: 10.1002/mds.22966
4. Dickson DW, Neuropathologic differentiation of progressive supranuclear palsy and corticobasal degeneration. *J Neurol.* (1999) 246:II6–15. doi: 10.1007/BF03161076
5. Ikeda K, Akiyama H, Haga C, Kondo H, Arima K, Oda T. Argyrophilic thread-like structure in corticobasal degeneration and supranuclear palsy. *Neurosci Lett.* (1994) 174:157–9. doi: 10.1016/0304-3940(94)90010-8
6. Arima K, Nakamura M, Sunohara N, Ogawa M, Anno M, Izumiyama Y, et al. Ultrastructural characterization of the tau-immunoreactive tubules in the oligodendroglial perikarya and their inner loop processes in progressive supranuclear palsy. *Acta Neuropathol (Berl).* (1997) 93:558–66. doi: 10.1007/s004010050652
7. Ling H, Kovacs GG, Vonsattel JP, Davey K, Mok KY, Hardy J, et al. Astrogliopathy predominates the earliest stage of corticobasal degeneration pathology. *Brain.* (2016) 139:3237–52. doi: 10.1093/brain/aww256
8. Upadhyay N, Suppa A, Piattella MC, Bologna M, Di Stasio F, Formica A, et al. MRI gray and white matter measures in progressive supranuclear palsy and corticobasal syndrome. *J Neurol.* (2016) 263:2022–31. doi: 10.1007/s00415-016-8224-y
9. Boxer AL, Geschwind MD, Belfor N, Gorno-Tempini ML, Schauer GF, Miller BL, et al. Patterns of brain atrophy that differentiate corticobasal degeneration syndrome from progressive supranuclear palsy. *Arch Neurol.* (2006) 63:81–6. doi: 10.1001/archneur.63.1.81
10. Spillantini MG, Yoshida H, Rizzini C, Lantos PL, Khan N, Rossor MN, et al. A novel tau mutation (N296N) in familial dementia with swollen achromatic neurons and corticobasal inclusion bodies. *Ann Neurol.* (2000) 48:939–43. doi: 10.1002/1531-8249(200012)48:6<939::AID-ANA17>3.0.CO;2-1
11. Kahlson MA, Colodner KJ. Glial Tau Pathology in Tauopathies: Functional Consequences. *J Exp Neurosci.* (2015) 9:43–50. doi: 10.4137/JEN.S25515

12. 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) 9:4225–30. doi: 10.1002/j.1460-2075.1990.tb07870.x
13. Dickson DW, Kouri N, Murray ME, Josephs KA. Neuropathology of frontotemporal lobar degeneration-tau (FTLD-tau). *J Mol Neurosci.* (2011) 45:384–9. doi: 10.1007/s12031-011-9589-0
14. Alonso AD, Grundke-Iqbal I, Barra HS, Iqbal K. Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. *Proc Natl Acad Sci USA.* (1997) 94:298–303. doi: 10.1073/pnas.94.1.298
15. Cleveland DW, Hwo SY, Kirschner MW. Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly. *J Mol Biol.* (1977) 116:227–47. doi: 10.1016/0022-2836(77)90214-5
16. Brunello CA, Merezhko M, Uronen RL, Huttunen HJ. Mechanisms of secretion and spreading of pathological tau protein. *Cell Mol Life Sci.* (*2*019) 77:1721–44. doi: 10.1007/s00018-019-03349-1
17. Wegmann S, Eftekharzadeh B, Tepper K, Zoltowska KM, Bennett RE, Dujardin S, et al. Tau protein liquid-liquid phase separation can initiate tau aggregation. *EMBO J.* (2018) 37:e98049. doi: 10.15252/embj.201798049
18. Qiang L, Sun X, Austin TO, Muralidharan H, Jean DC, Liu M, et al. Tau does not stabilize axonal microtubules but rather enables them to have long labile domains. *Curr Biol.* (2018) 28:2181–9.e4. doi: 10.1016/j.cub.2018.05.045
19. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol.* (2009) 11:909–13. doi: 10.1038/ncb1901
20. Paslawski W, Zareba-Paslawska J, Zhang X, Hölzl K, Wadensten H, Shariatgorji M, et al. α-synuclein–lipoprotein interactions and elevated ApoE level in cerebrospinal fluid from Parkinson's disease patients. *Proc Natl Acad Sci.* (2019) 116: 15226–35. doi: 10.1073/pnas.1821409116
21. Abramoff MD, Magelhaes PJ, Ram SJ. Image processing with ImageJ. *Biophotonics Int.* (2004) 11:36–42.
22. Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, et al. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem.* (2003) 86:582–90. doi: 10.1046/j.1471-4159.2003.01879.x
23. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, et al. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet.* (2000) 25:402–5. doi: 10.1038/78078
24. Xia Y, Prokop S, K.-Gorion MM, Kim JD, Sorrentino ZA, Bell BM, et al. Tau Ser208 phosphorylation promotes aggregation and reveals neuropathologic diversity in Alzheimer's disease and other tauopathies. *Acta Neuropathol Commun.* (2020) 8:88. doi: 10.1186/s40478-020-00967-w
25. Ahmed Z, Cooper J, Murray TK, Garn K, McNaughton E, Clarke H, et al. A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. *Acta Neuropathol.* (2014) 127:667–83. doi: 10.1007/s00401-014-1254-6
26. Boluda S, Iba M, Zhang B, Raible KM, Lee VM, Trojanowski JQ. Differential induction and spread of tau pathology in young PS19 tau transgenic mice following intracerebral injections of pathological tau from Alzheimer's disease or corticobasal degeneration brains. *Acta Neuropathol.* (2015) 129:221–37. doi: 10.1007/s00401-014-1373-0
27. Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. *Proc Natl Acad Sci USA.* (2013) 110:9535–40. doi: 10.1073/pnas.1301175110
28. Hu W, Zhang X, Tung YC, Xie S, Liu F, Iqbal K. Hyperphosphorylation determines both the spread and the morphology of tau pathology. *Alzheimers Demen.* (2016) 12:1066–1077. doi: 10.1016/j.jalz.2016.01.014
29. Narasimhan S, Changolkar L, Riddle DM, Kats A, Stieber A, Weitzman SA, et al. Human tau pathology transmits glial tau aggregates in the absence of neuronal tau. *J Exp Med.* (2020) 217:e20190783. doi: 10.1084/jem.20190783
30. Guo JL, Narasimhan S, Changolkar L, He Z, Stieber A, Zhang B, et al. Unique pathological tau conformers from Alzheimer's brains transmit tau pathology in nontransgenic mice. *J Exp Med.* (2016) 213:2635–54. doi: 10.1084/jem.20160833
31. Narasimhan S, Guo JL, Changolkar L, Stieber A, McBride JD, Silva LV, et al. Pathological tau strains from human brains recapitulate the diversity of tauopathies in nontransgenic mouse brain. *J Neurosci Nurs.* (2017) 37:11406–23. doi: 10.1523/JNEUROSCI.1230-17.2017
32. Audouard E, Houben S, Masaracchia C, Yilmaz Z, Suain V, Authelet M, et al. High-molecular-weight paired helical filaments from alzheimer brain induces seeding of wild-type mouse tau into an argyrophilic 4R tau pathology in vivo. *Am J Pathol.* (2016) 186:2709–22. doi: 10.1016/j.ajpath.2016.06.008
33. Ferrer I, Aguilo Garcia M, Carmona M, Andres-Benito P, Torrejon-Escribano B, Garcia-Esparcia, et al. involvement of oligodendrocytes in tau seeding and spreading in tauopathies. *Front Aging Neurosci.* (2019) 11:112. doi: 10.3389/fnagi.2019.00112
34. He Z, McBride JD, Xu H, Changolkar L, Kim S-j, Zhang B, et al. Transmission of tauopathy strains is independent of their isoform composition. *Nature Commun.* (2020) 11:7. doi: 10.1038/s41467-019-13787-x
35. Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* (2007) 53:337–51. doi: 10.1016/j.neuron.2007.01.010
36. Higuchi M, Zhang B, Forman MS, Yoshiyama Y, Trojanowski JQ, Lee VM. Axonal degeneration induced by targeted expression of mutant human tau in oligodendrocytes of transgenic mice that model glial tauopathies. *J Neurosci.* (2005) 25:9434–43. doi: 10.1523/JNEUROSCI.2691-05.2005
37. Tanemura K, Murayama M, Akagi T, Hashikawa T, Tominaga T, Ichikawa M, et al. Neurodegeneration with tau accumulation in a transgenic mouse expressing V337M human tau. *J Neurosci.* (2002) 22:133–41. doi: 10.1523/JNEUROSCI.22-01-00133.2002
38. Zhang B, Higuchi M, Yoshiyama Y, Ishihara T, Forman MS, Martinez D, et al. Retarded axonal transport of R406W mutant tau in transgenic mice with a neurodegenerative tauopathy. *J Neurosci.* (2004) 24:4657–67. doi: 10.1523/JNEUROSCI.0797-04.2004
39. Probst A, Götz J, Wiederhold KH, Tolnay M, Mistl C, Jaton AL, et al. Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. *Acta Neuropathol.* (2000) 99:469–81. doi: 10.1007/s004010051148
40. 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) 3:519–26. doi: 10.1016/0896-6273(89)90210-9
41. McMillan P, Korvatska E, Poorkaj P, Evstafjeva Z, Robinson L, Greenup L, et al. Tau isoform regulation is region- and cell-specific in mouse brain. *J Comp Neurol.* (2008) 511:788–803. doi: 10.1002/cne.21867
42. Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO J.* (1989) 8:393–9. doi: 10.1002/j.1460-2075.1989.tb03390.x
43. Nualart-Marti A, Solsona C, Fields RD. Gap junction communication in myelinating glia. *Biochim Biophys Acta.* (2013) 1828:69–78. doi: 10.1016/j.bbamem.2012.01.024
44. Vejar S, Oyarzún JE, Retamal MA, Ortiz FC, Orellana JA. Connexin and pannexin-based channels in oligodendrocytes: implications in brain health and disease. *Front Cell Neurosci.* (2019) 13:3. doi: 10.3389/fncel.2019.00003
45. Simons M, Trajkovic K. Neuron-glia communication in the control of oligodendrocyte function and myelin biogenesis. *J Cell Sci.* (2006) 119:4381. doi: 10.1242/jcs.03242
46. Kovacs GG, Yousef A, Kaindl S, Lee VM, Trojanowski JQ. Connexin-43 and aquaporin-4 are markers of ageing-related tau astrogliopathy (ARTAG)-related astroglial response. *Neuropathol Appl Neurobiol.* (2018) 44:491–505. doi: 10.1111/nan.12427
47. Reyes JF, Sackmann C, Hoffmann A, Svenningsson P, Winkler J, Ingelsson M, et al. Binding of α-synuclein oligomers to Cx32 facilitates protein uptake and transfer in neurons and oligodendrocytes. *Acta Neuropathol.* (2019) 138:23–47. doi: 10.1007/s00401-019-02007-x
48. Kim AC, Lim S, Kim YK. Metal ion effects on Aβ and tau aggregation. *Int J Mol Sci.* (2018) 19:128. doi: 10.3390/ijms19010128
49. Lim S, Haque MM, Kim D, Kim DJ, Kim YK. Cell-based models to investigate tau aggregation. *Comput Struct Biotechnol J.* (2014) 12:7–13. doi: 10.1016/j.csbj.2014.09.011

50. Earl C, Chantry A, Mohammad N, Glynn P. Zinc ions stabilise the association of basic protein with brain myelin membranes. *J Neurochem.* (1988) 51:718–24. doi: 10.1111/j.1471-4159.1988.tb01803.x
51. Mocchegiani E, Bertoni-Freddari C, Marcellini F, Malavolta M. Brain, aging and neurodegeneration: role of zinc ion availability. *Prog Neurobiol.* (2005) 75:367–90. doi: 10.1016/j.pneurobio.2005.04.005
52. Baran C, Smith GS, Bamm VV, Harauz G, Lee JS. Divalent cations induce a compaction of intrinsically disordered myelin basic protein. *Biochem Biophys Res Commun.* (2010) 391:224–9. doi: 10.1016/j.bbrc.2009.11.036
53. Lehotzky A, Oláh J, Szunyogh S, Szabó A, Berki T, Ovádi J. Zinc-induced structural changes of the disordered tppp/p25 inhibits its degradation by the proteasome. *Biochim Biophys Acta.* (2015) 1852:83–91. doi: 10.1016/j.bbadis.2014.10.015
54. Gotz J, Tolnay M, Barmettler R, Chen F, Probst A, Nitsch RM. Oligodendroglial tau filament formation in transgenic mice expressing G272V tau. *Eur J Neurosci.* (2001) 13:2131–40. doi: 10.1046/j.0953-816x.2001.01604.x
55. Lin W-L, Lewis J, Yen S-H, Hutton M, Dickson DW. Filamentous tau in oligodendrocytes and astrocytes of transgenic mice expressing the human tau isoform with the P301L mutation. *Am J Pathol.* (2003) 162:213–8. doi: 10.1016/S0002-9440(10)63812-6
56. Lin WL, Zehr C, Lewis J, Hutton M, Yen SH, Dickson DW. Progressive white matter pathology in the spinal cord of transgenic mice expressing mutant (P301L) human tau. *J Neurocytol.* (2005) 34:397–410. doi: 10.1007/s11068-006-8726-0
57. Ren Y, Lin WL, Sanchez L, Ceballos C, Polydoro M, Spires-Jones TL, et al. Endogenous tau aggregates in oligodendrocytes of rTg4510 mice induced by human P301L tau. *J Alzheimers Dis.* (2014) 38:589–600. doi: 10.3233/JAD-130986
58. Ferrer I. Oligodendrogliopathy in neurodegenerative diseases with abnormal protein aggregates: the forgotten partner. *Prog Neurobiol.* (2018) 169:24–54. doi: 10.1016/j.pneurobio.2018.07.004
59. Hoglinger GU, Melhem NM, Dickson DW, Sleiman PM, Wang LS, Klei L, et al. Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. *Nat Genet.* (2011) 43:699–705. doi: 10.1038/ng.859
60. Kouri N, Ross OA, Dombroski B, Younkin CS, Serie DJ, Soto-Ortolaza A, et al. Genome-wide association study of corticobasal degeneration identifies risk variants shared with progressive supranuclear palsy. *Nature Commun.* (2015) 6:7247. doi: 10.1038/ncomms8247
61. Valerio-Gomes B, Guimaraes DM, Szczupak D, Lent R. The absolute number of oligodendrocytes in the adult mouse brain. *Front Neuroanat.* (2018) 12:90. doi: 10.3389/fnana.2018.00090
62. Wilkins A, Chandran S, Compston A. A role for oligodendrocyte-derived IGF-1 in trophic support of cortical neurons. *Glia.* (2001) 36:48–57. doi: 10.1002/glia.1094
63. Nave KA. Myelination and the trophic support of long axons. *Nat Rev Neurosci.* (2010) 11: 275–83. doi: 10.1038/nrn2797
64. Yuen TJ, Silbereis JC, Griveau A, Chang SM, Daneman R, Fancy SPJ, et al. Oligodendrocyte-encoded HIF function couples postnatal myelination and white matter angiogenesis. *Cell.* (2014) 158:383–96. doi: 10.1016/j.cell.2014.04.052
65. Seo JH, Maki T, Maeda M, Miyamoto N, Liang AC, Hayakawa K, et al. Oligodendrocyte precursor cells support blood-brain barrier integrity via TGF-beta signaling. *PloS One.* (2014) 9:e103174. doi: 10.1371/journal.pone.0103174

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

MINI REVIEW
published: 10 February 2021
doi: 10.3389/fneur.2021.610330

Tau Pathology and Adult Hippocampal Neurogenesis: What Tau Mouse Models Tell us?

*Sarah Houben, Mégane Homa, Zehra Yilmaz, Karelle Leroy, Jean-Pierre Brion and Kunie Ando**

Laboratory of Histology, Neuroanatomy and Neuropathology, UNI (ULB Neuroscience Institute), Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium

Adult hippocampal neurogenesis (AHN) has been widely confirmed in mammalian brains. A growing body of evidence points to the fact that AHN sustains hippocampal-dependent functions such as learning and memory. Impaired AHN has been reported in *post-mortem* human brain hippocampus of Alzheimer's disease (AD) and is considered to contribute to defects in learning and memory. Neurofibrillary tangles (NFTs) and amyloid plaques are the two key neuropathological hallmarks of AD. NFTs are composed of abnormal tau proteins accumulating in many brain areas during the progression of the disease, including in the hippocampus. The physiological role of tau and impact of tau pathology on AHN is still poorly understood. Modifications in AHN have also been reported in some tau transgenic and tau-deleted mouse models. We present here a brief review of advances in the relationship between development of tau pathology and AHN in AD and what insights have been gained from studies in tau mouse models.

Keywords: neurogenesis, tauopathy, Alzheimer's disease, dentate gyrus, tau

OPEN ACCESS

Edited by:
Sonia Do Carmo, McGill University, Canada

Reviewed by:
Chaur-Jong Hu, Taipei Medical University, Taiwan
Li Zeng, National Neuroscience Institute (NNI), Singapore

****Correspondence:***
Kunie Ando
Kunie.Ando@ulb.ac.be

Specialty section:
This article was submitted to Dementia and Neurodegenerative Diseases, a section of the journal Frontiers in Neurology

Received: *25 September 2020*
Accepted: *06 January 2021*
Published: *10 February 2021*

Citation:
Houben S, Homa M, Yilmaz Z, Leroy K, Brion J-P and Ando K (2021) Tau Pathology and Adult Hippocampal Neurogenesis: What Tau Mouse Models Tell us? Front. Neurol. 12:610330. doi: 10.3389/fneur.2021.610330

INTRODUCTION

AD has two neuropathological hallmarks, amyloid plaques, and NFTs. Amyloid plaques are composed of amyloid ß peptides (1) derived from successive cleavages of amyloid precursor protein (APP) (2). NFTs are constituted of microtubule-associated protein tau (MAPT) (3). In a family of neurodegenerative diseases called tauopathies including AD, tau undergoes hyperphosphorylation and aggregation to develop pathological forms of tau species such as oligomers or highly insoluble filaments that form NFTs. The levels of NFTs are highly correlated with cognitive decline (4). Tauopathies include frontotemporal lobar degeneration (FTLD) with tau positive inclusions with or without gene mutation in *MAPT*, Pick disease, progressive supranuclear palsy, corticobasal degeneration, and others (5). In AD brains, tau deposition occurs in a stereotypical manner, with the hippocampus, limbic structures, brain stem, and the basal nucleus of Meynert being most affected at the early stages (6). The hippocampus is a crucial brain structure for the acquisition of new memories and retrieval of older memories. Afferent pathways to the dentate gyrus (DG) are affected by NFTs developing in the entorhinal cortex (6), and NFTs develop in the granule cell layer (GCL) (7, 8) in the DG in AD and in some tau transgenic mouse models (**Figures 1A–D**). Tau pathology in the DG might play a role in memory impairment. Whereas, abnormalities in AHN have been extensively investigated in AD mouse models based on *APP* or *PSEN1/2* familial AD mutations (13, 14), the impact of tau pathology on AHN remains largely unclear in AD and other tauopathies. We provide here a brief overview of recent advances on

the relationship between development of tau pathology and AHN in AD and what insights have been gained from studies in tau transgenic mouse models.

NORMAL AHN

Since its discovery in mammalian brain in 1965 (15), AHN has been documented in many species (16–20). In placental mammals and marsupials, adult neurogenesis is mainly limited to two areas: the subventricular zone (SVZ) along the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG). AHN is necessary for spatial memory and specific learning tasks and is related to mood regulation (21, 22).

Neural stem cells found in the SGZ of the hippocampus generate new neurons for the DG (23). The identity of adult neural stem cells remains still controversial. Growing evidence suggests that they have an astrocytic phenotype (24, 25) or they may be radial glial cells, able to give rise asymmetrically either to a glial cell or a neuron (26). There are five principal developmental stages of AHN starting from the radial glia-like cells, progenitor cells, neuroblast cells, immature neurons, and finally mature neurons as granular cells (27). These stages can be identified by specific markers such as GFAP, BLBP, SOX2, Nestin, Doublecortin (DCX), tau with three-repeats (3R) or four-repeats (4R) of microtubule-binding repeat domains (RD), NeuN, and Calbindin (**Figure 1E**) (23, 28). Newborn cells can be experimentally traced using exogenous cell tracers such as thymidine analogs that are incorporated into dividing cells during DNA synthesis (29). Newborn neurons can be also identified by other mitotic markers such as Ki67 in combination with neuronal markers (30). Studies have provided compelling evidence for the persistence of AHN in humans and non-human primates (31, 32). There are some contradictory findings pointing to hardly detectable levels of AHN in human brains due to a sharp decrease in childhood (33, 34). A breakthrough was made when a study provided evidence for the birth of ∼700 newborn neurons a day per one adult human hippocampus by measuring the concentration of nuclear bomb test-derived ^{14}C in genome DNA (35). By a similar approach, striatum has also been recently identified as a neurogenic zone in the adult human brain (36). Annual turnover rates are estimated as 1.75% of neurons in the hippocampus and 2.7% in the striatum in the human adult brain (35, 36). Although observed in other species than human (37, 38), the role of adult striatal neurogenesis remains largely elusive (39).

Stress, aging, and disease have a negative impact on AHN (40). On the contrary, AHN can be enhanced in rodents by lifestyle factors such as environmental enrichment (EE) (41), physical activity (e.g., running) (42, 43), anti-depressants (44), or electroconvulsive seizures (45).

TAU PROTEINS. "CANONICAL" AND "NON-CANONICAL" FUNCTIONS

Tau is a cytosolic protein predominantly expressed in neurons. Tau has physiological roles, the most studied being the regulation of the axonal transport and of the cytoskeleton by maintaining the stability of microtubules (46). Human *MAPT* gene is located on chromosome 17 and contains 16 exons. Exons 2, 3, and 10 are alternatively spliced to give rise to six different isoforms in the adult human central nervous system (**Figure 1F**) (47). Alternative splicing of exon 10 results in generating either tau with 3R or 4R microtubule-binding sequences in the half carboxyl domain. 3R and 4R tau isoforms include sequences of exon 2, exons 2 and 3, or none of them in their amino domain. Tau regulates axonal microtubule assembly but has also other functions (48) by interacting with many partners in addition to microtubules (49, 50). Among other functions, tau is implicated in pathways regulating synaptic plasticity, cell signaling, and DNA integrity (51). Tau is also secreted via several pathways (52), a process that is thought to play a role in the "Prion-like" propagation of tau pathology (53) but that is not well-understood in physiological conditions. This multifunctional aspect of tau might be involved in the regulation of AHN.

DEVELOPMENTAL EVOLUTION OF TAU PROTEIN EXPRESSION AND ROLE OF TAU IN AHN

While six isoforms are expressed in adult human brain, only 4R isoforms are predominantly detected in the mature neurons of mouse brains. During brain development, only the 0N3R isoform (fetal isoform) is expressed in human and rodent brains (54, 55). Owing to the lack of one microtubule-binding domain, 3R tau isoforms have less affinity for microtubules and consequently less efficiency to promote microtubule assembly compared to 4R isoforms (56). Expression of 3R tau isoforms is thus related to plasticity in neuronal development in neonatal stage and in neurogenesis for dynamic process formation, neurite elongation, and neuronal polarity (57–59). 3R tau isoform lacking exon 2 and 3 is also expressed in the adult brain in the immature neurons in the SGZ (60) and can be used as a specific marker to detect newborn neurons and newly generated axons in the adult mouse hippocampus (28, 61). The number of cells expressing 3R tau isoforms in the SGZ decreases with age in mice, but they are still detectable at 12 months (**Figure 1G**) (12). Tau in immature neurons in the SGZ shows a higher phosphorylation seemingly through activated GSK-3 (62), reducing its affinity for microtubules and providing these cells with a more dynamic microtubule network during dendritic and axonal outgrowth. In these immature neurons, tau is abundant in the somatodendritic domain (as during development) and appears to be at least partly in a microtubule-unbound form (63). Increased tau phosphorylation is associated with increased proliferation of newborn neurons (62).

AHN IN AD

Emerging evidence suggests that overall AHN (e.g., generation of fully functional new neurons) is reduced in AD (64). The detection of AHN markers by immunohistochemistry on *post-mortem* brain tissues has recently confirmed the existence of AHN in aged healthy subjects and a significant reduction of

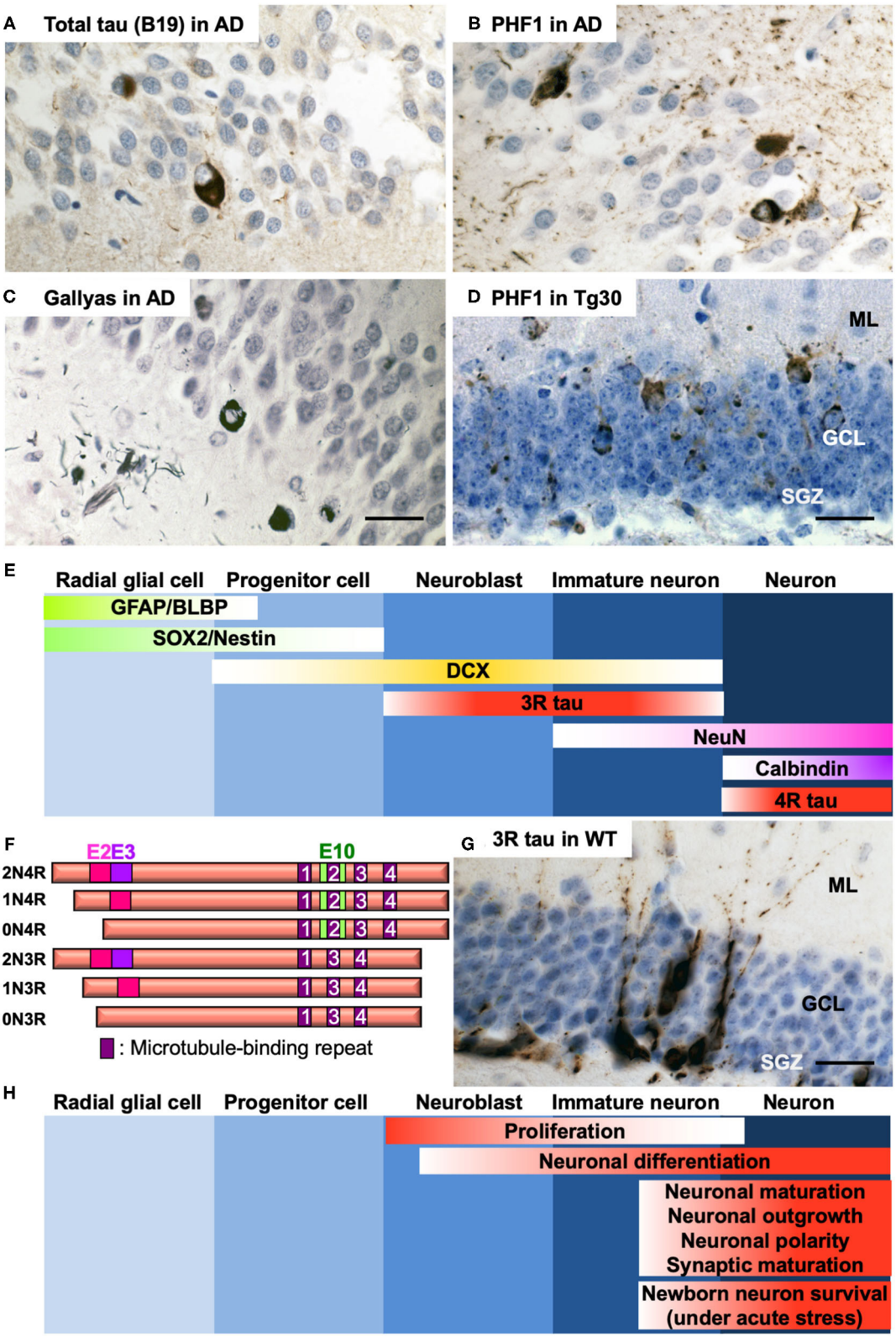


FIGURE 1 | (A–C) Representative photos of tau pathology detected in the DG of *post-mortem* brain section of a 65-year-old male AD patient (Braak VI). Tau pathology was detected by anti-total tau B19 antibody (9) **(A)**, anti-phospho Ser396/404 tau PHF1 antibody (10) **(B)**, or by Gallyas silver staining (11) **(C)**.

(Continued)

FIGURE 1 | (D) Representative photo of tau pathology detected by PHF1 in the DG of 12-month-old tau Tg30 mice (12). ML, molecular layer; GCL, granule cell layer; SGZ, subgranular zone. **(E)** Specific markers for five different stages of AHN in the dentate gyrus of the hippocampus. GFAP, glial fibrillary acidic protein; BLBP, brain lipid-binding protein; SOX2, SRY (sex determining region Y)-box 2; DCX, doublecortin; 3R tau, tau with 3 repeats of microtubule-binding sequences; NeuN, neuronal nuclei; 4R tau, tau with 4 repeats of microtubule-binding sequences. **(F)** Schematic representation of the human 6 isoforms of tau protein. Exon 2, 3, and 10 (E2, E3, and E10, respectively) are alternatively spliced. Alternative splicing leads to 0, 1 or 2 inserts near amino terminus (0N, 1N, or 2N, respectively) and 3 or 4 repeats (3R or 4R, respectively) of microtubule-binding sequences near carboxyl terminus. The shortest 0N3R isoform is predominantly detected in immature neurons of fetal brains and of adult hippocampus. While only 4R isoforms are principally expressed in adult mouse brains, all the 6 isoforms are expressed in adult human brains. **(G)** Immunostaining of immature neurons by anti-3R tau RD3 antibody (Merck Millipore #05-803) in the dentate gyrus of the hippocampus in a 12-month-old wild-type mouse. **(H)** Functional involvement of tau at different stages of AHN. Hematoxylin counterstaining for **(A–D,G)**. Detailed protocol on histological analyses is available in (12). Scale bars: 25 μm.

DCX-positive immature neurons in AD brains (65, 66). AHN drops sharply even at the early stage of cognitive decline in the patients with mild cognitive impairment (66). These studies imply that the reduction of AHN may directly compromise cognitive functions (67). Importantly, SOX2-positive neural stem cells were increased in some cognitively normal subjects but with extensive AD neuropathological lesions (68), implying that increased AHN may rescue cognitive deficits caused by AD lesions. Numerous genetic factors and variants implicated in AD (*Apolipoprotein E*, *PSEN1*, *APP*) have been identified with a modulating role on AHN in human AD patients (69). This observation is supported by the generalized decrease in newborn neuron generation observed in various AD transgenic mouse models overexpressing FAD-related mutant *APP* and/or *PS1* (13) or overexpressing APP intracellular C-terminal domain fragments (AICD) (70).

AHN IN TAU MOUSE MODELS

Studies of AHN in different tau transgenic mouse models have suggested that tau has critical roles in proliferation, neuronal differentiation/maturation, dendritic/axonal outgrowth, neuronal plasticity and synaptic maturation in DG. Tau is also involved in selective cell death of newborn granule neurons in case of acute stress (71) (**Figure 1H**). However there remain controversies in distinct tau models (**Table 1**). Whereas tau knockout mice are viable and macroscopically normal (72, 73, 92), behavioral studies have unraveled that they exhibit abnormalities such as hyperactivity (93) and deficits in short-time memory in an age-dependent manner (94). Deletion of endogenous tau also leads to delayed neuronal maturation in primary cultured neurons (73) and transcriptional repression of neuronal genes in the hippocampus (95). A significant reduction of DCX- and NeuroD- positive neuroblast cells in tau knockout mice was observed (62). On the contrary, Criado-Marrero et al. have recently reported that BrdU-positive newborn cells and DCX-positive immature neurons were increased in the DG and SVZ of tau knockout mice at 14 months (75). Yet, other two independent studies have reported that DCX-labeled neuroblast cell number was not altered in the DG of adult tau knockout mice (71, 74). Moreover, tau has critical roles in both stress-induced suppression of AHN and stimulatory effect of EE. Unlike wild-type mice, tau knockout mice are insensitive to the modulation of AHN by stress or EE (71).

Human non-mutant tau seems to have several roles in AHN such as suppressing proliferation and promoting neuronal differentiation. KOKI mice expressing human 2N4R tau isoform in the absence of murine tau (76) had an increase in DCX-positive immature neurons, hippocampal volume and cell number in DG and an improved cognitive function (77). Nevertheless, other studies suggest negative effect of human non-mutant tau on AHN in mouse brains. hTau mice expressing the 6 isoforms of non-mutant wild-type human tau (78) in the absence of murine tau had reduced DCX-positive immature neurons at 2 and 6 months (79). Hippocampal injection of soluble non-mutant 2N4R human tau led to morphological changes of newborn granule neurons without changing the total number of DCX-positive neuroblast cells (80). Adeno-associated virus-mediated specific overexpression of human tau in DG interneurons induced deficits in AHN by suppressing GABAergic transmission (81). Another recent study has reported an impact of glial tau accumulation on AHN. Lentiviral-mediated 1N3R tau accumulation in hilar astrocytes in mouse led to reduction of AHN accompanied by impaired spatial memory performances (82).

Abnormalities in AHN have been observed in FTLD-mutant tau transgenic mouse models. In THY-Tau22 and Tg30 mice that express a human 1N4R tau mutated at G272V and P301S under a Thy1.2-promoter, Gallyas-positive NFTs are detectable from 6 months in hippocampus (83, 85). An increase in AHN was observed with the DCX and BrdU markers in 6-month-old THY-Tau22 mouse (84). Nonetheless, Tg30 mice exhibited an impaired AHN at 12 months, an age in which some of the granule cells in DG have a severe somatodendritic tau pathology (**Figure 1D**) (12). By crossing Tg30 with tau knockout mice (72), we generated Tg30/tauKO mice that express only human mutant tau in the absence of murine tau (86, 87). The reduction of AHN observed in Tg30 mice at 12 months was rescued in the Tg30/tauKO mouse model as measured by DCX-positive cell number (12). Another independent study reported that Tau^{VLW} mice carrying G272V, P301L, and R406W mutant tau (88) also had decreased DCX-positive immature neurons (89). Interestingly, EE significantly increased the number of DCX-positive immature neurons in wild-type littermates but not in Tau^{VLW} mice (89). To our knowledge, this is the first and only report showing that tau pathology may inhibit the response to a positive factor enhancing AHN.

The overall controversies may derive from the variation in the age and from the heterogeneities of tau species in distinct models. Tau^{RDDKPP} mice expressing anti-aggregant tau RD showed increased number of DCX-positive cells in DG and a larger volume of hippocampus unlike tau^{RDDK} mice expressing pro-aggregant mutant tau RD (91). The latter findings support

TABLE 1 | Summary of neurogenesis changes in tau mouse models.

Mouse line	Tau expression	Neurogenesis assessment	References
Tau knockout models			
$tau^{-/-}$ Tucker et al. (72)	–	Decrease in the number of DCX- and NeuroD- positive cells (age not indicated)	(62)
$tau^{-/-}$ Dawson et al. (73)	–	No change in the number of BLBP, Sox2- and DCX positive cells at basal conditions but reduction of dendritic and synaptic maturation of newborn granule neuron (4 months)	(71)
$tau^{-/-}$ Dawson et al. (73)	–	No change in the number of DCX/BrdU double positive cells (9 weeks)	(74)
$tau^{-/-}$ Dawson et al. (73)	–	Increase in the number of DCX- and BrdU- positive cells (14 months)	(75)
Human non-mutant tau models			
KOKI Terwel et al. (76)	2N4R human non-mutant tau in the absence of murine tau	Increase in the number of DCX- and BrdU-positive cells (2 months)	(77)
hTau Andorfer et al. (78)	6 isoforms of human non-mutant tau in the absence of murine tau	Decrease in the number of DCX-, Ki67-, and BrdU-positive cells (2, 6, 12 months)	(79)
Injection of human tau-Cy5 in WT mice	Endogenous murine tau and injected monomeric 2N4R human non-mutant tau	No change in the number of DCX-positive cells but change in the morphology of newborn granule cells	(80)
AAV-mediated expression of human tau	Human tau overexpressed in DG interneurons	Decrease in the number of BrdU-positive cells and DCX-positive cells	(81)
Lentiviral expression of human tau in hilar astrocytes	1N3R human non-mutant tau overexpressed in hilar astrocytes in the presence of murine tau	Decrease in the number of DCX-positive cells	(82)
Human FTLD-mutant tau models			
THY-Tau22 Schindowski et al. (83)	IN4R human double mutant G272V/P301S tau in the presence of murine tau	Increase in the number of DCX- and BrdU-positive cells (6 months)	(84)
Tg30 Leroy et al. (85)	IN4R human double mutant G272V/P301S tau in the presence of murine tau	Decrease in the number of DCX-, Ki67-, and tau 3R-positive cells (12 months)	(12)
Tg30/tauKO Ando et al. (86, 87)	IN4R human double mutant G272V/P301S tau in the absence of murine tau	Increase in the number of DCX-positive cells (12 months) compared to Tg30 and wild-type mice	(12)
Tau^{VLW} Lim et al. (88)	2N4R human triple mutant G272V/P301L/R406W tau in the presence of murine tau	Decrease in the number of DCX- and IdU- positive cells (2 months)	(89)
Tau repeat-domain models			
Tau^{RDDK} and tau^{RDDKPP} (90)	Tau^{RDDK} expressing pro-aggregant mutant tau repeat domain and tau^{RDDKPP} expressing anti-aggregant mutant tau repeat domain	Decrease in hippocampal volume at 16 months in tau^{RDDK}. Increase in hippocampal volume at 16 months, in hippocampal stem cell proliferation and in the number of DCX-positive cells in tau^{RDDKPP}	(91)

the idea that distinct tau species seem to have different effects on neurogenesis.

DISCUSSION

There are conflicting reports as to whether AHN persists in late age in humans. Controversies may be partially due to the limited availability of adequately preserved *post-mortem* human brain samples. The technical and methodological issues can further add variability in detecting specific markers of neural stem and progenitor cells in human autopsy tissues. Some of the conflicting results are also presumably related to the heterogeneities in individual life stories: age, sex, lifestyle, physical activities, with or without previous disease histories, and medical status at the end of life. There is a great variability in the *post-mortem* delays and processing methods of human *post-mortem* brain tissues. In general, fixation is known to play a critical role in antigen preservation since some epitopes are more prone to denaturation during the fixation. For example, the immature neuron marker DCX undergoes rapid degradation during the *post-mortem* period (96). Some difficulties could be overcome by tightly documenting the brain samples and their processing, optimizing the methodologies (65), and standardization of detailed protocols (97).

Although tau seems involved in modulating AHN, there are controversies among the different tau mouse models about the effect of tau ablation or overexpression. As for human samples, controversial reports may derive from distinct protocols and

various parameters such as genetic background, age, gender, and tau species. Distinct time point of analysis could lead to data variation (98). A remarkable sex difference was observed in AHN of rodent brains (75, 99). Furthermore, data variability may be caused by the sensitivities of antibodies used for detection (29). Besides, the methods of analysis and quantification have significant impact on the results. One of the most commonly used approaches is to measure total proliferating cell number using optical fractionator, an unbiased stereological method, on serial sections of the whole hippocampus (100). Since the distributions of the proliferating cells are not homogeneous and are often in the form of clusters in the SGZ of hippocampus (30), measuring setup needs to be carefully optimized (101).

The mechanisms behind AHN impairment in AD are still poorly understood. Numbers of independent studies have shown that amyloid pathology, APP, and PSEN1/2 are involved in modulating AHN in AD transgenic mouse brains (13). Since tau pathology led to defects in AHN in several tauopathy mouse models (12, 89, 91), we support the idea that tau pathology impairs AHN independently from amyloid pathology. In this context, it would be highly informative to study AHN in the *post-mortem* human brains of primary tauopathies devoid of amyloid pathology (e.g., FTLD with tau pathology, etc.). Yet, more studies are necessary to better understand both physiological and pathological roles of tau in AHN.

Given that increased AHN is associated with preservation of cognitive functions in non-demented individuals with AD lesions (68), stimulation of AHN should be beneficial. However, tau pathology presumably plays a negative role in AHN: EE led to increased AHN in wild-type mice but not in tau^{VLW} transgenic mice (89). Taking into consideration that an ablation of murine tau rescued AHN impairment in Tg30 mice (12) and stress-induced suppression of neurogenesis (74), reduction of tau may be beneficial for AHN. Indeed, there is compelling evidence showing the efficacy of tau reduction via anti-sense oligonucleotides (ASOs) to prolong life expectancy, reduce tau pathology, and rescue behavioral deficits in tau transgenic mice (102). Cautions need to be taken as complete ablation of tau leads to deficits in cognitive function in an age-dependent manner (94). Tau is a multifunctional protein and the net benefit of long-term reduction of tau still remains unclear (48). There are numbers of factors that can boost AHN such as EE, physical activities, or pharmacological agents (44). Testing these in tau transgenic models of tauopathies in combination with modulation of tau expression may open a new window for future therapies.

AUTHOR CONTRIBUTIONS

SH, J-PB, and KA wrote the main manuscript. All the authors participated in constructing the concept and writing the manuscript, contributed to manuscript revision, and read and approved the submitted version.

FUNDING

This study was supported by grants from the Belgian Fonds de la Recherche Scientifique Médicale (T.0027.19) to J-PB and KL, the Fund Aline (King Baudoin Foundation) to J-PB, the Belgian Fondation Recherche Alzheimer/Stichting Alzheimer Onderzoek to J-PB and KL, and the Génicot Fund (ULB) to J-PB and KL.

ACKNOWLEDGMENTS

We sincerely thank Dr. Peter Davies for the generous gift of anti-PHF1 antibody and Dr. Luc Buée for Tg30 mice.

REFERENCES

1. Wong CW, Quaranta V, Glenner GG. Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related. *Proc Natl Acad Sci USA*. (1985) 82:8729–32. doi: 10.1073/pnas.82.24.8729
2. Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun*. (1984) 120:885–90. doi: 10.1016/S0006-291X(84)80190-4
3. Brion JP, Passareiro H, Nunez J, Flament-Durand J. Mise en évidence immunologique de la protéine tau au niveau des lésions de dégénérescence neurofibrillaire de la maladie d'Alzheimer. *Arch Biol.* (1985) 95:229–35.
4. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *J Neuropathol Exp Neurol*. (2012) 71:362–81. doi: 10.1097/NEN.0b013e31825018f7
5. Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev*. (2000) 33:95–130. doi: 10.1016/S0165-0173(00)00019-9
6. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* (1991) 82:239–59. doi: 10.1007/BF00308809
7. Dickson DW, Yen SH, Horoupian DS. Pick body-like inclusions in the dentate fascia of the hippocampus in Alzheimer's disease. *Acta Neuropathol*. (1986) 71:38–45. doi: 10.1007/BF00687960
8. Wakabayashi K, Hansen LA, Vincent I, Mallory M, Masliah E. Neurofibrillary tangles in the dentate granule cells of patients with Alzheimer's disease, Lewy body disease and progressive supranuclear palsy. *Acta Neuropathol*. (1997) 93:7–12. doi: 10.1007/s004010050576
9. Brion JP, Hanger DP, Bruce MT, Couck AM, Flament-Durand J, Anderton BH. Tau in Alzheimer neurofibrillary tangles. N- and C-terminal regions are differentially associated with paired helical filaments and the location of a putative abnormal phosphorylation site. *Biochem J.* (1991) 273:127–33. doi: 10.1042/bj2730127
10. Otvos LJr, Feiner L, Lang E, Szendrei GI, Goedert M, Lee VM. Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404. *J Neurosci Res*. (1994) 39:669–73. doi: 10.1002/jnr.490390607
11. Kuninaka N, Kawaguchi M, Ogawa M, Sato A, Arima K, Murayama S, et al. Simplification of the modified Gallyas method. *Neuropathology*. (2015) 35:10–5. doi: 10.1111/neup.12144
12. Houben S, Leroy K, Ando K, Yilmaz Z, Widomski C, Buee L, et al. Genetic ablation of tau in postnatal neurons rescues decreased adult hippocampal neurogenesis in a tauopathy model. *Neurobiol Dis*. (2019) 127:131–41. doi: 10.1016/j.nbd.2019.02.021
13. Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol Neurodegener*. (2011) 6:85. doi: 10.1186/1750-1326-6-85
14. Hollands C, Tobin MK, Hsu M, Musaraca K, Yu TS, Mishra R, et al. Depletion of adult neurogenesis exacerbates cognitive deficits in Alzheimer's disease by compromising hippocampal inhibition. *Mol Neurodegener*. (2017) 12:64. doi: 10.1186/s13024-017-0207-7

15. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol*. (1965) 124:319–35. doi: 10.1002/cne.901240303
16. West MJ. Stereological studies of the hippocampus: a comparison of the hippocampal subdivisions of diverse species including hedgehogs, laboratory rodents, wild mice and men. *Prog Brain Res*. (1990) 83:13–36. doi: 10.1016/S0079-6123(08)61238-8
17. Barnea A, Nottebohm F. Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc Natl Acad Sci USA*. (1994) 91:11217–21. doi: 10.1073/pnas.91.23.11217
18. Cai Y, Xiong K, Chu Y, Luo DW, Luo XG, Yuan XY, et al. Doublecortin expression in adult cat and primate cerebral cortex relates to immature neurons that develop into GABAergic subgroups. *Exp Neurol*. (2009) 216:342–56. doi: 10.1016/j.expneurol.2008.12.008
19. Wullimann MF. Secondary neurogenesis and telencephalic organization in zebrafish and mice: a brief review. *Integr Zool*. (2009) 4:123–33. doi: 10.1111/j.1749-4877.2008.00140.x
20. Kempermann G. New neurons for 'survival of the fittest'. *Nat Rev Neurosci*. (2012) 13:727–36. doi: 10.1038/nrn3319
21. Stone SS, Teixeira CM, Devito LM, Zaslavsky K, Josselyn SA, Lozano AM, et al. Stimulation of entorhinal cortex promotes adult neurogenesis and facilitates spatial memory. *J Neurosci*. (2011) 31:13469–84. doi: 10.1523/JNEUROSCI.3100-11.2011
22. Gage FH. Adult neurogenesis in mammals. *Science*. (2019) 364:827–8. doi: 10.1126/science.aav6885
23. Ming GL, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*. (2011) 70:687–702. doi: 10.1016/j.neuron.2011.05.001
24. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. (1999) 97:703–16. doi: 10.1016/S0092-8674(00)80783-7
25. Alvarez-Buylla A, Garcia-Verdugo JM, Tramontin AD. A unified hypothesis on the lineage of neural stem cells. *Nat Rev Neurosci*. (2001) 2:287–93. doi: 10.1038/35067582
26. Kriegstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. *Annu Rev Neurosci*. (2009) 32:149–84. doi: 10.1146/annurev.neuro.051508.135600
27. Kozareva DA, Cryan JF, Nolan YM. Born this way: hippocampal neurogenesis across the lifespan. *Aging Cell*. (2019) 18:e13007. doi: 10.1111/acel.13007
28. Fuster-Matanzo A, Llorens-Martin M, Jurado-Arjona J, Avila J, Hernandez F. Tau protein and adult hippocampal neurogenesis. *Front Neurosci*. (2012) 6:104. doi: 10.3389/fnins.2012.00104
29. Leuner B, Glasper ER, Gould E. Thymidine analog methods for studies of adult neurogenesis are not equally sensitive. *J Comp Neurol*. (2009) 517:123–33. doi: 10.1002/cne.22107
30. Wojtowicz JM, Kee N. BrdU assay for neurogenesis in rodents. *Nat Protoc*. (2006) 1:1399–405. doi: 10.1038/nprot.2006.224
31. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. *Nat Med*. (1998) 4:1313–7. doi: 10.1038/3305
32. Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, et al. Human hippocampal neurogenesis persists throughout aging. *Cell Stem Cell*. (2018) 22:589–99 e585. doi: 10.1016/j.stem.2018.03.015
33. Cipriani S, Ferrer I, Aronica E, Kovacs GG, Verney C, Nardelli J, et al. Hippocampal radial glial subtypes and their neurogenic potential in human fetuses and healthy and Alzheimer's disease adults. *Cereb Cortex*. (2018) 28:2458–78. doi: 10.1093/cercor/bhy096
34. Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature*. (2018) 555:377–81. doi: 10.1038/nature25975
35. Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, et al. Dynamics of hippocampal neurogenesis in adult humans. *Cell*. (2013) 153:1219–27. doi: 10.1016/j.cell.2013.05.002
36. Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, et al. Neurogenesis in the striatum of the adult human brain. *Cell*. (2014) 156:1072–83. doi: 10.1016/j.cell.2014.01.044
37. Dayer AG, Cleaver KM, Abouantoun T, Cameron HA. New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. *J Cell Biol*. (2005) 168:415–27. doi: 10.1083/jcb.200407053
38. Luzzati F, De Marchis S, Fasolo A, Peretto P. Neurogenesis in the caudate nucleus of the adult rabbit. *J Neurosci*. (2006) 26:609–21. doi: 10.1523/JNEUROSCI.4371-05.2006
39. Inta D, Cameron HA, Gass P. New neurons in the adult striatum: from rodents to humans. *Trends Neurosci*. (2015) 38:517–23. doi: 10.1016/j.tins.2015.07.005
40. Lazarov O, Mattson MP, Peterson DA, Pimplikar SW, Van Praag H. When neurogenesis encounters aging and disease. *Trends Neurosci*. (2010) 33:569–79. doi: 10.1016/j.tins.2010.09.003
41. Kempermann G, Gast D, Gage FH. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol*. (2002) 52:135–43. doi: 10.1002/ana.10262
42. Van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci*. (1999) 2:266–70. doi: 10.1038/6368
43. Dostes S, Dubreucq S, Ladeveze E, Marsicano G, Abrous DN, Chaouloff F, et al. Running per se stimulates the dendritic arbor of newborn dentate granule cells in mouse hippocampus in a duration-dependent manner. *Hippocampus*. (2016) 26:282–8. doi: 10.1002/hipo.22551
44. Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci*. (2000) 20:9104–10. doi: 10.1523/JNEUROSCI.20-24-09104.2000
45. Schloesser RJ, Orvoen S, Jimenez DV, Hardy NF, Maynard KR, Sukumar M, et al. Antidepressant-like effects of electroconvulsive seizures require adult neurogenesis in a neuroendocrine model of depression. *Brain Stimul*. (2015) 8:862–7. doi: 10.1016/j.brs.2015.05.011
46. Wang Y, Mandelkow E. Tau in physiology and pathology. *Nat Rev Neurosci*. (2016) 17:22–35. doi: 10.1038/nrn.2015.1
47. 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) 3:519–26. doi: 10.1016/0896-6273(89)90210-9
48. Sotiropoulos I, Galas MC, Silva JM, Skoulakis E, Wegmann S, Maina MB, et al. Atypical, non-standard functions of the microtubule associated Tau protein. *Acta Neuropathol Commun*. (2017) 5:91. doi: 10.1186/s40478-017-0489-6
49. Brandt R, Trushina NI, Bakota L. Much more than a cytoskeletal protein: physiological and pathological functions of the non-microtubule binding Region of Tau. *Front Neurol*. (2020) 11:590059. doi: 10.3389/fneur.2020.590059
50. Sinsky J, Majerova P, Kovac A, Kotlyar M, Jurisica I, Hanes J. Physiological Tau interactome in brain and its link to tauopathies. *J Proteome Res*. (2020) 19:2429–42. doi: 10.1021/acs.jproteome.0c00137
51. Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. *Acta Neuropathol*. (2017) 133:665–704. doi: 10.1007/s00401-017-1707-9
52. Merezhko M, Uronen RL, Huttunen HJ. The cell biology of tau secretion. *Front Mol Neurosci*. (2020) 13:569818. doi: 10.3389/fnmol.2020.569818
53. Mudher A, Colin M, Dujardin S, Medina M, Dewachter I, Alavi Naini SM, et al. What is the evidence that tau pathology spreads through prion-like propagation? *Acta Neuropathol Commun*. (2017) 5:99. doi: 10.1186/s40478-017-0488-7
54. Kosik KS, Orecchio LD, Bakalis S, Neve RL. Developmentally regulated expression of specific tau sequences. *Neuron*. (1989) 2:1389–97. doi: 10.1016/0896-6273(89)90077-9
55. Brion JP, Smith C, Couck AM, Gallo JM, Anderton BH. goe. *J Neurochem*. (1993) 61:2071–80. doi: 10.1111/j.1471-4159.1993.tb07444.x
56. 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) 9:4225–30. doi: 10.1002/j.1460-2075.1990.tb07870.x
57. Caceres A, Kosik KS. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature*. (1990) 343:461–3. doi: 10.1038/343461a0

58. Shea TB, Beermann ML, Nixon RA, Fischer I. Microtubule-associated protein tau is required for axonal neurite elaboration by neuroblastoma cells. *J Neurosci Res*. (1992) 32:363–74. doi: 10.1002/jnr.490320308
59. Goode BL, Feinstein SC. Identification of a novel microtubule binding and assembly domain in the developmentally regulated inter-repeat region of tau. *J Cell Biol*. (1994) 124:769–82. doi: 10.1083/jcb.124.5.769
60. Bullmann T, De Silva R, Holzer M, Mori H, Arendt T. Expression of embryonic tau protein isoforms persist during adult neurogenesis in the hippocampus. *Hippocampus*. (2007) 17:98–102. doi: 10.1002/hipo.20255
61. Llorens-Martin M, Teixeira CM, Fuster-Matanzo A, Jurado-Arjona J, Borrell V, Soriano E, et al. Tau isoform with three microtubule binding domains is a marker of new axons generated from the subgranular zone in the hippocampal dentate gyrus: implications for Alzheimer's disease. *J Alzheimers Dis*. (2012) 29:921–30. doi: 10.3233/JAD-2012-112057
62. Hong XP, Peng CX, Wei W, Tian Q, Liu YH, Yao XQ, et al. Essential role of tau phosphorylation in adult hippocampal neurogenesis. *Hippocampus*. (2010) 20:1339–49. doi: 10.1002/hipo.20712
63. Fuster-Matanzo A, De Barreda EG, Dawson HN, Vitek MP, Avila J, Hernandez F. Function of tau protein in adult newborn neurons. *FEBS Lett*. (2009) 583:3063–8. doi: 10.1016/j.febslet.2009.08.017
64. Gatt A, Lee H, Williams G, Thuret S, Ballard C. Expression of neurogenic markers in Alzheimer's disease: a systematic review and metatranscriptional analysis. *Neurobiol Aging*. (2019) 76:166–80. doi: 10.1016/j.neurobiolaging.2018.12.016
65. Moreno-Jimenez EP, Flor-Garcia M, Terreros-Roncal J, Rabano A, Cafini F, Pallas-Bazarra N, et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat Med*. (2019) 25:554–60. doi: 10.1038/s41591-019-0375-9
66. Tobin MK, Musaraca K, Disouky A, Shetti A, Bheri A, Honer WG, et al. Human hippocampal neurogenesis persists in aged adults and Alzheimer's disease patients. *Cell Stem Cell*. (2019) 24:974–82 e973. doi: 10.1016/j.stem.2019.05.003
67. Choi SH, Tanzi RE. Is Alzheimer's disease a neurogenesis disorder? *Cell Stem Cell*. (2019) 25:7–8. doi: 10.1016/j.stem.2019.06.001
68. Briley D, Ghirardi V, Woltjer R, Renck A, Zolochevska O, Taglialatela G, et al. Preserved neurogenesis in non-demented individuals with AD neuropathology. *Sci Rep*. (2016) 6:27812. doi: 10.1038/srep27812
69. Van Der Lee SJ, Wolters FJ, Ikram MK, Hofman A, Ikram MA, Amin N, et al. The effect of APOE and other common genetic variants on the onset of Alzheimer's disease and dementia: a community-based cohort study. *Lancet Neurol*. (2018) 17:434–44. doi: 10.1016/S1474-4422(18)30053-X
70. Jiang M, Vanan S, Tu HT, Zhang W, Zhang ZW, Chia SY, et al. Amyloid precursor protein intracellular domain-dependent regulation of FOXO3a inhibits adult hippocampal neurogenesis. *Neurobiol Aging*. (2020) 95:250–63. doi: 10.1016/j.neurobiolaging.2020.07.031
71. Pallas-Bazarra N, Jurado-Arjona J, Navarrete M, Esteban JA, Hernandez F, Avila J, et al. Novel function of Tau in regulating the effects of external stimuli on adult hippocampal neurogenesis. *EMBO J*. (2016) 35:1417–36. doi: 10.15252/embj.201593518
72. Tucker KL, Meyer M, Barde YA. Neurotrophins are required for nerve growth during development. *Nat Neurosci*. (2001) 4:29–37. doi: 10.1038/82868
73. Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci*. (2001) 114:1179–87.
74. Dioli C, Patricio P, Trindade R, Pinto LG, Silva JM, Morais M, et al. Tau-dependent suppression of adult neurogenesis in the stressed hippocampus. *Mol Psychiatry*. (2017) 22:1110–8. doi: 10.1038/mp.2017.103
75. Criado-Marrero M, Sabbagh JJ, Jones MR, Chaput D, Dickey CA, Blair LJ. Hippocampal neurogenesis is enhanced in adult tau deficient mice. *Cells*. (2020) 9:210. doi: 10.3390/cells9010210
76. Terwel D, Lasrado R, Snauwaert J, Vandeweert E, Van Haesendonck C, Borghgraef P, et al. Changed conformation of mutant Tau-P301L underlies the moribund tauopathy, absent in progressive, nonlethal axonopathy of Tau-4R/2N transgenic mice. *J Biol Chem*. (2005) 280:3963–73. doi: 10.1074/jbc.M409876200
77. Sennvik K, Boekhoorn K, Lasrado R, Terwel D, Verhaeghe S, Korr H, et al. Tau-4R suppresses proliferation and promotes neuronal differentiation in the hippocampus of tau knockin/knockout mice. *Faseb J*. (2007) 21:2149–61. doi: 10.1096/fj.06-7735com
78. Andorfer C, Kress Y, Espinoza M, De Silva R, Tucker KL, Barde YA, et al. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem*. (2003) 86:582–90. doi: 10.1046/j.1471-4159.2003.01879.x
79. Komuro Y, Xu G, Bhaskar K, Lamb BT. Human tau expression reduces adult neurogenesis in a mouse model of tauopathy. *Neurobiol Aging*. (2015) 36:2034–42. doi: 10.1016/j.neurobiolaging.2015.03.002
80. Bolos M, Pallas-Bazarra N, Terreros-Roncal J, Perea JR, Jurado-Arjona J, Avila J, et al. Soluble Tau has devastating effects on the structural plasticity of hippocampal granule neurons. *Transl Psychiatry*. (2017) 7:1267. doi: 10.1038/s41398-017-0013-6
81. Zheng J, Li HL, Tian N, Liu F, Wang L, Yin Y, et al. Interneuron accumulation of phosphorylated tau impairs adult hippocampal neurogenesis by suppressing GABAergic transmission. *Cell Stem Cell*. (2020) 26:331–345 e336. doi: 10.1016/j.stem.2020.01.021
82. Richetin K, Steullet P, Pachoud M, Perbet R, Parietti E, Maheswaran M, et al. Tau accumulation in astrocytes of the dentate gyrus induces neuronal dysfunction and memory deficits in Alzheimer's disease. *Nat Neurosci*. (2020) 23:1567–79. doi: 10.1038/s41593-020-00728-x
83. Schindowski K, Bretteville A, Leroy K, Begard S, Brion JP, Hamdane M, et al. Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. *Am J Pathol*. (2006) 169:599–616. doi: 10.2353/ajpath.2006.060002
84. Schindowski K, Belarbi K, Bretteville A, Ando K, Buee L. Neurogenesis and cell cycle-reactivated neuronal death during pathogenic tau aggregation. *Genes Brain Behav*. (2008) 7 (Suppl. 1):92–100. doi: 10.1111/j.1601-183X.2007.00377.x
85. Leroy K, Bretteville A, Schindowski K, Gilissen E, Authelet M, De Decker R, et al. Early axonopathy preceding neurofibrillary tangles in mutant tau transgenic mice. *Am J Pathol*. (2007) 171:976–92. doi: 10.2353/ajpath.2007.070345
86. Ando K, Leroy K, Heraud C, Kabova A, Yilmaz Z, Authelet M, et al. Deletion of murine tau gene increases tau aggregation in a human mutant tau transgenic mouse model. *Biochem Soc Trans*. (2010) 38:1001–5. doi: 10.1042/BST0381001
87. Ando K, Leroy K, Heraud C, Yilmaz Z, Authelet M, Suain V, et al. Accelerated human mutant tau aggregation by knocking out murine tau in a transgenic mouse model. *Am J Pathol*. (2011) 178:803–16. doi: 10.1016/j.ajpath.2010.10.034
88. Lim F, Hernandez F, Lucas JJ, Gomez-Ramos P, Moran MA, Avila J. FTDP-17 mutations in tau transgenic mice provoke lysosomal abnormalities and Tau filaments in forebrain. *Mol Cell Neurosci*. (2001) 18:702–14. doi: 10.1006/mcne.2001.1051
89. Terreros-Roncal J, Flor-Garcia M, Moreno-Jimenez EP, Pallas-Bazarra N, Rabano A, Sah N, et al. Activity-dependent reconnection of adult-born dentate granule cells in a mouse model of frontotemporal dementia. *J Neurosci*. (2019) 39:5794–815. doi: 10.1523/JNEUROSCI.2724-18.2019
90. Mocanu MM, Nissen A, Eckermann K, Khlistunova I, Biernat J, Drexler D, et al. The potential for beta-structure in the repeat domain of tau protein determines aggregation, synaptic decay, neuronal loss, and coassembly with endogenous Tau in inducible mouse models of tauopathy. *J Neurosci*. (2008) 28:737–48. doi: 10.1523/JNEUROSCI.2824-07.2008
91. Joseph M, Anglada-Huguet M, Paesler K, Mandelkow E, Mandelkow EM. Anti-aggregant tau mutant promotes neurogenesis. *Mol Neurodegener*. (2017) 12:88. doi: 10.1186/s13024-017-0230-8
92. Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, et al. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature*. (1994) 369:488–91. doi: 10.1038/369488a0

93. Ikegami S, Harada A, Hirokawa N. Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice. *Neurosci Lett*. (2000) 279:129–32. doi: 10.1016/S0304-3940(99)00964-7
94. Biundo F, Del Prete D, Zhang H, Arancio O, D'adamio L.A role for tau in learning, memory and synaptic plasticity. *Sci Rep*. (2018) 8:3184. doi: 10.1038/s41598-018-21596-3
95. De Barreda EG, Dawson HN, Vitek MP, Avila J. Tau deficiency leads to the upregulation of BAF-57, a protein involved in neuron-specific gene repression. *FEBS Lett*. (2010) 584:2265–70. doi: 10.1016/j.febslet.2010.03.032
96. Boekhoorn K, Joels M, Lucassen PJ. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. *Neurobiol Dis*. (2006) 24:1–14. doi: 10.1016/j.nbd.2006.04.017
97. Zhao X, Van Praag H. Steps towards standardized quantification of adult neurogenesis. *Nat Commun*. (2020) 11:4275. doi: 10.1038/s41467-020-18046-y
98. Chen Q, Nakajima A, Choi SH, Xiong X, Sisodia SS, Tang YP. Adult neurogenesis is functionally associated with AD-like neurodegeneration. *Neurobiol Dis*. (2008) 29:316–26. doi: 10.1016/j.nbd.2007.09.005
99. Yagi S, Splinter JEJ, Tai D, Wong S, Wen Y, Galea L, et al. Sex differences in maturation and attrition of adult neurogenesis in the hippocampus. *eNeuro*. (2020) 7. doi: 10.1523/ENEURO.0468-19.2020
100. West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. *Anat Rec*. (1991) 231:482–97. doi: 10.1002/ar.1092310411
101. Noori HR, Fornal CA. The appropriateness of unbiased optical fractionators to assess cell proliferation in the adult hippocampus. *Front Neurosci*. (2011) 5:140. doi: 10.3389/fnins.2011.00140
102. Devos SL, Miller RL, Schoch KM, Holmes BB, Kebodeaux CS, Wegener AJ, et al. Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. *Sci Transl Med*. (2017) 9:eaag0481. doi: 10.1126/scitranslmed.aag0481

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership