Factors Related to Alzheimer's Disease, Tau Pathology in Alzheimer's Disease: Possible Treatments for Tau Pathology

Vityala Yethindra[1], Narsimharaj Alenur[2], Lakkam Saicharan[3]

[1]MBBS 4th Year and Young Scientist, [2]MBBS 2nd Year, [3]MBBS 4th Year, International Higher School of Medicine, International University of Kyrgyzstan, 1f Intergelpo Street, Bishkek, Kyrgyzstan

Abstract

The Tau protein is a microtubule-associated protein that acts as a three-dimensional "railroad tie" for the microtubule. Accumulation and Aggregation of the Tau is the pathogenesis of Alzheimer's disease. Risk factors like ApoE risk alleles, changes in the endoplasmic reticulum, and Kinases and phosphatases dysregulation have identified as the most critical factors. In Tau pathology, the abnormal hyperphosphorylation of tau appears as its accumulation in the affected neurons in Alzheimer's disease.

Neurofibrillary Tangles has shown truncated tau in both Glu-391 and Asp-421. Truncated tau associated with apoptosis in cultured cells. All six molecules of tau are the hyperphosphorylated state in PHF. In AD, hyperphosphorylated tau is present as a cytosolic protein and PHF. Treatments related to tau pathology are under research. Tau phosphorylation inhibitors and Tau aggregation inhibitors tested in people with AD. In tau phosphorylation inhibitors, Lithium has multiple targets and inhibits GSK-3b, and in tau aggregation inhibitors, many drugs block aggregation of tau in cell-free conditions. Methylene blue has multiple targets; it slows disease progression. Tau pathology appears to be a primary cause of neurodegeneration in AD. Risk factors showed a relation between AD and Tau pathology clearly. Abnormal hyperphosphorylation of tau leads to AD, and truncated tau is the main finding in tau pathology. Tau phosphorylation inhibitors and Tau aggregation inhibitors are emerging treatments.

Keywords: *Alzheimer's disease, Paired Helical Filaments, Neurofibrillary tangles, Tau pathology, Abnormal Hyperphosphorylation, Truncation, Conformation and Isoforms, Toxicity, Tau Phosphorylation Inhibitors and Tau Aggregation Inhibitors.*

Introduction

Tau is the essential Microtubule-Associated proteins (MAP) in the neurons and acts as a three-dimensional "railroad tie" for the microtubule. Accumulation and Aggregation of the Tau is the pathogenesis of Alzheimer's disease. Phosphorylation of tau binds it to the microtubules and helps in maintaining the structure, stability of neurons[1]. Accumulation of phosphate (Hyperphosphorylation) on the tau proteins cause "paired helical filaments" (PHFs) that accumulate and lead to the neurofibrillary tangles (NFTs)[2]. PHFs are the main component in NFTs. Abnormally hyperphosphorylated, insoluble, and filamentous tau was the main component of Neurofibrillary Tangles[3,4,5]. NFTs are neurological hallmark of AD[6]. It expressed in the central and peripheral nervous system and less amount observed in the kidney, lungs, and testis[7]. Abundantly seen in neuronal axons[8]. The human tau gene is located over 100 kb on the long arm of chromosome 17 at band position 17q21 and has 16 exons[9]. Tau divided into four regions: N-terminal Projection Region, a Proline-Rich Domain, a Microtubule-Binding Domain (MBD), a C-terminal region[10]. Tau can bind to outside and inside of microtubules by N- and C- terminal regions projecting outwards[11,12].

Corresponding Author:
Vityala Yethindra
International Higher School of Medicine, International University of Kyrgyzstan, 1f Intergelpo
Street, Bishkek, Kyrgyzstan, 720054.
Medical Student (MBBS 4th year) and Young Scientist.
Telephone: +91 9121925658 (Whatsapp)
Email: yethindravityala10@gmail.com

Factors related to Alzheimer's disease

ApoE Risk alleles

ApoE is a risk factor related to AD in relation to tau pathology. ApoE, as a vital component of chylomicrons and IDLs involved in the catabolism of lipoproteins[13]. It has a crucial role in the metabolism of fats in the body. ApoE is a cholesterol carrier in the brain. ApoE transfers lipoproteins, fat-soluble vitamins, cholesterol to the lymph vessels, and into the blood. ApoE is abundant in CNS but synthesized in the liver[14].

Accumulated cholesterol in the neurons converted into 24OHC (24S-hydroxycholesterol) in neurodegeneration[15]. The CSF tau levels related to neurodegeneration in AD[15]. The association between ApoE, 24S-hydroxycholesterol, and tau shows its direct involvement in the generation of NFTs[15].

Damage in Endoplasmic reticulum

Damage in Endoplasmic reticulum is a significant risk factor related to AD in relation to tau pathology. The Endoplasmic reticulum functions are energy production, folding, and trafficking of proteins and apoptosis[16]. Defects of ER results in cell stress response and triggers apoptosis. Role of ER in the development and progression of neurodegenerative diseases. Inositol-Requiring kinase 1 (IRE1) starts the ER stress pathway by triggering Apoptosis signal-regulating Kinase 1 (ASK 1), activates c-Jun N-terminal Kinase (JNK) signaling route[17]. This cascade has the strength to trigger AD pathology through hyperphosphorylation of tau and aggregation of neurofibrillary tangles[18]. ER stress causes deposition of misfolded like tau[19], and long-time existence of these toxic misfolded proteins triggers intrinsic apoptosis pathways[20]. Overexpression increase the intracellular concentration of tau may inhibit the plus-end-directed transport of vesicles along microtubules by kinesin, and the minus-end-directed transport by dynein becomes dominant[21].

Tau degradation decreased by 20% in ER stress due to a decrease in the tau binding to CHIP (carboxyl-terminus of Hsc70-interacting protein), which delayed the tau degradation through the ubiquitin-proteasome pathway[22].

Kinases and phosphatases dysregulation

Dysregulation of kinases and phosphatases is a critical risk factor related to AD in relation to tau pathology. Several protein kinases like cyclic AMP-dependent Protein Kinase A (PKA), Glycogen Synthase Kinase-3 (GSK-3), cyclin-dependent protein kinase-5 (cdk5), Calcium/Calmodulin-dependent Protein Kinase-II (CaMKII), Mitogen-Activated Protein (MAP) kinase, Extracellular signal-regulated kinase (ERK 1/2), Stress-activated protein kinases are involved in abnormal hyperphosphorylation of tau[23]. Some kinases like Protein Kinase A (PKA), Protein Phosphatases 2A (PP2A), Glycogen Synthase Kinase-3 (GSK-3), Calcium/Calmodulin-dependent Protein Kinase-II (CaMKII) plays a role in Tau phosphorylation and de-phosphorylation[24]. Abnormally hyperphosphorylated sites in tau are proline-directed, serine, and threonine, followed by proline are official sites of proline-directed protein kinases (PDPKs). GSK-3, cdk5, PDPKs, and ERK 1/2 are subjected to phosphorylate tau the sites in AD[25].

The preparation of tau by PKA or CAMKII is enough to initiate the abnormal hyperphosphorylation of tau. Dysregulation of Tau kinase CAMKII effects AD progression[26], and CAMKII inhibits tau-microtubule interaction by tau phosphorylation[27]. When GSK-3 overexpressed and it results in hyperphosphorylation of tau[28]. GSK-3 activation results in brain aging and AD initiates detrimental events like NFT formation and neuronal death pathways[29]. PP-2A and PP-1 do 90% of serine/threonine protein phosphatase activity[30]. Phosphorylation of ERK inhibits activity in response to neuronal stimuli[31].

Tau pathology

In AD, the regular role of tau impaired because this protein loses its capacity to bind to microtubules. Tau pathology starts from the entorhinal cortex and continues to the hippocampus, neocortex[32], frontal, temporal cortices, and all isocortex areas. Tau pathology observed in the form of abnormal Hyperphosphorylated protein. There is a very robust correlation between tau pathology and clinical measures of dementia. In AD, tau pathology also plays a significant role because this has the potential to trigger AD in humans.

Abnormal hyperphosphorylation of tau

The main characteristic feature of tau pathology is the abnormal phosphorylation of tau. The abnormal hyperphosphorylation of tau and significant protein subunit of PHF, which results in neurodegeneration by sequestration of MAPs, self assembles bundles of

PHF and forms NFTs. In Tau pathology, accumulation of abnormal Hyperphosphorylated tau associated with neurofibrillary degeneration and dementia. The abnormal hyperphosphorylation of tau observed in both NFTs[33 and] the cytosol of AD brains[34]. Mutations in the tau gene and their cosegregation linked to chromosome-17 (FTDP-17) have abnormalities in tau as a leading, and the first event leads to neurodegeneration and dementia[35]. The study of mAb Tau1 revealed deposits of abnormally hyperphosphorylated tau in neurons without tangles (stage "0" tangles) in AD[36]. In tau, Ghost tangles are ubiquitinated[37,38],andinabnormallyhyperphosphorylated tau, cytosol has no ubiquitin reactivity. Abnormal hyperphosphorylation of tau might be due to a conformational change in tau, and tau conformationally altered in AD[39,40]. On hyperphosphorylation, murine tau self assembles into tangles of filaments (PHF/SF)[41]. Abnormal hyperphosphorylation of tau comes before its accumulation into NFT's[42]. In our research, tau hyperphosphorylation seen at many sites and even HMW-tau hyperphosphorylated at many locations. An essential finding of the study is overexpressing p25 initiates and promotes hyperphosphorylation. This abnormal hyperphosphorylation leads to filaments self-assembly.

Truncated tau

Presence of truncation connected to neurofibrillary pathology in the brain of AD patients[43]. Neurofibrillary Tangles has shown truncated tau in both Glu-391 and Asp-421. Truncated Tau associated with apoptosis in cultured cells[44]. There is no report on what percentage of tau truncated at affected sites in different stages of AD. Neurodegeneration in AD seen for a long time from months to years, and there is a fair chance to view truncation in tau. Truncation is seen in both affected neurons and ghost tangles (extracellular space) when NFTs exposed to hydrolases[45]. Truncation of tau was observed by immunolabeling with the monoclonal antibody MN423[46]. MN423 recognizes Glu-391. Glu-391 was associated with NFTs and progression related to neurofibrillary pathology described by Braak's stages[47]. Braak stages I and II – NFT involvement confined to the transentorhinal region of the brain, Stages III and IV – Also involvement of limbic regions (hippocampus) and Stages V and VI – Extensive neocortical involvement[47].

In vitro studies described that truncated tau at Glu-391 showed elevated rates of polymerization over full-length tau, promoted by arachidonic acid[48]. Truncation of tau was observed by immunolabeling with the monoclonal antibody Tau-C3[49]. Tau-C3 recognizes Asp-421. Asp-421 was associated with the neurofibrillary pathology in the AD brain[50]. *In vitro* studies described that truncated tau at ASP-421 showed elevated rates of polymerization over full-length tau[49]. Cells transfected with truncated tau protein, it has demonstrated that both soluble and insoluble forms can induce toxicity[51,52]. In COS7 cells that Asp-421 is highly phosphorylated and abnormally redistributed. In HEK-293T cells that Glu-391 has a problem in binding with microtubule[51], and there is toxicity resulted in cell death. In the progression of the disease, Early event truncation of Asp-421 and preceded by truncation of C-terminal of Glu-391, further occurring from intermediate to advanced stages of NFTs evolution[53]. In truncation, this research specially confined to truncated tau in Glu-391 and Asp-421. In our findings, COS7 cells highly phosphorylated in Asp-421 and HEK cells have a binding problem with microtubule.

Isoforms of tau

Six isoforms of tau differ by contents of three (3R) or four (4R) tubulin-binding domains of 31 or 32 amino acids in C-terminal and one (1R), two (2R) or inserts of 29 amino acids each in N-terminal of tau. All six molecules of tau are the hyperphosphorylated state in PHF 3,4,54 . 441-residue tau is the longest tau isoform seen in the human CNS. In AD, hyperphosphorylated tau is present as a cytosolic protein[42] and PHF[3,4,54]. Cytosolic hyperphosphorylated tau (P tau) has 5-9 mol of phosphate per mole of the protein (contains 2-3 phosphate groups)[42]. Tau self-assembles by intermolecular hydrophobic interaction and microtubule-binding repeat R3 (3R taus), and R2 and R3 (4R taus), when the rest of the molecule neutralized[41]. Inhibitory amino-terminal and carboxyl-terminal regions neutralized by abnormal hyperphosphorylation in AD[41]. Cytosolic and PHF are abnormally hyperphosphorylated taus are readily dephosphorylated by phosphatases *in vitro* [33,54,55,56]. Tau molecule changing its conformation to form a paperclip-like conformation by folding the N- and C-terminal portions back on the microtubule-binding repeats[57]. FTDP-17 mutations alter the conformation of the protein, and it becomes a more favorable substrate to brain protein kinases[58]. Mutated taus are soon hyperphosphorylated and self-assemble at a lower level of hyperphosphorylation[58]. In tau, Dephosphorylation inhibits self-assembly, and hyperphosphorylation promotes self-assembly, but Deglycosylation of AD promotes self-assembly. This study found that all six tau

isoforms self-assembled into PHF/SF. This result clearly explains that hyperphosphorylation is enough to initiate self-assembly of tau into filaments. The 3R isoforms are more toxic than 4R.

Possible treatments for tau pathology

Treatments targeting different aspects of tau pathology are under research. Tau phosphorylation Inhibitors and Tau Aggregation Inhibitors are in clinical trials for people with AD, while tau reduction strategies are still in preclinical trials.

Tau Phosphorylation Inhibitors

In tau phosphorylation inhibitors, Lithium has multiple targets and inhibits GSK-3b[59]. Lithium has a narrow safety margin[60]. Studies in geriatric patients taking chronic Lithium for BPAD showed a reduced risk of developing AD. Lithium inhibits chemical changes in tau that lead to the formation of NFT's. Studies on Lithium shown benefit with low doses in mild cognitive impairment, Reduction of GSK-3b impairs NMDAR-mediated long-term depression[61] and memory consolidation[62], raising concerns about potential side effects of GSK-3b inhibitors. CDK5 is essential for multiple cell signaling pathways and adult neurogenesis, limiting its appeal as a tau-targeting approach in AD. However, CDK5 and p25, a truncated form of the CDK5 subunit p35, promote neurodegeneration through mechanisms that are independent of tau phosphorylation[63].

Tau Aggregation Inhibitors

Filamentous tau aggregates are damaging forms of tau and Filamentous tau is toxic. Tau enters dendritic spines, and adverse effects of tau aggregates are seen only in intracellular compartments[64]. Many of the drugs that block the aggregation of tau also prevent the pathological aggregation of other proteins under cell-free conditions[65]. Some tau aggregation inhibitors are effective in Neuro2A cell lines overexpressing a 4R tau microtubule repeats domain fragment with a K280 deletion and promotes its aggregation[66]. In AD patients, the Methylthioninium Chloride (methylene blue) showed slowing disease progression[67]. Methylene blue is the first drug targeting tau. The drug derived from the dye used to stain NFT's in neuropathological studies. Primarily inhibits tau aggregation and showed cognitive benefits. Methylene blue inhibits tau-tau interactions[68] and reduces soluble tau by mechanisms[69]. Inhibition of tau aggregation mediated by direct binding of tau to the FK506 binding protein 52[70]. It is unknown that tau assembly is responsible for tau-dependent neuronal dysfunction and degeneration[71].

Other treatments

Hyperacetylation increases half-life[72] and due to this microtubule-binding impairs and initiates aggregation[73]. Lysosomal pathway degrades proteasome, removes aggregated tau[74], and inhibition of this pathway produces NFT-like tau deposition[75]. Lysosomal pathway of tau degradation involved in Niemann-Pick type C disease (NPC), seen with neurological symptoms and NFT formation[76]. NPC caused by loss of function of NPC1 (Lysosomal trafficking protein)[77]. We found that that degradation of tau occurs in Lysosomal and ubiquitin-proteasome pathways. Destabilization of microtubules and problem binding to microtubules are two main problems related to overexpression of tau. Microtubule stabilizers have shown excellent results in preclinical and clinical trials of AD. Epothilone D has better BBB permeability, improved microtubule density, and cognition[78]. Peptide NAP stabilizes microtubules[79] and reduces tau phosphorylation[80]. In our study, Microtubule stabilizers have shown more than one mechanism of action.

Conclusion

Tau pathology is the leading and primary cause of neurodegeneration in AD. In the brain of AD, the level tau expression has no change concerning healthy aging, and toxicity depends on the contribution of many factors, like loss of normal function, the impermanent aggregation, and state of tau processing. Abnormal posttranslational modifications, like hyperphosphorylation and truncation, are responsible for altered tau structure in AD. Abnormal hyperphosphorylation and truncation are supported by *in vitro* experiments explain modifications elevate fibrillization of tau and induce cell toxicity[81]. Conformational changes of tau promoted by posttranslational modifications and the role of fibrillization important for checking the potential of tau-directed therapies[81]. Inhibition of the abnormal Hyperphosphorylation of tau and sequestration of MAPs by the hyperphosphorylated tau[41] is the most critical therapeutic targets for AD. The pathogenic tau slowed or prevented by treatments that reduce levels of extracellular tau, so neurons cannot internalize it[82]. Identification and characterization of mechanisms of tau release and uptake

are essential for therapeutic interventions that may slow or prevent neurodegeneration in AD. Drugs are needed to develop more effectively to slow or prevent tau pathology. Methods to be prepared to restrict or inhibit the hyperphosphorylation of tau because inhibiting abnormal hyperphosphorylation does not self-assemble tau, and there will be no formation of PHF/SF.

Abbreviations

AD: Alzheimer's disease, MAP: Microtubule Associated Protein, PHF: Paired Helical Filaments, SF: Straight Filaments, NFT'S: Neurofibrillary tangles, MBD: Microtubule-Binding Domain, IDLs: Intermediate-Density Lipoproteins, CNS: Central Nervous System, CSF: Cerebrospinal fluid, 24OHC: 24S-hydroxycholesterol, ER: Endoplasmic reticulum, IRE1: Inositol-Requiring kinase 1, ASK1: Apoptosis signal-regulating Kinase 1, JNK: c-Jun N-terminal Kinase, CHIP: Carboxyl-terminus of Hsc70-interacting protein, Camp: cyclic AMP-dependent, PKA: Protein Kinase A, GSK3: Glycogen Synthase Kinase-3, cdk5: cyclin-dependent protein kinase-5, CaMKII: Calcium/ Calmodulin-dependent Protein Kinase-II, MAP kinase: Mitogen-Activated Protein kinase, ERK 1/2: Extracellular signal-regulated kinase, PP2A: Protein Phosphatases 2A, PP1: Protein Phosphatases 1, FTDP-17: Frontotemporal dementia with parkinsonism-17, MN423 – Recombinant Mouse Antibody, COS-7: CV-1 in Origin with SV40 genes, HEK293T cells: Human embryonic kidney 293T cells, NMDAR: N-methyl-D-aspartate receptor, NPC: Niemann-Pick type C, BBB: Blood-Brain barrier.

Conflict of Interest: The authors declare no conflict of interest

Source of Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Considerations: Compliance with ethical guidelines

There is no ethical principle to be considered during this research.

Acknowledgment: As Vityala Yethindra, Iam very thankful to my parents Vityala Anitha and Vityala Thirupathi, for their valuable support in continuing research. We all authors wish to thank Dr. Tugolbai Tagaev, Dr. Cholpon Dzhumakova, Dr. Elmira Mainazarova, and Dr. Asel Namazbekova for their useful criticisms and discussion of this manuscript.

References

1. 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. Proceedings of the National Academy of Sciences 2015; 112(24): 7501-7506.
2. Harrison JR, & Owen MJ. Alzheimer's disease: The amyloid hypothesis on trial. The British Journal of Psychiatry 2016; 208(1): 1-3.
3. Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, and Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J. Biol. Chem. 1986; 261: 6084–6089.
4. Lee VM, Balin BJ, Otvos L Jr, and Trojanowski JQ. A68: A major subunit of paired helical filaments and derivatized forms of normal Tau. Science 1991; 251: 675–678.
5. Kondo J, Honda T, Mori H, Hamada Y, Miura R, Ogawara M, and Ihara Y. The carboxyl third of tau is tightly bound to paired helical filaments. Neuron 1988; 1: 827–834.
6. Cox PA, Davis DA, Mash DC, Metcalf JS, & Banack SA. Dietary exposure to an environmental toxin triggers neurofibrillary tangles and amyloid deposits in the brain. Proceedings of the Royal Society B: Biological Sciences 2016; 283(1823): 2015-2397.
7. Gu Y, Oyama F, and Ihara Y. Tau is widely expressed in rat tissues. J. Neurochem. 1996; 67: 1235–1244.
8. Trojanowski JQ, Schuck T, Schmidt ML, and Lee VM. Distribution of tau proteins in the normal human central and peripheral nervous system. J. Histochem. Cytochem. 1989; 37: 209–215.
9. Sergeant N, Delacourte A, and Buée L. Tau protein as a differential biomarker of tauopathies. Biochimica et Biophysica Acta vol. 1739; no. 2, 2005. pp: 179–197.
10. Mandelkow EM, Schweers O, Drewes G, Biernat J, Gustke N, Trinczek B, and Mandelkow E. Structure, microtubule interactions, and phosphorylation of tau protein. Ann. N. Y. Acad. Sci. 1996; 777: 96–106.
11. Kar S, Fan J, Smith MJ, Goedert M, and Amos

LA. Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. EMBO. J. 2003; 22: 70–77.

12. Santarella RA, Skiniotis G, Goldie KN, Tittmann P, Gross H, Mandelkow EM, Mandelkow E, and Hoenger A. Surface-decoration of microtubules by human tau. J. Mol. Biol. 2004; 339: 539–553.
13. Huang Y, & Mahley RW. Apolipoprotein E: Structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. Neurobiology of Disease 2014; 72: 3-12.
14. Mahley RW. Central nervous system lipoproteins: ApoE and regulation of cholesterol metabolism. Arteriosclerosis Thrombosis and Vascular Biology 2016; 36(7): 1305-1315.
15. Leoni V, Solomon A, & Kivipelto M. Links between ApoE, brain cholesterol metabolism, tau and amyloid β-peptide in patients with cognitive impairment. Biochemical Society Transactions 2010: 38(4); 1021-1025.
16. Cao SS, & Kaufman RJ. Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. Antioxidants & Redox Signaling 2014; 21(3): 396-413.
17. Okazawa H, & Estus S. The JNK/c-Jun cascade and Alzheimer's disease. American Journal of Alzheimer's Disease & Other Dementias 2002; 17(2): 79-88.
18. Viana RJ, Nunes AF, & Rodrigues CM. Endoplasmic reticulum enrollment in Alzheimer's disease. Molecular Neurobiology 2012; 46(2): 522-34.
19. Kanemoto S, & Wang H. Roles of endoplasmic reticulum stress in neurodegenerative diseases. Translational Medicine; 2012, 2:e108.
20. Fribley A, Zhang K, & Kaufman RJ. Regulation of apoptosis by the unfolded protein response. Methods in Molecular Biology 2009; 559: 191-204.
21. Ebneth A, Godemann R, Stamer K, et al. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. Journal of Cell Biology vol. 143; no.3, 1998. pp. 777–794.
22. Sakagami Y, Kudo T, Tanimukai H, Kanayama D, Omi T, Horiguchi K, et al. Involvement of endoplasmic reticulum stress in tauopathy. Biochemical and Biophysical Research Communications 2013; 430(2): 500-504.
23. Pei JJ, Gong CX, An WL, Winblad B, Cowburn RF, Grundke-Iqbal I, Iqbal K, Okadaic-acid-induced inhibition of protein phosphatase 2A produces activation of mitogen-activated protein kinases ERK 1/2, MEK 1/2, and p70 S6, similar to that in Alzheimer's disease. Am. J. Pathol. 2013; 163: 845–858.
24. Shanavas A, & Papasozomenos SC. τ kinases in the rat heat shock model: Possible implications for Alzheimer disease. Proceedings of the National Academy of Sciences 2000; 97(26): 14139-14144.
25. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida H, Titani K, Ihara Y. Proline-directed and non-proline-directed phosphorylation of PHF-tau. J. Biol. Chem. 1995; 270: 823–829.
26. Ghosh A., & Giese KP. Calcium/calmodulin-dependent kinase II and Alzheimer's disease. Molecular Brain 2015; 8(1): 78.
27. Singh TJ, Wang JZ, Novak M, Kontzekova E, Grundke Iqbal I, & Iqbal K. Calcium/Calmodulin-dependent protein kinase II phosphorylates tau at Ser-262 but only partially inhibits its binding to microtubules. FEBS Letters 1996; 387(23): 145-148.
28. Lucas JJ, Hernandez F, Gomez-Ramos P, Moran MA, Hen R, Avila J. Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. EMBO. J. 2001; 20: 27–39.
29. Takashima A. GSK-3 is essential in the pathogenesis of Alzheimer's disease. Journal of Alzheimer's Disease 2006; 9(3 Suppl): 309-17.
30. Oliver CJ, Shenolikar S. Physiologic importance of protein phosphatase inhibitors, Front. Biosci. 1998; 3: 961–972.
31. Grewal SS, York RD, & Stork PJ. Extracellular-signal regulated kinase signalling in neurons. Current Opinion in Neurobiology 1999; 9(5): 544-553.
32. Arriagada PV, Growdon JH, Hedley-Whyte ET, and Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology vol. 42; no. 3, 1992. pp. 631–639.
33. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal

phosphorylation of the microtubule associated protein (tau) in Alzheimer cytoskeletal pathology. Proc. Natl. Acad. Sci. U.S.A. 1986; 93: 4913–4917.

34. Iqbal K, Grundke-Iqbal I, Zaidi T, Merz PA, Wen GY, Shaikh SS, Wisniewski HM, Alafuzoff I, Winblad B. Defective brain microtubule assembly in Alzheimer's disease. Lancet 1986; 2: 421–426.
35. Hutton M, Lendon CL, Rizsazu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, et al. Association of missense and 5V-splice-site mutations in tau with the inherited demential FTDP-17. Nature 1998; 393: 702–705.
36. Bancher C, Brunner C, Lassmann H, Budka H, Jellinger K, Wiche G, Seitelberger F, Grundke-Iqbal I, Iqbal K, Wisniewski HM. Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. Brain Res. 1989; 477: 90–99.
37. Mori H, Kondo J, Ihara Y. Ubiquitin is a component of paired helical filaments in Alzheimer's disease. Science 1987; 235: 1641–1644.
38. 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. U.S.A. 1987; 84: 3033–3036.
39. Jicha GA, Berenfeld B, Davies P. Sequence requirements for formation of conformational variants of tau similar to those found in Alzheimer's disease. J. Neurosci. 1999; Res 55: 713–723.
40. Jicha GA, Rockwood JM, Berenfeld B, Hutton M, Davies P. Altered conformation of recombinant frontotemporal dementia-17 mutant tau proteins. J. Neurosci. Lett. 1999; 260: 153–156.
41. Iqbal K, Alonso Adel C, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Iqbal I. Tau pathology in Alzheimer disease and other tauopathies. Biochimica et Biophysica Acta 2005; 1739: 198–210.
42. Kopke E, Tung YC, Shaikh S, del C. Alonso A, Iqbal K, Grundke-Iqbal I. Microtubule associated protein tau: abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. J. Biol. Chem. 1993; 268: 24374–24384.
43. García-Sierra F, Wischik CM, Harrington CR, Luna-Muñoz J, and Mena R. Accumulation of C-terminally truncated tau protein associated with vulnerability of the perforant pathway in early stages of neurofibrillary pathology in Alzheimer's disease. Journal of Chemical Neuroanatomy vol. 22; no. 1-2, 2001. pp. 65–77.
44. Fasulo L, Ugolini G, Visintin M, Bradbury A, Brancolini C, Verzillo V, Novak M, Cattaneo A. The neuronal microtubule associated protein tau is a substrate for caspase-3 and an effector of apoptosis J. Neurochem. 2000; 75: 624–633.
45. 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: 178–182.
46. Novak M, Jakes R, Edwards PC, Milstein C, and Wischik CM. Difference between the tau protein of Alzheimer paired helical filament core and normal tau revealed by epitope analysis of monoclonal antibodies 423 and 7.51. Proc. Natl. Acad. Sci. U.S.A. 1991; 88: 5837–5841.
47. Braak H, and Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathologica vol. 82, no. 4, 1991. pp. 239–259.
48. Abraha A, Ghoshal N, Gamblin TC, Cryns V, Berry RW, Kuret J and Binder LI. C-terminal inhibition of tau assembly in vitro and in Alzheimer's disease. J. Cell. Sci. 2000; 113 (Pt 21): 3737–3745.
49. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI and Cryns VL. Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. Proc. Natl. Acad. Sci. U.S.A 2003; 100: 10032–10037.
50. García-Sierra F, Jarero-Basulto JJ, Kristofikova Z, Majer E, Binder LI, and Ripova D. Ubiquitin is associated with early truncation of tau protein at aspartic acid421 during the maturation of neurofibrillary tangles in Alzheimer's disease. Brain Pathology vol. 22; no. 2, 2012. pp. 240–250.
51. Fasulo L, Ovecka M, Kabat J, Bradbury A, Novak M and Cattaneo A. Overexpression of Alzheimer's PHF core tau fragments: implications for the tau truncation hypothesis. Alzheimers Res. 1996; 2: 195–200.
52. Fasulo L, Visintin M, Novak M and Cattaneo A. Tau truncation in Alzheimer's disease: expression of a fragment encompassing PHF core tau induces apoptosis in COS cells. Alzheimer's Rep. 1998; 1:

25–32.

53. Basurto-Islas G, Luna-Muñoz J, Guillozet-Bongaarts AL, Binder LI, Mena R, and García-Sierra F. Accumulation of aspartic acid421- and glutamic acid 391-cleaved tau in neurofibrillary tangles correlates with progression in Alzheimer disease. J. of Neuropathology and Experimental Neurology vol. 67; no. 5, 2008. pp. 470–483.
54. Iqbal K, Grundke-Iqbal I, Smith AJ, George L, Tung YC, Zaidi T. Identification and localization of a tau peptide to paired helical filaments of Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 1989; 86: 5646–5650.
55. Iqbal K, Zaidi T, Bancher C, Grundke-Iqbal I. Alzheimer paired helical filaments: restoration of the biological activity by Dephosphorylation. FEBS Lett. 1994; 349: 104–108.
56. Wang JZ, Gong CX, Zaidi T, Grundke-Iqbal I, Iqbal K. Dephosphorylation of Alzheimer paired helical filaments by protein phosphatase-2A–2B. J. Biol. Chem. 1995; 270: 4854–4860.
57. Jeganathan S, von Bergen M, Brutlach H., Steinhoff HJ, and Mandelkow E. Global hairpin folding of tau in solution. Biochemistry 2006; 45: 2283–2293.
58. Alonso Adel C, Mederlyova A, Novak M, Grundke-Iqbal I, Iqbal K. Promotion of hyperphosphorylation by frontotemporal dementia tau mutations. J. Biol. Chem. 2004; 279: 34878–34881.
59. Noble W, Planel E, Zehr C, Olm V, Meyerson J, Suleman F, Gaynor K, Wang L, LaFrancois J, Feinstein B, et al. Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. Proc. Natl. Acad. Sci. U.S.A. 2005; 102: 6990–6995.
60. Grandjean EM, and Aubry JM. Lithium: updated human knowledge using an evidence-based approach: Part III: Clinical safety. CNS Drugs 2009; 23: 397–418.
61. Peineau S, Taghibiglou C, Bradley C, Wong TP, Liu L, Lu J, Lo E, Wu D, Saule E, Bouschet T, et al. LTP inhibits LTD in the hippocampus via regulation of GSK3b. Neuron 2007; 53: 703–717.
62. Kimura T, Yamashita S, Nakao S, Park JM, Murayama M, Mizoroki T, Yoshiike Y, Sahara N, and Takashima A. GSK-3beta is required for memory reconsolidation in adult brain. PLoS ONE; 2012, 20083, e3540.
63. Kim D, Frank CL, Dobbin MM, Tsunemoto RK, Tu W, Peng PL, Guan JS, Lee BH, Moy LY, Giusti P, et al. Deregulation of HDAC1 by p25/Cdk5 in neurotoxicity. Neuron 2008; 60: 803–817.
64. Zempel H, Thies E, Mandelkow E, and Mandelkow EM. Ab oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J. Neurosci. 2010; 30: 11938–11950.
65. Masuda M, Suzuki N, Taniguchi S, Oikawa T, Nonaka T, Iwatsubo T, Hisanaga S, Goedert, M., and Hasegawa, M. Small molecule inhibitors of alpha-synuclein filament assembly. Biochemistry 2006; 45: 6085–6094.
66. Pickhardt M, Gazova Z, von Bergen M, Khlistunova I, Wang Y, Hascher A, Mandelkow EM, Biernat J, and Mandelkow E. Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments *in vitro* and in cells. J. Biol. Chem. 2005; 280: 3628–3635.
67. Gura T. Hope in Alzheimer's fight emerges from unexpected places. Nat. Med. 2008; 14: 894.
68. Wischik C. TauRX Group Fact Sheet. 2000; (Singapore) http://www.taurx.com/fact.html.
69. O'Leary JC, 3rd Li Q, Marinec P, Blair LJ, Congdon EE, Johnson AG, Jinwal UK, Koren, J, 3rd Jones JR, Kraft C, et al. Phenothiazine-mediated rescue of cognition in tau transgenic mice requires neuroprotection and reduced soluble tau burden. Mol. Neurodegener. 2019; 5: 45.
70. Chambraud B, Sardin E, Giustiniani J, Dounane O, Schumacher M, Goedert M, and Baulieu EE. A role for FKBP52 in Tau protein function. Proc. Natl. Acad. Sci. U.S.A. 2010; 107: 2658–2663.
71. Morris M, Maeda S, Vossel K, and Mucke L. The Many Faces of Tau. Cell press. Neuron 2011; 70: 410-425.
72. Min SW, Cho SH, Zhou Y, Schroeder S, Haroutunian V, Seeley WW, Huang EJ, Shen Y, Masliah E, Mukherjee C, et al. Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron 2010; 67: 953–966.
73. Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, and Lee VM. The acetylation of tau inhibits its function and promotes pathological tau aggregation. Nat. Commun. 2011; 2: 252.
74. Wang Y, Martinez-Vicente M, Kru ¨ger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM, and Mandelkow E. Tau fragmentation, aggregation and

clearance: The dual role of lysosomal processing. Hum. Mol. Genet. 2009; 18: 4153–4170.

75. Bi X, Zhou J, and Lynch G. Lysosomal protease inhibitors induce meganeurites and tangle-like structures in entorhinohippocampal regions vulnerable to Alzheimer's disease. Exp. Neurol. 1999; 158: 312–327.
76. Auer IA, Schmidt ML, Lee VM, Curry B, Suzuki K, Shin RW, Pentchev PG, Carstea ED, and Trojanowski JQ. Paired helical filament tau (PHFtau) in Niemann-Pick type C disease is similar to PHF tau in Alzheimer's disease. Acta. Neuropathol. 1995; 90: 547–551.
77. Pacheco CD, and Lieberman AP. The pathogenesis of Niemann Pick type C disease: A role for autophagy? Expert. Rev. Mol. Med. 2008, 10, e26.
78. Brunden KR, Zhang B, Carroll J, Yao Y, Potuzak JS, Hogan AM, Iba M, James MJ, Xie SX, Ballatore C, et al. Epothilone D improves microtubule density, axonal integrity, and cognition in a transgenic mouse model of tauopathy. J. Neurosci. 2010; 30: 13861–13866.
79. Divinski I, Holtser-Cochav M, Vulih-Schultzman I, Steingart RA, and Gozes I. Peptide neuroprotection through specific interaction with brain tubulin. J. Neurochem. 2006; 98: 973–984.
80. Vulih-Shultzman I, Pinhasov A, Mandel S, Grigoriadis N, Touloumi O, Pittel Z and Gozes I. Activity-dependent neuroprotective protein snippet NAP reduces tau hyperphosphorylation and enhances learning in a novel transgenic mouse model. J. Pharmacol. Exp. Ther. 2007; 323: 438–449.
81. Kolarova M, Garc´ıa-Sierra F, Bartos A, Ricny J, and Ripova D. Structure and Pathology of Tau Protein in Alzheimer Disease. Int. J. Alzheimers. Dis. 2012; 2012:731526.
82. Pooler AM, Polydoro M, Wegmann S, Nicholls SB, Spires-Jones TL and Hyman BT. Propagation of tau pathology in Alzheimer's disease: identification of novel therapeutic targets. Alzheimers. Res. Ther. 2013; 5(5): 49.