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Tau as a Therapeutic Target for Alzheimer's Disease

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Abstract

Neurofibrillary tangles (NFTs) are one of the pathological hallmarks of Alzheimer's disease (AD) and are primarily composed of aggregates of hyperphosphorylated forms of the microtubule associated protein tau. It is likely that an imbalance of kinase and phosphatase activities leads to the abnormal phosphorylation of tau and subsequent aggregation. The wide ranging therapeutic approaches that are being developed include to inhibit tau kinases, to enhance phosphatase activity, to promote microtubule stability, and to reduce tau aggregate formation and/or enhance their clearance with small molecule drugs or by immunotherapeutic means. Most of these promising approaches are still in preclinical development whilst some have progressed to Phase II clinical trials. By pursuing these lines of study, a viable therapy for AD and related tauopathies may be obtained.

Keywords

Tau; therapy; kinases; phosphatase; aggregation; immunotherapy

Introduction

Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disorder characterized by two major neuropathologic hallmarks: extracellular senile plaques composed of amyloid beta (Aβ) and intracellular neurofibrillary tangles (NFT). The cause of this disease is still unknown and is most likely multifactorial. Several genetic and environmental risk factors have been proposed including age, apolipoprotein E4 genotype, diabetes, cardiovascular disease, mutations in the amyloid precursor protein (APP), education levels, elevated cholesterol, SORL1, depression, head injury, mutations in the presenilin genes and others (1). NFT's were first described by Alois Alzheimer in 1906. In the early sixties, ultrastructural analysis by electron microscopy showed that NFT's were composed of paired helical filaments (PHF) (2). Tau is a heat stable protein which was found to be essential for microtubule assembly and was identified by its ability to co-purify with microtubules through repeated cycles of polymerization (3). In 1984, Iqbal and colleagues isolated PHF's from pathologically confirmed AD brain (4). Thereafter, in 1985 it was discovered that tau was a major component and antigenic determinant of PHF (5). This finding was quickly confirmed by several other groups (6–8). At the same time immunoblotting of a supernatant preparation from AD brain with an antibody known as Alz

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50, was found to label a band with an apparent molecular weight of 68 kDa. The authors suggested that the Alz 50 antigen was unlikely to be tau (9), but it was later shown that Alz 50 did in fact recognize a phosphorylated tau epitope (10). The novel idea that PHF's from AD brain were composed of not just tau but phosphorylated tau was first confirmed by Grundke-Iqbal and colleagues and Ihara and colleagues in 1986 (11;12). This finding is of added significance since tau's phospho-status can affect its microtubule binding ability (13;14), thus many groups have sought to identify the kinases that play a role in PHF tau phosphorylation. More recently, the role of phosphatases leading to abnormal tau phosphorylation has also been investigated (15).

Our current understanding of tau's function and dysfunction has been aided with the discovery of mutations in the tau gene which are responsible for a range of neurodegenerative disorders collectively termed tauopathies – since they are characterized by the presence of aggregated tau deposits. However, no mutations in the tau gene are associated with AD. With an increasing number of disorders proposed to be caused by tau pathology, there is a very urgent need to develop viable therapeutics. The focus of this minireview will be to assess some of the currently available and potentially novel strategies aimed at targeting tau pathology in neurodegenerative disease.

Phosphorylation of Tau

Tau, a member of the family of microtubule associated proteins (MAPs), is widely expressed in both the central and the peripheral nervous system. Along with various other MAPs, tau is involved in modulating microtubule assembly and maintaining stability within neurons (16). There are over 80 Ser/Thr-Pro motifs in tau which can act as potential phosphorylation sites and it has been shown that increased phosphorylation diminishes tau's ability to effectively bind microtubules (14). Tau phosphorylation is developmentally regulated, with increased tau phosphorylation in fetal brain compared to adult brain. The phospho-status of tau is controlled by the activities of various kinases and phosphatases. Several kinases including proline directed kinases such as mitogen activated protein kinase (14;17), stress activated kinases (17;18), glycogen synthase kinase 3β(GSK3β) (14);(19), and cyclin dependant kinases cdc2 and cdk5 (20), and non-Ser/Pro kinases, such as calcium-calmodulin dependant protein kinase II (CaMKII), casein kinases I and II and protein kinases A and C (21;22), have been shown to phosphorylate tau *in vitro* or *in situ*. The most likely candidates for physiological and pathological conditions are proposed to be glycogen synthase kinase 3 (GSK3), cyclin-dependent kinase 5 (cdk5) and the microtubule-affinity-regulating kinase (MARK) (23). Phosphatases PP1, PP2A, PP2B and PP2C have also been shown to have a role in modulating the phosphorylation state of brain tau. Although tau can undergo other post translational modifications such as glycosylation, ubiquitination and glycation, most of our interest has been directed to the study of tau phosphorylation since tau in an abnormally phosphorylated state is the main component of one of the pathological hallmarks of AD - the intraneuronal neurofibrillary tangle (NFT) that accumulates in the brain of AD patients (24;25). Also, it was found that levels of NFTs rather than amyloid burden, correlated better with the presence of dementia (26;27)

The following has been proposed as the pathogenic series of events that lead to neuronal death. Abnormal tau hyperphosphorylation leads to decreased microtubule binding. This results in destabilized microtubules and a loss of axonal integrity. Detachment of tau results in an increase in the pool of free tau which is available to form misfolded protein aggregates. Based on this model, there are several potential tau based therapeutic targets. For example, the imbalance between kinase and phosphatase activities induces changes in tau phosphorylation, suggesting kinase inibitors and phosphatase activators as a potential therapy targeting tau pathology.

Tau Based Therapies

Since 1990, therapeutic approaches in the AD field have mainly focused on targeting Aβ pathology. The discovery that mutations in the tau gene alone could result in diseases collectively termed tauopathies (28);(29) has reinvigorated efforts on the development of treatment strategies aimed at rectifying tau dysfunction. Tau mutations fall under two categories, a) those that affect the alternative splicing of Exon 10, thereby affecting the ratio of 3 repeat versus 4 repeat tau isoforms, and b) those that affect tau microtubule interactions. Thus, it has been proposed that in the tauopathies there is a loss of tau function i.e. microtubule binding ability, followed by a gain of toxic effect – the enhanced ability to form tau aggregates. To clarify our understanding of the pathophysiology of tau, several transgenic mouse models have been generated. They include the JNPL3 line, which expresses the most common P301L mutation found in frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). This model and other similar ones have been shown to develop extensive neurodegeneration (30);(31) and provide a valuable *in vivo* system to test therapeutic strategies.

Kinases as a Therapeutic Target

More than 20 protein kinases can phosphorylate tau *in vitro* and in cells. The imbalance of the activities of kinases and phosphatases most likely leads to the aberrant hyperphosphorylation of tau seen in tauopathy. Hence an obvious and desirable therapeutic target would be to inhibit tau kinases, but it is essential to identify which ones to target (32–35).

GSK3β, cdk5 and MARK, have been proposed as the three main tau kinases responsible for phosphorylating the majority of epitopes that are present in PHF-tau (36);(37). Of these three kinases GSK3β and cdk5 have received particular attention, and are the primary targets for drug discovery efforts.

GSK3 Inhibitors

GSK3 is a serine-threonine kinase first identified as an enzyme that phosphorylates glycogen synthase in the glycogen synthesis pathway. There are two isoforms of GSK3 encoded by two independent genes, GSK3α and GSK3β. GSK3β is especially abundant in brain (38). GSK is highly conserved, ubiquitously expressed and found in all eukaryotes. It can phosphorylate tau, neurofilament protein and several transcription factors such as CREB, c-Jun, c-myc, c-Myb and HSF-1 (Heat shock transcription factor) *in vitro* and *in vivo* (39;40). Furthermore, it has been identified as a participant in diverse cellular processes including promoting cell death (41) or survival (42;43), signaling pathways, metabolic control, and oncogenesis. In addition, dysregulation of GSK3β has been implicated in a wide range of neurological and degenerative diseases such as bipolar mood disorder and AD (44;45). It has also been shown that inhibition of GSK3 facilitates long term potentiation (46). In AD brain GSK3β has been shown to co-localize with dystrophic neurites and NFT's (47–49). The active form of GSK3 was found in neurons with pre-tangles (50). Based on these findings it is evident that GSK3β is a promising candidate to be targeted for developing new drugs for AD and other tauopathies (51). Several classes of GSK inhibitors have been identified (52), in addition to peptides (53) and metal ions (54). Some of these molecules have shown efficacy in different animal models (55).

Paullones, indirubines and bisindol-maleimides represent some of the first class of GSK3 inhibitors discovered by high throughput screening. However since these inhibitors compete with ATP for its binding site, these compounds could readily inhibit other kinases such as CDK1 and CDK2. Importantly, knowledge gained from the crystal structure of GSK3β

(56;57), has allowed various potential structure based pharmacophores to be designed (58;59).

Lithium as a GSK3 Inhibitor

The alkali metal lithium was discovered in 1817, and utilized as a remedy for multiple human diseases (60), including as effective therapy for manic–depressive illness (61;62). Lithium is selective for GSK3, but its mechanism of action is not well understood (63). Cell culture and *in vivo* studies have clearly shown that lithium treatment can effectively inhibit the enzyme and reduce tau phosphorylation levels (63–65), thus suggesting lithium as a potential therapeutic agent for AD and other tauopathies (66). However, a recent study found no effect of lithium on Aβ load or memory deficits, despite the reduction of phosphorylated-tau in a triple transgenic mouse model of AD (67).

Lithium has been used as a treatment for various psychiatric disorders for over 50 years, but its use as a potential therapy for AD has only been explored more recently. A pilot study examined the feasibility and tolerability of lithium carbonate in mild to moderate AD. Patients were treated for upto a year and their cognition and adverse effects were assessed (68). The pilot study showed no difference in deaths, drop outs or change in mini mental state exam (MMSE) between those receiving lithium and the comparison group, however discontinuation rates were high and many study participants reported contraindications; thus the authors concluded that lithium therapy for AD may have limited potential (68).

The interest in finding inhibitors of GSK has resulted in the development of several classes of small molecule inhibitors. Bhat and colleagues from AstraZeneca have written a very comprehensive review on GSK3 inhibitors for the treatment of AD and readers are encouraged to refer to this review for details about the various inhibitor classes. In this review we will briefly outline these classes of inhibitors (69).

Other GSK3 Inhibitors

The amino-thiazole small molecule known as AR-A0114418 (Astra Zeneca) has been described as a selective GSK3 inhibitor which acts in an ATP competitive manner (70). It has been shown to inhibit tau phosphorylation on Ser 396 in cells overexpressing the long isoform of human tau (4 repeat tau) (70). It also reduces tau phosphorylation, accumulation and axonal degeneration in a tauopathy mouse model (71). Inhibition of cell death by this molecule correlates with reduced GSK3 activity. These results indicate that GSK-inhibitors can be neuroprotective.

Aminopyrazinyl-2-carboxamide (AZ11125357; Astra Zeneca) is a novel small-molecule GSK3 inhibitor identified through a high-throughput biochemical screen using purified recombinant human GSK3. It inhibits GSK3 mediated tau phosphorylation in cells by competing with ATP binding and it inhibits CDK2 as well (69).

Oxindolequinazoline (AZ10316813; Astra Zeneca) inhibits GSK3β by competing with ATP binding and demonstrates selectivity against more than ten other kinases tested, including IGF1R, ZAP70, PTK2, SRC, JAK3, Abl, p38, JNK1, PKA, MAP2K1 and CHK1 but not CDK2.

Thiadiazolidindiones (TDZD's) represents the first selective GSK3 inhibitor entering phase I clinical trial (Neuropharma) as a new therapeutic agent which inhibits GSK3 and tau phosphorylation in AD (72;73). Oral treatment of animals with TDZD's decrease tau phosphorylation in a dose and time dependent way (74).

SRN-003-556 (Sirenade) inhibits ERK2/cdc2, GSK3β, PKA and PKC, and has been shown to reduce soluble hyperphosphorylated and aggregated tau in a P301L tau mouse model (75).

SB-216763 and SB-415286 developed by SmithKline Beecham are maleimide ATP-competitive inhibitors that inhibit GSK3 (76). In primary cerebellar granule neurones, an inhibition of Thr181 and Ser202 tau phosphorylation was shown with both these compounds. In HEK293 cells overexpressing recombinant tau and GSK3β, SB-216763 and SB-415286 treatment led to a reduction in tau phosphorylation at Ser202 (77). SB-216763 and SB-415286 have been shown to cause a decrease in inhibitory Ser9 phosphorylation of GSK3β. This effect is possibly via activation of an upstream kinase p90rsk, that has been shown to inhibit tau phosphorylation at the GSK3-specific site Ser396 (78;79).

Cdk 5 Inhibitors

In 1993, Kobayashi et al., discovered that Tau protein kinase II is cdk5 (20). It is expressed ubiquitously (80;81) and along with GSK, it has been suggested to play a significant role in tau phosphorylation and contribute to the formation of NFT's in AD brain (82;83). Cdk5 phosphorylates several substrates, and is involved in such processes as insulin exocytosis (84), neurotransmitter release (85), dopamine signaling pathways (86), and neurite outgrowth (87). Cdk5 is a 33 kDa proline-directed protein kinase that phosphorylates serine and threonine residues immediately preceding a proline residue (88–92). Previous studies have suggested that cdk5 activity plays an important role in axonal growth, neuronal migration, synaptic function, neurite outgrowth, synapse formation at the neuromuscular junction and myogenesis (93;94). Many of the substrates of cdk5 are cytoskeleton proteins, such as microtubule associated protein tau. Purified cdk5 was shown *in vitro* to phosphorylate several tau epitopes using multiple methods explored by different laboratories, such as mass spectrometry, two dimensional gel electrophoresis using radioisotope labeling and phosphotau dependent antibodies (95–97). It can phosphorylate tau at several pathologically relevant residues such as: Ser202, Thr205, Thr212, Thr217, Ser235, Ser396 and Ser404 (98–100).

Cdk5 requires phosphorylation of the activation loop for full activation (101), and depends on the presence of p35 (102;103) or p39 (104). p25 is generated from p35 by proteolytic cleavage (102;105;106), that can lead to neuronal cell death by unknown mechanisms and has been implicated in the progression of AD (107–111). p25 is more effective than p35 in activating cdk5 to phosphorylate tau (112). Overexpression of cdk5 and its activators in neurons and cell lines can induce tau phosphorylation on multiple epitopes (113–115). These findings have been validated in mouse models, in which overexpression of activated cdk5 or p25 leads to the formation of NFTs and impairment of cognitive functions (116–118). P301L mice crossed with p25 mice have a fivefold increase in NFT (119). Interestingly, triple Tg mice that overexpress cdk5, human p35 and tau do not show an increase in tau phosphorylation (120). While non-selective cdk inhibitors have been profiled in preclinical and clinical studies, clinical trials have yet to be initiated on selective cdk5 inhibitors.

The crystal structure of active cdk5/p25 kinase complexed with the three inhibitors, R-roscovitine (121–123), aloisine (124), and indirubin-3′-oxime (125) has been reported. These chemicals are ATP antagonists, and R-roscovitine is entering phase I and II clinical trials against glomerulonephritis and cancer, respectively (123).

MARK/PAR1 as a Target for Inhibition

The microtubule affinity regulating kinase (MARK/PAR1) phosphorylates microtubule associated proteins (MAPs) such as MAP2, MAP4 and tau, resulting in their dissociation

from microtubules (126;127). Human PAR-1 is encoded by four genes, giving rise to the isoforms MARK1 (PAR-1c), MARK2 (PAR-1b/EMK), MARK3 (PAR-1a/p78/C-TAK1) and MARK4 (PAR-1d/MARKL1), encoded respectively on chromosomes 1, 11, 14, and 19 (128). Members of the MARK/PAR-1 kinase family play crucial roles in cellular functions such as polarity and cell-cycle control (129;130). MARK 1 is involved in axonal transport and neurite growth (131;132). MARKs phosphorylate tau, MAP2 and MAP4 at KXGS motifs in the repeat regions, and reduce their affinity for microtubules which then become more unstable (131). Phosphorylation of KXGS motifs in the repeat region of tau was found to occur at an early pre-tangle state in AD brains (133). Also, fluorescence resonance energy transfer experiments showed that MARK was responsible for phosphorylation of the Ser262 epitope in PHF's from AD brain (134). The Mandelkow group has created an inducible mutant tau transgenic mouse (ΔK280), which replicates MARK phosphorylation of the Ser262 site at an early age (135).

Due to its role in phosphorylating tau within the microtubule binding region, MARK maybe another target for therapeutic intervention in AD and other tauopathies. Staurosporine and hymenialdisine have been used *in situ* as non-selective pharmacological inhibitors of MARK and its activating kinase (MARKK) (131;136).

Interestingly, GSK3β was shown to phosphorylate MARK and inhibit its activity. Since GSK3β can also phosphorylate tau, the net effect of these two kinases would be to alter the status of tau phosphorylation in AD (137).

Phosphatases

The restoration of tau phosphatase activity by targeting its subunits is another approach to reduce hyperphosphorylated tau. Tau is dephosphorylated by protein phsophatase 2A (PP2A), and to lesser effect by PP1, PP2B and PP5 (138;139). PP2A is a major phosphatase implicated in tau phosphorylation *in vitro*. In addition to dephosphorylating the kinase substrate, PP2A regulates the activity of protein kinase. Kinases and phosphatases act synergistically on tau phosphorylation because the phosphorylation of tau by GSK3 or Cdk5 is overridden by inhibition of PP2A (140).

Phosphatase activity (PP2A and PP2B) is lower in AD brain compared to controls (141;142). Okadaic acid inhibits PP2A and PP1, and in rat brain slice cultures increases tau phosphorylation but does not lead to the formation of tangles (143). Transgenic mice with reduced PP2A activity show a somatodendritic accumulation of hyperphosphorylated and aggregated tau in cortical pyramidal cells and cerebellar purkinje cells (144). Memantine, a noncompetitive N-methyl-D-Aspartate (NMDA) receptor antagonist already approved for the treatment of AD, many also influence PP2A. Using a hippocampal slice culture model, Li et al., showed that Memantine could reverse tau hyperphosphorylation induced by pre-treatment with okadaic acid, most likely through its effect on PP-2A signaling pathway (145). This novel finding from a slice culture model should be evaluated further clinically.

Other Proteins Affecting Tau Phosphorylation

Another potential target for tau therapy is Pin1 (protein interacting with NIMA1) which regulates tau phosphorylation. Tau and Pin1 bind when tau is phosphorylated at Thr231 (146). In AD, more tangles were observed in pyramidal neurons with a lower amount of Pin1, and no tangles were detected in neurons expressing high levels of Pin1 (147). Pre-incubation of phosphorylated tau with Pin1 was shown to restore the ability of abnormally phosphorylated tau to promote microtubule assembly (146). Furthermore, Pin1 knockout mice have been shown to develop hyperphosphorylated tau, tau aggregates and neuronal

degeneration (147) suggesting that Pin1 activators could be a potential therapy for tauopathies.

Aggregation of Tau

Under normal conditions, tau is a very soluble protein that does not readily aggregate into filaments and phosphorylated tau does not spontaneously form PHF's *in vitro*. However, its fibrillogenicity is influenced by post translational modifications such as enzymatic cleavage (148), and phosphorylation can promote or inhibit aggregation depending on which epitopes are involved (149).

Methylene Blue

In 2008 Wischik and colleagues piqued the interest of the AD field when they presented data from a Phase II trial using the drug methylene blue (MB) (RemberTM). This was the first clinical study to examine inhibitors of tau aggregation as a potential therapy for treating AD. MB is a heterocyclic aromatic which belongs to a class of compounds known as phenothiazines. Its uses are varied and numerous. They include as a dye for staining, a redox indicator, a cheap and effective treatment for malaria, as an antiseptic and even as an antipsychotic drug (150;151). In 1996 it had been determined that MB (phenotiazines) could be used to inhibit tau-tau binding through the repeat domain (152). This finding suggested that the self assembly of tau into pathological PHF's could be prevented.

The data presented at the ICAD in 2008 examined 321 people with mild or moderate AD and compared the effect of three different doses of MB versus placebo on cognitive abilities as measured by ADAS-Cog (153). Amazingly, patients with moderate AD and treated with MB had a significant improvement in tests of cognitive function at six months. Three different MB doses were compared and by 50 weeks of treatment 60 mg MB resulted in an 81% reduction in rate of decline compared to placebo treated.

More recently, it has been reported that Wischik's team has developed and patented a new form of MB called leuco-methylthioninium or LMT, which is no longer blue and renders the drug more bioavailable and less toxic at higher dose (Alzforum 2009 and web http://www.alzforum.org/new/detail.asp?id=2203).

N744

The lab of Jeff Kuret has also made headway in identifying compounds to prevent tau oligomerizarion or fibrillization. They have screened small molecule libraries to find fibrillization antagonists. One of these is a cyanine dye compound called N744. It was found to inhibit tau fibrillization and promote disaggregation of mature synthetic filaments (154).

Another way to identify potential tau aggregation inhibitors has been to screen large compound libraries which have sufficient structural diversity. This then allows any potential "hits" to be optimized by medicinal chemists and further developed to have appropriate inhibitory properties and pharmacokinetics (155). The Mandelkow group have screened a random library of 200,000 compounds, from which they were able to identify 77 compounds that were found to inhibit PHF formation and inhibit tau aggregation as validated by using a thioflavine S fluorescence assay. Further screening identified rhodanines, antraquinones and N-phenylamines as classes of aggregate inhibitors. The efficacy of these compounds was tested using a neuronal cell model and the compounds were found to successfully prevent tau aggregation (156–160).

Taniguchi and colleagues also identified phenothiazines, porphyrins and polyphenols as classes of compounds that were successful at inhibiting heparin induced tau filament formation (161).

One caveat that must be borne in mind when contemplating the potential benefits of tau aggregation and fibrillization inhibitors is that more toxic oligomers may be generated, in the course of preventing the formation of PHF like tau aggregates. If this is the case then the usefulness of these aggregate inhibitors would be limited.

Microtubule Stabilising Drugs

Tau in a pathological state is abnormally phosphorylated and this is proposed to result in a loss of one of its classical functions – the binding of microtubules leading to decreased microtubule stability (162). In fact it was shown immunohistochemically that levels of acetylated α-tubulin, an indicator of stable microtubules, were decreased in tangle bearing neurons in AD brain tissue (163). This impairment in tau–cytoskeleton interactions may have consequences on axonal integrity and cellular transport of organelles and vesicles (164). Thus, another valid treatment strategy that has gained some attention is the use of drugs to complement the microtubule stabilising function of tau such as paclitaxel. Early studies by Michaelis et al., showed the promise of this approach in culture models using low doses of microtubule stabilizing drugs including paclitaxel (Taxol®) (165), which is already widely used as an anti cancer drug. Further work carried out in the group of Virginia Lee and John Trojanowski showed that injections of paclitaxel could restore fast axonal transport deficits and improve motor impairments in a murine tauopathy model (166). However paclitaxel does not readily cross the blood brain barrier (BBB), and its beneficial effect was proposed to be a result of uptake at the neuromuscular junction and retrograde transport to spinal motor neurons (167). Its ability to access the brain is limited because of its high affinity to the efflux transporter ABCB1 (p-glycoprotein 170, p-gp) (168–170). By blocking ABCB1 mediated efflux, it would be possible to increase the concentration of ABCB1 substrates such as paclitaxel in the brain (171–173). New third generation p-gp modulators have been developed, which aim to achieve the delivery of efficacious brain concentrations of paclitaxel and overcome potential systemic toxic side effects of these powerful antimitotic agents (174). Recently, Brunden and colleagues tested the efficacy of Epothilone D (EpoD), a brain penetrant microtubule stabilizing compound. PS19 tau transgenic mice were treated for EpoD for three months. Low doses of EpoD were well tolerated and resulted in significantly fewer dystrophic axons and increased microtubule density in the optic nerve. Additionally, EpoD treated PS19 mice appeared to perform better than vehicle treated mice in the Barnes maze - a test of cognitive function (175).

The octapeptide NAPVSIPQ (NAP) has also been proposed to be a microtubule stabilizing agent, it can cross the BBB and has been shown to promote microtubule stability (176), as discussed in more detail below.

NAP (NAPVSIPQ) is an eight amino acid peptide derived from activity-dependent neuroprotective protein (ADNP) (177–179). The group of Illana Gozes has carried out most of its *in vitro* and *in vivo* studies. NAP treatment in tissue culture and *in situ* models has been shown to protect neurons against a wide variety of toxins and stresses such as Aβ peptide, oxidative stress and zinc intoxication (180;181). NAP was also found to associate with tubulin and enhance proper microtubule assembly (181). Based on these *in situ* studies it was suggested that NAP may be a promising drug candidate for the treatment of tauopathy *in vivo*, as tau dysfunction may lead to loss of microtubule integrity. Using the triple transgenic AD model [31], Matsuoka et al., demonstrated that presymptomatic treatment with NAP protected against increased tau hyperphosphorylation and lowered levels of Aβ 1-40 and 1-42 (182). Additionally, triple transgenic mice that received a six month course of

NAP treatment showed improved cognitive performance in the Morris water maze (MWM) and an increase in heat-stable soluble tau (presumably containing microtubule-associated tau) (183). Studies with a "pure" tauopathy mouse model expressing two tau mutations (P301S and K257T), reinforced that mice treated with NAP performed better in the Morris water maze, had reduced levels of phosphorylated tau and NFT pathology and had increased levels of soluble tau (184). Allon pharmaceuticals have developed *Davunetide,* a compound based on NAP, delivered as a nasal spray, and have evaluated its effectiveness in patients with mild cognitive impairment. Their study reported at ICAD 2008 indicated that patients treated with AL108 (NAP) had a significant improvement over placebo treatment in two measures of memory, but not on the primary or secondary endpoints in other cognitive tests. Headache and nasopharyngeal events were reported more frequently by NAP-treated subjects. However, the overall incidence of adverse events was similar between placebo- and NAP-treated groups ((185); www.allontherapeutics.com).

Targeting Glycosylation and Chaperones

Thus far we have discussed the major post-translational modification that tau undergoes – namely phosphorylation. Another modification that tau undergoes and that has been shown to reciprocally affect tau's phosphorylation state is glycosylation, for example abnormally hyperphosphorylated tau and PHF tau have been found to be glycosylated (186). A specific form of glycosylation, O-linked β-N-acetylglucosaminylation (O-GlcNAc), which was only discovered in 1983 (187), may have a significant role in AD pathophysiology. In the healthy adult brain, tau is extensively O-GlcNAcylated at more than 12 sites but the O-GlcNAcylation level in AD brain extracts has been shown to be decreased compared to controls (188). Interestingly, O-GlcNAcylation of human tau was found to negatively regulate its phosphorylation in a site-specific manner both *in vitro* and *in vivo* (188). Thus it has been suggested that by increasing tau O-GlcNAc levels, the hyperphosphorylation of tau could be blocked and the accumulation of toxic tau species prevented (189). To test this, Yuzwa and colleagues have synthesized a potent inhibitor of human O-GlcNAcase, thiamet-G. Thiamet-G decreased phosphorylation of tau in PC-12 cells at pathologically relevant sites including Thr231 and Ser396. Thiamet-G also efficiently reduced tau phosphorylation at Thr231, Ser396 and Ser422 in both rat cortex and hippocampus, and thereby provides an alternative route to block pathological hyperphosphorylation of tau in AD (189).

Since AD is characterized by the accumulation of abnormally aggregated proteins, several labs have examined the role of chaperones in contributing to misfolded protein disorders and also as a potential site for therapeutic intervention. Molecular chaperones are proteins that regulate the correct handling, conformation and transport of other proteins, as well as facilitating the removal of "misbehaving" proteins by transporting them to the proteosome to be degraded (190;191). One class of chaperones are the heat shock proteins (HSP). Immunohistochemical and biochemical studies showed that HSP27 expression was elevated in AD brains, particularly in astrocytes (192). HSP27 has also been shown to directly bind to hyperphosphorylated tau, decreasing its concentration and thus protect against hyperphosphorylated tau induced cell death in a neuronal cell line (193). Levels of HSP70 and HSP90, the two main chaperone scaffolds, which protect cytosolic proteins against stress-induced unfolding and aggregation (194), were also elevated in affected regions from AD brain tissue (195;196). The upregulation of these proteins may suggest that the brain is attempting to actively clear the buildup of aggregated or pathological proteins. Thus the interest in modulating HSP's as a therapeutic target for AD and other neurodegenerative diseases. The Petrucelli and Chiosis groups have been actively pursuing the development of HSP inhibitors. Dickey et al., used a mutant P301L tau expressing CHO cell line to assess the effect of various small HSP90 inhibitors. These compounds reduced levels of tau phosphorylated at proline-directed Ser/Thr sites (pSer202/Thr205, pSer396/Ser404) and

conformationally altered (MC-1) tau species, but tau phosphorylated at Ser262/Ser356 within the tau microtubule binding domain was more resistant to degradation (197). Luo et al., used a novel HSP90 inhibitor, PU24FCl, to treat cortical neurons and a derivative, PU-DZ8, which can more readily enter the brain, to treat JNPL3 mice, and showed that levels of phosphorylated and aggregated tau could be reduced (198).

Tau Immunotherapy

A novel therapeutic approach for Alzheimer's disease (AD) that is currently being intensively pursued is immune modulation to clear amyloid-β (Aβ). Studies in transgenic mice showed that immunization with aggregated Aβ1-42 was able to reduce Aβ levels and associated pathology (199). Additional studies indicated that the mechanism for this effect was likely to be antibody-mediated (200;201). The promise shown by these vaccination studies led to the initiation of a Phase I clinical study in AD patients using Aβ1-42 (AN 1792). The subsequent Phase IIa study had to be halted because 6% of the patients developed meningoencephalitis (202). Despite the suspension of the clinical trial, there were several positive outcomes. Neuropathological examination of three cases stunningly showed that Aβ had been removed and there was a resolution of dystrophic neurites (203). Also, cognitive testing of the Zurich cohort indicated that of the patients who were immunized and generated antibodies to Aβ there was a significantly slower rate of decline of cognitive functions and activities of daily living (204). But this promising finding was not detected in the overall analysis of all the study sites, although a trend for cognitive stabilization was observed (205). And disappointingly, more recent autopsy data from a few subjects from the trials indicates that complete clearance of Aβ plaques does not appear to halt the progression of AD dementia (206). This approach is being refined and further trials are underway. Interestingly, passive immunization targeting Aβ was recently shown to reduce amyloid detected with *in vivo* PET imaging (207). However, Aβ clearance in the AN 1792 trial did not appear to affect tangle pathology (208;209), although plaque-associated tau pathology was decreased (203;210;211). Autopsy data is not yet available from the second generation Aβ immunotherapy trials. Using the 3XTg mouse model, immunotherapy targeting Aβ cleared early tau pathology but hyperphosphorylated or aggregated tau was not affected (212). Together, these findings further emphasize the need for therapy targeting neurofibrillary tangles.

As detailed above, therapeutic approaches targeting tau pathology have concentrated on reducing its level of phosphorylation primarily by targeting tau kinases. An alternative novel approach, from our lab has used active immunization with peptides containing pathologically hyperphosphorylated tau epitopes or corresponding antibody, resulting in a decrease in levels of aggregated tau which importantly includes prevention of cognitive impairments and reduction in other functional impairments in two tauopathy mouse models (213–218). Hence, clearance of NFTs or their precursors could reduce the amount of synaptic and neuronal loss associated with AD. Importantly, the functional outcome correlated well with reduction of tau aggregates (219). Studies from related fields have helped to add credence to our tau immunotherapy approach as a viable treatment strategy. While our studies were underway, vaccination with recombinant α-synuclein was shown to lead to clearance of intraneuronal aggregates of the same sequence in transgenic Parkinson's disease model mice (220), and subsequently anti-Aβ antibodies were shown to be taken up into neurons in culture and promote the clearance of intracellular Aβ aggregates via the endosomal-lysosomal pathway (221). The idea that an immunological approach could be used to target an intracellular protein has met with a fair deal of skepticism. However, we have showed that following intracarotid injections of FITC-labeled anti-tau antibodies in tangle model mice, these antibodies are detected within brain neurons bound to tau aggregates (213). Furthermore, clearance of extracellular tau may also be beneficial, as it

may be toxic and/or have a role in the spread of tau pathology throughout the brain. The addition of various recombinant tau constructs to the culture media of SH-SY5Y cells has been shown to increase cell death (222). More recently it was reported that extracellular tau aggregates, but not monomer, can be taken up by cultured cells and displace tubulin, co-localize with dextran, a marker of fluid-phase endocytosis, and induce fibrillization of intracellular full-length tau (223). This interesting finding was extended by an *in vivo* study that indicated that extracellular tau mediates the spread of tau pathology through anatomically connected regions (224). These findings clearly enhance the value of an immunotherapeutic approach targeting tau as these antibodies should have better access to the extracellular pool of tau compared to its intracellular one. The potential mechanism of tau immunotherapy is discussed extensively in (219;225).

Conclusion

Currently there is no cure for a multifactorial disease like AD. In this mini review we have described some of the extensive data that supports a causative role for tau protein in the pathogenesis of tauopathies. For targeting the dysregulation of tau protein in an appropriate temporal and spatial manner, the various potential treatment options discussed are likely to have a therapeutic value. Further work on improving the specificity, efficacy and minimizing potential side effects of these treatments remains to be completed, but these are exciting times for the tau research community.

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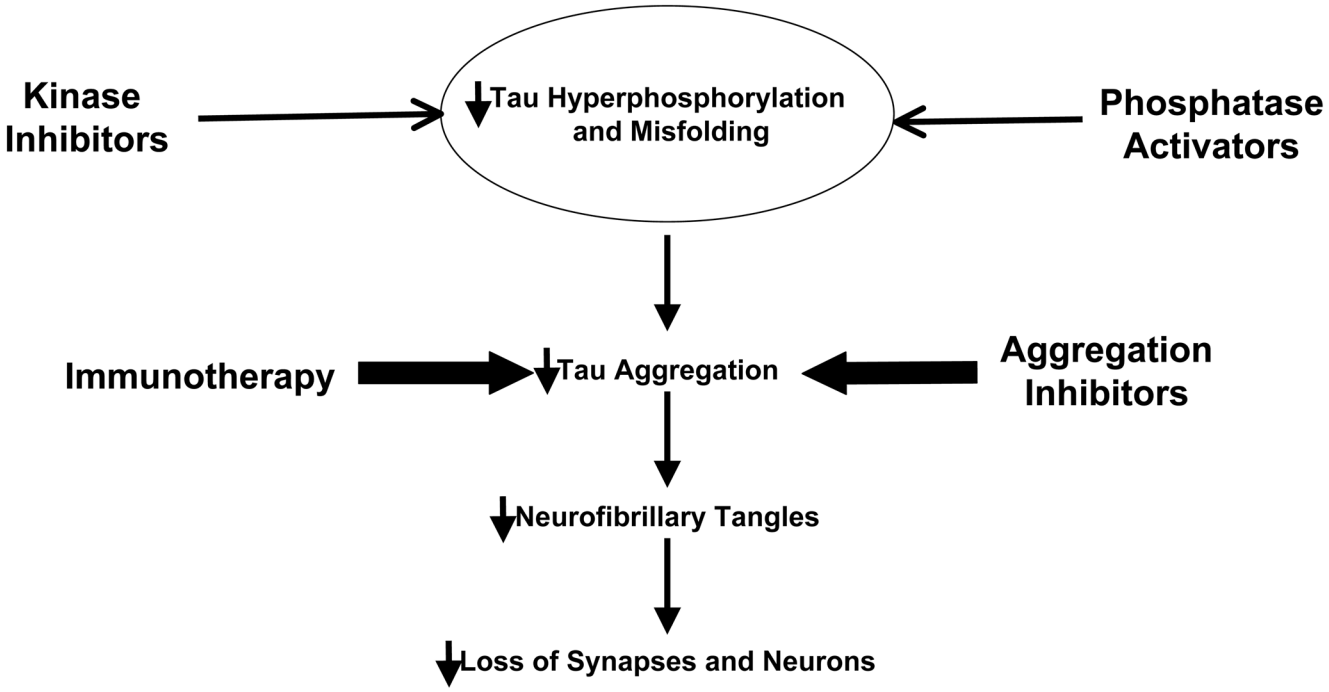

Figure 1. Tau based therapeutic strategies under development

Kinase inhibitors targeting GSK3β, MARK and cdk5 or phosphatase activators may reduce pathological tau phosphorylation. Small molecule aggregation inhibitors or immunotherapy may prevent the build-up and spread of toxic tau aggregates. These therapies should reduce the levels of tau oligomers, paired helical filaments and/or neurofibrillary tangles. Additionally, microtubule stabilizing drugs may compensate for loss of tau function by maintaining the integrity of the cytoskeleton. The overall aim of these direct or indirect approaches is to maintain synaptic connections and prevent neuronal loss.