

Targeting tau for Alzheimer's disease and related neurodegenerative disorders

There is no effective treatment for Alzheimer's disease (AD) even though the prevalence of this neurodegenerative disorder and its associated costs are growing along with an ageing population. This article discusses whether tau could become a target for the development of disease modifying therapeutics for Alzheimer's and other neurodegenerative disorders.

As several drug discovery programmes based on the amyloid cascade hypothesis have not been successful in meeting their clinical endpoints in late stage clinical studies, alternate targets for AD are being given closer attention. Tau protein has become an increasingly important target based on advances in understanding its role in AD progression. The importance of neurofibrillary tangles (NFT) as a histopathological marker of AD was established more than 100 years ago. Although NFT are composed primarily of aggregated tau protein, it was thought that tau aggregation was primarily a consequence of the disease process and not a major contributing factor for the initiation and progression of neurodegenerative disease. Progress in the field indicates that tau can be a causative factor in neurodegenerative diseases and is directly involved in the spread of pathology to neighbouring neurons making it an attractive target for development of disease modifying therapeutics for AD and numerous additional neurodegenerative diseases with intracellular aggregates of tau collectively called tauopathies.

Normal tau biology and function

In normal neurons tau is found mostly in the central nervous system in axons, the long processes that conduct impulses to other neurons, where it modulates the length and stability of microtubules, the major structural component of axons. An anal-

ogy often used to describe the function of tau on microtubules is that tau monomers are like ties on the microtubule track that is used to transport material to and from the cell body to the synapse at the end of the axon. Loss of synapses is correlated with cognitive decline in AD.

That tau protein is also found in other locations in cells and in other parts of the body besides the brain, suggesting that it has additional functions. Tau was found to accumulate in neuronal nuclei in response to stressful growth conditions where it protected DNA from damage. It has been known for some time that there is normally a low concentration of tau in the cerebrospinal fluid surrounding the brain, but it was only recently found that tau is transported out of neurons by mechanisms unrelated to cell damage leading to a new field of research studying its extracellular function.

Tau protein is unusual in that it normally lacks a stable folded structure but has domains that allow it to interact with itself and other proteins. There are six isoforms of tau produced in the brain that include different regions encoded by its gene produced by alternative splicing. The C-terminal region has either three or four microtubule binding repeats (3R or 4R) depending on the isoform that modulate the affinity of tau to the microtubule and its stabilising function. Disruption of the ratio of the isoforms produced can lead to the development of tauopathies. The

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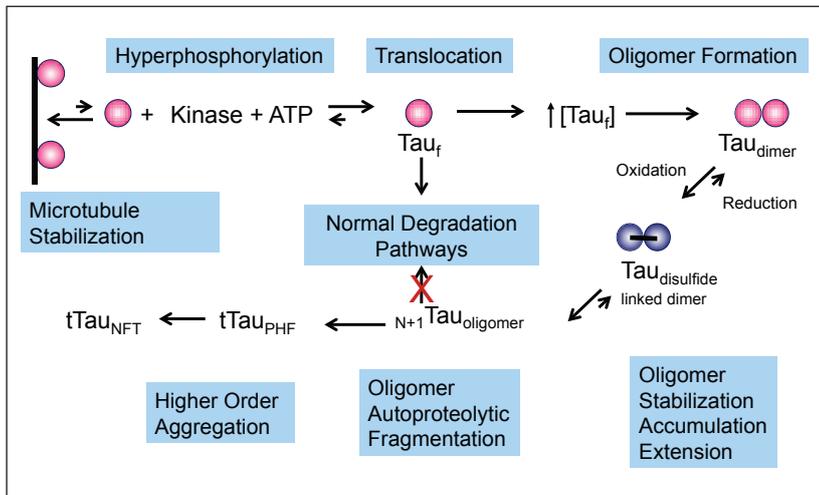


Figure 1

Tau aggregation mechanisms where Tau_f = free tau (not bound to microtubules); $\uparrow [Tau_f]$ = intracellular accumulation of tau; Tau_{dimer} = tau non-covalent self interaction (monomer-monomer); Tau_{dimer} = disulfide linked dimer; $N_{+1} Tau_{oligomer}$ = disulfide linked dimer (4R, 3R/4R, 3R), disulfide linked trimer, tetramer, etc (4R Tau, and 3R/4R tau); $tTau$ = truncated and full length 3R and 4R tau

3R isoforms have only one cysteine amino acid allowing them to self associate into disulfide linked dimers, but the 4R isoforms have two cysteines enabling them to form an intramolecular disulfide linkage or extended disulfide linked oligomers depending on the concentration of tau. Several reversible biochemical modifications of tau are also involved in modulating its function including phosphorylation, nitration, glycosylation, glycation, acetylation, ubiquitination and aggregation into small soluble aggregates called oligomers and progressively larger aggregates forming filaments and tangles. Proteolytic cleavage of tau is an irreversible modification that can produce toxic fragments and modulate the propensity of tau to aggregate. The novel finding that tau acquires a proteolytic function upon self-association into oligomers leading to its self-cleavage and its ability to cut other proteins also highlights that tau has multiple functions that play a role in normal and disease mechanisms.

Tau in neurodegenerative disease

Genetic confirmation for a neurodegenerative function for tau in disease came from the discovery that mutations in tau are sufficient to cause frontotemporal dementia. Work using mouse models of AD showed that tau mediates the synergistic cognitive decline caused by beta amyloid and the enzyme Fyn kinase involved in the regulation of synaptic function. Reduction in the levels of tau prevented the loss of memory in this model suggesting that tau reduction may have a therapeutic benefit. Although NFT are a pathological hallmark of AD, it is now recognised that smaller soluble aggregates of tau called oligomers are the structures causing toxicity to neurons, inhibition

of synaptic signalling and impairment of memory formation. In AD, the formation and distribution of tau aggregates follows a reproducible pattern progressing through the hippocampus, where short-term memory is formed, to the surrounding cortex. A number of recent experiments in cell and animal models have shown that tau can transmit its own pathology to neighbouring neurons demonstrating a direct role for tau in the progression of disease.

Tau forms disulfide linked oligomers under oxidative conditions such that a ladder of species is observed with non-reacted monomer, dimer (composed of two monomer subunits), trimer (composed of three monomer subunits), tetramer (composed of four monomer subunits) and higher order species (Figure 1). The proteolytic activity co-purifies with oligomers, but not tau monomer, demonstrating a structural requirement for the activity and a mechanism linking tau aggregation and tau loss of function and gain of toxicity. After purification, higher order oligomers undergo autoproteolysis such that the c-terminus and n-terminus regions are removed, while the central region that contains the microtubule binding domain maintains its self-association via disulfide linkages (tau protease). Amino acid sequences of cut sites generated by tau autoproteolytic activity have been characterised, and it has been demonstrated *in vitro* that tau protease can cleave peptides derived from other proteins containing the cut site motif such as tubulin, amyloid precursor protein (APP), and the neuropeptide alpha-endorphin. A disease model incorporating tau's autoproteolytic activity is shown in Figure 2. If proven to be part of the disease mechanism, the proteolytic activity of tau may be the central mechanism for its gain of toxic function. It is intriguing to speculate that tau proteolytic activity may play a role in the generation of amyloid pathology.

Therapeutic strategies

The concentration-dependent aggregation of tau in the cell body of neurons is facilitated by the hyperphosphorylation of tau at specific sites causing reduced affinity to microtubules and its displacement from axons. The consequences are loss of tau function as microtubule stabilisers and regulators of axonal transport, as well as gain of toxicity caused by the sequestration of normal tau into aggregates, the formation of toxic oligomers, as well as synaptic dysfunction in dendritic spines. A number of strategies are being pursued to counter tau hyperphosphorylation and to reduce tau aggregates.

Microtubule-stabilising agents

To compensate for the loss of tau function microtubule-stabilising small molecules, some of which were developed for treating cancer, neuroprotective peptides are being tested for AD. Although this approach may compensate for loss of tau function on microtubules it does not address the accumulation of toxic tau aggregates.

Modulation of tau phosphorylation

Hyperphosphorylation of tau leads to its dissociation from microtubules and accumulation in the cell body where it forms aggregates. The phosphorylation state of tau results from the combined activity of kinases adding phosphates and phosphatases removing phosphates. Both set of enzymes are being targeted to reduce tau phosphorylation.

Several kinases are involved in the phosphorylation of tau at numerous sites containing amino acids serine, threonine and tyrosine. Toxicity problems with this approach derive from the involvement of these enzymes in numerous biological processes and inhibitor specificity. Additionally, non-targeted kinases can compensate for the reduced activity of the targeted kinases.

A promising approach for increasing phosphatase activity on tau is to modulate the specificity of phosphatase PP2A for tau by upregulating the methylation of PP2A by inhibition of PP2A methyltransferase, the enzyme that demethylates PP2A. Normally PP2A is almost all methylated but in AD PP2A methylation is reduced in half. A non-toxic small molecule found in coffee was discovered as an inhibitor for PP2A methyltransferase and has shown efficacy in improving motor and cognitive performance in mouse models with tau pathology.

Tau aggregation inhibitors

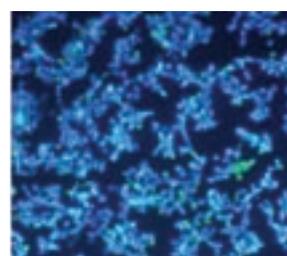
Although oligomeric species of tau are now thought to be the acutely toxic aggregates most assays for tau aggregation are based on fibril formation due to the initial characterisation of paired helical or straight filaments of tau in association with AD. A potential problem with this approach is that if compounds are selected that dissociate preformed large aggregates into smaller toxic oligomers then they may be detrimental. Fibril formation assays require relatively high concentrations of tau and a facilitator of aggregation such as heparin. The tau constructs in these assays are often limited to the microtubule binding region that contains the motifs necessary for fibril formation. Mutations found



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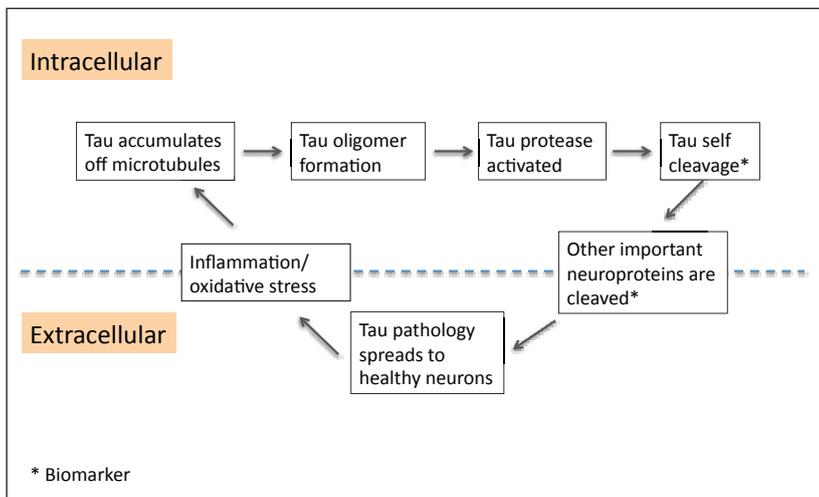


Figure 2
Model of tau pathology in AD stimulated by cellular stress and the spread of its pathology that occurs via an extracellular step

in frontotemporal dementia causing tau to become more prone to aggregation are also often introduced in the constructs. Fibril formation is measured by a shift in fluorescence of an intercalating reporter dye binding to beta sheet structures within tau fibrils. Many of these assay attributes are not physiologically relevant. There are no mutations in tau associated with AD, tau aggregation occurs within neurons but heparin is located outside of cells, and only a small region of tau is being used discounting the effects of the remaining parts of the protein. However, secondary assays and testing candidates in animal models producing tau aggregates help address some of these issues.

Recently an assay was developed to specifically screen for inhibitors of tau oligomer formation at the initial stages of tau self-association. The assay uses full length tau protein without mutations and without an extraneous aggregation facilitator or reporter dye. Oligomer formation occurs at room temperature within a few hours using tau concentrations more than an order of magnitude lower than the fibril formation assay. The assay has good signal to background and reproducibility characteristics and was used to screen a diverse library of drug-like compounds to generate hits for further development.

The challenges of this approach are that tau aggregation occurs intracellularly and tau concentrations in the somatodendritic compartments of a diseased neuron are presumably high. Thus a small molecule therapeutic not only has to cross the blood brain barrier, but must achieve a significantly high concentration within neurons and thus may be more prone to off target problems.

Extracellular tau oligomers

Purified tau oligomer species have been demonstrated to be neurotoxic in cell culture and to impair synaptic function *in vitro* in a dose dependent manner in long-term potentiation studies in mouse hippocampal slices, and memory formation *in vivo* in wild type (normal) mice, when introduced into the hippocampi. The observations that tau oligomers cause synaptic impairment in a dose dependent manner and impair memory formation *in vivo* demonstrate that extracellular tau oligomers are a therapeutic target for AD. It is presumed a drug that eliminates these species, or neutralises their affect would improve cognitive function at all stages of the disease.

Extracellular tau levels in AD are more than four orders of magnitude lower than intracellular tau concentrations and for this reason may represent a better pharmacological target. Small molecule approaches could be used to target extracellular tau oligomers but must have the ability to dissociate disulfide linked tau oligomers.

Clearance

Macroautophagy

Large intracellular protein aggregates can be degraded within membrane bound structures called autophagosomes. The immunosuppressant drug rapamycin has been found to induce the macroautophagy of human tau aggregates in a fly model supporting the approach for small molecule clearance of tau aggregates.

Hsp90 inhibitors

Cells contain a mechanism for either properly refolding misfolded proteins or targeting them for degradation. Hsp90 is a protein called a chaperone which binds to misfolded proteins and recruits other protein components of the chaperone system to refold these proteins. Hsp90 binds to hyperphosphorylated tau preventing its degradation by the proteasome. Small molecule inhibitors of Hsp90 preventing its interaction with tau have shown efficacy in mouse models of human tau aggregation.

Immunotherapeutic approaches

Both active and passive immunotherapeutic approaches for treatment of tauopathies are being studied to clear tau pathology and reverse behavioural deficits in mouse models. The active approach uses a tau antigen as a vaccine to stimulate an immune response, whereas the passive approach uses administration of purified antibodies against specific tau epitopes. A potential

harmful result of using an active approach is the induction of a neuroinflammatory response. However, a number of peptides containing hyperphosphorylated epitopes found in AD have been developed as vaccines and used in mouse models to successfully reduce the amount of NFT without causing neuroinflammation. Passive immunisation using antibodies against disease-specific epitopes have shown promise as well. Many AD specific modifications such as hyperphosphorylation are reversible and dynamic, but other modifications such as cleavage of tau are irreversible. Targeting the cut ends of tau specific for disease has the advantage of being not only a more stable target for clearance but also a less variable biomarker for measuring efficacy in pre-clinical and clinical studies. Targeting the cut ends of self-truncated tau oligomers containing the proteolytic activity may be particularly efficacious in clearing this toxic species. An immunotherapeutic approach targeting tau oligomers was recently validated in an animal model of tauopathy.

Tau proteolysis as a drug target

Cellular enzymes implicated in cutting tau include caspases, calpains, cathepsins and a thrombin-like protease in addition to tau's autoproteolytic activity. Regardless of the protease, there is a presumable irreversible loss of normal function of tau once it is truncated. Disulfide linked oligomers of tau can be observed in AD brain and cerebral spinal fluid (CSF) specimens, and show significant fragmentation. Small molecule approaches are under way to identify inhibitors of tau oligomer's intrinsic proteolytic activity. An advantage of this approach is that it is more straightforward to inhibit an enzymatic mechanism than aggregation. Additionally, inhibition of truncation can also prevent the formation of aggregation prone fragments and consequently tau aggregates.

Targeting the spread of tau pathology

There is mounting experimental evidence that tau plays a role in the spread of pathology from diseased to healthy neurons in AD and in chronic traumatic encephalopathy (CTE). It is presumed that extracellular tau plays a role in the mechanism of the transfer of pathology from a diseased to a healthy neuron. It is thus possible that inhibiting extracellular tau's intrinsic proteolytic activity directly using specific protease inhibitors, or indirectly via targeting tau oligomer formation or immunotherapeutic intervention, holds promise

for interrupting the mechanism of transfer of pathology. Such an approach, if successful, would be a disease modifying therapeutic intervention.

Conclusion

There are multiple approaches available for targeting abnormal tau species contributing to AD and related tauopathies. It is anticipated that these strategies will be advanced at a faster rate as a more direct role for tau in AD progression is now understood and as targets other than beta amyloid are receiving heightened interest. **DDW**

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