Potential neuroprotective strategies against tauopathy

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Abstract

Tauopathies are neurodegenerative diseases, including AD (Alzheimer’s disease) and FTLD-T (tau-positive frontotemporal lobar degeneration), with shared pathology presenting as accumulation of detergent-insoluble hyperphosphorylated tau deposits in the central nervous system. The currently available treatments for AD address only some of the symptoms, and do not significantly alter the progression of the disease, namely the development of protein aggregates and loss of functional neurons. The development of effective treatments for various tauopathies will require the identification of common mechanisms of tau neurotoxicity, and pathways that can be modulated to protect against neurodegeneration. Model organisms, such as Caenorhabditis elegans, provide methods for identifying novel genes and pathways that are involved in tau pathology and may be exploited for treatment of various tauopathies. In the present paper, we summarize data regarding characterization of MSUT2 (mammalian suppressor of tau pathology 2), a protein identified in a C. elegans tauopathy model and subsequently shown to modify tau toxicity in mammalian cell culture via the effects on autophagy pathways. MSUT2 represents a potential drug target for prevention of tau-related neurodegeneration.

Introduction

Tauopathies are diseases defined by accumulation of insoluble deposits of tau in the central nervous system. They are also characterized by an increase in hyperphosphorylated tau and degeneration of neurons [1]. Tau dysfunction is implicated in several neurodegenerative diseases, which exhibit overlapping, but distinct, sets of clinical and pathological symptoms [2]. For instance, CBD (corticobasal degeneration) is characterized by astrocytic tau deposits and neuronal degeneration in the basal ganglia. The main clinical signs of CBD are various movement disorders, including parkinsonism, apraxia and aphasia. On the other hand, FTLD (frontotemporal lobar degeneration) primarily affects neurons in the frontal and temporal lobes and presents behaviourally as loss of executive function. However, there is variability in the clinical syndrome exhibited by individual patients, and many cases do not fit the classical definition of any one tauopathy. Even in genetic forms of FTLD, the same tau mutation can cause a pure behavioural syndrome in one family member and motor symptoms in another [3].

The aetiology of tauopathies is as diverse as the clinical syndromes. Some are known to be caused by environmental factors, such as head trauma (chronic traumatic encephalopathy) or viral infection (postencephalitic parkinsonism). Others are caused by rare single gene mutations, as in the case of tau mutations in FTLD [4–6]. However, in most cases of sporadic tauopathy, the aetiology is unknown and much research is currently focused on the molecular basis for neurodegeneration. AD (Alzheimer’s disease), which affects approximately 26 million people worldwide, is the most common tauopathy and the leading cause of dementia. Currently available treatments for AD only address some of the symptoms, and do not significantly alter the progression of the disease, namely the development of protein aggregates and neuronal loss. The development of effective treatments for various tauopathies will require identification of common mechanisms of tau neurotoxicity, and pathways that can be modulated to protect against neurodegeneration.

Tau aggregation and neurotoxicity

Since insoluble deposits of tau are the common pathological finding in all tauopathies, it is reasonable to hypothesize that tau aggregation causes neurodegeneration. Indeed, the amount of tau NFTs (neurofibrillary tangles) correlates more closely with severity of AD than does the number of amyloid plaques [7]. Alternatively, it has been suggested that large tau aggregates are protective during early disease stages, and that increased numbers of aggregates late in disease are merely a marker of disease progression, rather than causative for neurodegeneration [8]. In this case, tau oligomers are likely to be the toxic species that causes neurodegeneration and...
other clinical symptoms. The mechanism of tau toxicity is not currently understood, but could be due to loss of normal tau function, gain of abnormal function or some combination of both. Tau was identified as an MT (microtubule)-associated protein that binds MTs and stabilizes the cytoskeleton [9,10]. It is involved in the maintenance of axonal and dendritic processes and in the regulation of axonal transport and other cellular trafficking functions [11]. When tau is sequestered in aggregates, it is not able to interact with MTs, and loss of MT stabilization may contribute to loss of neuronal function. However, other MT-binding proteins are redundant with tau in vivo, and complete knockout of the tau gene in mice does not produce overt developmental or behavioural phenotypes [12]. Several other tau-binding partners have been identified, such as the kinase Fyn, PLCγ (phospholipase Cγ) and HDAC6 (histone deacetylase 6) [13]. Tau pathology may be mediated through these other pathways, distinct from the role of tau in cytoskeletal maintenance.

If neuronal dysfunction is caused by tau aggregation, then it is important to examine the causes of tau aggregation. Some tau mutations (e.g. P301L) increase the rate of tau aggregation [14–16]. Other mutations (e.g. N279K) alter tau splicing, thus increasing the ratio of 4R:3R tau isoforms [17,18]. The 4R tau isoform forms aggregates more avidly than 3R tau, and thus altering the 4R:3R ratio could contribute indirectly to tau aggregation [19]. Some tauopathies are associated with selective aggregation of tau, such as Pick’s disease where 3R tau aggregates or CBD in which 4R tau aggregates. Post-translational modifications also contribute to tau aggregation. When tau is phosphorylated, its affinity for MTs is decreased and propensity to aggregate is increased [20,21]. Tau can also be cleaved by caspases and other proteases, and the resulting fragments are more prone to aggregation [22,23]. It is not known whether production of tau fragments by caspases is relevant to disease progression in humans, since they are variably present in both control and AD brain samples [24].

Regardless of the neurotoxic mechanism, tau pathology in AD shows a stereotypical pattern of progression from the transentorhinal to the entorhinal to the neocortical regions, which are connected synaptically [25]. Studies in tau transgenic animals have demonstrated that spreading of tau pathology can occur via transmission of fibrillar tau from a mouse line with abundant NFTs into mice that do not normally exhibit NFTs [26]. Cellular studies have confirmed the cell-to-cell transmission of tau aggregates and conversion of normal tau into pathological tau fibrils by a seeding-like mechanism [27–29]. Taken together, these studies do not clarify what the tau neurotoxic mechanism is in AD or other tauopathies. However, these studies do highlight a potential avenue for intervention and clarify how tau pathology propagates from one brain region to the next. Tau fibrils transmit the neurotoxic process from diseased cells to healthy cells thus recapitulating the pattern observed by Braak and Braak [25]. Consequently one neuroprotective approach would be to block cell-to-cell spreading. Further understanding of the mechanisms by which cells take up tau aggregates is needed.

Identification of protective pathways using Caenorhabditis elegans

Several treatment strategies based on current understanding of tau neurotoxicity have reached clinical trials. These treatments are intended to reduce tau phosphorylation, tau aggregation or total levels of tau. Although the results of these trials remain to be seen, deeper understanding of the pathways that contribute to tau neurotoxicity (and which may therefore be manipulated to provide protection) should be beneficial for development of future treatments. Model organisms provide the opportunity to examine the effects of tau on a simple nervous system with a rapid turnaround time. The nematode C. elegans provides the additional benefits of a completely mapped nervous system, short life cycle and ease of transgenic modification or in vivo RNAi (RNA interference) gene knockdown. A C. elegans model for tauopathy was generated by expressing the human FTLD mutant tau allele V337M in all neurons [30]. Despite significant differences between nematode and mammalian neurons, this C. elegans model exhibits hyperphosphorylation of tau similar to that observed in AD brains. In addition, progressive accumulation of aggregated tau and neurodegeneration develops with age. Behaviourally, this manifests in the animals as severely impaired movement abilities [Unc (uncoordinated) phenotype].

This model has been used in screens to identify genes and pathways that modify tau neurotoxicity. Using a library containing RNAi clones corresponding to every predicted gene in the C. elegans genome, a screen was conducted to identify genes for which reduction of function leads to enhancement of tau-induced movement defects [31]. These are genes whose normal function presumably provides some protection against the toxic effects of tau. A total of 60 genes were identified that specifically modify tau-induced movement defects, 38 of which have human homologues. Some of these genes (e.g. GSK-3β (glycogen synthase kinase 3β) [32]) were previously implicated in tau pathology, but the majority were novel. The tau modifiers fall into several functional classes, including phosphorylation, protein folding and stress response, proteolysis and neurotransmission. Seven genes were examined for levels of soluble and insoluble tau. In all cases, knockdown of gene function did not increase levels of either soluble or insoluble tau, indicating that the enhancement of tau toxicity is based on modification of the cellular response to tau rather than a simple increase in tau levels.

Another screening was conducted using the C. elegans V337M tau model to identify suppressors of the tau-induced movement defects [33,34]. Animals carrying the mutant tau transgene were mutagenized, and their offspring were examined for individuals with improved locomotion. In this way, sut (suppressor of tau) genes were identified.
Presumably, the normal function of these genes either contributes to tau toxicity or is permissible for the development of tau toxicity. This class of genes is particularly attractive as candidate drug targets for treatment of tauopathies. The first gene identified in this manner was sut-1, which encodes a novel nematode-specific protein [33]. sut-1 interacts genetically with unc-34, a conserved member of the Ena/VASP (vasodilator-stimulated phosphoprotein) family of proteins, and SUT-1 protein physically binds to UNC-34 in a region of the protein responsible for cytokoskeleton interactions. UNC-34 and other Ena/VASP family members regulate actin dynamics by inhibiting actin fibril capping, and are important for the control of dynamic actin structures (e.g. lamellipodia) involved in processes such as cell migration and axonal pathfinding [35]. Thus, although sut-1 does not have a mammalian homologue, its identification indicates the possible importance of unc-34/Mena and cytoskeletal functions in tau toxicity. Indeed, an interaction between tau and actin has been demonstrated in a Drosophila model of tauopathy that expresses human R406W mutant tau [36]. Tau was found to co-precipitate with filamentous actin, and actin aggregates (similar to Hirano bodies) were identified in the brains of the transgenic flies. In addition, overexpression of actin exacerbated neurodegeneration in this model, whereas reduction of actin was observed to be protective.

A second gene, sut-2, was also identified as a suppressor of tau-induced movement defects [34]. sut-2 encodes a zinc finger protein with conserved homologues in all species from yeast to humans. Homozygous loss of function of sut-2 in C. elegans reduces aggregation of V337M tau and protects against neurodegeneration. The locomotion of sut-2 mutant animals carrying the V337M tau transgene is almost indistinguishable from normal animals that do not express any human tau. In addition, overexpression of sut-2 exacerbates all of the tau-related phenotypes. The identification of sut-1 and sut-2 suggests that it should be possible to prevent tau neurotoxicity in humans using drugs to reduce the function of human homologues of sut genes. The screen for sut genes is ongoing in our laboratory, and the identification of additional genes will deepen our understanding of tau toxicity as well as provide a wider range of potential drug targets.

**Possible role of MSUT2 (mammalian suppressor of tau pathology 2) in tau clearance by autophagy**

MSUT2 is the mammalian homologue of the nematode SUT-2 protein. In both nematodes and mammals, these proteins are predominantly nuclear and co-localize with SC35-positive nuclear speckles, suggesting a possible function in RNA processing [34]. Analysis of post-mortem tissue from AD cases shows a clear reduction in neuronal MSUT2 levels in brain regions affected by tau pathology, but little change in regions lacking tau pathology. Conversely, regions lacking MSUT2 are not vulnerable to tau pathology in AD. Overexpression of human tau in both nematodes and HEK (human embryonic kidney)-293 cells results in a marked increase in MSUT2 protein. Both cell culture and post-mortem tissue studies suggest that MSUT2 levels may influence neuronal vulnerability to tau toxicity and aggregation [37].

In cultured human cells overexpressing tau (HEK-293 cells/tau), tau is recruited to aggresomes in response to proteasome inhibition. RNAi knockdown of MSUT2 in HEK-293/tau cells results in a dramatic reduction of insoluble tau from aggresome-containing cells. This suggests that loss of MSUT2 function allows increased clearance of aggregated tau (Figure 1A). To test whether increased tau clearance might be mediated by autophagy pathways, we examined HEK-293/tau cells expressing a fluorescent LC3 reporter that labels autophagosomes. We observed increased flux through the autophagy pathway with MSUT2 knockdown, mimicking the effects of proteasome inhibition. This indicates that the protective effect of reduced MSUT2 function against tau...
pathology may be due to increased tau clearance via increased autoprophagy of insoluble tau aggregates or oligomers.

Several different treatment strategies for reduction of tau toxicity are currently under investigation, which target different stages of the progression from tau dysfunction to neuronal death (Figure 1B). MSUT2 is a promising target for modulating the clearance of aggregated tau from neurons in various tauopathies. Future research should continue to characterize the function of MSUT2 in the brain, its interactions with tau in disease states and the identification of pharmacological interventions that reduce MSUT2 function in human tauopathy patients.

**Funding**

This work was supported by a Department of Veterans Affairs Merit Review Grant and by the National Institute of Neurological Disorders and Stroke [grant number R01NS064131] (to B.C.K.).

**References**


Received 12 March 2012
doi:10.1042/BST20120017