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The role of MSUT-2 in tau neurotoxicity: a target for neuroprotection in tauopathy?

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Abstract

We previously developed a transgenic *Caenorhabditis elegans* model of human tauopathy disorders by expressing human tau in nematode worm neurons to explore genetic pathways contributing to tau-induced neurodegeneration. This animal model recapitulates several hallmarks of human tauopathies, including altered behaviour, accumulation of detergent-insoluble phosphorylated tau protein and neurodegeneration. To identify genes required for tau neurotoxicity, we carried out a forward genetic screen for mutations that suppress tau neurotoxicity. We ultimately cloned the *sut-2* (suppressor of tau pathology-2) gene, mutations in which alleviate tau neurotoxicity in *C. elegans*. SUT-2 encodes a novel subtype of CCCH zinc-finger protein conserved across animal phyla. SUT-2 shares significant identity with the mammalian SUT-2 (MSUT-2). We identified components of the aggresome as binding partners of MSUT-2. Thus we hypothesize that MSUT-2 plays a role in the formation and/or clearance of protein aggregates. We are currently exploring the role of MSUT-2 in tauopathy using mammalian systems. The identification of *sut-2* as a gene required for tau neurotoxicity in *C. elegans* suggests new neuroprotective strategies targeting MSUT-2 that may be effective in modulating tau neurotoxicity in human tauopathy disorders.

Keywords

aggresome; Alzheimer's disease; neurodegeneration; neurofibrillary tangle; protein aggregation; tau

Introduction

Protein aggregation is a common pathological hallmark of many neurological disorders. In a subset of these diseases, including AD (Alzheimer's disease), the protein tau has been identified as the main component of aggregates called NFTs (neurofibrillary tangles) [1]. Specifically, NFTs contain insoluble deposits of hyperphosphorylated tau. Normal tau binds to tubulin and promotes MT (microtubule) formation and stability in neuronal axons. MTs are a principal component of the cytoskeleton, and are crucial for maintenance of neuronal morphology and cellular trafficking activities. Although mutations in tau have not been linked to AD, tau mutations have been shown to cause some cases of FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17) [2,3]. In some of these FTDP-17 cases, tau mutations appear to enhance NFT formation by increasing free tau

concentration, either by decreasing the ability of tau to bind MT [4–6], or by reducing the rate of tau degradation [7]. Other tau mutations have been shown to accelerate NFT formation by enhancing the rate of tau aggregation [8,9]. Although NFT formation is not sufficient to cause behavioural impairment and neuronal death [10], the common theme of protein aggregation in diverse neurodegenerative diseases suggests that an understanding of protein aggregation pathways will be crucial for the development of future disease treatments.

An invertebrate model of tau pathology

Several years ago, Kraemer et al. [11] developed a model for studying tauopathy in the nematode *Caenorhabditis elegans*. These animals express human tau containing the FTDP-17 V337M mutation in all neurons (Figures 1A and 1B). They exhibit movement defects and neurodegeneration that progressively worsens with age. Progressive accumulation of aggregated tau species and worsening of behavioural defects go hand in hand, suggesting that tau aggregation may drive progression of behavioural defects. Phosphorylation of tau was also observed at many of the same sites known to be modified in AD. Electron microscopy revealed degenerating neurons containing non-fibrillar tau aggregates.

One of the major benefits of *C. elegans* as a model organism is the ease of carrying out large genetic screens for modifiers of any phenotype of interest. By mutagenizing large populations of nematode worms expressing human tau, genes that modify the movement defect of these animals were identified [12,13]. One such gene is *sut-2* (suppressor of tau pathology-2), which, when homozygous for loss-of-function alleles, restores the movement of tau-expressing nematode worms to wild-type levels (Figure 1C).

sut-2 is a conserved ZF (zinc-finger) protein required for tau neurotoxicity

Loss of a single gene, *sut-2*, can nearly eliminate the toxic effects of mutant tau in our *C. elegans* model, including reducing aggregated tau, alleviating neurodegenerative changes, and ameliorating motor dysfunction [13]. This suggests that functional inhibition of a single protein could be a treatment for neurodegeneration in tauopathy disorders. SUT-2 has a homologous human protein, which we have named MSUT-2 (mammalian SUT-2) (Figure 2). However, little is known about SUT-2, its human homologue MSUT-2 or any of the closely related proteins which are conserved in other organisms. Investigation into SUT-2 may suggest how the entire class of SUT-2 homologues functions throughout the animal kingdom. Likewise, understanding how loss of *sut-2* can prevent tau neurotoxicity in nematode worms may suggest how human MSUT-2 participates in authentic tau pathogenesis of tauopathy disorders.

Nematode *sut-2* encodes a 740-amino-acid protein that contains five CCCH-type ZF domains, an NLS (nuclear localization signal) and an NES (nuclear export signal) [13]. Homology searches revealed a subfamily of ZF proteins that are distinguished by conservation of unique spacing between the predicted zinc-chelating residues. A single family member is present in all animal species for which sequence data are available. All identified SUT-2 homologues, including human MSUT-2, appear to contain the NLS and NES sequences, although the location of these sequences within the proteins is not conserved. In addition, there is a novel homology domain at the N-terminus which is unique to this subfamily of ZF proteins.

SUT-2 and MSUT-2 may function in mRNA processing and/or aggresome formation

Limited evidence exists regarding the biological function of SUT-2 family members. One study of MSUT-2 (previously known as ZC3H14) indicated that the ZF domains of MSUT-2 bind specifically to poly(A) RNA [14]. Poly(A)-binding proteins influence gene expression by regulating the stability, translation, and localization of mRNA transcripts. Both SUT-2 [13] and MSUT-2 [15] have been shown to localize to the nucleus, but the presence of the NES in both proteins may suggest shuttling between the nucleus and the cytoplasm. MSUT-2 was also shown to co-localize with nuclear speckles [15], structures that represent sites of active transcription and mRNA processing [16].

Additional clues to the function of the SUT-2 family of ZF proteins have come from protein-binding assays. SUT-2 was shown to interact *in vitro* with ZYG-12, the only *C. elegans* HOOK protein, and loss of ZYG-12 causes up-regulation of SUT-2 protein *in vivo* [13]. Of the three HOOK proteins present in humans, MSUT-2 only binds specifically to HOOK2 [13]. HOOK proteins contain a MT-binding domain and a coiled-coil protein-interaction domain, and function as a link between various cellular structures and the cytoskeleton [17–21]. HOOK2 has been implicated specifically in centrosome function and aggresome formation [22]. Aggresomes form when misfolded proteins and protein degradation machinery are transported along MTs and accumulate at the centrosome [23]. It has been suggested that aggresome formation is a protective mechanism for sequestering toxic proteins and promoting their degradation via an autophagy-mediated transfer to the lysosome [24].

Possible role of aggresomes in neurodegenerative disease

Aggresomes have been implicated in neurodegenerative disease because of similarities between inclusion bodies observed in diseased human brain tissue and the aggresomes generated in cell culture models of protein misfolding [24,25]. Proteins that are mutated in several hereditary neurodegenerative diseases have been shown to accumulate in aggresomes, including presenilin-1 [23], huntingtin [26], α -synuclein [27] and superoxide dismutase [28]. In addition, mutations in DJ-1 known to cause Parkinson's disease have been shown to interfere with aggresome formation [29]. Finally, abnormal laforin and malin proteins cause Lafora disease, an inherited epilepsy with progressive neurodegeneration, and are recruited to the aggresome where they are normally required for clearance of misfolded protein aggregates [30].

Removal of misfolded proteins in neuronal cells is particularly important as these cells are post-mitotic, making them more susceptible to accumulation of protein aggregates. The first line of defence against accumulation of misfolded proteins is the ubiquitin–proteasome system [31]. When this system is overwhelmed, or is inhibited experimentally, misfolded proteins accumulate into potentially toxic aggregates [32]. The aggresome–autophagy system represents the next line of protection against accumulation of these protein aggregates [24].

Aggresome formation requires a functional MT cytoskeleton for transport of aggregated proteins and proteasome components to the centrosome [23]. Since tau is responsible for stabilizing MTs, disruption of tau's ability to bind MTs may impair the transport of toxic species (including tau itself) into aggresomes. Furthermore, it was recently observed that tau interacts with HDAC6 (histone deacetylase 6), a protein required for normal aggresome formation [33]. Evidence is accumulating implicating HDAC6 as a major regulator of aggresome formation [34]. One mechanism by which HDAC6 exerts control is by providing

a link between polyubiquitinated proteins and MTs via the dynein motor complex [35]. Excess tau protein inhibits HDAC6 function and disrupts aggresome function [36]. Thus abnormal tau would be expected to induce a feedback loop whereby aggresome formation is inhibited, reducing the ability of the cell to eliminate tau aggregates.

A model for tau aggregation and neurotoxicity

Normal tau is predominantly bound to MTs, but disruption of this association due to phosphorylation, tau mutation or the presence of misfolded proteins such as pathological amyloid β -peptide leads to an increase in free tau concentration (Figure 3). Free tau can be degraded by the ubiquitin–proteasome system, but if this system is overwhelmed by an excess of misfolded proteins, some tau will aggregate into oligomers and protofilaments [37]. Given enough time, tau protofilaments will form the NFTs observed in the brains of AD patients. However, some protofilaments will be transported via MTs to aggresomes, and subsequently eliminated by autophagy and lysosomal proteolysis. Since NFT formation is not sufficient to cause disease symptoms [10,38], and aggresome formation appears to be a protective mechanism [24], it has been proposed that tau oligomers, rather than larger aggregates, are toxic to the cell. Consistent with this, tau oligomer formation has been correlated with the severity of behavioural defects in mouse models of AD [39].

Loss of SUT-2 in *C. elegans* prevents the accumulation of tau protein. We hypothesize that *sut-2* ameliorates tau pathology by affecting centrosome and/or aggresome function, via its interaction with the HOOK protein ZYG-12. One possibility is that, in *sut-2* mutant animals, the efficiency of tau degradation via aggresome autophagy is increased. On the basis of the conservation between MSUT-2 and SUT-2, and the observation that MSUT-2 also interacts with HOOK2, MSUT-2 is an attractive candidate for the treatment of tauopathies in humans. Future work will examine the phenotype of MSUT-2-knockout mice and whether loss of MSUT-2 function can suppress the behavioural defects of various mouse AD models. In addition, the potential role of MSUT-2 in cell culture models of aggresome formation will be determined. MSUT-2 represents a promising target for pharmacological interventions that inhibit tau pathology.

Abbreviations used

AD	Alzheimer's disease
FTDP-17	frontotemporal dementia with parkinsonism linked to chromosome 17
HDAC6	histone deacetylase 6
MT	microtubule
NES	nuclear export signal
NFT	neurofibrillary tangle
NLS	nuclear localization signal
sut-2	suppressor of tau pathology-2
MSUT-2	mammalian SUT-2
ZF	zinc finger

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Figure 1. Loss of *sut-2* rescues neurotoxicity caused by expression of mutant human tau in *C. elegans*

(A) The *aex-3* promoter drives expression of tau isoform 1N4R in all neurons. The tau cDNA contains the V337M mutation which was identified in FTDP-17 patients and reduces the ability of tau to bind MTs. (B) Animals expressing the tau transgene exhibit movement defects and neurodegeneration that progressively worsens with age. Clearing of the bacterial lawn around the head and the presence of several eggs next to the animal indicate that it has not moved away from this area for an extended period of time. (C) Mutations in *sut-2* restore the movement of tau-expressing animals to wild-type levels. Sinusoidal tracks in the bacterial lawn indicate normal locomotion.

Figure 2. Domain structure of SUT-2 and MSUT-2 proteins

Nematode SUT-2 and human MSUT-2 contain a region of homology (S2NH, SUT-2 N-terminal homology domain) near their N-termini that is unique to this family of proteins. Both proteins contain putative NLS and NES sequences, but the location of these is not conserved. Five ZF domains are located near the C-termini and are distinguished from other ZFs by unique spacing of the zinc-chelating residues. The first two ZFs have the sequence CX₅CX₅CX₃H, and the last three have the sequence CX₅CX₄CX₃H.

Figure 3. Proposed model for tau metabolism

Free tau that is not degraded by the ubiquitin–proteasome system will form highly toxic tau oligomers and protofilaments. Protofilaments will form into NFTs given enough time, unless they can be degraded by the aggresome–autophagy system. We propose that MSUT-2 regulates the formation of aggresomes via its interaction with HOOK2. *Low neurotoxicity, low surface area per aggregated molecule; **moderate neurotoxicity, moderate surface area per aggregated molecule; ***high neurotoxicity, high surface area per aggregated molecule. Aβ, amyloid β-peptide.