Caenorhabditis elegans and Tau Toxicity

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Relevant information on the molecular basis of human neurodegeneration in vivo can be obtained using the nematode *Caenorhabditis elegans* (*C. elegans)*. Two main approaches can be applied: the overexpression of genes/proteins leading to neuronal dysfunction and death and studies in which proteins prone to misfolding are exogenously administered to induce a neurotoxic phenotype. These approaches can be employed to screen drugs and small molecules that can interact with the biogenesis and dynamics of formation of tau aggregates and to analyze their interactions with other cellular proteins.

Keywords: tau ; tauopathy ; Caenorhabditis elegans

1. Introduction

For many neurodegenerative diseases—including Alzheimer's disease (AD), progressive supranuclear palsy, Pick's disease, cortico-basal degeneration, and post-encephalitic parkinsonism—the ability of proteins to change their conformation and generate insoluble deposits in the brain of patients is believed to play a crucial role in etiopathology [1][2]. This is also true for frontotemporal dementia (FTD), a heterogeneous group of progressive brain disorders due to selective frontal and/or temporal lobe atrophy and frontotemporal lobar degeneration (FTLD) and associated with changes in behavior, personality and language. These diseases are grouped under the common name of "tauopathies" because filamentous tau inclusions, called neurofibrillary tangles [3], were identified as the major hallmark.

Tau is a natively unfolded, microtubule-binding protein that promotes tubulin polymerization and stabilizes microtubules. This protein, encoded by the microtubule-associated protein tau (*MAPT*) gene, plays a crucial role in preserving the integrity of neurons and axonal transport [4][5]. Six tau isoforms are present in the human brain, which differ in terms of the presence of three (R3) or four (R4) repeated sequences placed at the carboxy-terminal end. These repeated regions are encoded by exons 9–12 of the *MAPT* gene and represent functional microtubule binding domains. The ratio between the 3R and 4R isoforms is equal in normal subjects, whereas it is altered in the brain under pathological conditions [6].

The key role of tau in driving tauopathies is supported by the fact that mutations in *MAPT* cause autosomal dominant FTD and Parkinsonism linked to chromosome 17 [7]. Among the different pathogenic *MAPT* mutations described, most fall into the genetic regions encoding for tau repeats leading to changes of the R3/R4 ratio and the functional properties of the protein, thus increasing its aggregation propensity [8]. Substitutions or deletions of a single tau amino acid in the microtubule-binding domain modify the capability of the protein to bind to microtubules [9]. The most widespread and best characterized *MAPT* mutation is the one resulting from the substitution of the proline at position 301 with lysine (P301L) [10].

The roles of the majority of *MAPT* mutations in driving tauopathies has been demonstrated employing genetic analysis, neuropathological tests and biochemical studies on tau. However, the pathogenic effects of some other mutations remain controversial. The substitution of a single amino acid on the same *MAPT* codon may differently affect the early tau mislocalization and solubility in vivo. Increasing evidence supports the active role of tau oligomers, more than fibrillar inclusions, in causing the detrimental neurodegeneration of tau pathologies. Thus, *MAPT* mutations affecting tau oligomerization can significantly modify its toxicity.

Another relevant aspect of the tauopathy progression is represented by the ability of conformational abnormal tau to spread from cell to cell in a prion-like manner [11]. However, the cellular mechanisms mediating this spread and their involvement in the neurodegeneration process remain to be clarified. Our limited understanding of whether the molecular identity of tau, its phosphorylation degree, and its different isoforms can actively contribute to the clinical phenotype are in part the reason for the lack of an effective therapy for tauopathies.

In an attempt to rapidly obtain relevant information in vivo, we and others have recently established novel pre-clinical models involving the use of the non-parasitic nematode *C. elegans*. Although distant from vertebrates, some neuronal functions and genes encoding enzymes leading to the production of various neurotransmitters—such as acetylcholine, glutamate, serotonin, dopamine, and α-aminobutyric acid—and other elements involved in synaptic transmission, are conserved in worms [12].

2. Transgenic *C. elegans* Models of Tauopathy

Various transgenic *C. elegans* strains expressing human WT or mutated tau have been generated in the past few decades as new models of tauopathy [13][14][15][16][17]. Although *C. elegans* has a tau homolog protein with tau-like repeats (PTL-1) and a high level of sequence homology with the repeat region of mammalian microtubule-associated protein (MAP)2, MAP4, and tau [18][19][20], these transgenic strains are useful for deciphering the role of hyperphosphorylation and conformational changes in human tau within the context of neurodegeneration. In fact, PTL-1 is expressed only in a small subset of neurons, and its loss or mutation does not recapitulate tau pathology [21][22][23]. The motility of *C. elegans* is coordinated by a precise interaction between sensory and motor neurons and body wall muscle cells. Different promoters were employed to express the human tau WT at the pan-neuronal level, and the transgenic worms exhibited progressive uncoordinated movement/locomotion accompanied by accumulation of insoluble tau [17][19][24] (Table 1). When human tau was expressed in six mechanosensory neurons—a subset of neurons governing the touch response of worms—a decrease in the touch response, accompanied by neuritic abnormalities and microtubular loss, was observed [18] (Table 1). Worms expressing mutant human tau associated with cases of FTD and Parkinsonism linked to chromosome 17 (P301L, V337M, R406W, F3ΔK280, and A152T), as well as hyperphosphorylated tau, had more severe phenotypes and a greater accumulation of insoluble tau than *C. elegans* expressing tau WT [25][26][27]. More recently, Butler et al. reported that the phosphorylation of threonine 152 in transgenic worms expressing A152T tau exerts a relevant role in neuronal toxicity via impaired axonal trafficking. On the one hand, these findings helped to elucidate the mechanism underlying the ability of mutated tau to mediate the neurodegeneration in vivo; on the other, they confirmed the complexity of the mechanisms that regulate tau proteotoxicity.

Table 1. Transgenic *C. elegans* models of tauopathies related to microtubule-associated protein tau (*MAPT)* gene mutation.

Promoter::transgene	Expression pattern	Phenotype	Reference
Paex-3::tau WT *Paex-3::tau* V337M *Paex-3::tau* P301L	Pan-neuronal	Uncoordinated movement Nerve cord degeneration Insoluble tau accumulation	*Kraemer et al., 2003*
Pmec-7::tau WT *Pmec-7::tau* P301L *Pmec-7::tau* R406W	Mechanosensory neurons	Decrease in touch response Neuritic abnormalities and microtubular loss Tau accumulation	*Miyasaka et al., 2005*
Prgef-1::tauWT *Prfef-1::tau PHP* °	Pan-neuronal	Uncoordinated locomotion Defect in motor neuronal development	*Brandt et al., 2009*
Prab-3::tau F3ΔK280 *Prab-3::tau*F3ΔK280-PP*	Pan-neuronal	Locomotion impairment Motor neuron damage Tau aggregation	*Fatouros et al., 2012*

Punc-119::tau WT *Punc-119::tau* R406W	Pan-neuronal	Uncoordinated movement Neuritic abnormalities and microtubular loss	*Miyasaka et al., 2016*
Psnb-1::tauWT *Psnb-1::tauA152T*	Pan-neuronal	Locomotion impairment Paralysis Neuronal dysfunction	*Pir et al., 2016*
Paex-3::tauWT *Paex-3::tauV363A* *Paex-3::tauV363I*	Pan-neuronal	Locomotion impairment Pharyngeal dysfunction Insoluble tau accumulation Synaptic impairment	*Morelli et al., 2018*
Paex-3::tauWT *Paex-3::tauA152T* *Paex-3::tauA152E*	Pan-neuronal	Developmental toxicity Impaired retrograde axonal transport	*Butler et al., 2019*

° PHP tau = Hyperphosphorylated tau. Codons for S198, S199, S202, T231, S235, S396, S404, S409, S413 were changed to glutamate.* F3ΔK280-PP is and anti-aggregating strain due to the I277P and I308P substitutions.

We have recently generated two additional strains expressing at the pan-neuronal level human 2N4R tau carrying a V363A or V363I substitution (Table 1). These mutations, identified in patients with FTLD presenting different atypical clinical phenotypes, are caused by two mutations on the same codon of the *MAPT* gene. V363I substitution was previously described in asymptomatic subjects, thus raising uncertainties about its relevance in pathogenesis [28][29]. Studies in vitro have indicated that the two substitutions differently affected microtubule polymerization and, unlike other *MAPT* mutations, resulted in the production of tau with a high propensity to form oligomers. These two new *C. elegans* strains were employed to elucidate whether the expression of the mutant genes resulted in gene-specific disease hallmarks. To this end, their functional and biochemical characteristics were compared to those of transgenic nematodes expressing human 2N4R tau WT. We found that the V363A and V363I mutations differently affected in vivo, in transgenic worms, the expression of tau at neuronal level, the amount of protein produced, and its phosphorylation degree. Like other transgenic *C. elegans* strains expressing human tau whose sequence was deduced from patients with FTD and Parkinsonism linked to chromosome 17, tau carrying a V363A or V363I mutation caused a motility defect and a comparable shortening of the worm's lifespan [27]. In addition, in V363A nematodes, we observed a peculiar pharyngeal dysfunction as a consequence of an effect on neurons grouped in the nerve ring around the pharynx. This outcome had never been previously observed with other mutations, and it was later also reported in A152T and A152E strains. V363A and V363I tau differently affected the synaptic transmission: V363A caused a presynaptic defect involving both motor and pharyngeal neurons, whereas V363I induced only a postsynaptic defect that resulted in motor neuronal dysfunction. As observed in vitro, the two mutations differently influenced the ability of tau to misfold in vivo and form soluble oligomers. In particular, V363A substitution promotes the formation of more dimeric/trimeric tau assemblies and fewer monomers than V363I. Given that V363A mutation is the most toxic and phosphorylated, this finding points to the key role of soluble tau oligomers in driving the proteotoxic process involved in tauopathy.

3. *C. elegans* Recognizes the Toxic Component of Tau

To study the relationship between the toxicity and structure of amyloidogenic proteins, as well as the pathogenetic mechanisms responsible for the formation of tissue deposits, different experimental approaches can be applied. These mainly involve in vitro studies aimed at recognizing and characterizing conformational states and evaluating the toxicity of different protein assemblies. Particular attention is paid to the formation of soluble oligomers, very reactive intermediates formed during the protein aggregation process, which are more toxic than the end-stage fibrillar species. We have developed a totally innovative method in which *C. elegans* can be used as a "biosensor" able to react quickly and

specifically to the toxic forms of amyloidogenic proteins by developing specific behavioral dysfunctions. This method involves the direct administration to ancestral N2 nematodes of an amyloidogenic protein obtained by recombinant production or purified from the biological fluids of diseased patients, such as blood and urine. Alterations in *C. elegans* behavior are then assayed to monitor the onset of proteotoxic effect. The biophysical and biochemical characterization of the protein is fundamental in linking the toxicity with a specific conformational state.

The exogenous administration of amyloidogenic proteins to *C. elegans* has also been recently applied to elucidate the possible relationship between the conformational state of tau and its biological effects. First, soluble or insoluble aggregated forms of recombinant WT or P301L-mutated protein, whose conformational state was fully characterized using different biophysical and biochemical approaches, were employed. Interestingly, worms treated with oligomeric tau but not monomeric protein did not develop a pharyngeal defect but exhibited a neuromuscular dysfunction that appeared 48 h after the administration and worsened over time. The reason why tau does not act as pharyngeal stressor and results in a peculiar motoneuronal toxic phenotype remains to be clarified. The roles of ingestion, absorption and/or digestion also remain to be explained. WT and P301L tau caused a comparable dose- and time-dependent toxic effect, suggesting that the conformational state of tau, more than sequence, is relevant to the onset of motility deficit in worms. Using aldicarb and levamisole, two pharmacological compounds employed to establish the involvement of synaptic dysfunction in neuromuscular defects, we also demonstrated that oligomeric tau impaired the synaptic transmission in *C. elegans*, affecting both pre- and post-synaptic cholinergic neuronal function. These defects are comparable to those scored in transgenic worms expressing WT or mutated tau [18], suggesting that the toxicity observed using the two models may involve similar mechanisms. To better validate our observations, we conceived the use of brain homogenates from nine-month-old P301L transgenic mice, which express high levels of human hyperphosphorylated tau and represent a well-characterized animal model of tauopathy. Brains from non-transgenic mice of the same age were employed as negative controls. We observed that the administration to worms of brain homogenates from P301L mice, but not non-transgenic ones, caused a neuromuscular defect comparable to that observed with oligomeric recombinant tau. The connection between misfolded/oligomeric tau and neuronal impairment was supported by the fact that P301L homogenates were no longer toxic when incubated with anti-tau antibodies. These results indicate that the toxic form of tau that accumulates over time in an animal model of tauopathy damages the *C. elegans'* neuromuscular control, indicating a crucial role of abnormal tau conformers in chronic neurodegeneration. This worm-based approach can be employed to gain insights into the complex molecular interactions that drive neurological dysfunction and may represent an experimental platform to screen for pharmacological agents.

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