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Tau aggregation and toxicity in tauopathic neurodegenerative diseases

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Abstract

Since its discovery as a structural component of neurofibrillary lesions of Alzheimer's disease more than twenty years ago, tau protein has been implicated in the cascade of events associated with neurodegeneration. Specifically, the “tau hypothesis” posits that dysfunction of tau, which occurs in response to unknown stimuli, results in its intracellular assembly into filaments that eventually prove toxic to the cells that produce them. The tau hypothesis is supported by numerous neuropathological and genetic observations of authentic human disease cases. However, experiments designed to study aggregate toxicity in biological models suggest that some aggregate species may be inert or could potentially serve a neuroprotective function. Distinguishing these possibilities experimentally has been complicated by currently available biological models, which do not fully recapitulate aggregation conditions seen in disease. Additional model systems which better approximate physiological conditions may help elucidate the molecular mechanisms involved in aggregation associated toxicity. Here we examine the accumulated evidence linking aggregation and neurodegeneration, and experimental approaches to the problem of tau aggregation-mediated toxicity.

Keywords

tau; aggregation; protein structure; neurofibrillary tangle; microtubules

1. Tau aggregates as markers of neurodegeneration

Aggregation of protein monomers into filamentous inclusions accompanies many human disorders, including neurodegenerative diseases [12]. Classically, the proteins in these aggregates adopt cross- β -sheet conformation, characterized by parallel, in-register β -sheets that run approximately perpendicular to the long axes of the filaments [53]. Mature tau filaments isolated from Alzheimer's disease (AD) brain also conform to this structural model [6]. They are termed “paired helical filaments” (PHFs) because they appear to consist of two protofilaments wound around each other [36]. Consistent with this morphology, PHFs have a mass-per-unit length corresponding to ~ 2 tau molecules per β -sheet spacing [60]. Other, less-well characterized granular tau aggregates also can be isolated from AD tissue [43]. Although these differ morphologically from PHFs, they appear to share enriched β -sheet structure [43]. PHF formation is a useful marker for AD progression, because intracellular deposition of tau filaments in the form of neurofibrillary lesions correlates temporally with cognitive decline and neurodegeneration [23,25]. The correlations are stronger than those involving other aggregation products observed in AD, such as between neurodegeneration and β -amyloid

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plaque burden [25]. In fact, neurofibrillary lesion formation precedes plaque deposition in early stage disease [54]. The lesions reflect tau aggregation occurring in neuronal cell bodies (neurofibrillary tangles) and processes (neuropil threads and dystrophic neurites). As disease progresses, aggregates entirely fill cell bodies and apical dendrites, and eventually survive the death of the neuron, appearing as extracellular “ghost tangles”. Tau aggregation also correlates spatially with the onset of dementia, implicating loss of neuronal function in the frontal cortex as being most closely associated with the loss of “executive control” function that characterizes dementia [50]. The spatial and temporal correlations between tau aggregate deposition and dementia have been leveraged to differentially diagnose and stage AD in postmortem brain [8]. Together, these correlations suggest that tau aggregation is positioned to serve as a potential mediator of AD-associated neurodegeneration, by either creating novel toxic species (gain-of-function toxicity), or by interfering with the normal function of tau protein (loss-of-function toxicity).

2. Complexities of the tau system

Despite the strengths of these correlations, the tau system presents unique complications for elucidating pathways of aggregation and for critically assessing whether gain-of-function or loss-of-function toxicity is associated with a specific tau species formed during disease. First, tau proteins contain extensive structural diversity. Transcripts of the tau gene (*MAPT*, located on chromosome 17) are alternatively spliced to yield the six human tau isoforms found in the central nervous system [24]. These isoforms, which vary in size from 352 to 441 amino acid residues, are related by the presence or absence of sequences encoded by exons 2, 3, or 10. Inclusion of the imperfect repeat region encoding exon 10 leads to expression of tau containing four microtubule-binding repeats (4R tau: 0N4R, 1N4R, 2N4R), while exclusion of exon 10 results in splicing products expressing tau with three microtubule-binding repeats (3R tau: 0N3R, 1N3R, 2N3R). Isolated tau isoforms differ in binding affinity for their physiological binding partner, tubulin polymer [26], and also in their aggregation propensity [37]. In addition, each isoform is extensively modified post-translationally at >20 phosphorylation sites, as well as multiple ubiquitination and O-GlcNAcylation sites [18,42]. For example, mature tau aggregates composed of all six isoforms contain 7 – 8 mol/mol covalently bound phosphate, representing a 3 – 4 fold increase relative to normal tau [38,40]. The presentation of certain phosphoepitopes could be a critical component of gain-of-function toxicity irrespective of effects on aggregation propensity. The interplay among these diverse modifications, and their effects on toxicity, is not fully understood and difficult to model in a comprehensive way.

Second, tau is found in the cytoplasm where it interacts with tubulin polymers in a crowded molecular environment. Normal tau is present at low micromolar concentrations in the presence of a vast excess of tubulin dimer [19]. Under physiological conditions, therefore, most tau should be microtubule associated, and unable to enter into aggregation reactions. However, tau-tubulin binding affinity is depressed by tau phosphorylation [7,9], providing a mechanism for regulation of bound tau levels and for liberation of free tau so that it can enter into aggregation, turnover, and other reactions. Occupancy of at least a subset of phosphorylation sites precedes aggregation, but others appear to accompany or follow lesion maturation [3, 35]. Once liberated, tau monomers aggregate in both cell bodies and processes, with the latter predominating in AD [44]. It is conceivable that aggregates are more toxic to one compartment than to another. In AD frontal cortex, for example, neuronal processes retract well before cell death [52]. In fact, cell bodies may survive for decades while harboring aggregates [46], and so disruption of intracellular transport or other aspects of normal biology in neuronal processes may be especially relevant for the synaptic loss and cognitive decline seen in disease.

Third, tau can enter into reactions other than aggregation, including binding reactions with the SH3 domains of various signal transduction enzymes [56], with other microtubule associated

proteins [1], and with the active sites of proteases leading to tau fragmentation [2,48]. These interactions can generate toxicity unrelated to aggregation and therefore complicate interpretation of modeling studies. For example, under conditions that promote these interactions, sequestration of tau in the form of aggregates would appear to be neuroprotective.

Fourth, tau aggregate morphology is not restricted to PHFs, with significant differences in twist frequency and mass-per-unit length found in some frontotemporal lobar degeneration diseases relative to AD [41]. Oligomeric aggregates also have been detected in AD brain, including species with the migration pattern of dimers when analyzed by SDS-polyacrylamide electrophoresis [5]. Although the effect of aggregate morphology on toxicity is unknown, aggregate size may be a major variable in gain-of-function toxicity. For example, β -sheet enriched oligomers composed of poly-glutamine proteins or β -amyloid peptide exhibit cytotoxicity [14,47], perhaps via pathological β -sheet interactions with cellular proteins or membranes [57]. Filamentous morphologies can arise from nucleation dependent kinetics, which favor filament extension over nascent filament formation, and the accumulation of large aggregates over small oligomers [16]. Nonetheless, the pathway of tau aggregation *in vivo* is not established, and it is possible that an isodesmic or other non-nucleated pathway capable of supporting dimer or small oligomer accumulation operates in parallel. Overall, the complexity of tau biology suggests that tau misfunction could contribute to toxicity through multiple mechanisms that are not necessarily limited to aggregate formation.

3. Evidence supporting aggregation-associated toxicity

Although tau mutations have not been directly linked to AD, genetic studies of frontotemporal lobar degeneration have identified >30 *MAPT* mutations that lead to tau aggregation and neurodegeneration [59]. Tau pathology in these cases consists of filamentous hyperphosphorylated tau inclusions in the absence of other amyloid lesions, demonstrating that tau misfunction alone can trigger tau pathology. Tau mutations cluster into two categories: 1) exonic mutations which change tau primary structure, and 2) intronic mutations which alter exon 10 splicing [59]. Most intronic mutations increase inclusion of exon 10 at the mRNA level, leading to increased expression of 4R relative to 3R tau species. Intronic mutations favoring expression of 3R tau may reflect polymorphisms not linked to disease [49]. 4R isoforms are far more aggregation prone than 3R isoforms, and within a single isoform background, many exonic mutations increase aggregation propensity still further [22]. These data support a link between aggregation propensity and disease onset. Indeed, aggregation propensity may explain why tau haplotypes favoring 4R expression patterns associate with tauopathy [59], whereas expression patterns that favor 3R tau, such as found in some primates [31], are resistant to the development of tauopathy. Tau isoform expression may be perturbed in AD as well, where inclusion of exon 10 is preferentially found in some brain regions susceptible to neurofibrillary lesion formation [17]. Thus, the aggregation promoting activity of 4R tau may contribute to AD pathogenesis as well. The effects of aggregation propensity appear to synergize with aging, since tauopathy mutations typically lead to disease in the sixth or later decades of life.

Additional support for a role of aggregation in toxicity comes from observations on human fetal tau, which is hyperphosphorylated to similar stoichiometries and on similar sites as in AD [32,55], but does not form aggregates or lead to tauopathy. Fetal tau is exclusively 3R, and so has a lower propensity to aggregate. These data suggest that hyperphosphorylation (and loss of microtubule-binding activity) in the absence of aggregation is inadequate to support the cascade of events contributing to tauopathic neurodegeneration. Together these data suggest that tau aggregation may be an important facet of tauopathic neurodegeneration, but do not conclusively invoke a mechanism of toxicity.

4. Biological Models

To gain insight into mechanism, both loss of tau function and formation of tau aggregates have been modeled in cells and animals. The loss-of-function hypothesis has been tested in tau knock-out mice. These are viable, have normal rates of axonal transport [62], and do not show signs of cytoskeletal collapse predicted from the loss-of-function hypothesis [30]. The data are consistent with AD tissue, where microtubule length correlates poorly with tau aggregate deposition [11]. These data suggest that the effects of tau sequestration on microtubule function should be minor when the activities of redundant microtubule-associated proteins are preserved, and that loss of tau function by itself cannot be a direct cause of cytoskeletal collapse seen in late-stage disease. In fact, substantial amounts of normal tau persists in affected brain regions despite the accumulation of abnormal tau aggregates [33,39].

Biological models have also been created to test whether aggregate formation leads to gain-of-function toxicity (for a review on tau transgenic animal models, see [28]). The approach faces the challenge that full-length wild-type tau isoforms are resistant to aggregation even when present at supraphysiological concentrations [21,36]. Thus, most models depend on high level overexpression of tau alone or in combination with aggregation promoting mutations, both to increase aggregation rate and to overcome the binding capacity of tubulin polymers. Wild-type tau transgenic mice overexpressing 3R and 4R human tau exhibit some of the phenotypes associated with human tauopathy, such as hyperphosphorylation and pretangle formation [10,27]. However, they also foster neurodegeneration irrespective of aggregation state [58,61]. Because levels of normal tau protein do not increase in AD [34], the significance of overexpression-mediated toxicity found in transgenic systems has been ambiguous. For example, a transgenic mouse model overexpressing tau mutation P301L by more than an order of magnitude was observed to form and accumulate neurofibrillary lesions in parallel with neuronal loss and memory impairment [51]. But the toxic phenotype correlated with tau overexpression rather than aggregate formation, with suppression of tau expression to only 2-fold above normal reversing losses in cognitive performance. Although tau pathology can be generated from models such as these, their associated phenotypes are complicated by artificially raised tau levels. Above all, the toxicity generated in overexpression systems may not be relevant to authentic disease.

Two strategies have been developed to overcome these limitations. The first leverages an aggressive aggregation promoting mutation (Δ K280) that supports spontaneous fibrillization. Aggregation is further favored by artificially placing the mutation in a 4R background (in authentic human disease, the Δ K280 mutation affects splicing to favor 3R isoform expression; [59]) [20], and still further by truncating N- and C-terminal sequences that interfere with aggregation [45]. The latter construct supports aggregation in transgenic mice at bulk tau concentrations only 1.75 - fold above physiological. Under these conditions, aggregation correlates with both neurodegeneration and synapse loss, suggesting a direct link between tau aggregate formation and toxicity [45].

The second strategy leverages exogenous tau aggregation inducers to promote the fibrillization of full-length wild-type tau isoforms [15]. The inducers are small-molecule dyes capable of diffusing across cell membranes, and so can drive tau aggregation within the cytoplasm of living cells. The first demonstration of this approach employed human embryonic kidney cells (HEK293) constitutively overexpressing 2N4R human tau in the presence of Congo red inducer [4]. The resultant model recapitulated several features of authentic disease, including a significant increase in bulk tau levels owing to aggregate accumulation, loss of tubulin binding activity, and generation of a modest tau-dependent toxic phenotype over a period of days [4]. Although intracellular tau concentrations in these pilot studies were abnormally high, small-molecule agonists support the aggregation of 2N4R tau down to 200 nM [13], and so it may

be possible to apply the approach to the physiologically relevant tau concentrations found in neurons. The approach of using membrane-permeable exogenous inducers may facilitate dissection of the temporal relationship between aggregation and post-translational modifications, their mechanisms of generating toxicity, and their synergism with defects in other microtubule associated proteins. In addition, the approach is amenable to microfluidic strategies [29], which can isolate neuronal structures so that differential effects of aggregation in neurites and cell bodies can be resolved.

5. Conclusions

The “tau hypothesis” was formulated in part from the correlations between neurofibrillary lesion appearance and neurodegeneration that are used for diagnosis and staging of human disease. The hypothesis is indirectly supported by genetic studies showing that expression of tau proteins having increased aggregation propensity synergize with aging to promote neurofibrillary lesion formation late in life. How the aggregation reaction contributes to neurodegeneration is not clear, and may be a function of aggregate size, morphology, and state of post-translational modification, rather than simple mass concentration. It may also depend on the location of aggregate deposition, with consequences in cell processes that are distinct from direct effects on cell viability. Robust biological models will be required to elucidate the molecular mechanisms involved in tau aggregation associated toxicity, and will help identify toxic mediators involved in the aggregation pathway. In particular, there is a need to deconvolute the phenotypes associated with aggregation from the effects of tau overexpression, truncation, and post-translational modification. Small-molecule aggregation agonists may be helpful in this regard, since they are capable of promoting aggregation of 4R tau at submicromolar concentrations in the absence of post-translational modification, act rapidly, and can gain access to the intracellular compartment.

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