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Review

Transcriptional and conformational changes of the tau molecule in Alzheimer's disease

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Abstract

Mutations in the tau gene cause frontotemporal dementia with parkinsonism, presumably by affecting the balance between tau isoforms (with either three or four microtubule-binding repeats) or by impairing tau-tubulin binding. Although to date no mutations have been found for Alzheimer's disease, it is plausible that tangle pathology in this disorder is also driven by similar molecular modifications. Investigations of Alzheimer brain tissue with new technologies such as laser capture microscopy, quantitative PCR and fluorescence lifetime imaging will shed light on whether transcriptional or conformational alterations play a role in Alzheimer pathogenesis.

Keywords: Tau; Microtubule; Alternative splicing; qPCR; Conformational changes; FRET

1. Dysregulation of tau in neurodegeneration

Classification of neurodegenerative disorders has traditionally been based on a gross neuroanatomical characterization in combination with light microscopy-derived descriptions of intra- and extracellular abnormalities of the affected brain. However, the accelerating advancement during the last decades in terms of developing and applying techniques to study molecular biological mechanisms in human diseases has gradually promoted a new understanding of the cause and progression of dementias and other primary degenerative brain disorders.

One of the most highlighted molecules in the context of neurodegeneration has been the microtubule-associated protein tau. In this review, we will highlight new technical approaches to examining tau expression and tau conformation, providing future opportunities to examine alterations in tau mRNA levels and tau protein structure/function at a single cell level.

Ever since tau was found to be the principal component of neurofibrillary tangles in the Alzheimer's disease (AD) brain in the mid 1980s [1–3], its importance for the disease process has been investigated with numerous biochemical and cell biological methodologies. However, it was not until the discovery in 1998 of the first pathogenic mutations in the tau gene, causing familial forms of frontotemporal dementia (FTD) [4], that evidence for tau having a primary role in the pathogenesis of a neurodegenerative illness could be presented. In the following years, fueled by the discovery of further tau mutations [5–21] (and reviewed in Refs. [22,23]) (Table 1) and additional insight into tau molecular biology, the field has witnessed a steady increase in the number of "tauopathies", brain disorders having underlying tau molecular pathology as a common denominator, regardless of the gross anatomical picture or whether tangle formation is present or not (Table 2).

2. Tau mutations affect alternative splicing and/or microtubule affinity

Major research efforts have been made in order to understand the molecular consequences of the tau mutations. The human tau gene is located on the long arm of chromosome 17 and consists of 16 exons, of which 11 are

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Table 1
Loci, mutational effects, clinical phenotype and biochemical/neuropathological features for the pathogenic tau mutations published to date [4–21]

Mutation locus	Mutational effects	Clinical Phenotype	Neuropathological features
K257T	reduces microtubule assembly	FTD	FT atrophy, tau-positive Pick bodies
I260V	increases 4R tau	FTD	FT atrophy, cortical NFTs
G272V	reduces microtubule assembly	FTD/Pick's disease	FT atrophy, neuronal loss, tau-positive inclusions, 'Pick-like' inclusions
N279K	increases 4R tau	parkinsonism, progressing to dementia	mild FT atrophy, NFTs with hyperphosphorylated 4R tau
delK280	decreases 4R tau	FTD	not known
L284L	increases 4R tau	FTD with visuospatial dysfunction	FT atrophy, deposits of both amyloid and tau
N296N	increases 4R tau	FTD	FT atrophy, neuronal loss, tau-positive inclusions
P301L	reduces microtubule assembly	FTD	pronounced FT atrophy, NFTs with mainly 4R tau
P301S	increases 4R tau	FTD and CBD	filaments of hyperphospho tau
S305N	increases 4R tau	FTD with very early onset	pronounced FT atrophy, glial and neuronal NFTs
S305S	increases 4R tau	FTD with PSP-like phenotype	mild cortical atrophy, mainly subcortical NFTs
L315R	reduces microtubule assembly	FTD/Pick's disease	FT atrophy, Pick bodies
V337M	reduces microtubule assembly	FTD with psychotic components	FT atrophy, cortical NFTs
E342V	increases 4R tau	FTD/Pick's disease	FT atrophy, Pick bodies
G389R	reduces microtubule assembly	FTD/Pick's disease	FT atrophy, tau-positive pick bodies
R406W	reduces microtubule assembly	FTD, some with parkinsonism	FT atrophy, NFTs
3′Ex10+3	increases 4R tau	FTD with parkinsonism	diffuse cortical atrophy
3′Ex10+12	increases 4R tau	FTD	neuronal and glial tau aggregates with hyperphosphorylated 4R tau
3′Ex10+13	increases 4R tau	FTD	not known
3′Ex10+14	increases 4R tau	FTD with parkinsonism	FT atrophy, 4R tau filaments
3′Ex10+16	increases 4R tau	FTD with aphasia	FT atrophy, 4R tau filaments
3′Ex10+19	decreases 4R tau	FTD	FT atrophy
3′Ex10+29	decreases 4R tau	FTD	FT atrophy

expressed in the human brain; exons 2, 3 and 10 are alternatively spliced, leading to the presence of six different tau isoforms of 48–67 kDa [24]. Moreover, tau exon 4a is alternatively expressed in peripheral neurons as well as other non-brain tissues and encodes additional tau isoforms between 110 and 125 kDa [25–27]. The functional significance of "big tau" isoforms is unknown.

In human brain, the splicing of tau exon 10 is of particular interest, since this gene sequence encodes one of the protein's four microtubule-binding repeat regions. By rt-PCR and exon-trapping methods, it could be demonstrated that all of the initially identified intronic mutations associated with FTD caused exon 10 to be excessively expressed, leading to an overrepresentation of "four-repeat tau" (4R tau) as compared to "three-repeat tau" (3R tau) [4,7,10,18,28] (Table 1). As it had been demonstrated that 4R tau isoforms have a higher affinity for microtubules, it was hypothesized that an excess of 4R tau would result in a rigid and inflexible microtubular structure that hence is more prone to destabilize [29,30].

Table 2
Tauopathies

Alzheimer's disease
Frontotemporal dementia
Progressive supranuclear palsy
Corticobasal degeneration
Down's syndrome
Amyotrophic lateral sclerosis/Parkinson-dementia complex of Guam
Postencephalitic parkinsonism
Subacute sclerosing panencephalitis
Dementia pugilistica/head trauma
Niemann–Pick disease
Pick's disease

3. The role of tau and tangles in AD

Although to date no tau mutations have been identified in AD cases, the most common of all dementing disorders unquestionably qualifies as a tauopathy because tau-positive neurofibrillary tangles, in addition to amyloid plaques, are a neuropathological hallmark of the diseased brain. Tangles in the AD brain affect neurons in a hierarchical fashion and always appear first in large pyramidal neurons of the entorhinal cortex, subiculum and the CA1 subfield of the

hippocampus formation [31] (Fig. 1). As the disease progresses, increasing numbers of tangles are formed in layers III and V throughout temporal and parietal cortical association areas and their overall numbers have, in several studies, been shown to correlate well with disease severity [32–37] although the reason for this unique pattern of vulnerability is unknown. The class of neurons most likely to develop tangles are large cortico-cortical projection neurons in limbic and association cortices. These neurons are generally believed to be glutamatergic, excitatory and immunopositive for nonphosphorylated neurofilaments.

Tau is normally mainly located in axons but accumulates in various fibrillar morphologies within the perikarya (as neurofibrillary tangles) or within dendrites (as neuropil threads) in brains with intracytoplasmic tau inclusions. This redistribution of the intracellular compartmentalization of tau is associated with a change in tau conformation and the appearance of multiple phosphoepitopes. Whether cellular redistribution, conformation change, or phosphorylation is the primary pathogenic event is uncertain.

The extensive phosphorylation of tau by the action of several kinases (reviewed in Ref. [38]) typically results in less affinity between tau and microtubules [39]. However, phosphorylation of certain epitopes seems to protect tau from aggregating [40], indicating that tau phosphorylation may also have a protective effect on the neuron. More than 40 different phosphorylation sites on tau have been identified, mainly on serine and threonine residues followed by proline [41]. We have previously described that the phosphoepitopes of tau are phosphorylated in a hierarchical manner during the progression of the disease, leading to a classification of the respective tau phosphoepitopes into "early" and "late" sites [42].

On the ultrastructural level, AD tangles are composed of multiple pairs of twisted ribbon-like filaments, mainly adopting a β-sheet conformation. The relative presence of tau isoforms seems to be a crucial determinant for which configuration the filaments will have in the different tauopathies. For example, instead of the paired helical filaments (PHF) in AD, the pathological tau formations in progressive supranuclear palsy (PSP) form straight filaments, which are believed to result from the marked predominance of 4R tau in the brains of subjects with PSP [43]. Apart from PSP and some of the FTDP-17 variants, corticobasal degeneration (CBD) and certain forms of Pick's disease are other examples of tauopathies that display a relative abundance of both protein and mRNA levels of 4R tau in the affected brain regions [43–45]. Yet other variants of FTDP-17 and Pick's disease are known to have mainly 3R tau pathology [45].

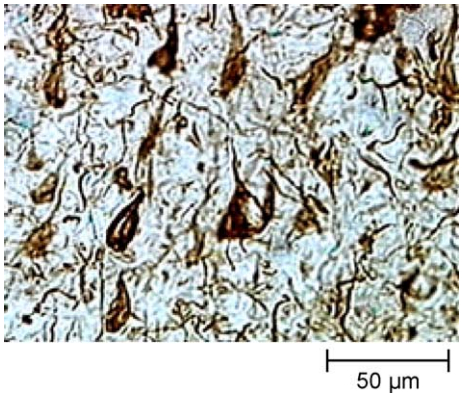

Fig. 1. Light microscopy picture of tangles in pyramidal cells from the superior temporal sulcus of a severely affected Alzheimer brain (×40).

4. Altered tau expression also in AD?

We and others have asked the question whether there is also an altered expression of tau underlying the tangle pathology in the AD brain. The commonly held notion, based on Western blot, is that overall tau protein levels are elevated with all six tau isoforms being equally represented in AD PHFs [24]. However, by using antibodies that discriminate 3R tau from 4R tau isoforms, several groups have found that tangles in the AD brain seem to be predominantly 3R tau-immunoreactive [46,47]. More specifically, immunohistochemistry of AD brains seems to reveal distinct classes of tangles with either only 3R tau or a combination of 3R tau and 4R tau reactivity, whereas it seems as if only very few AD tangles display 4R tau pathology alone.

We examined whether tau mRNA expression is increased in AD by quantitative PCR (qPCR) methods, initially by measuring 3R tau mRNA levels. When comparing the amount of 3R tau mRNA detected in 12 AD vs. 16 control temporal neocortices, we found decreased levels in the AD brains when compared to total mRNA (Fig. 2a). It is possible that this reflects the general reduction of neurons known to occur in the diseased brain; when normalizing the tau levels to GAPDH, a housekeeping gene, the difference between AD and control brains was no longer statistically significant (Fig. 2b). In addition, preliminary data suggests a similar expression pattern for 4R tau. However, regardless of whether there is an overall decrease or unchanged expression of tau in the AD brains, the increase of tau protein as observed on Western blot must reflect enhanced stabilization of the posttranslationally modified protein, rather than increased synthesis.

Albeit most studies to date have failed to find any major alterations in the levels of and ratios between the various tau mRNA species in AD, one previous study indicated that transcripts containing tau exon 3 were overrepresented in the AD brain [48], while findings by Yasojima et al. [49] suggested that 4R tau mRNA species occur in a relative surplus in brain regions that are more severely affected in AD, such as the entorhinal cortex. The previous investigations were mainly carried out by Northern blot or traditional PCR, techniques that only can distinguish fairly

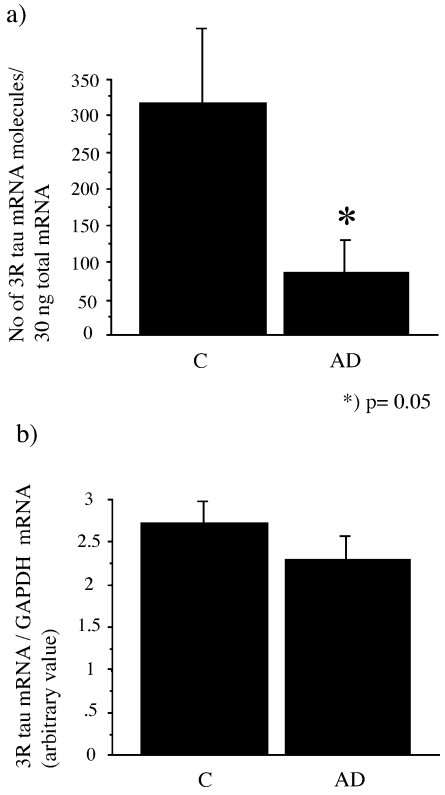

Fig. 2. (a) Levels of 3R tau mRNA (±1 S.E.) are decreased in AD brains ($n=12$) as compared to normal control brains ($n=16$). (b) No statistically significant decrease in 3R tau mRNA levels (±1 S.E.) between AD ($n=12$) and control brains ($n=16$) after normalizing to the housekeeping gene GAPDH.

robust differences between disease and control groups. More sensitive techniques, like qPCR and exon-typing by the polony method [50], will be more likely to detect less significant but nevertheless potentially physiologically important shifts in expression of tau isoforms in human brain diseases. Apart from examining AD and control tissue homogenates from various brain regions, analyzing nerve and glial cells separately by the combined use of qPCR and laser capture microscopy (LCM), a technique that permits microdissection of individual cell populations from ultrathin tissue slices [51], will determine if tangle-bearing neurons differ as compared to normal neurons in their tau expression profiles.

5. Tau conformation and tangle formation

One of the remaining questions of significance is to understand how the various tau mutations cause nerve cell dysfunction and disease. Is the relative excess of 4R tau the direct mediator of an altered neuronal physiology or is it possible that there is another underlying factor that also would be applicable to mutations that do not cause changes in the splicing of tau? In fact, when assessing tau mutations in microtubule-binding assays, it has been shown that some of the mutations (for which no shift in alternative splicing could be seen) instead seemed to cause an impairment in tau-tubulin interactions (reviewed by Refs. [22,23]), possibly by inducing a conformational shift of the tau molecule.

Isolated tau does not seem to have a distinct secondary structure but appears as a random coil in isolated preparations [52,53]. Several in vitro studies suggest that tau misfolding can lead to aggregation. It has, for example, been illustrated that tau, when binding tubulin, adopts a folded conformation that is guided by a domain–domain interaction between the microtubule-binding (MTB) region of tau and the C-terminus of tubulin [54]. Moreover, studies of isolated PHF preparations have demonstrated that folded conformations of either α-helical or β-sheeted structures occur under different conditions [55–57]. In a review of the data on in vitro tau aggregation, a "unified model" was suggested, in which conformational alterations are the main factor in the tau fibrillization process [58]. More specifically, the model proposes soluble tau monomers to have a conformation in which the microtubule-binding domains form core β-sheet structures that are blocked by a hairpin structure either in the C-terminus or within the repeat domain [59]. According to this model, a change in tau conformation would lead to a release of such inhibitory folds, followed by formation of tau dimers and subsequent polymers.

The existence of corresponding changes in the secondary structure of tau in vivo initially gained support by the finding of subpopulations of morphologically intact neurons in the AD brain that were immunopositive for Alz50, an antibody whose epitope is conformation-specific and requires a proximity between the N-terminus and the MTB-region of tau [60,61] (Fig. 3). The Alz50-positive neuron may be at a "pre-tangle" stage, suggesting that it represents an early alteration in tau prior to frank NFT formation and neuronal death [62,63]. The fact that Alz50 also stains the tau lesions in Pick's disease and PSP suggests a commonality of conformational changes in several diseases with tangle pathology [64–67]. The exact timepoint during the tangle development at which Alz50 reactivity appears has not been firmly established but our own preliminary cell-based data, in which tau inclusions are induced by transfection with tau constructs, suggest that conformational change, as detected by Alz50, precedes the pathological phosphorylation of most, if not all, tau phosphoepitopes (unpublished observations). Ongoing studies aim at investigating whether 4R tau isoforms are more prone than 3R tau to induce altered molecular conformations.

Efforts to pinpoint the exact sequence of pathological tau misfolding can be undertaken by using techniques that permit a precise assessment of intramolecular distances between the various tau epitopes. In our laboratory, we have developed an approach based on fluorescence lifetime imaging (FLIM), which is one of several ways to take advantage of a physical principle termed fluorescence

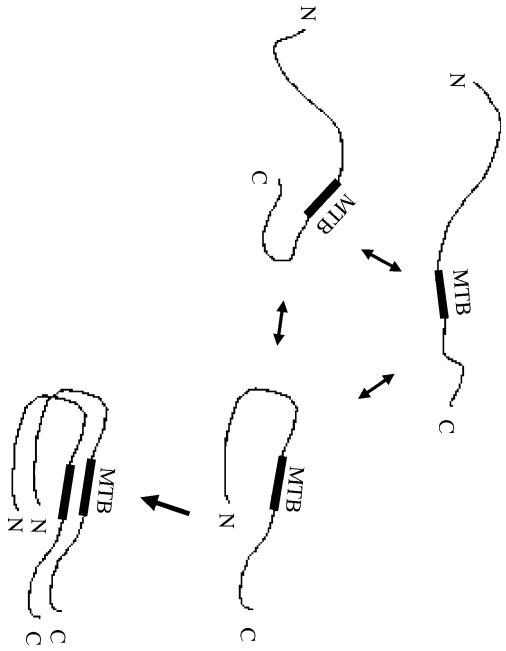

Fig. 3. Schematic drawing of how a conformational change in tau may mediate dimerization and subsequent tangle formation. The proximity between the C-terminus and the MTB region may block fibrillization, whereas misfolding and aggregation may be mediated by interaction between the N-terminus and MTB.

resonance energy transfer (FRET) [68,69]. FRET occurs when two fluorophores with matching excitation–emission properties come within 10 nm of one another. After excitation, one of the fluorophores in such a pair, the *donor*, will emit light and return to its baseline level after a certain amount of time. This lifetime will be shorter in the presence of an *acceptor*, a fluorophore whose excitation wavelength is equivalent to the emission wavelength of the donor. A shorter lifetime of the donor fluorophore thus reflects a closer proximity to the acceptor fluorophore. For FLIM, any two intra- or intermolecular epitopes for which antibodies raised in two different species are available (allowing for parallel immunostaining by two differently labeled secondary antibodies) could thus be studied with this approach.

Our preliminary FLIM-based results on tau conformation in vivo so far have corroborated the notion that the N-terminus and the MTB-region have a very close proximity in the AD tangle [60,61,70] (Fig. 4). Ultimately, a tau epitope interaction map could be generated for tangles at different stages in terms of tau phosphorylation.

Moreover, the FLIM technique can be used to assess interactions between tau and other molecules in cellular processes of relevance. Since more and more studies indicate multifunctional aspects of tau, the choice of proteins should not be restricted to only α and β tubulin, its traditional binding partners, but also to targets such as molecular motors. Previous studies have, for example, indicated that a surplus of tau molecules may impair axonal transport by competing with kinesin [71,72] (and reviewed in Ref. [73]).

6. Summary and future directions

The discovery of mutations in the tau gene that lead to inherited neurodegenerative dementias challenges us to understand the molecular mechanisms involved and whether analogous mechanisms occur in AD.

The suggested increase in tau protein levels, as indicated by previous Western blot analyses, does not seem to be reflected on the mRNA level; our preliminary qPCR-based data indicate either slightly lower levels of tau in AD temporal cortex or comparable levels between Alzheimer and control brains. These findings imply a stabilization of the tau protein, likely through posttranslational modifications.

Initial studies using modern fluorescence methods confirm previously predicted conformational changes of tau in neurofibrillary tangles, with the N-terminus apparently folded closely to the microtubule-binding domain, thus covering the domain and inhibiting microtubule binding. The predicted molecular consequences of such tau misfolding may include increased cytoplasmic sequestration, hyperphosphorylation and most likely a reduction in its microtubule-binding capacity.

Although analyses of functional effects for the tau mutations have been successful in terms of elucidating alterations in alternative splicing and defective microtubule affinities, many questions remain unanswered as to why the mutations lead to neuronal damage, and ultimately, degeneration of the affected brains. If the increase in 4R tau isoforms with their stronger microtubule-binding affinity

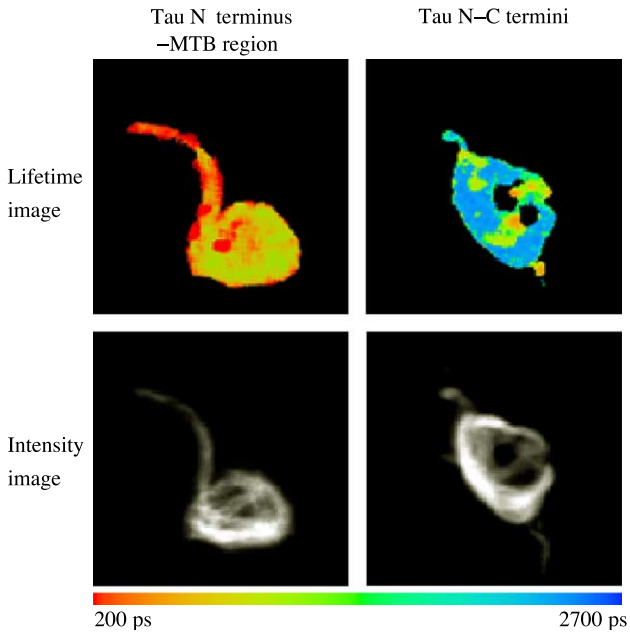

Fig. 4. The upper panel shows pseudocolored lifetime images reflecting a close interaction between the MTB region and the N-terminus (left) but no interaction between the MTB region and the C-terminus (right). The lower panel shows the intensity images for the N- (left) and C- (right) terminal tau staining.

would be the direct causative factor for some of the tau mutations, it seems paradoxical that other mutations lead to disease by lowering the tubulin-binding affinity of the tau molecule. If a common mechanism were to be found for tau mutations, conformational changes of tau that weaken the interaction between tau and tubulin may well prove to be such a unifying factor. As more and more refined tools to investigate molecular interactions are being developed, we will be able to address this question in-depth and elucidate the potential relationship between altered splicing and conformational changes of tau in the tauopathies.

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