Tau and Intracellular Transport in Neurons

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Abstract Among the early changes in the brains of Alzheimer’s disease patients is the loss of synapses, which is accompanied by the abnormal phosphorylation of tau protein, its missorting into the somatodendritic compartment of neurons, and its incipient aggregation. The physiological function of tau is to stabilize axonal microtubules, which enables them to carry out their role as tracks for the transport of vesicles and organelles. By implication, perturbations in the functions of tau could be related to the loss of synapses and neuronal degeneration. Cell and transgenic animal models of tauopathy reveal that tau can indeed cause an impairment of transport in neurons. As a result, cell processes of neurons become starved, leading first to the decay of synapses and then to the loss of axons and dendrites.

1 Tau Protein: Properties and Functions

The loss of synapses observed during incipient Alzheimer’s disease (AD) corresponds to the beginning loss of memory during the mild cognitive impairment phase (Coleman and Yao 2003). The synapse decay precedes the abnormal protein aggregation of the Aβ peptide in senile plaques or of tau protein in neurofibrillary tangles (Walsh and Selkoe 2004). It has been suspected that the highly elongated structure of neurons is one reason for their vulnerability. Most synapses are distant from the cell body, the major site of protein synthesis, and therefore rely on an efficient transport system. In cells, the traffic system consists of microtubules and microfilaments along which cargoes can be moved (Hollenbeck and Saxton 2005; Baas et al. 2006). This transport is achieved by means of motor proteins that can be subdivided into three classes: myosins (for the microfilament tracks), kinesins and dyneins (for microtubule tracks; Hirokawa and Takemura 2005). The directionality of movement
is determined by the polarity of the tracks and the directionality of the motors. The “plus” ends of microtubules point to the cell periphery, so that plus end-directed motors (kinesin) carry out anterograde transport and minus end-directed motors (dynein) achieve retrograde movements towards the cell body. The “ties” for the tracks are represented by microtubule-associated proteins (MAPs; Cassimeris and Spittle 2001). In neurons, the most important MAPs are MAP2 (mostly dendritic), tau and MAP1b (mostly axonal). The interaction of MAPs with microtubules is controlled by phosphorylation and involves several protein kinases and phosphatases (Mandellkow et al. 2007). Microtubules can assemble from their subunits (α-β-tubulin heterodimers) under the regulation by GTP. Additional control is achieved by MAPs, such as tau, whose detachment can induce microtubule breakdown, and by the microtubule-disassembling proteins, katanin, spastin, or kinesin-13 (Howard and Hyman 2007).

Tau has received attention in the field of several neurodegenerative disorders (“tauopathies”) because of its anomalous behavior (Ballatore et al. 2007, Schneider and Mandellkow 2008), which is most conspicuously seen as aggregation into neurofibrillary tangles, consisting of paired helical filaments (PHFs) and straight filaments (Crowther and Goedert 2000; Mandellkow et al. 2007). Tau also becomes highly phosphorylated, missorted into the somatodendritic compartment, partly cleaved by proteases, and otherwise modified (Watanabe et al. 2004; Binder et al. 2005). The H1 haplotype of tau shows a genetic association with certain tauopathies, e.g., progressive supranuclear palsy, corticobasal degeneration, AD and Parkinson disease, which may be caused by a perturbation of tau isoform homeostasis resulting in a relative increase of 4-repeat tau isoforms (inclusion of exon 10) and decrease of N-terminal inserts (especially lack of exon 3; Myers et al. 2007; Caffrey et al. 2007). Biochemically, AD-tau is found to be detached from microtubules and no longer stabilizes microtubules. The consequences are the destabilization of transport tracks and the aggregation of tau in the cytosol, both of which can disrupt intracellular traffic. AD-tau aggregates show a well-defined pattern of spreading in the brain, from the transentorhinal region to the hippocampus and later throughout the cortex. This pattern corresponds to the progression of clinical symptoms from mild cognitive impairment to severe dementia (Braak stages 1–6; Braak and Braak 1991).

The gene of tau (MAPT) is located on chromosome 17; the protein occurs in the CNS as six main isoforms arising from alternative splicing (352–441 residues; Andreadis 2005). The repeat domain (3 or 4 pseudo-repeats of ∼31 residues, depending on splice isoforms) and the domains flanking the repeats are responsible for microtubule binding. The repeat domain also forms the core of Alzheimer PHFs (Wille et al. 1992; Novak et al. 1993). The overall character of tau is basic and hydrophilic, due to the many lysine or arginine and polar residues, which makes tau highly soluble, up to the point that tau is heat and acid stable without losing its biological function (Lee et al. 1988). A further consequence is that tau is not compactly folded as most proteins but rather is a natively unfolded protein (Schweers et al. 1994). Several mutations in the tau gene can cause different forms of neurodegeneration (FTDP-17; Ballatore et al. 2007), presumably due to a change in protein...
function or an altered distribution of isoforms caused by modifications in the pattern of alternative splicing (D’Souza and Schellenberg 2005).

Tau from AD brains is extensively phosphorylated, ∼4-fold higher than in normal brain and at numerous sites (Khatoon et al. 1992; Morishima-Kawashima et al. 1995). The consequences are heterogeneous. Phosphorylation at certain sites can affect microtubule binding and/or PHF aggregation; other sites appear to be functionally neutral (Schneider et al. 1999). Phosphorylation at the KXGS motifs in the repeat domain by the kinase MARK strongly disrupts tau-microtubule binding and leads to dynamic microtubules (Drewes et al. 1997). The interplay between tau and MARK becomes particularly noticeable in the case of neurite outgrowth, where activation of MARK has a similar effect as nerve growth factor signalling (Biernat et al. 2002).

The most unusual property of tau in AD is its aggregation, which is counterintuitive because of the high solubility of tau. The aggregation is based on certain hexapeptide motifs in the sequence that have an increased propensity for β-sheet interactions (275VQINK280 and 306VQIVYK311; von Bergen et al. 2000). Therefore, the aggregation of tau is based on an “amyloid” principle, although the major part of the protein remains disordered, even when it is assembled into PHFs. This finding is borne out by recent structural results. X-Ray crystallography reveals that amyloidogenic peptides derived from different proteins form hairpin-like “amyloid spines” that assemble into cross-β-sheets, stabilized by internal hydrophobic interactions and hydrogen bonds and paired by hydrophilic interactions (Sawaya et al. 2007). Nuclear magnetic resonance studies reveal that the amyloidogenic subdomains have an enhanced tendency for extended conformation with β-propensity even in solution, which is stabilized in hairpin-like conformations during fiber assembly (Mukrasch et al. 2005; Andronesi et al. 2008).

2 Tau and Transport Inhibition in Neurons

The traffic systems in neurons can be regulated at different levels, for example at the level of tracks (microtubules, tau), motors (kinesin, dynein), or cargo adaptors (kinesin or dynein light chains or associated proteins), or by posttranslational modifications (phosphorylation; Stokin and Goldstein 2006). In this context, proteins closely related to AD include tau and protein kinases that can regulate tracks, motors, or adaptors. In cells, one observes that elevation of tau causes a stabilization of microtubules as well as a general inhibition of intracellular traffic, particularly in the anterograde direction (Stamer et al. 2002; Fig. 1), which can be explained by the fact that tau inhibits both forward motors (kinesin) and reverse motors (dynein), but the inhibition of kinesin is more pronounced, resulting in a net retrograde bias in the transport of vesicles and organelles (Seitz et al. 2002; Dixit et al. 2008). The observations are consistent with the view that the attachment of motors is obstructed by tau bound to the microtubule tracks. In addition, tau may interact directly with kinesin or dynein motors (Magnani et al. 2007; Cuchillo-Ibanez et al. 2008). The
Fig. 1 Diagram of microtubules, tau, and kinesin motors, illustrating several possible modes of dysregulation of the neuronal transport system. Lower left, normal state with intact microtubule tracks, sparse decoration by tau (which suffices for stabilization), and kinesin motor with vesicle cargo moving in the anterograde direction. (1) Too much tau bound to the microtubule surface can restrict the access of motor proteins, retard axonal transport, and overstabilize microtubules (leading to insufficient dynamic instability and excess microtubule polymerization). (2) Tau may become hyperphosphorylated (e.g., in the repeat domain by the kinase MARK), which causes detachment from microtubules. This may lead to destabilization of microtubules and thus to loss of transport tracks. (3) Tau detached from microtubules may aggregate into paired helical filaments, which coalesce into neurofibrillary tangles that obstruct the cell interior.
Fig. 2 Transport inhibition by tau in retinal ganglion cell axons. (a) Retinal ganglion cell axons growing out from an explant. Mitochondria are stained with MitoTracker Red; one cell is transfected with CFP-tau by adenovirus (blue). There are numerous highly mobile mitochondria in most cells, but the tau-transfected cell has already lost most of its mitochondria, and the remaining ones are almost immobile and are in the process of degenerating. (b) Quantification of mitochondrial movements. In control growing axons, the majority of mitochondria move anterogradely (55%). In tau-transfected cells, this fraction drops to 5%, and most mitochondria either move retrogradely or are stationary within the window of observation. When cells are additionally transfected with MARK, the microtubule-bound tau becomes phosphorylated and detaches from microtubules, and anterograde movement of mitochondria is largely restored.
Fig. 3 Decay of dendritic spines and synapses under the influence of tau. Top: Hippocampal neurons were cultured for 25 days in vitro, leading to numerous synapses. The neurons were transfected with CFP-tau (blue), which enters the dendritic compartment, including spines, due to missorting of tau. Bottom: 20 hours later most spines have withered away, concomitant with a loss of energy (ATP), loss or displacement of synaptic markers, and translocation of F-actin into the dendritic shaft (not shown).

Organelles such as mitochondria from cell processes by inhibition of anterograde transport will cause deficiencies in local metabolism, leading to reduced adenosine triphosphate (ATP) levels, Ca\(^{++}\) buffering capacity, and defense against oxidative stress. These reductions will impair the overall viability of the cells, and among the first victims are the dendritic spines (Thies and Mandelkow 2007), reminiscent of the early decay of synapses in AD (Fig. 3).

A special aspect of tau-induced traffic inhibition is that vesicles carrying amyloid precursor protein (APP) are affected as well, suggesting a potential link between the two major pathological hallmarks in AD. However, there appears to be no direct link to the generation of the A\(_\beta\) amyloid peptide, and vesicles carrying APP are distinct from those carrying the protease BACE1 (responsible for the first cleavage of APP leading to A\(_\beta\)), making a direct interaction between APP and BACE1 during transport unlikely (Goldsbury et al. 2006; Lazarov et al. 2005).

While the inhibition of axonal traffic by tau is easily observable in vitro and in cell models, the results from studies on organisms are heterogeneous. Mouse models, where the tau gene is expressed under the pan-neuronal promotor Thy-1, suffer from motor neuron disease, which makes it difficult to test any effects on memory. This disease has been ascribed to the expression of tau in motor neurons that are particularly long and therefore vulnerable to traffic inhibition by tau (Terwel et al. 2002; Götz et al. 2006; Lee et al. 2005). This problem can be circumvented by using promoters restricted to the forebrain, e.g., the CaMKII promoter. Similarly, expression of tau in the axons of flies causes traffic deficits and damage to the neuromuscular junction (Chee et al. 2005; Fulga et al. 2007). In Aplysia, overstabilization of microtubules by tau can take the form of misoriented microtubules that obstruct axons (Shemesh et al. 2008). On the other hand, a tau-induced inhibition of traffic has not been observed in extruded squid giant axoplasm (Morfini et al. 2007).
or in vivo in retinal ganglion cells from mice overexpressing tau (Yuan et al. 2008). These variable results may be related to differences in regulatory systems operating in the experimental systems used.

One of the puzzling features of the behavior of tau in neurons is that, on the one hand, it inhibits anterograde traffic of microtubule-dependent cargo (vesicles, organelles, neurofilaments) but, on the other hand, tau itself, when elevated, enters axons and dendrites with apparent ease. Thus, while vesicles and organelles tend to disappear from cell processes, tau moves out, seemingly “against the tide” (Konzack et al. 2007). The solution to this paradox resides in two features of tau. First, anterograde transport of tau occurs on small microtubule fragments that can be transported not only on microtubules but also on actin filaments (Wang and Brown 2002; Baas et al. 2006). In the latter case, a dynein-based movement of tau-tubulin complexes relative to a stationary actin network would achieve the required anterograde directionality (note that dynein is much less affected by tau than kinesin; see above). This finding would explain the observed rates of tau within the slow component b (Scb) of axonal transport (Mercken et al. 1995; Roy et al. 2008). Secondly, tau is much more mobile than anticipated for a “microtubule-associated” protein. It spends only ∼70% of the time on the microtubule, associates and dissociates rapidly (residence time ∼4 sec) and, in the unbound state diffuses freely in the cytoplasm (Konzack et al. 2007). Thus, over periods of ∼days and distances of ∼mm, diffusion appears to be adequate to supply the axon with the required level of tau.

3 Conclusions

In this review we have considered some mechanism by which tau could gain toxic functions, based on the known functions of tau in neurons. They can be summarized as follows:

1. Elevated tau, when bound to the microtubule surface, can inhibit the attachment of motor proteins and thus slow down transport rates in the cell processes of neurons (Stamer et al. 2002; Figs. 1, 2).

2. Elevated tau can overstabilize microtubules and generate excess microtubules in axons and dendrites, with two consequences: the excess microtubules can fill out the cytosolic space and thus prevent the transit of vesicles and organelles (Thies and Mandelkow 2007; Fig. 4).

3. In addition, the overstabilization of microtubules by excess tau can suppress the dynamic instability of microtubules, which is necessary for maintaining the capacity for remodelling the neuronal cytoskeleton (Baas et al. 2006). In this context, it is notable that the 4-repeat tau isoforms (which bind and stabilize microtubules better than the fetal 3-repeat isoforms) suppress microtubule dynamics in a fashion reminiscent of taxol, a microtubule poison used in cancer chemotherapy (Panda et al. 2003).
Fig. 4 Thin-section electron microscopy of dendrites before and after tau transfection. In the control cells (top), the microtubules are spaced wide apart and allow passage of transport vesicles and organelles. After two days of transfection with tau, microtubules become more numerous (four-fold) because tubulin synthesis is initially upregulated. Microtubules become more densely spaced, thus blocking transit (note the mitochondrion pushed to the upper side of the cell). After four days, microtubules have mostly disappeared (due to lack of ATP and GTP) and mitochondria are swollen and in a state of degeneration. The tau-induced changes can be halted, at least temporarily, by phosphorylating tau (by kinase MARK) and thereby detaching it from microtubules (Thies and Mandelkow 2007).

4. Tau could interfere with transport by inactivating complexes of motor proteins directly (e.g., kinesin, dynactin; Magnani et al. 2007; Cuchillo-Ibanez et al. 2008).

5. Hyperphosphorylation of tau and subsequent detachment from microtubules could destabilize microtubules, leading to a loss of transport tracks (Zhang et al. 2004; Fig. 1).

6. Tau could interfere with the functions of other cellular proteins, e.g., the actin network (Fulga et al. 2007), and signalling molecules (e.g., Pin-1, Lippens et al. 2007; kinases and phosphatases, Stoothoff and Johnson 2005; chaperones, Shimura et al. 2004).

On the level of the tau gene and the mutations known from FTDP-17 and other tauopathies, it is notable that the shift in the splicing pattern generates an imbalance between 4-repeat and 3-repeat tau isoforms in favor of 4-repeat forms. The 4-repeat forms bind and stabilize microtubules more strongly, but aggregate into PHFs less readily than 3-repeat isoforms. This finding is suggestive of a mechanism of toxicity.
based on blocking traffic and/or suppressing microtubule dynamics (points 1–3 above). Consistent with this, the H1 haplotype of MAPT causes a higher level of 4-repeat tau protein in neurons, which might explain why this haplotype represents a risk factor for several tauopathies (Myers et al. 2007).

In this review we focussed on the question of how tau might disturb the neuronal transport system and thus contribute to neurodegeneration. We have not considered the causes and effects of the abnormal tau aggregation that is prominent in tauopathies. However, it is notable that certain cell and animal models display a strong tau-dependent toxicity that is specifically related to aggregation (Wang et al. 2007; Mocanu et al. 2008). This mode appears to be independent of the mode of transport-related toxicity, consistent with the observation that transport defects and aggregation defects occur at different stages of the AD process (Götz et al. 2006).

Acknowledgement

Work from our laboratory discussed here was supported by the Max-Planck-Gesellschaft (MPG) and the Deutsche Forschungsgemeinschaft (DFG).

References


