‘Prion-Like’ Propagation of Mouse and Human Tau Aggregates in an Inducible Mouse Model of Tauopathy

Astrid Sydow  Eva-Maria Mandelkow
Max-Planck-Unit for Structural Molecular Biology, Hamburg, Germany

Key Words
Alzheimer’s disease  ·  Tau  ·  Aggregation  ·  Transgenic mouse models  ·  Tauopathy

Abstract

Background: Aggregates of the tau protein are a hallmark of Alzheimer’s and several other neurodegenerative diseases. Various transgenic mouse models have been generated to study the aggregation process. Since wild-type tau is highly soluble and does not aggregate readily, most models make use of tau mutations that occur in human frontotemporal dementias and are more prone to aggregate. These mouse models show neurofibrillary tangles similar to those of Alzheimer’s disease. However, since the mice contain both endogenous wild-type mouse tau and exogenous human mutant tau, the relative contribution of these components to the aggregates has been a matter of debate. Objective: Using a new set of regulatable transgenic mouse models, we sought to determine whether mouse tau coaggregates with human tau when it is switched on. Furthermore, we asked what type of tau remains in the aggregates after switching off the expression of exogenous tau. Methods: We generated doxycycline-inducible transgenic mice expressing either full-length human tau or the tau repeat domain (tauRD). In addition, both types of human tau derivatives were expressed in a ‘proaggregant’ form (with the frontotemporal dementia with parkinsonism linked to chromosome 17 mutation ΔK280), or in an ‘antiaggregant’ form (with additional proline mutations to block β-structure and aggregation).

Results: The proaggregant tauRD mice develop tangles rapidly after induction, the antiaggregant mice do not. Analysis by biochemistry and immunohistology reveals that the tangles contain both exogenous and endogenous mouse tau. After switching off the proaggregant tauRD, tangles persist for extended periods. However, they are composed entirely of mouse tau. Conclusions: Mouse tau and exogenous human tau can coaggregate in transgenic models of tauopathy. The aggregates are in dynamic equilibrium with their subunits, so that exogenous tau disappears when its expression is switched off. Once the seeds of aggregation are generated by the foreign tau species, they propagate in a ‘prion-like’ fashion within the cell even after the foreign tau has disappeared.

Introduction

Tau is a microtubule-associated protein which occurs mainly in neuronal axons and stabilizes microtubules as tracks for axonal transport. Tau is a highly soluble, natively unfolded protein, yet it forms pathological ‘paired-helical filaments’ in neurodegenerative diseases such as Alzheimer’s disease (AD), frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP17), PSP, Pick’s disease and others. The tau-microtubule interaction is based on tau’s ‘repeat domain’ of ~100 residues which also forms the core of Alzheimer paired helical filaments (fig. 1). This illustrates the close relationship be-
Prion-Like Propagation of Tau Aggregates in Tauopathy

Between physiological and pathological functions of tau [for reviews, see 1, 2]. Because of the good solubility of tau, it has been difficult to design cell or mouse models of tau pathology; overexpression of tau alone generally does not yield aggregation. Researchers have therefore made use of tau mutations that occur in cases of FTDP17 and tend to be more prone to aggregation than wild-type tau. A well-known example is the mutation P301L [3]. In our work, we have chosen the mutant ΔK280 because of its rapid aggregation in vitro [4].

We generated several transgenic mouse lines based on this mutation, expressing either the repeat domain of human tau (human tau RD), or full-length human tau [5, 6]. The expression was made regulatable by doxycycline using the tetracycline-responsive transactivator (tet-off system [7]). Expression was restricted to the forebrain by means of the CaMKIIα-promoter [8]. In the presence of doxycycline, no expression of tau RD and no aggregation were detected after 5 months, indicating that the system was tightly regulated. In order to distinguish the effects of tau expression from aggregation, we designed two types of protein, containing either the ΔK280 mutation alone, or in combination with two prolines in the hexapeptide motifs that control the transition to β-structure and therefore the aggregation of tau [9]. In the latter case, no aggregation occurs because prolines are not compatible with β-structure. These two forms are termed ‘proaggregant’ or ‘antiaggregant’ tau.

When expressing human tau in a mouse, one has to consider the relationship with endogenous mouse tau. Both types of protein are very similar, especially in their microtubule-binding and repeat domains. In vitro, both bind tightly to microtubules, and both are able to copolymerize into paired helical filaments, regardless of isoform [10]. One would therefore expect this to happen in vivo as well. However, this has been a matter of debate for several reasons. One is that wild-type mouse tau does not aggregate by itself (e.g., in models of AD based on APP mutations), and wild-type human tau, even when expressed at high levels, does not aggregate either [11]. This has led to the assumption that endogenous mouse tau or the environment in a mouse neuron is protective against aggregation. In apparent support of this view, human wild-type tau (comprising all isoforms) was shown to aggregate only when mouse tau was eliminated [12]. A second problem was the detectability. Both mouse and human tau exist as multiple isoforms (3 and 6, respectively) which approach each other closely on an SDS gel, especially since all of them can be phosphorylated at multiple sites, with shifts in MR values causing overlaps of bands. Thus, the analysis of the composition by immunoblotting is ambiguous because most tau antibodies cross-react between human and mouse tau.

In this report, we show that these ambiguities can be circumvented by expressing human tau RD variants in transgenic mice which differ in their aggregation propensity and are clearly distinguishable from mouse tau by their lower molecular weight. The results show that proaggregant human tau RD can induce normal mouse tau to coaggregate when they are coexpressed within a neuronal cell, and that the aggregation can be propagated within the cell even when the foreign tau disappears.

Results and Discussion

In the mouse model used here [5], we chose to express a human tau construct (the four-repeat domain, tau RD) that is much smaller than the endogenous mouse isoforms (fig. 1). It is therefore readily distinguished in biochemical assays. Nevertheless, tau RD contains all the sequence motifs required for aggregation (the hexapeptide motifs in R2 and R3). Furthermore, since most of the AD-diagnostic antibody epitopes lie outside the repeat domain, the composition of aggregates in brain sections and immunoblots can be readily determined.

These tools allow one to demonstrate that exogenous human tau and mouse tau can indeed coaggregate. The
mutant tauRD appears in Western blots as a band of 12–14 kDa, clearly distinct from the endogenous mouse tau [see also 5]. This allows one to determine the ratios of the proteins. Both the proaggregant and antiaggregant mouse lines had similar levels of exogenous tau, ~70% of endogenous tau protein. The aggregation can be evaluated by the sarkosyl extraction method [13] which reveals tangles as well as pretangle aggregates. Most exogenous tauRD proteins and the three adult mouse tau isoforms are in the soluble fraction, both for pro- and antiaggregation mutants. Sarkosyl-insoluble fractions contain pronounced tau only in the proaggregation mouse line. The ratio of tauRD to endogenous tau in the sarkosyl-insoluble and soluble fractions is similar (0.4–0.5), showing that endogenous mouse tau is incorporated into the aggregates. By contrast, the antiaggregation mouse line, like the wild-type line, shows no or very little tau in the sarkosyl-insoluble pellet.

Immunohistochemistry of the brains yields a similar picture. In the proaggregant mice, tangle formation starts at ~2 months of gene expression, and Gallyas-positive neurons appear in the entorhinal cortex and amygdala. At 12–15 months, neurofibrillary tangle (NFT) pathology spreads across the entorhinal cortex and neocortex [see also 5]. By contrast, the antiaggregation tau mice show no NFTs even at 22 months. The aggregates of the proaggregant mice can be immunostained with antibodies whose epitopes lie outside the repeat domain and occur only in mouse tau; this provides confirmation of the coaggregation between endogenous tau and exogenous proaggregant tauRD (fig. 2).

A key question in the AD field is whether the aggregation of tau is reversible. When the expression of proaggregant tauRD is switched on for 9 months, and then off for 5 months, the exogenous sarkosyl-soluble and insoluble protein nearly disappears (fig. 3). As shown elsewhere, the degradation of aggregated tau is largely dependent on
macroautophagy [14]. By contrast, the aggregated endogenous mouse tau in the sarkosyl-insoluble fractions decreases only moderately, indicating that the tangles formed from mouse tau persist. This can be explained by the continued synthesis of endogenous mouse tau and the presence of seeds of aggregation once they have been generated within a cell.

Several conclusions can be drawn from these experiments:

- The beta propensity of tau in the repeat domain determines its aggregation behavior in transgenic mice. Thus, proaggregant tauRD aggregates, antiaggregant tauRD does not.
- Once the seeding of aggregates is achieved by proaggregant tau species within a cell, aggregation can spread to the pool of normal endogenous tau that is neither elevated nor mutated. In other words, the ‘poisonous’ conformation can be propagated to ‘healthy’ endogenous tau in a fashion reminiscent of prion propagation. As a result, mouse tau coaggregates with human tauRD.
- Aggregation is surprisingly persistent once it has been initiated. Tau aggregates survive long after the initial proaggregant culprit (tauRD) has been switched off and disappeared. The remaining aggregates consist entirely of wild-type mouse tau (which cannot be switched off). The result has several implications:

  - The neuron’s self-defense mechanisms are still active, even after harboring tau aggregates for several months, i.e., when tauRD is switched off, it can be eliminated from the soluble and insoluble pool of proteins.
  - Since tauRD is gradually released from the aggregates and eliminated, the aggregates must be in a dynamic equilibrium with their subunits.

An important consequence of the last point is that it should be possible to shift the equilibrium between aggregates and subunits by low molecular weight compounds that lock tau in a nonaggregating conformation. A number of such tau inhibitor compounds have been developed and are currently examined for their therapeutic potential [for a review, see 15].

Acknowledgements

We thank Dagmar Drexler, Olga Petrova and Anne Hofmann for excellent technical assistance, Dr. Jacek Biernat and Dr. Eckhard Mandelkow for stimulating discussions. This research was supported by the Max-Planck Society, Deutsche Forschungsgemeinschaft, Breuer Foundation, and EU/Memosad.

References