Our Working Point of View of Tau Protein

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Accepted 3 August 2017

Abstract. Tau protein, which was discovered in Prof. Kirschner’s laboratory in 1975, has been the focus of my research over the last 40 years. In this issue of the Journal of Alzheimer’s Disease commemorating its 20th year of publication, I will provide a short review of some of the features of my relationship with tau.

Keywords: Alzheimer’s disease, microtubules, neurons, tau

INTRODUCTION

Cellular shape is determined mainly by a cytoskeletal component, namely microtubules. These are fibrillar polymers composed of tubulin and their polymerization-depolymerization is a highly dynamic process (for a review, see [1]). If the probability of polymerization or depolymerization were equal in every direction inside a cell, the resulting shape would be a sphere [1]. However, when microtubules are stabilized without depolymerization in a specific direction, a cytoplasmic extension forms [2]. In cells with a complex morphology, like neurons, these cytoplasmic extensions are known as axons and dendrites, and they are characterized by the presence of stable (less dynamic) microtubules. Thus, it was of interest to determine the reason why neuronal microtubules show greater stability.

Tubulin is the main protein found in brain cells (Fig. 1), accounting for around 20% of the total soluble protein present in a brain homogenate. This huge amount of protein facilitates the in vitro polymerization of microtubules from a brain extract [3]. Protein characterization of these polymerized brain microtubules revealed the presence of tubulin and some microtubule-associated proteins (MAPs). Later, it was shown that MAPs, which are responsible for brain microtubule stabilization, can maintain the assembled polymers [4]. Among these MAPs, the one with the fastest electrophoretic mobility is known as tau factor [5].

BINDING OF TAU TO TUBULIN

In 1986, it was found that the C-terminal region of tubulin subunits are cleaved by digestion with the protease subtilisin and that the resulting truncated tubulin is unable to bind MAPs, including tau protein [6]. This C-terminal region of tubulin is rich in acidic residues and is thus negatively charged. Two years later, tau cDNA was cloned and the sequence of tau protein was revealed. It was then shown that the tau region involved in the binding to tubulin contained some similar, but not identical, repeated sequences enriched in basic (positively charged) residues [7]. On the basis of these observations, it was proposed that the tau-tubulin interaction was an ionic interaction between a basic and an acidic region of the tau and tubulin molecules, respectively (Fig. 2).
Fig. 1. *In vitro* polymerization of brain microtubules. Protein characterization. A) A porcine brain extract shows that tubulin (Tb) is the major protein present. Also, actin (Ac) is found in a high proportion. B) Electron micrograph of *in vitro* polymerized microtubules from porcine brain. C) Protein characterization of porcine brain polymerized microtubules by gel electrophoresis.

THE BINDING OF TAU ISOFORMS TO TUBULIN

Human tau is expressed from a single gene (mapt) located at chromosome 17 that is translated into nuclear RNA and, after RNA splicing, it yields 16 exons. However, two of these (0 and 14) are not translated into protein [8]. Mapt nuclear RNA is spliced in different ways and results in the appearance of various protein isoforms. This alternative splicing is regulated by several proteins [9].

Tau in the central nervous system contains isoforms that include exons 1, 4, 5, 7, 9, 11, 12, and 13. In addition, some isoforms contain or lack exons 2, 3, and 10 [8]. Those containing exon 10 are known as tau 4R isoforms while those lacking it are referred to as tau 3R. Tau present in the peripheral nervous system contains exons 4a, 6, and 8 [8].

Tau protein has various isoforms that are translated from different mRNAs generated by alternative splicing [10]. To test the tubulin-binding capacity of the different isoforms, we used gel electrophoresis to fractionate all the isoforms isolated from a brain cell extract and that arose from alternative splicing or by post-translational modifications. We were able to fractionate tau isoforms into eight distinct electrophoretic bands (Fig. 3A). The nature of each band was further characterized. Curiously, those with a lower electrophoretic mobility (odd numbers) (Fig. 3B) showed a higher affinity for microtubules than the others (even numbers). These microtubule-binding isoforms are probably modified by phosphorylation, since their electrophoretic mobility increases upon phosphatase treatment and the isoforms with odd numbers become even numbers. However, the site modified and the kinase involved in the modification remain unknown. This preferential binding [11] could be explained by the modification causing the opening of the so-called tau paper-clip confirmation [12]. However, other conformational changes, involving the ends of the tau molecule, cannot be excluded [13].

LOCALIZATION OF TAU IN NEURONS

Tau, a microtubule-binding protein, is found mainly in the cytoplasm, although its presence in the
cell nucleus [14, 15], where it can bind to nucleic acids [16, 17], and at the membrane [18, 19] has also been reported.

In neurons, tau is found mostly in the axon [20], although its localization in the somatodendritic compartment, including dendritic spines, has been described [21].

In axons, tau regulates the localization and function of end-binding protein 1 and 3 (EB1/3), a protein involved in axonal navigation [22], and also mitochondrial axonal transport [23].

TAU BINDING TO OTHER MOLECULES

We have analyzed the binding of tau to tubulin or to itself, but also its interaction with actin [24], heparin [25–27], muscarinic receptors M1/M3 [28], zeta 14-3-3 protein [29], EB 1/3 [22, 30], deacetylase HDAC6 [31, 32] and ferritin [33]. A scheme of the tau regions involved in some of these interactions is shown in Fig. 4.

TAU IN PAIRED HELICAL FILAMENTS

From 1975 (tau discovery [5]) to 1986, only a small number of groups worldwide were working on tau. However, the seminal discovery made by Iqbal’s group in 1986 [34] describing the presence of tau in the paired helical filaments (PHFs) of the brains of Alzheimer’s disease (AD) patients—an observation that was rapidly confirmed [35, 36]—changed the scenario. To determine whether tau is a PHF-associated protein or the core protein of PHFs, it was then tested whether highly purified tau protein in vitro was able to polymerize into filaments similar to PHFs. Thus, in 1986, we achieved a positive result indicating the assembly of highly purified tau into PHF-like structures [37, 38]. Later on, this result was confirmed [39], and it was also reported that the main component of PHFs isolated from AD patients is tau protein [40, 41].

Two laboratories almost simultaneously showed that tau polymerization is facilitated by the presence of heparin [26, 42] and also that the tubulin binding-
tau region is involved in tau self-assembly [26]. We reported that VQIVYK hexapeptide (residues 306–311) or the similar one VQIINK (residues 275–280) plays an important role in tau self-assembly. However, in the presence of heparin, we found that peptide KSKIGSTENLKHQPGGGKV (residues 257–275), which lacks these hexapeptides, forms fibrillar polymers [26].

Furthermore, we described that another post-translational modification, namely glycation, facilitates the assembly of PHFs into larger structures [43], like neurofibrillary tangles, one of the two main aberrant structures found in the brains of AD patients [44].

TAU TOXICITY

A main feature of some tauopathies, like AD, is an increase in the level of intracellular tau [39]. In some of these tauopathies, aging is a major risk factor. During aging, a decrease in tau protein turnover may result in protein accumulation—which in turn favors post-translational modifications, such as phosphorylation (see below) and/or protein aggregation—and leads to increased proteotoxicity. At the level of protein aggregation, it has been widely debated whether smaller tau aggregates are more toxic than larger ones [45]. The jury is still out on this question.

EXTRACELLULAR TAU

The brains of AD patients show an increase in tau protein (in the unmodified and phosphorylated or aggregated form) [46]. This increase could occur mainly though a decrease in tau turnover rather than tau expression. It was proposed that such an increase results in cell death or tau secretion to the extracellular medium [47, 48]. Tau secretion occurs through membrane vesicles or in a naked form [48]. In both cases, the result is the presence of extracellular tau [47]. This extracellular protein (in monomeric form) binds to neuron receptors; an interaction that results in increased the levels of intracellular calcium [28]. The neuronal receptors that bind to tau protein were identified as muscarinic (M1, M3) receptors [28, 49].

On the other hand, extracellular aggregated or truncated tau is also toxic for neurons [50, 51] and can propagate from one brain region to another [51]. In this case, the entry of extracellular tau to the neuron may occur via macropinocytosis [52] (Fig. 5).

Indeed, in 2006, we proposed that extracellular tau propagates from neuron to neuron [47], a research field (tau propagation) in which we have also been working [28, 47, 53] (Bolos M et al., unpublished).

TAU PHOSPHORYLATION

One of the main features of AD is increased tau phosphorylation [46, 54]. Several protein kinases are involved in this process [55], but one of them, GSK3β, also known as tau kinase I [56], is the one that modifies a greater number of sites in the tau molecule [57]. In this regard, we raised a conditional transgenic mouse overexpressing GSK3β, under a promoter that facilitates the expression of the kinase at the forebrain [58].

In this transgenic mouse, tau was phosphorylated and clear age-related damage at the dentate gyrus was found [58]. This damage correlated with cognitive impairment [59] and some morphological changes in the newborn neurons present at the dentate gyrus [60]. The morphological changes in dendrites of granular cells (present at dentate gyrus) led to decreased connectivity of the newborn neurons with the neuronal network [60]. Also, a clear decrease in the number of dendritic spines was observed [60], although when
the transgenic mice were placed in an enrichment environment some spine loss was reversed [60, 61].

Thus, an increase in the level of intracellular or extracellular tau is toxic for neurons. In this regard, a therapeutic strategy to decrease (or eliminate?) the presence of tau would be valuable.

THE CONSEQUENCES OF TAU ABSENCE

To date, mainly two tau knockout (KO) models have been used [62, 63]. While general changes in phenotype were not observed in either, clear differences between the expression of phosphorylated (non-functional) tau in transgenic (Tg GSK3) mice and the lack of tau in tau KO mice were found. With respect to the newborn neurons of the tau KO, no major alterations in dendritic morphology were found [64], but some changes in the number and localization of dendritic spines were observed [64]. However, these changes differed to those reported for the Tg GSK3 mouse model [60]. In Tg GSK3 mice, the loss of dendritic spines was reversed when these animals were exposed to an enrichment environment [60]. However, the absence of tau impaired the adaptation of newborn neurons to such an environment and, also, tau protected newborn neurons from acute stress-induced impairments that may affect spine number [64]. In other words, tau protein is necessary to allow the plastic modulation of adult hippocampal neurogenesis (which takes place in the dentate gyrus) exerted by both positive and negative external stimuli.

This lack of synaptic plasticity in tau KO mice correlates with a decrease in the number of spines at the distal region of the apical dendrites of newborn granule neurons (Fig. 6) [64, 65] (see also Kimura et al. [66]).

It is known that tau localizes in spines [21] and that the absence of tau in spines bearing glutamate (NMDA) receptor subunit GluN2B prevents the toxic effect of amyloid-β peptide (Aβ) when it binds to these NMDA receptors [21]. Curiously, not only does the absence of tau prevent Aβ toxicity but also tau phosphorylation by GSK3 [67, 68] or another kinase (p38k) [69] at a specific residue (serine 205) [69].

More recently, it has been proposed that phosphorylation of tyrosine 18 of tau by fyn kinase also blocks Aβ toxicity [70].

Also, it has been shown that tau deletion does not result in lethality or in neurodegeneration. At the level of the whole organism, the lack of tau can result in an increase in the duration of wakefulness and a decrease in NREM sleep time [71], brain insulin resistance [72] (see also the pioneer work of Planel et al. [73] and the comment on it [74]), and the development of some features related to Parkinson’s disease [75]. However, I do not wish to focus this review on the role of tau in this or other disorders like Huntington’s disease [76] or other tauopathies. What I will briefly mention is a recent study involving the presence of a specific SNP of tau gene in educational attainment [77]. It has been suggested that the presence of this SNP facilitates the expression of a non-coding RNA (ncRNAMAPT-AS1) [78] that may regulate (decrease) tau RNA level [79]. This notion would support the idea that low levels of tau preserve cognition [80]. However, to maintain protein homeostasis [81], not only should the amount of tau be considered but also the “quality” or origin of the tau. Recently, the possible causes for the exceptional vulnerability of humans to AD have been discussed [82]. Given that one of these causes is postulated to be the presence of a specific feature in the structure of human tau, we are currently analyzing these structural differences, testing tau from human, cow, mouse syrian hamster, etc. [83], following the studies of other laboratories [84, 85].

TAU AT THE SYNAPSES

Intracellular tau, present at the dendritic spines, facilitates Aβ toxicity [21], and extracellular tau may have a toxic effect on the presynaptic region [86, 87]. Furthermore, other compounds, in addition to tau or Aβ peptide, may be involved in synaptic dysfunction or, in general, in the development of
AD. In this regard, recent studies are looking into the involvement of somatic mutations in the onset of the disease [88]. Preliminary results in brain tissue from AD patients have indicated the presence of mutations in some genes related to protein degradation [89].

CONCLUSIONS AND ACKNOWLEDGEMENTS

This is a short review of some of the work on tau performed by my group. In this regard, I wish to acknowledge the contributions of all those who have passed through my laboratory since it was set up in 1977 for their invaluable contributions. Also, I wish to express my deepest gratitude to all the members of my laboratory. In some of our research lines on tau/AD, we collaborate with Dr. Perry, an outstanding scientist and person.

Also, much of our work has been based on reading and learning from articles after attending talks or discussions or listening to invited speakers in conferences. In this regard, I have known many scientists whose work I have followed at least once. I also wish to acknowledge them, even when some of them sometimes rejected our papers (hopefully for a good reason). Sorry, if I have not included more of their articles in the list of references.

DISCLOSURE STATEMENT

The author’s disclosure is available online (http://j-alz.com/manuscript-disclosures/17-0600r1).

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