Tau phosphorylation in hippocampus results in toxic gain-of-function

Jesús Ávila†, Elena Gómez de Barreda*, Tobias Engel‡, Jose J. Lucas† and Félix Hernández*

*Centro de Biología Molecular Severo Ochoa (CSIC-UAM), C/Nicolás Cabrera 1 Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain, †Centro de Investigación en Red de Enfermedades Neurodegenerativas (CIBERNED), 28049 Madrid, Spain, and ‡Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland

Abstract

The MAP (microtubule-associated protein) tau binds to tubulin, the main component of MTs (microtubules), which results in the stabilization of MT polymers. Tau binds to the C-terminal of tubulin, like other MAPs (including motor proteins such as kinesin) and it therefore may compete with these proteins for the same binding site in the tubulin molecule. In pathological conditions, tau is the main component of aberrant protein aggregates found in neurodegenerative disorders known as tauopathies where tau is present in its hyperphosphorylated form. GSK3 (glycogen synthase kinase 3, also known as tau kinase I) has been described as one of the main kinases involved in tau modifications. We have analysed the role of phospho-tau as a neurotoxic agent. We have analysed a transgenic mouse model which overexpresses GSK3β. In this transgenic mouse, a clear degeneration of the dentate gyrus, which increases with age, was found. In a double transgenic mouse, which overexpresses GSK3 and tau at the same time, dentate gyrus degeneration was dramatically increased. This result may suggest that phospho-tau may be toxic inside neurons of the dentate gyrus. Once neuronal degeneration takes place, intracellular tau is secreted to the extracellular space. The present review discusses the toxicity of this extracellular tau for surrounding neurons.

Introduction

Neuronal morphology is determined by its cytoskeleton which is composed of three components: MTs (microtubules), microfilaments and intermediate filaments. Tubulin, the main component of the MTs, is the most abundant protein in the cytoplasm of neurons. Usually, MTs are very dynamic polymers in the cells, but, in neurons, MTs become stabilized in specific directions, generating the cytoplasmic extensions which will become the axons and dendrites [1]. Among the MT-stabilizing proteins are the MAPs (MT-associated proteins) [2], of which the protein tau is one [3]. Additionally, tau has also been shown to facilitate MT assembly in vitro [4].

Tau has no recognizable secondary structure in its native state [5], thus all of its residues can be accessible to interact with other proteins, including itself. The majority of tau residues are conserved across mammalian species and, since they do not contribute to the formation of any ternary structure, they may be involved in some conserved protein–protein interactions [6]. In fact, tau can bind to tubulin, actin, presenilin-1, α-synuclein, phospholipase Cγ, PP (protein phosphatase) 1, ferritin or itself [7,8]. In some cases, tau protein interactions with other proteins can play a role in MT dynamics, such as the interaction of tau with hGas7b [9]. Also, some of these interactions can be regulated by tau phosphorylation (see below). Almost 20% of the total amino acids from the longest tau isoform of the central nervous system are phosphorylatable residues. It is known that tau can bind (being modified) several protein kinases such as GSK3 (glycogen synthase kinase 3), Cdk5 (cyclin-dependent kinase 5), MARK (MAP-regulating kinase), JNK (c-Jun N-terminal kinase), PKA (protein kinase A), protein kinase CK2, CaMKII (Ca2+/calmodulin-dependent protein kinase II) and PKC (protein kinase C) [7]. Hence tau is usually found as a phosphoprotein. Thus phospho-tau can also bind to other proteins such as 14-3-3 protein, JIP-1 (JNK-interacting protein 1), Pin-1 or phosphatases such as PP1, PP2A, PP2B or PPJ [7,10].

Tau binds to the C-terminal region of tubulin [11], whereas tubulin binds to tau at a region containing three (tau 3R) or four (tau 4R) similar, but not identical, repetitive sequences of 31 or 32 residues [12]. Inside the primary structure of tubulin, close to the GTP-binding region, a sequence similar to these repetitive tau sequences [13] is located, which might play a role in MAP-induced tubulin assembly [13,14].

The function of tau inside the cell can probably be replaced by the presence of other MAPs, since mice lacking tau survive and show a similar phenotype to that of their wild-type counterparts [15]. However, overexpressing tau seems to be toxic [15]. This tau overexpression may also result in increased levels of phospho-tau [16].

Aberrant tau structures in AD (Alzheimer’s disease) and other tauopathies present in the brains of patients are known as neurofibrillary tangles [17]. These structures are aggregates of filamentous polymers called PHFs (paired helical filaments) [18], where the main component is tau in...
its phosphorylated form \[19,20\], although phosphorylation of tau is not essential for its assembly \textit{in vitro} \[21\].

In the present article, we review some of the functions and dysfunctions of tau.

**Tau binding to MTs and the consequences for MT dynamics**

Tubulin (composed of two subunits, $\alpha$ and $\beta$) requires the presence of GTP for its \textit{in vitro} assembly into MTs. Tubulin binds to GTP and polymerizes to form MTs in its GTP-bound form. After polymerization, tubulin subunits hydrolyse GTP to GDP. The resultant GDP-bound polymer is weaker than the GTP-bound polymer and depolymerizes. Tubulin has two binding sites (one for each subunit). One in the $\alpha$ subunit that is not exchangeable with exogenous GTP and the other one (exchangeable) in the $\beta$ subunit. Close to the exchangeable GTP-binding site ($\beta$-tubulin), we find the sequence QLTHSLGGG, which is similar to the motif NITHVPGG present in the first tubulin-binding repeat on the tau molecule. It has been suggested that the C-terminal region of the $\beta$-tubulin subunit interferes with the GTP binding to this subunit, preventing, in part, MT polymerization. MT-associated proteins, such as tau, bind to the C-terminal region of the tubulin subunits \[22\] which may facilitate binding of tubulin to GTP \[23\]. For the assembly of MTs, GTP has to bind to the $\beta$-tubulin subunit. MTs are dynamic polymers which polymerize and depolymerize by adding and removing subunits at the end of the polymers. In neurons, MTs are less dynamic (or more stable) than in other cells owing to the presence of MAPs. Tau plays an important role in MT dynamics and a mechanism has been proposed for this role. Tau binds (through four repeated sequences) to the C-terminal region of the $\beta$-tubulin subunit \[11\]. Also, it has been described that the C-terminal region of $\beta$-tubulin (a relatively mobile region \[24\]) could bind, in an intracellular interaction, to the region containing the sequence QLTHSLGGG, indicated previously \[13\], and that this may prevent GTP binding to $\beta$-tubulin \[23\] (Figure 1). Thus the interaction of tau with the C-terminal region of $\beta$-tubulin will prevent the previously described intramolecular interaction of $\beta$-tubulin, facilitating the binding of GTP to the protein and MT assembly. There are other MAPs, such as MAP2, which contain similar tubulin-binding regions to those found in tau and this could also facilitate MT assembly \[2\]. This similar function present in different MAPs could explain the fact that the absence of tau \textit{in vivo} could be complemented by the presence of other proteins \[7\]. Tau interaction with MTs appears to be regulated through phosphorylation. Broadly, phosphorylation reduces the affinity between tau and MT.

**Tau is the main component of protein aggregates present in tauopathies**

In AD, the most common and best-studied tauopathy, tau forms fibrillar polymers known as PHFs \[18\]. These filaments, in their aggregated form, are the neurofibrillary tangles observed in the brains of AD patients \[17\]. Historically, the presence of tau in PHFs was described in 1986 \[19\]. In the same year, it was also described that tau was found to be hyperphosphorylated in these aggregates \[20\], and that pure homogenous tau can be assembled into PHF-like polymers in the absence of other proteins \[21\]. The region of tau–tau interaction was located at the tubulin-binding region of the tau molecule \[25\]. Since tau is present in its hyperphosphorylated form in AD, this modification was studied by analysing the action of different kinases and phosphatases on tau \[7\]. GSK3 (or tau kinase I) is one of the main tau kinases \[26\] and several of the sites modified by this kinase on the tau molecule are also found in its phosphorylated form in AD \[27\]. Consequently, a GSK3 transgenic mouse model was designed, overexpressing the kinase in those brain regions damaged in AD, such as the hippocampus and the cortex \[28\].
Figure 2 | Correlation of phospho-tau with dentate gyrus volume

(A) Phospho-tau levels [measured by using an antibody which recognizes phospho-tau (abAT8)] in Tet GSK3 and in tauVLW/Tet GSK3 mice compared with phospho-tau levels in wild-type (wt) mice.

(B) Dentate gyrus volume of wt, Tet GSK3 and in tauVLW/Tet GSK3 mice. There is a correlation between an increase in tau phosphorylation and a decrease in dentate gyrus volume.

Characterizations of this transgenic mouse model have shown increased phosphorylation of tau in regions such as the dentate gyrus [28,29].

**Tau phosphorylation by GSK3 correlates with neuronal degeneration in the dentate gyrus**

A main feature of GSK3 transgenic mice is memory impairment [30] which could be the consequence of progressive dentate gyrus degeneration [29,31]. To test whether the presence of tau (or phospho-tau) could regulate dentate gyrus degeneration, mice overexpressing GSK3 and lacking the tau gene were cross-bred, and preliminary experiments suggest decreased dentate gyrus degeneration when tau is lacking [31]. This observation agrees with the finding that in mice overexpressing GSK3 and a variant of tau (a better substrate than normal tau for GSK3), a more dramatic increase in tau phosphorylation and a faster dentate gyrus degeneration has been observed than in mice which also overexpressed GSK3 but lacked the tau gene [32] (Figure 2).

In conclusion, the previous observations suggest a toxic role for phospho-tau and this toxic function could result in neurodegeneration. When neurons die, tau can reach the extracellular space and, consequently, this extracellular tau might be toxic for the surrounding neurons, spreading tau toxicity [33–35].

**Extracellular tau**

Extracellular tau in its monomeric [35] or aggregated form [35,36] might be toxic for neurons. In its monomeric form, it has been shown to interact with muscarinic cell receptors, raising intracellular Ca²⁺ to toxic levels in neurons [33,35].

On the other hand, it has been suggested that extracellular tau aggregates induce the formation of new intracellular tau aggregates in the surrounding neurons in a prion-like fashion [36,37]. Future studies should reveal whether the spreading of tau pathology through the brain is due to the presence of tau in monomeric or in its aggregated form.

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**References**


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23 Padilla, R., Lopez Otin, C., Serano, L. and Avila, J. (1993) Role of the carboxy-terminal region of β tubulin on microtubule dynamics through its interaction with the GTP phosphate binding region. FEBS Lett. 325, 173-176


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