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Tau phosphorylation and assembly

Review

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Abstract. Neurofibrillary tangles, one of the aberrant structures found in the brain of Alzheimer's disease patients are mainly composed of tau in hyperphosphorylated form.Thus, a possible relation between phosphorylation and assembly of tau proteins has been analysed. By doing *in vitro* studies we have observed that in certain conditions, where compounds from oxidative stress are present, the capacity of tau for self assembly increases upon phosphorylation.

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INTRODUCTION

Tau protein is a microtubule associated protein (MAP) (Fellous et al. 1977, Weingarten et al. 1975) that is primarily restricted to neurons where it plays an important role in microtubule stabilization (Caceres and Kosik 1990, Drubin and Kirschner 1986, Kanai et al. 1992). As a result of this stabilization, neurite formation takes place (Caceres and Kosik 1990). However, a mutant mouse lacking tau (isolated by gene-targeting) is very similar to its wild type counterpart (Harada et al. 1994). An explication for this behavior is that the lack of tau function could be complemented by the presence of other MAPs, like MAP1B (Takei et al. 2000).

Tau protein is coded by a unique gene (Andreadis et al. 1992) that upon alternative splicing of the transcript generates different tau isoforms. In neurons from the central nervous system (CNS) up to six tau isoforms (from 352 to 441 aminoacids) are expressed (Baudier et al. 1987, Goedert et al. 1988). Some of these tau isoforms, which differ by the presence (or absence) of two exons located at the N-terminal and on the presence (or absence) of an exon located close of the C-terminal (Goedert et al. 1989), are developmentally regulated (Kosik et al. 1989). The C-terminal exon codes for sequences involved in the binding of tau to microtubules and is adjacent to three similar, but not identical, sequences with a similar function. All together these sequences constitute the tubulin-binding repeats (Goedert et al. 1989, Himmler et al. 1989) (Fig. 1).

TAU PHOSPHORYLATION

Fractionation of CNS tau isoforms by gel electrophoresis indicates the presence of more than the expected six isoforms (García de Ancos et al. 1993). This is due to the existence of additional tau isoforms arising by postranslational modification (phosphorylation) of the coded ones (García de Ancos et al. 1993). The level of tau phosphorylation is the consequence of the action of protein kinases that modify tau protein and that of phosphatases that dephosphorylate the previously modified tau. Several tau kinases have been described and they are grouped into two different types: proline (PDPK) and non-proline (NPDPK) directed protein kinases (Morishima-Kawashima et al. 1995a). PDPK modified Ser-Pro or Thr-Pro tau motifs and three main PDPK have been describe to phosphorylate tau, GSK3, also known as tau kinase I (Baum et al. 1996, Hanger et al. 1992, Ishiguro et al. 1993, Mandelkow et al. 1992,

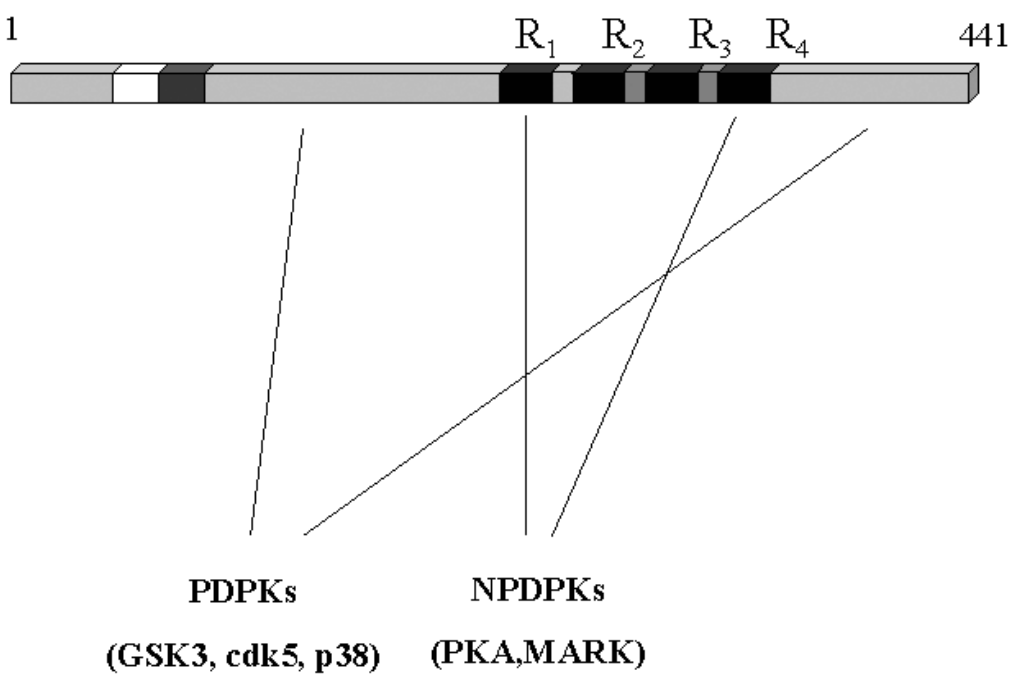

Fig. 1. Scheme of tau molecule, showing the localization of some of the sites that can be phosphorylated by proline directed protein kinases (PDPKs) and non-proline dependent protein kinases (NPDPKs). The location of the repeated sequences (R_1-R_4) is also shown.

Munoz-Montano et al. 1997, Sperber et al. 1995), cdk5 (or tau kinase II) (Baumann et al. 1993, Kobayashi et al. 1993, Liu et al. 1995, Yamaguchi et al. 1996) and stress kinases like JNK and p38 (Goedert et al. 1997, Mielke and Herdegen 2000, Reynolds et al. 1997, 2000, Zhu et al. 2000, 2001a,b). NPDPK modify Ser or Thr residues where not followed by prolines. Among them are: cyclic-AMP dependent kinase (Johnson 1992); Ca^{2+}/calmodulin dependent kinase (CaMPKII) (Baudier and Cole 1987), protein kinase C (PKC) (Correas et al. 1992) or microtubule affinity regulating kinase (MARK) (Trinczek et al. 1995). The localization of the region containing residues modified by PDPK or NPDPK is indicated in Fig. 1.

It has been suggested that tau phosphorylation could regulate tau binding to microtubules and in pathological situations to its self aggregation (Biernat et al. 1993, Bramblett et al. 1993, Cleveland et al. 1977, Drubin and Kirschner 1986, Lindwall and Cole 1984, Yamamoto et al. 1988). As indicated in Fig. 1, NPDPK phosphorylation mainly occurs at the tubulin-binding region of the tau molecule. Therefore, it has been suggested that this type of modification could result in a decrease in the binding of tau to microtubules (Biernat et al. 1993) as studies of phosphorylation of serine 262 support (Biernat et al. 1993). In pathological situations, like Alzheimer's disease (AD), where phosphorylation of the microtubule binding region takes place, a decreased binding of tau to microtubules has been observed (Cash et al. 2003).

On the other hand, modification of tau by PDPK apparently mainly affects tau self-aggregation (García de Ancos et al. 1993, Perez et al. 2000, 2002). Also, for some kinases like GSK3, two different modes of phosphorylation can occur dependent on prior phosphorylation (Cho and Johnson 2003).

Phosphatases also act on phospho-tau to regulate phosphorylation state (Iqbal et al. 1997), with primarly protein phosphatase 2A (PP2A) being involved (Iqbal et al. 1997). As some residues modified by PDPK, PP2A could require the action of a chaperone, Pin-1, to affect dephosphorylation (Lu et al. 1999). Also, it should be noted that PP2A binds to tau through the tubulin binding region (Goedert et al. 2000).

TAU PATHOLOGY

AD is pathologically characterized by the presence of two aberrant structures: senile plaques (SP) and neurofibrillary tangles (NFT) (Alzheimer 1907, Braak and Braak 1997, Selkoe 1989). Senile plaques are extracellular structures composed of amyloid-β (Aβ) peptide (Glenner and Wong 1984), which is a proteolytic cleavage product of peptide of the amyloid-β protein precursor (AβPP) (Dyrks et al. 1988).

Neurofibrillary tangles are intracellular structures composed of paired helical filaments (PHF) (Kidd 1963) and the PHFs are composed by hyperphophorylated tau protein (Grundke-Iqbal et al. 1986, 1988, Ihara et al. 1986, Wood and Zinsmeister 1989). Both senile plaque and NFT formation are related to aging even among normal patients (Alzheimer 1907). However, the frequency of NFT correlates to dementia better than does that of senile plaques (Arriagada et al. 1992). Therefore, it is not surprising that disorders leading to hyperphosphorylated tau accumulation are associated with dementia. Tauopathies involve different neuronal types. For example, in progressive supranuclear palsy (PSP), neurons from brain stem and basal ganglia are affected (Bergeron et al. 1997, Hauw et al. 1990). The aberrant structures in this disease are mainly straight filaments (Arrasate et al. 1997, Cervos-Navarro and Schumacher 1994) composed of tau isoforms with the four tubulin binding regions (Mailliot et al. 1998, Sergeant et al. 1999) which are hyperphosphorylated form (Schmidt et al. 1996).

In the most well known tauopathy, AD, hippocampal and cortical neurons are affected and PHF are found. These PHF are composed by the six CNS tau isoforms (Lee et al. 2001). Finally, in frontotemporal dementia linked to chromosome 17 (FTDP17) the frontotemporal cortex is affected and again aberrant aggregates of hyperphosphorylated tau are found. In some cases the aggregates containing the six CNS tau isoforms while in other cases mainly those containing all the tubulin binding regions are found (Spillantini et al. 1998).

POSSIBLE AGENTS THAT COULD INDUCE TAU PHOSPHORYLATION IN DIFFERENT TAUOPATHIES

It has been suggested that, in AD, Aβ could activate tau phosphorylation (Yankner 1996), possibly by GSK3 (Alvarez et al. 1999). Recently, it has been suggested that Aβ could act as an antagonist for the insulin receptor (Xie et al. 2002). Thus, the PKB kinase should be inactivated and as a consequence of that, GSK3 could be active leading to tau hyperphosphorylation. The stress kinase, p38, is also activated in AD (Hensley et al. 1999, Zhu et al. 2000) and in other tauopathies (Ferrer et al. 2001, Hartzler et al. 2002). This kinase, p38, can

phosphorylate tau on some residues that are modified in AD (Anderton et al. 2001, Gomez-Ramos et al. 2003). Also, this kinase could be activated in stress conditions (Anderton et al. 2001).

Oxidative stress has been implicated in AD (Nunomura et al. 2001, Pagocki 2003, Smith et al. 1996). A consequence of this stress is lipid peroxidation. In this way, modification of arachidonic acid yields compounds like 4-hydroxynonenal (HNE) (Sayre et al. 1997) or acrolein (Uchida et al. 1998). Both compounds are present in the brain of AD patients and colocalize with NFT (Lovell et al. 2001, Sayre et al. 1997). Also, HNE inhibits tau dephosphorylation in cultured hippocampal neurons (Mattson et al. 1997) and its adduction to phospho-tau, but not unphosphorylated tau, allows the *in vitro* formation of filaments (Perez et al. 2000) and also the appearance of filaments in cultured human neuroblastoma cells (Perez et al. 2002).

Recently, it has been shown that acrolein favors tau phosphorylation by the stress kinase p38 (Gomez-Ramos et al. 2003). This result suggests that oxidative stress could facilitate tau phosphorylation through the activation of p38. This process could take place in tauopathies like PSP where oxidative stress appears to play a role (Odetti et al. 2000) (see Fig. 2).

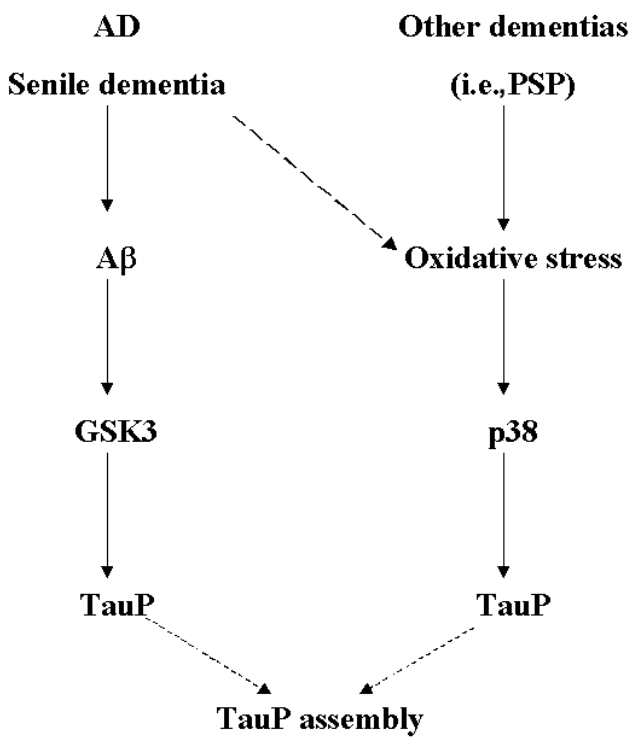

Fig. 2. Working hypothesis suggesting a role of oxidative damage in the formation of tau aggregates, through the action of the kinase p38 (right part of the Figure); or alternatively, phosphorylation (and further assembly) of tau by GSK3.

Finally, in another tauopathy, FTDP17, tau hyperphosphorylation could occur through inhibition of its dephosphorylation. In this way, it has been shown that the binding of the main phosphatase, PP2A, acting on phosphotau occurs through the tubulin-binding region of tau, and that mutation in that region (and present in FTDP17 patients) decreases PP2A-tau interaction (Goedert et al. 2000).

Thus, tau could be modified by phosphorylation in different tauopathies by different kinases and, probably, as a consequence of that phosphorylation, tau could change its conformation (Takeda et al. 2000) and increase its capacity to assemble into aberrant polymers.

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