

Tau as a Molecular Marker of Development, Aging and Neurodegenerative Disorders

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Abstract: The purpose of this work is to review the changes that take place in the microtubule associated protein tau during neuronal development, aging and neurodegeneration. Human tau protein is expressed from a single gene located on chromosome 17. The DNA is transcribed into nuclear RNA and this RNA, by alternative splicing, yields different mRNA species which are developmentally regulated. In aging, or in neurodegenerative disorders, post translational modifications of tau, such as phosphorylation, could take place, and new tau isoforms may appear. Thus, tau isoforms can be used as markers to follow neuronal development, aging or neurodegeneration.

INTRODUCTION

It has been widely commented the relatively small number of genes that are present in the human genome and how such few genes can regulate all cellular functions taking place in the human being. However, this is an incomplete view because a single nuclear RNA can yield, by alternative splicing, different mRNA species. The translation of these distinct spliced mRNAs results in the production of different isoforms. In addition to these isoforms, different post translational modifications can also yield additional protein isoforms. Thus, during the life of an organism, including aging, the product of a single gene could result in the expression of different protein isoforms. In this review we discuss the changes in tau protein during the life of an organism as well as how different tau protein isoforms could be used as markers for different developmental stages. In addition, the presence of specific tau isoforms in some pathological processes will be discussed.

TAU PROTEIN

A single human tau gene can be transcribed into nuclear RNA. This nuclear RNA yields different mRNA species by alternative splicing. The translation of these distinct spliced mRNAs results in the production of the different tau isoforms with different numbers of exons [1].

Goedert *et al* described the presence of six different tau isoforms in the human central nervous system (CNS) [2] (Fig. (1)). However, the pioneer work in this field was carried out on bovine tau where at least 14 tau exons are expressed [1]. We now know that the human tau gene contains 16 exons [3] and is present on chromosome 17. However, not all of these exons are commonly expressed in the human tau protein.

Tau protein is enriched in four amino acids: proline, glycine, lysine and serine, which account for 40% of the total residues of the protein (in the largest CNS tau isoform). The high proline and glycine content suggests the existence of a

random-coil conformation and, indeed, tau adopts this conformation allowing it to be a very soluble protein in different buffer conditions. The abundance of lysine residues indicates the presence of basic regions and, indeed, there is a basic region in the tau molecule which contains some repeated sequences that are involved in the binding of tau to microtubules via the acidic C-terminus of tubulin [4]. Finally, the presence of many serine residues suggests that the protein can be extensively modified by phosphorylation, at these residues [5].

Tau protein can be divided into four regions: 1) residues 1-103, the acidic amino terminal region with a variable number of exons. There are three types of isoforms, those containing only exon 1 (first type), those with exon 1 and 2 (second type), and those with exon 1, 2 and 3 (third type); 2) residues 104-239, the second region, rich in proline; 3) amino acids 240-370, the third region, is a basic region, rich in lysine residues (and arginine residues too) that contains the repeated sequences involved in microtubule binding. Some of the tau isoforms lack exon 10, corresponding to the second repeated sequence; 4) residues 371-441, the last region, is the acidic carboxy terminal region rich in serines.

The expression of some of these tau isoforms is developmentally regulated. Thus, isoforms lacking exon 10 (Tau 3R) are found at early developmental stages whereas tau isoforms containing exon 10 (Tau 4R) are mainly found in neurons at mature developmental stages. However, in human adult brain Tau 3R can be also found [2] in new-born neurons such as those in the hippocampal dentate gyrus.

Alternative splicing of nuclear tau RNA, occurs during brain development but this point will be only briefly commented, since an excellent review on tau RNA splicing has been already published [6]. The expression of tau isoforms derived from different numbers of exons is characteristic during brain development: those isoforms lacking exon 10 being expressed at early developmental stages [5, 6]. Human tau isoforms contain some exons present in every tau isoform. These exons (with reference to those first described in bovine tau) are exons 1, 4, 5, 7, 9, 11, 12 and 13. In human tau, there is a little (if any) expression of exons 6 and 8. On the other hand exons 2, 3 and 10 are present in some isoforms and absent in others. In the peripheral nervous system (PNS), there is a high molecular weight tau isoform derived

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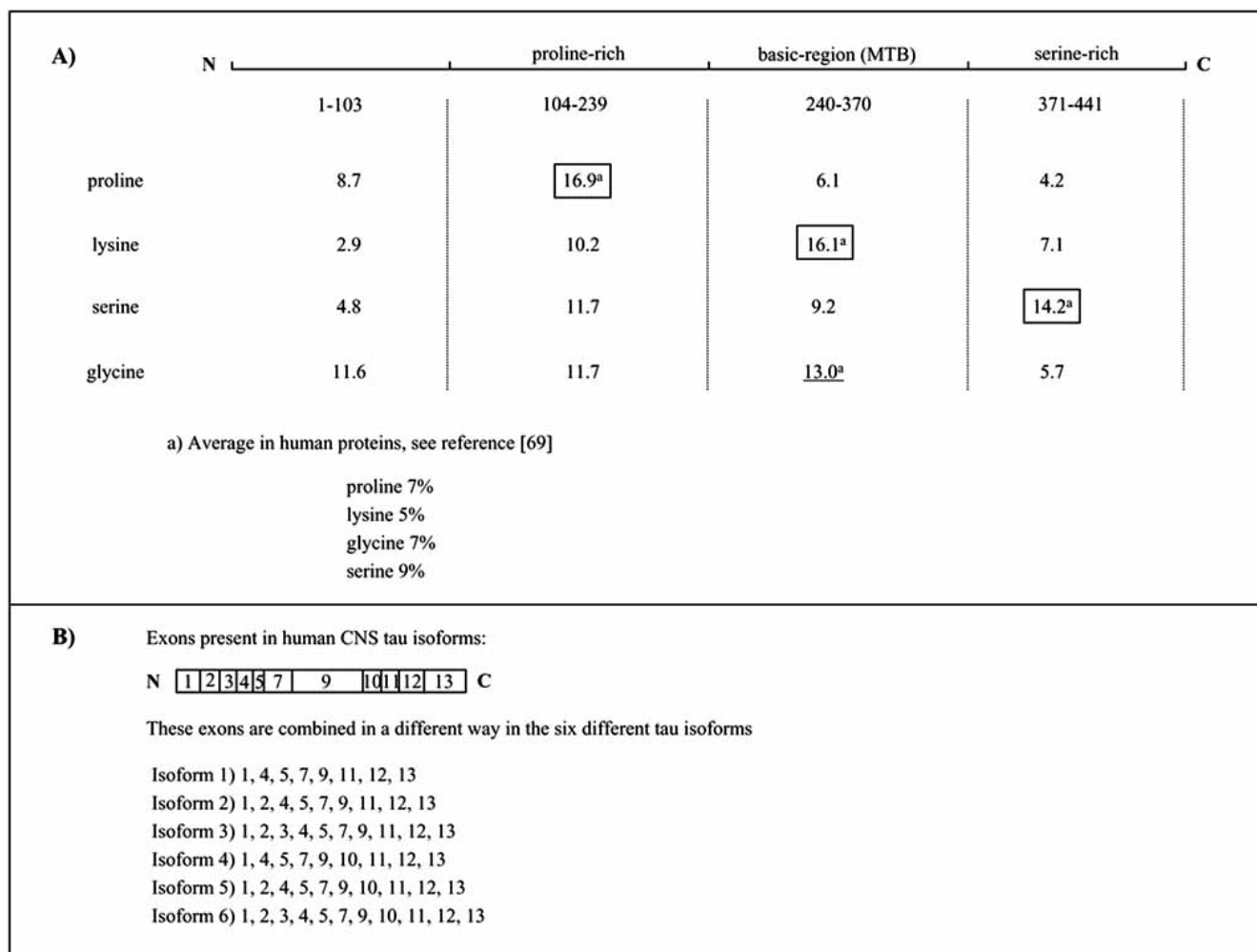


Fig. (1). A. Percentage of proline, lysine, serine and glycine residues respect to the total residues present in different tau regions (N-terminal, proline rich, basic and serine-rich regions). As a comparison (a), the average proportion of these aminoacids present in human proteins is also shown [69].

B. Exon number present in each of the six tau isoforms found in human brain.

from the expression of exon 4A. This exon is not expressed in CNS tau isoforms [7].

On the other hand, the inclusion of exons 2/3, in the tau molecule, is higher in those individuals showing the tau H1 haplotype [8] and it is not related to brain development. Splicing factors like CELF and MBNL appear to regulate the inclusion of exons 2/3 in tau protein [9]. The lack of exon 6 in human tau protein is probably due to the difficult and intricate interplay of trans factors and cis elements required for its expression [10]. The absence of exon 8 in human tau has also been reported [11, 12].

Since, the presence or absence of exon 10, in tau protein, could be used as a developmental marker (and also as a marker for some neurodegenerative disorders [5, 12-14]), the determinants of exon 10 splicing have been studied in detail [13, 15, 16].

Some splicing regulators like TRA2-BETA 1 and CLK2 [17], RBM4 [18] or SFRS11 [19] have been involved in tau exon 10 alternative splicing. Other serine-arginine rich pro-

teins [20, 21] have also been implicated. In addition, mutations in the tau gene could interfere with the presence of tau exon 10 [20, 22, 23].

Little is known about the relation between the previously indicated factors and the developmental regulation of tau isoforms containing or lacking exon 10. It has been shown that the phosphorylation of splicing factors like SC35-like protein [21] can be implicated and it has been suggested that such phosphorylation could be developmentally regulated by kinases such as GSK3β [24]. However, more work is needed to analyze this possible connection. Finally, a specific post-translational modification -phosphorylation- has been described in fetal tau [25]. Phosphorylation decreases with progressive developmental stages (Fig. (2)).

In summary, the presence of tau protein, lacking exon 10, could be suggested as a marker of young neurons. The presence of tau having exon 10 results in a possible increase in the binding of tau protein to microtubules, since exon 10 contains one of the repeated sequences involved in the binding of tau protein to microtubules. Thus, tau isoforms, pre-

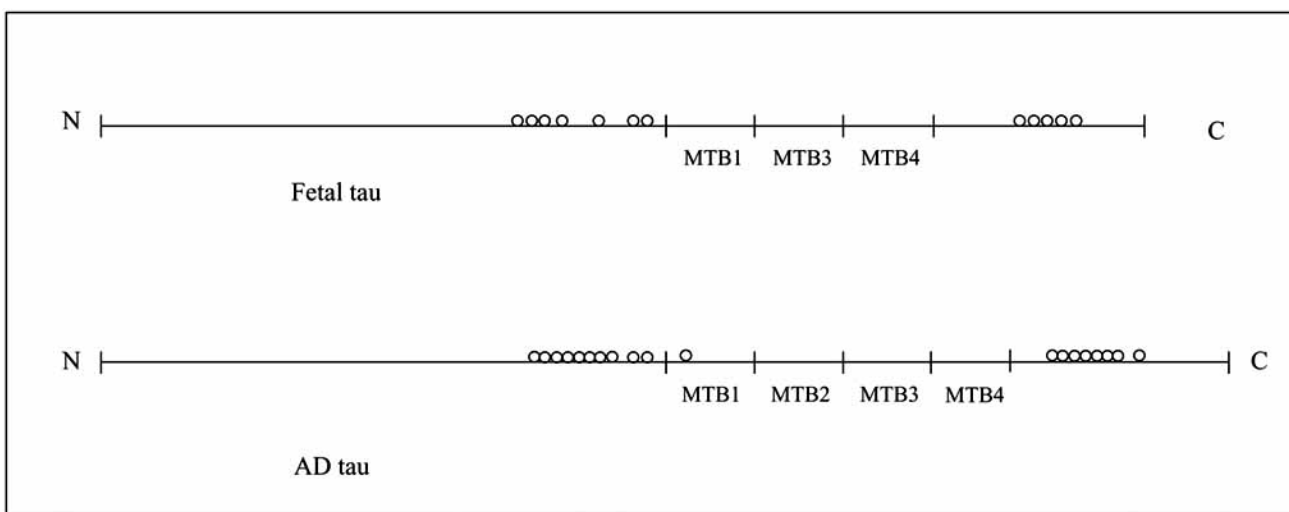


Fig. (2). Phosphorylated residues (circles) present in fetal tau and tau protein from Alzheimer disease patients (AD). Although both tau isoforms are highly phosphorylated, there are some modified residues in AD that are not present in fetal tau. The repeated sequences containing the microtubule binding motifs (MTB) in tau molecule are shown.

sent in mature neurons, are probably bound with a higher affinity to microtubules than in fetal neurons and this stronger binding may facilitate the stabilization of these cytoskeletal polymers.

AGING AND TAU ISOFORMS

Aging, and longevity, have been studied at genetic and epigenetic levels. At the genetic level the study has been performed in simple models such as yeast or worms [26]. It has been found that when yeast is grown in low glucose medium, an increase in life-span is observed [27]. Analysis carried out in *Caenorhabditis elegans* has shown that the deficient functioning of the insulin-like pathway results in an extremely long life of the worm [28].

Activation of the insulin transduction pathway results in the inhibition of GSK3 and reduction in the phosphorylation of its substrates, like tau protein. Additionally, activation of the pathway results in a decrease of the activity of the transcription factor FoxO which regulates cellular oxidative stress resistance [29], since it up-regulates several antioxidant enzymes like catalase [30] or manganese superoxide dismutase. It has been suggested that the sporadic activation of FoxO could favor longevity [31]. However, prolonged activation results in apoptosis in cultured neuronal cells [32].

It has been proposed that cell vulnerability to different forms of chronic stress increases with age. This has been part of the study at the epigenetic level. In this way, an increased vulnerability of aged neurons to oxidative damage has been proposed [33], suggesting that dysfunction in aged neurons is a result of deregulation of the removal of the toxic products arising from oxygen metabolism.

Oxygen is needed for cell viability, and, significantly, oxygen consumption is much higher in brain than in other tissues. Some of the metabolic products are toxic compounds, like the reactive species (ROS). If these toxic compounds are not removed, there is a lack of balance between

the generation and removal of ROS (oxidative stress) that may promote neuronal degeneration, and this degeneration will increase with aging.

Oxidative damage could also result in the appearance of lipid peroxidation products like 4-hydroxy-2-nonenal [34], acrolein or malondialdehyde [35]. On the other hand, oxidation of sugars could yield the formation of compounds like methylglyoxal. These products could react with proteins, like tau protein, [35, 36] to form advanced glycation-end products. Thus, proteins could be modified by these products, by ROS, (free radicals like the anion superoxide, the hydroxyl radical or nitric oxide), or by oxidative products like hydrogen peroxide. Modifications present in tau protein could also appear as a consequence of oxidative damage. In this way, mitochondrial oxidative stress may cause tau hyperphosphorylation [37].

Other consequences, at the protein level, promoted by oxidative damage are the formation of disulfide bridges, that favour tau aggregation [38], and tau glycation [35, 36], that also may result in the formation of large tau aggregates [39]. The reaction of tau with acrolein may favor tau phosphorylation [24]. Also, the reaction with acrolein, or other carbonyl compounds, could facilitate tau aggregation [40]. 4-hydroxy 2 nonenal facilitates tau assembly into fibrillar polymers [41] and it has been reported that peroxy-nitrite-mediated tau modifications facilitate tau assembly and destabilize microtubules [42]. In addition, the site for tau nitration, has already been identified [43].

One compound that could facilitate tau assembly in a more efficient way is the oxidized form of coenzyme Q₀ (CoQ₀). Coenzyme Q₀ could bind to tau protein in a non-covalent or covalent form and favor the assembly of unmodified tau as well as the phosphorylated form [44]. It should be indicated that quinones represent a class of toxicological intermediates which can create a variety of hazardous effects *in vivo*. Indeed, quinones are highly redox active molecules which can redox cycle with their semiquinone radicals, lead-

ing to formation of reactive ROS, including superoxide, hydrogen peroxide and the hydroxyl radical.

In addition to oxidative damage, other types of stress could also result in modifications of tau protein. These take place mainly by activation of stress kinases or by inhibition of specific phosphatases, which results in hyperphosphorylation of tau protein. Thus, exposure to a range of environmental insults, or stress, may result in induction of tau phosphorylation. Among these environmental changes are hibernation, cold water stress, anesthesia, alterations in glucose metabolism or electric-shock [45-52].

Nevertheless, oxidative damage appears to be one of the main causes related to neuron aging. This damage could be originated from a situation like activation of growth factor receptors which result in the appearance of toxic compounds like H₂O₂. If there is a deregulation in the pathways used to remove those toxic products, oxidative damage could take place. Also, the period of calcium entry in neurons, upon activation of some receptors, is generally brief, but unrelenting calcium entry constitutes a stress that makes neurons particularly susceptible to oxidative damage [53]. An excess of calcium can be absorbed by mitochondria, but a continuous calcium entry would produce mitochondrial stress. In fact, mitochondria house the oxidative phosphorylation machine and many metabolic pathways. Essentially, all the production of cellular energy takes place in mitochondria, where most of the reactive oxygen species (ROS) are generated. Thus, chronic mitochondria stress results in increased ROS production and oxidative damage. Also, calcium entry could activate proteases like calpain that could activate GSK3 [54] and this, in turn, may increase tau phosphorylation. Finally, it should be also noted that an increase in ROS levels could take place during hypoxia [55].

To generalize, cell vulnerability to different forms of chronic stress could increase with age and the aging process could be associated with major stress response systems. It has been suggested that the ability to cope with stress in adulthood is an predictive indicator of life expectancy and quality of life at senescence [56], since higher vulnerability to stress is associated with accelerated senescence.

As indicated, stress conditions could result in tau modifications like glycation, nitration, oxidation or phosphorylation by stress kinases [35, 36, 40, 42, 57, 58].

TAU ISOFORMS AND SENILE NEURODEGENERATIVE DISORDERS

There are some neurodegenerative disorders which involve changes in tau phosphorylation or aggregation. These disorders are known as tauopathies [23], Alzheimer’s disease being the most prevalent. Alzheimer’s disease is a senile dementia characterized by the presence of two histopathological hallmarks: senile plaques composed of a peptide called beta amyloid (a fragment of the amyloid precursor protein [59]), and neurofibrillary tangles composed essentially of the microtubule associated protein tau in a hyperphosphorylated form [60]. This tau phosphorylation takes place mainly by tau kinase I (GSK3) and tau kinase II (cdk5) [61, 62]. Beta-amyloid peptide (the main component of senile plaques) has been implicated in the activation of GSK3 [63, 64] which facilitates tau phosphorylation [65]. The localization of phosphorylation sites on tau protein from Alzheimer’s disease patients has been analyzed [25] and is shown in Fig. (2).

As indicated above, another feature of tau protein in neurodegenerative disorders is its aberrant aggregation into different types of polymers; paired helical and straight filaments in Alzheimer’s disease, globular aggregates like Hirano bodies (also present in Alzheimer’s disease cases) or in the formation of Pick bodies in Pick Disease. Other types of fibrillar tau polymers are present in other tauopathies like Corticobasal Degeneration or Progressive Supranuclear Palsy [5]. Thus, highly soluble tau protein becomes insoluble, forming tau aggregates in these neurodegenerative disorders. Truncation is another tau posttranslational modification that has been suggested to take place during tau aggregation [66]. The analysis of all these modified tau isoforms could yield markers of the indicated tauopathies. In addition, the presence of a higher proportion of modified tau 3R respect to tau 4R, or viceversa, in different tauopathies has been used to classify different tauopathies such as Corticoba-

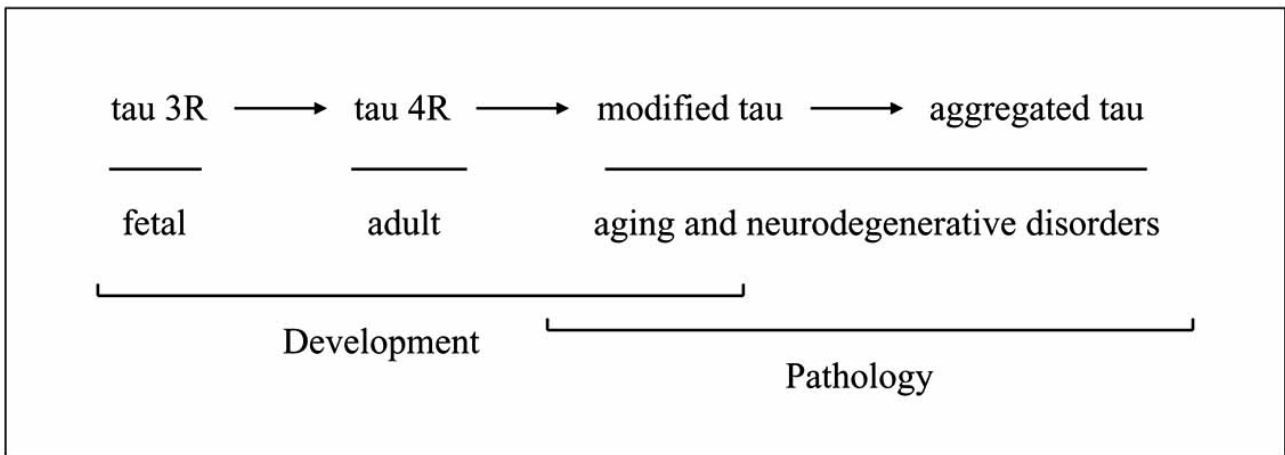


Fig. (3). Tau isoforms present in Central Nervous System during brain development and in neurodegenerative disorders with tau pathology. At fetal stage, tau 3R, with three microtubule binding motifs, is expressed. In the adult organism tau 4R, with four microtubule binding motifs, is present. This tau 4R can be modified by phosphorylation in neurodegenerative disorders like AD.

sal degeneration, Progressive Supranuclear Palsy or Pick disease [67]. The proportion of different tau isoforms in these tauopathies could be due to the fact that the affected neurons mainly express tau 4R or tau 3R isoforms in these different proportions [67, 68].

In summary, tau protein could be used as a marker to indicate the different brain stages during the life of a human being (Fig. (3)). In fetal stage, it is mainly composed of a short isoform which shows lower affinity for microtubules compared to mature brain tau isoforms. This decrease in microtubule binding capacity could be due to the absence of one of the sequences involved in microtubule binding as well as to the higher phosphorylation found in fetal tau compared with that present in mature brain tau isoforms. In mature brain, new tau isoforms are expressed and the whole tau protein shows a higher microtubule binding capacity than of that of fetal stages. However, in neurodegenerative processes, like AD, in which the main risk factor is aging, there is a dramatic decrease in the capacity of tau protein to bind to microtubules, mainly due to its hyperphosphorylated status, although other modifications could be also important. In consequence, tau protein changes its solubility characteristics and forms different types of aggregates in these neurodegenerative disorders.

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