Are Intracellular Paired Helical Filaments (PHFs) from Alzheimer's Disease Toxic for a Neuron?

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Abstract

In this review we discuss the possible role of paired helical filaments (PHFs) as a toxic agent for a neuron, and we hypothesize that the amino-terminal end of tau molecule, that is not involved in tau-tau interaction in PHFs, could be available for the binding of proteins like prolyl isomerase-1 (Pin1) or ferritin that could be essential for cell metabolism. Depletion of these proteins could result in a toxic effect.

Keywords: Alzheimer's disease; ferritin; neurodegeneration; PHFs; Pin1; tauopathies

Introduction

Alzheimer's disease (AD) is the most usual neurodegenerative disorder leading to dementia in the aged human population. It is characterized by the presence of two main brain pathological hallmarks: senile plaques and neurofibrillary tangles. Neurofibrillary tangles are composed of fibrillar polymers of the abnormally phosphorylated cytoskeletal protein tau. Tau filaments accumulate in dystrophic neurites as fine neuropil threads or as bundles of paired helical filaments (PHFs) in neuronal bodies forming the neurofibrillary tangles (NFTs) which become extracelular ghost tangles after the death of the neuron[1,2]. The severity of dementia has been correlated with accumulation of NFTs in different brain regions[3] while in the case of the other hallmark of AD, the senile plaques (extracelular aggregates formed by the amyloid peptide), such correlation has not been demonstrated[4].

The accumulation of PHFs might induce neuronal dysfunction leading to neurodegeneration. There exist an inverse correlation between the number of extracelular tangles and the number of surviving neurons, demonstrating that the nerve cells that degenerate developed neurofibrillary lesions. However, there are not a direct evidence showing that the PHFs are toxic for the neurons or if, as has been proposed for other neurodegenerative diseases (i.e. Huntington´s disease), the formation of aggregates is a defense mechanism. In this review, we will comment some evidences suggesting that PHFs could be indeed toxic for the cells.

Paired helical filaments: tau polymers

The morphology of PHFs show a twisted ribbon structure with widths between 10 and 20 nm and half period of 65 to 80 nm[5-8]. Another type of filaments (about 5 %) can also be found in AD samples. They are not twisted but straight and do not show variations in width along their length, these filaments are called straight filaments[9].

Tau is the main component of PHFs but in a hyperphosphorylated form[10]. As a consequence of that hyperphosphorylation, tau shows a loss of microtubule binding capacity[11] and is accumulated in neuronal bodies. AD-tau suffers posttranslational modifications as ubiquitination[12], proteolysis[13], glycation[14,15] and oxidation[16,17].

After the discovery that tau protein was the main component of PHFs, several attempts have been made to obtain PHFs *in vitro*. It was reported that tau protein purified from brain extracts was able to aggregate *in vitro* although at high concentration[18-21]. This observation suggested that some other molecules might contribute to tau assembly. Sulfoglycosaminoglycans (sGAG) received especial attention mainly because they are present in the NFT[22]. Heparin induces a conformational change in tau protein[23] and two heparin binding sites have been proposed to be present in tau molecule[24]. Tau polymerization is facilitated in the presence of sGAG[25,26], being heparin, as an example of sGAG, the most studied. Similarly other polyanions such as RNA[27] and polyglutamic acid[25,28] also favor tau aggrega-

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tion. PHFs contain some unidentified sGAG as evidenced by the fact that heparinase and condroitinase treatments of PHFs result in untwisting of these filaments[24]. How the interaction among tau and sGAG can take place in neurons is a matter of controversy (for a discussion see [29]). Another category of agents suggested to alter assembly are fatty acids that can facilitate aggregation either directly[30-32] and/or additionally through 4-hydroxynonenal (HNE) a highly reactive product of lipid oxidation[33,34], a compound that is increased in AD[35].

The most studied mechanism of tau polymerization is phosphorylation although under many conditions, hyperphosphorylated tau does not show a high capacity for *in vitro* polymerization as compared to unmodified tau[36]. This represents a pivotal paradox since mutations linked to familiar Alzheimer's disease (the most common tauopathie) like those found in presenilin-1 and amyloid η protein precursor, result in an increase in both the level of phosphorylation of tau protein[37,38] and in its aggregation leading to neurofibrillary tangles. Nonetheless, it is clear that phosphorylated, but not unmodified tau, is able to polymerize *in vitro* in the presence of HNE[39].

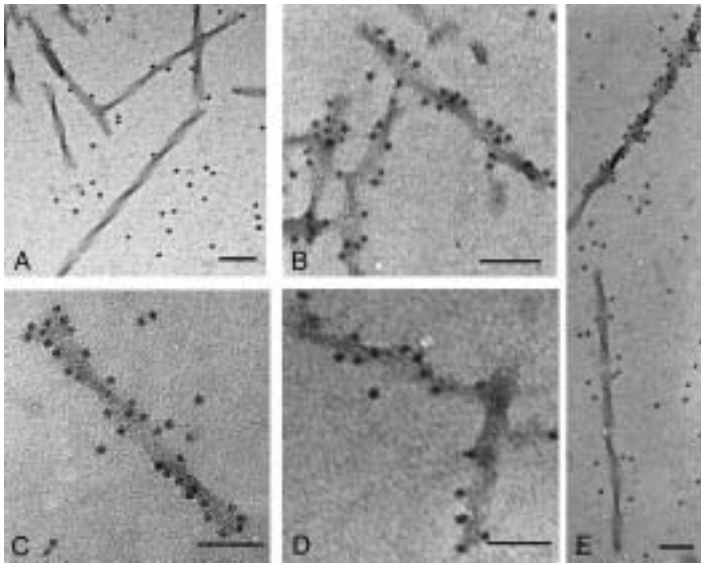

Figure 1.

Ferritin is bound to PHFs with a periodic ordination through the filament.

Electron microscopy of negatively stained PHFs before (A) and after (B-E) five months at 4ºC. Brain samples, supplied by Dr. Ravid (Netherlands Brain Bank), from AD patients were used to isolate PHFs by following the procedure described previously[51]. The PHFs samples were visualized by electron microscopy. Thus, after adsorption of the samples to electron microscopy carbon-coated the samples were stained with 2 % uranyl acetate for 1 min. Transmission electron microscopy was performed in a JEOL model 1200EX electron microscope operated at 100 kV. Scale bars represent 50 nm. Ferritin molecules containing electron-dense cores can be observed distributed randomly (no bound to PHFs) through the sample (A) or bound to PHFs (B-E). In some filaments ferritin is bound to PHFs with an helical distribution (C-E).

Paired helical filaments are able to bind Pin1 and ferritin. The origin of toxicity?

As previously indicated, there is not a direct evidence showing that PHFs could be toxic for a neuron. By comparison with other aberrant protein aggregates, it could be suggested that tau polymers could trap proteasomes reducing the total capacity for protein degradation and, as consequence of that, producing the toxic effect[40]. However, this possibility has to be further tested and other more feasible possibilities can be taking into account to explain PHF toxicity in neurons.

PHFs are able to sequestrate prolyl isomerase-1 (Pin1), a chaperone protein that binds to phosphoproteins containing phosphoserine or phosphothreonine followed by proline[41]. Pin1 enhances the rate of *cis* to *trans* isomerization of the peptide bond on the amino-terminal side of the proline. Phosphorylated tau is able to bind Pin1 being the phosphorylated threonine 231 necessary for that interaction[41]. Hyperphosphorylated tau cannot bind to microtubules but in the presence of Pin1 tau phosphorylated by Cdc2Kinase is able to bind to microtubules. Lu et al.[41] also showed that PHFs copurified with Pin1 and that soluble levels of Pin1 are reduced in AD. Thus, Lu et al.[41] suggested that Pin1 is trapped by PHFs, likely through the accessible N-terminal region of tau molecule that is not involved in tau-tau interaction. It results in depletion of soluble Pin1 and since this protein could be needed for the survival of the cell, neuronal death occurs.

Another protein that binds to PHFs is ferritin. PHFs copurify with ferritin varying greatly from one preparation to other the amount of ferritin found in PHF preparations[42]. We have observed that in fresh PHFs samples ferritin is distributed homogeneously through the sample mainly unbound to PHFs (Figure 1A); however, after storage at 4ºC, ferritin is mainly present bound to PHFs, as can be seen in Figure 1B-E. Ferritin binds to some filaments with a random distribution (Figure 1B) while in the binding to other filaments it was possible to distinguish a periodic binding pattern of ferritin particles through the filament with an "helical" distribution (Figure 1C-E). However, it was also possible to find filaments with no ferritin particles associated with them (Figure 1E). Curiously, these filaments are not immunolabeling with antibodies that bind to epitopes present in the N-terminal region of tau molecule. It suggests that such a region of tau molecule could be involved in the binding to ferritin.

Ferritins control iron homeostasis by storing large amounts of iron (up to 4500 atoms/molecule). Ferritins store the excess of iron that could be is involved in the induction of oxidative stress and neuronal death, due to its ability to promote formation of oxygen radicals. Ferritins have a cytoplasmatic localization and are composed by two

kinds of subunits (H and L) existing considerable differences in iron sequestration between neurons and glia and among neuronal and glial subtypes[43]. Taking into account that neurons have less ferritin that glia cells, neurons should be more sensitive to iron depletion.

An imbalance in H/L subunits has been observed in AD[44] as well as an increase in levels of ferritin. The ferritin accumulation observed in AD was almost exclusively associated with the microglia[45]. Alterations in iron regulatory proteins and their iron responsive element in Alzheimer's diseased brains have been reported[46]. In additon, the presence of ferrous ion, Fe^{2+}, has been observed in AD-ferritin[47], suggesting some dysfunction in these pathological ferritins that might contribute to production of free radicals involved in neurodegeneration. All these observations are a circumstantial support for the oxygen radical hypothesis induced by alteration of iron homeostasis that could take place in AD[48].

On the other hand, ferritin is also associated with the aberrant tau filaments present in progressive supranuclear palsy (PSP), another tauopathy[49]. Tau can be assembled through an oxidation process involving iron[17]. Also, It has been suggested that ferritin could be the source for iron in a redox reaction leading to tau assembly in PSP or other tauopathies[49,50].

In summary, taking into account our data, it can be proposed that PHFs, as well as another tau filaments present in different tauopathies, might be a dead-end trap for ferritin, as it seems to be for Pin1 and probably for another proteins, altering, in the case of ferritin, the homeostasis of iron. That alteration can induce oxidative stress and neuronal death, due to the ability of iron to promote formation of oxygen radicals.

Biochemical analysis of the proteolytically stable core of the PHFs and immunolabelling of PHF preparations show that N-terminal end of the tau molecule is located in the outer coat of the PHFs while the C-terminal half is in an occluded conFigureuration within the core of the PHFs[13]. Thus, the N-terminal end is the domain that most likely interacts with proteins as ferritin or Pin1. N-terminal end is lost during the process of neurofibrillary tangle formation when PHFs become extracelular after the death of the neuron. In our PHF preparations some filaments do not bind ferritin likely because they are N-terminal truncated (extracellular PHFs).

Conclusions

A growing number of evidences show that the formation of aggregates in several neurodegenerative diseases may be a way to neutralize aberrant/toxic proteins generated during the process of neurodegeneration. It has been proposed that the formation of aggregates could be a defense mechanism. However, these aggregates can bind some cellular compounds needed for cell metabolism and they can finally promote the dead of neurons. We have suggested in this review that one of these protein that bind to PHFs is ferritin and that as consequence of the binding the homeostasis of iron is altered, resulting the induction of oxidative stress and neuronal death. Thus, PHFs may have a direct toxic effect in a neuron.

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