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Tau phosphorylation in Alzheimer's disease: pathogen or protector?

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During the past decade, hypotheses concerning the pathogenesis of most neurodegenerative diseases have been dominated by the notion that the aggregation of specific proteins and subsequent formation of cytoplasmic and extracellular lesions represent a harbinger of neuronal dysfunction and death. As such, in Alzheimer's disease, phosphorylated tau protein, the major component of neurofibrillary tangles, is considered a central mediator of disease pathogenesis. We challenge this classic notion by proposing that tau phosphorylation represents a compensatory response mounted by neurons against oxidative stress and serves a protective function. This novel concept, which can also be applied to protein aggregates in other neurodegenerative diseases, opens a new window of knowledge with broad implications for both the understanding of mechanisms underlying disease pathophysiology and the design of new therapeutic strategies.

Tau: feared by the bad, loved by the good

The appearance of neurofibrillary tangles (NFTs), primarily composed of aggregated phosphorylated tau protein within specific neuronal populations, is a known neuropathological feature in several diseases known as 'tauopathies' [1]. These include many different types of diseases, such as Alzheimer's disease (AD), Down's syndrome, progressive supranuclear palsy, corticobasal degeneration, Parkinsonism-dementia complex of Guam and frontotemporal dementias, including Pick's disease and frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). In AD, because the pathological diagnosis of AD is dependent upon NFTs and the brain areas affected by NFTs correlate to disease progression, it is widely assumed that NFTs are central mediators of AD pathogenesis. However, such correlations are insufficient to conclude that NFTs are harbingers of cell death in AD. Here, we propose a novel model for the roles played by tau phosphorylation and aggregation in AD by arguing that the accumulation of phosphorylated tau might actually be a protective (antioxidant) response that serves as a manifestation of cellular adaptation to save endangered neurons.

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Tau protein: function and physiological roles

Neuronal morphology and structural integrity are maintained largely by the cytoskeleton, which is partially composed of microtubules. The assembly and stability of microtubules, in turn, are maintained by microtubule-associated proteins. One such microtubule-associated protein, tau protein, participates in the association–dissociation cycle of microtubules in neurons [2,3]. This protein is found primarily in the cytosol, but is also associated with the cell membrane [4], and it is present mainly, but not exclusively, in axons. Tau protein appears as a series of polypeptides of differing lengths on electrophoresis gels [5–7], which is a characteristic phenomenon of alternative RNA splicing [8–15] and/or various phosphorylation levels [5]. The gene encoding tau, consisting of at least 16 exons [16], is located on chromosome 17 [11,17].

The adult brain expresses six isoforms of tau, which differ by the presence of three or four repeats of 31 or 33 amino acids in the C-terminal portion, and none, one or two inserts in the N-terminal region (see [18] for details). The three or four tandem repeats contain domains that are important for microtubule binding. Two proline-rich regions, the phosphorylation of which affect the ability of tau to bind to microtubules, flank the microtubule-binding domain. It has been suggested that the four-repeat forms favor fibril formation, whereas the three-repeat forms do not [19]. A high molecular weight tau protein, containing a region encoded by an extra exon, has been described in the peripheral nervous system [20].

The importance of tau as a microtubule-associated protein was first realized during a search for factors that affect microtubule assembly [2,3]. Tau stabilizes microtubules by promoting their polymerization [21,22] and suppressing their dissociation [23]. However, there is evidence that tau is not necessary for normal cell function. A tau-deficient mouse produced by gene targeting is viable and phenotypically similar to tau-containing mice [24]. The only morphological change observed was a reduction in the number and density of axons in parallel fibers from the cerebellum. Furthermore, acknowledging that

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knocking out a gene might lead to compensatory effects, tau-deficient mice develop increased levels of alternative microtubule-associated proteins, such as MAP1A, to compensate for the loss of tau [24]. The binding of tau to microtubules, in addition to cell membranes, is highly regulated by phosphorylation [4,25]. Tau contains four distinct domains, including the microtubule (tubulin)-binding region, which becomes highly phosphorylated in neurodegenerative diseases. Hypophosphorylated tau binds with high affinity to microtubules, whereas hyperphosphorylated tau, similar to that present in AD, shows a low capacity for binding to microtubules [5,26].

The classic concept: the harmful side of NFTs in AD

NFTs are intracellular fibrillar structures composed of aggregations of paired helical filaments (PHFs) [27], which are made up of abnormally phosphorylated tau [28,29]. Tau filaments accumulate in dystrophic neurites as fine neuropil threads or as bundles of PHFs in neuronal bodies, forming NFTs which become extracellular 'ghost' tangles after the death of the neurons [30,31]. The number and localization of NFTs has been correlated with the level of dementia; by contrast, such a correlation has not been demonstrated for senile plaques [32]. Therefore, phosphorylated tau has been suggested to have a key role in the mental deficits associated with AD pathophysiology.

In PHFs, tau shows an abnormal and high level of phosphorylation localized at the C-terminus [28], which is associated with a loss of microtubule-binding capacity and a consequent accumulation in neuronal bodies. Similarly, in FTDP-17, mutations at or near the region of the tau gene that encodes the microtubule-binding region are thought to be responsible for tau aggregation and the loss of motor function [33,34]. After aggregation, PHF-tau undergoes posttranslational modifications, including ubiquitination [35], glycation [36,37] and oxidation [38].

PHF-tau is usually assumed to be a neurotoxic agent and several mechanisms have been suggested for its role in neurodegeneration. First, because it has been shown that phosphorylated tau inhibits microtubule assembly and causes the disassembly of microtubules [39], PHF-tau is also thought to compromise microtubule stability and function, resulting in a loss or decline in axonal or dendritic transport in disease [40,41]. Furthermore, PHF-tau disrupts intracellular compartments that are essential for normal metabolism. Cell culture studies show that the overexpression of tau causes a change in cell shape, retards cell growth and dramatically alters the distribution of various organelles transported by microtubule-dependent motor proteins. In these studies, mitochondria fail to be transported to peripheral cell compartments and cluster in the vicinity of the microtubule-organizing center. Similarly, the endoplasmic reticulum no longer extends to the cell periphery and becomes less dense [42,43]. Moreover, transgenic mice that overexpress the four-repeat human tau protein isoform specifically in neurons develop axonal degeneration in the brain and spinal cord and have notable axonal dilations due to the accumulation of neurofilaments, mitochondria and other vesicular structures [44].

Together, the aforementioned studies have fostered the notion that tau phosphorylation and aggregation represent a key pathogenic mechanism that is directly involved in neurodegeneration.

The alternative concept: looking for the benefit of NFTs in AD

Several studies associate the neuronal loss observed in AD brain with NFT formation. However, the correlation between NFT presence and the incidence of disease does not necessarily dictate a causal relationship. Indeed, because NFTs are produced in response to a variety of disease conditions [45,46], there is the distinct possibility that tau phosphorylation has an alternative role in disease – one that proceeds rather than precedes disease (Box 1).

NFT as an antioxidant stress response

What evidence is there to support an alternative role for NFTs in AD? First, PHF or PHF-like fibril formation itself might not have a significant impact on neuronal viability because NFT-bearing neurons appear to survive for decades [47]. In the same study, the authors generated a model that enables the quantification of neuronal loss in the hippocampus that is associated with NFT formation. This model showed that hippocampal neurons with NFTs survive for ~20 years and, therefore, NFTs might not be central mediators of neuronal death in AD. It has been shown that the amount of neuronal loss exceeds, by many fold, the number of NFTs accumulated [48,49]. No direct correlation exists between age at death and hippocampal-neuron number or the amount of NFTs. Nonetheless, these findings suggest that the formation of NFTs might occur many decades before neuronal death and, therefore, a relationship between NFT formation and the duration of disease symptoms seems unlikely. Moreover, there is no association between apoptotic morphology, such as DNA fragmentation, and tau deposition [50], suggesting that NFTs are not directly related to neuronal degeneration.

So, if NFTs are not directly responsible for neuronal cell loss, what is the role of these formations and why do they, or similar intracellular inclusions, appear in so many neurodegenerative diseases? Our view is that the presence of NFTs in AD serves to protect crucial cellular components from attack by reactive oxygen species (ROS) (Figure 1) [51]. Oxidative damage is one of the earliest events in AD and actually decreases with disease progression and NFT formation [52]. These findings indicate that NFTs might represent a compensatory response aimed at reducing ROS-associated damage. Indeed, both tau and neurofilament proteins, another major component of NFTs [53], are uniquely adapted to

Box 1.

Pathology in disease can be likened to Newton's Third Law of Motion – for every action there is an equal and opposite reaction. In Alzheimer's disease, although tau phosphorylation and neurofibrillary tangles or amyloid-β and senile plaques are often viewed as pathogenic (i.e. action), we suspect them to be secondary protective events (i.e. reaction). Deciphering action from reaction is as crucial in biology as it is in the physical world.

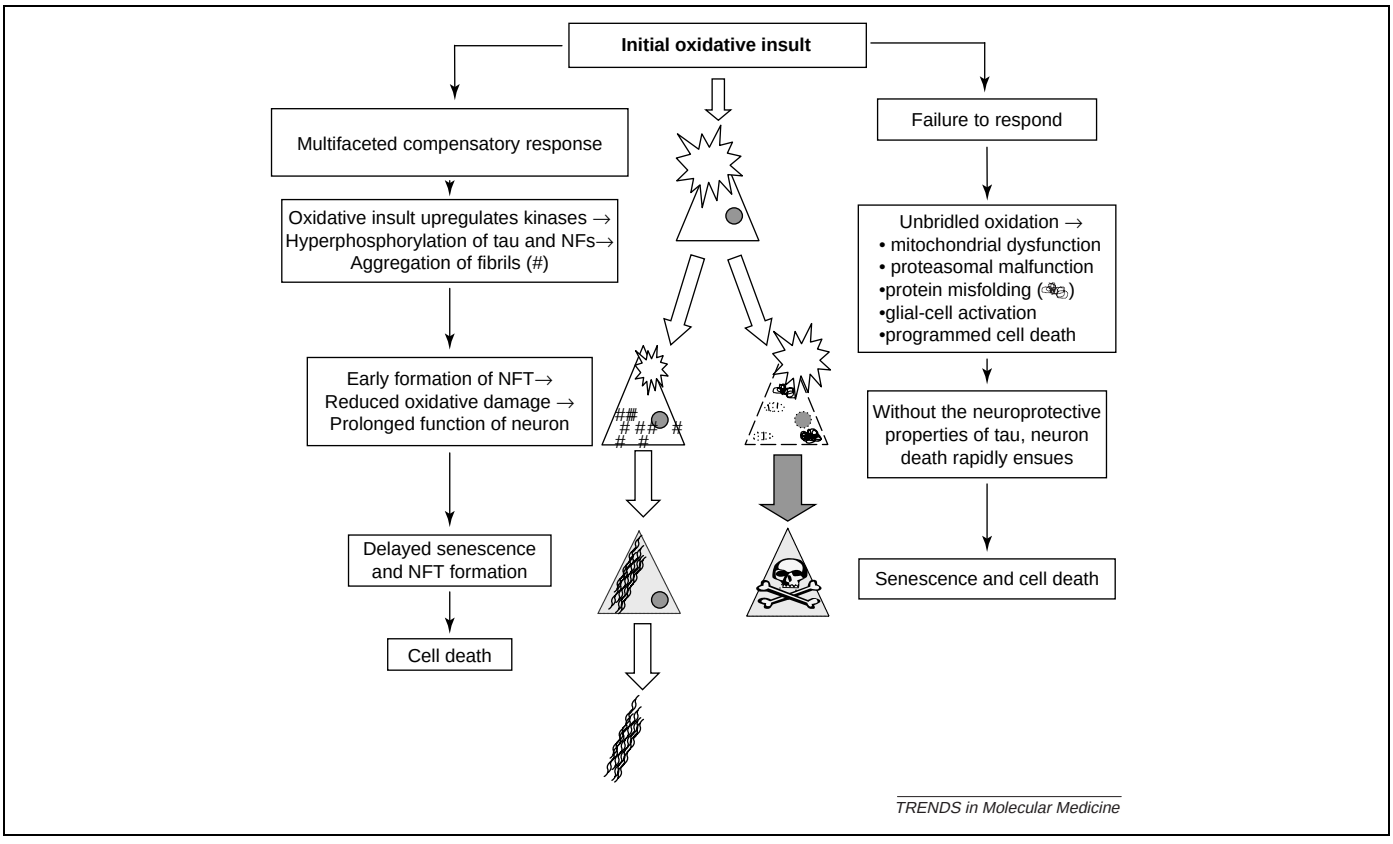

Figure 1. The hypothetical survival strategy of neuronal cells under oxidative stress. Oxidative insult to neurons in AD results in two possible outcomes. First, the neuron mounts an extensive and multifaceted compensatory response including the phosphorylation of cytoskeletal elements, such as tau and neurofilaments (NFs). In spite of leading to inclusion formation, this pathway leads to protracted survival. Neurons that fail to respond rapidly succumb to oxidative insult.

oxidative attack as a result of their high content of lysine–serine–proline (KSP) domains. The exposure of these domains on the protein surface facilitates the extensive phosphorylation of serine residues, resulting in an oxidative sponge of surface-modifiable lysine residues [54]. Tau phosphorylation is upregulated by oxidative stress [55] and tau and neurofilaments are modified by the products of oxidative stress, including 4-hydroxy-2-nonenal (HNE) [56] and other cytotoxic carbonyls [57], leading to protein aggregation as NFTs [58]. Moreover, following oxidative modification, phosphorylated tau, but not other tau forms, polymerizes more easily [58,59].

Oxidative stress activates several kinases, including glycogen synthase kinase-3 (GSK3) and mitogen-activated protein kinases (MAPKs), such as extracellular receptor kinase (ERK), p38 MAPK and Jun-N-terminal kinase (JNK), which are activated in AD [60–62] and are capable of phosphorylating tau [63] and neurofilaments [64]. Additionally, once phosphorylated, tau and neurofilaments are vulnerable to modification by carbonyl products of oxidative stress [54,58,59] and, consequently, aggregation into fibrils [58]. Because phosphorylation has a pivotal role in the redox balance, it is not surprising that the oxidative-stress-associated activation of MAPK pathways leads to the phosphorylation of tau and neurofilaments [60,61,65]. Therefore, conditions associated with chronic oxidant stress [66] are invariably associated with the extensive phosphorylation of cytoskeletal elements. Indeed, progressive supranuclear palsy and frontotemporal dementia, neurological conditions in which phosphorylated tau and neurofilament protein accumulations occur, also show evidence of oxidative adducts [67,68]. A protective role for tau phosphorylation is further supported by the fact that embryonic neurons that survive after treatment with oxidants have more phospho-tau immunoreactivity relative to those that die [69]. It was also shown that PHF-like tau phosphorylation occurs during hibernation [70], a neuroprotective phenomenon [71]. Therefore, the regulation of tau phosphorylation in the adult mammalian brain appears to represent a naturally occurring process that is associated with neuroprotective mechanisms [70]. In support of this, it has been observed that cellular antioxidant induction and tau expression are opposing [56,72], suggesting that the reduced oxidative damage in neurons showing tau accumulation [73] might be a direct consequence of an antioxidant function of phosphorylated tau [51,54].

Reduction in microtubule assembly is independent of tau abnormalities occurring in AD

The classic notion is that phosphorylated tau loses its capacity to bind to microtubules and leads to the destruction of microtubule structure and, consequently, to neurodegeneration [40,41]. However, this is not borne out in intact animal or human studies. For example, it has been shown that mice lacking tau protein develop normally and do not present major phenotypic changes. In fact, the nervous system of these tau-deficient animals is immunohistologically normal and cultured hippocampal neurons prepared from these mice show that axonal

elongation is unaffected [24]. Furthermore, knockout mice for neurofilament subunits also do not show any overt behavioral phenotype or gross structural defects in the nervous system [74,75]. The fact that knockout animals for tau and neurofilament subunits do not present alterations of neuronal function suggests that NFT formation, and a consequent microtubule disorder, is not likely to be a cause of neurodegeneration in AD. In fact, tau phosphorylation is not even likely to be a factor in microtubule stability because there is no relationship between PHF-tau and microtubule alterations in the AD brain [76]. Both the number and total length of microtubules are significantly and selectively reduced in pyramidal neurons from AD in comparison with control cases, but this decrement in microtubule density is unrelated to PHFs. These findings suggest that the reduction in microtubule assembly is independent of tau abnormalities occurring in AD.

NFTs might protect neurons: learning from other types of intracellular inclusions

Despite all the evidence, the question remains: are NFTs a harbinger of death or a manifestation of cellular adaptation for neurons? Furthermore, can we say that all intracellular inclusions are actually protective and compensatory responses to cell stressors [46,51]? Although much remains unknown, it is clear that cytoskeletal phosphorylation and other inclusions could, in fact, be beneficial because similar phosphorylation occurs in cytokeratins in response to various stressors, such as heat shock and toxins [77]. In particular, the administration of hepatotoxins to mice causes the phosphorylation of cytokeratin and its aggregation into Mallory bodies, which are highly insoluble inclusions [78]. Cytokeratins, which are normally expressed in a tissue-specific manner, are, like neurofilaments, members of the large family of intermediate-filament cytoskeletal proteins [79,80]. Although the mechanism by which stress-induced cytokeratin phosphorylation protects against certain types of liver injuries remains unknown, an association between increased phosphorylation of intermediate filaments and a variety of cell stressors has been clearly demonstrated [81,82]. The significance of cellular inclusions in Huntington's disease has also received much scrutiny. Recently, neuronal inclusion bodies of aggregated huntingtin were shown to serve as a 'coping' mechanism against neurodegeneration [83]. In a Huntington's disease cell-culture model, inclusion-body formation is associated with increased cell survival and decreased levels of huntingtin throughout the neuron. Similarly, cells that failed to form inclusion bodies were more likely to die. The inclusion-body formation appeared to decrease cell death by reducing the amount of free huntingtin, a protein with abnormal polyglutamine expansion. Thus, it is also possible that tau has a protective role for neurons not only by its phosphorylation but also by its aggregation, as with huntingtin. The long-term consequences of intracellular inclusions such as NFTs and huntingtin might be deleterious and lead to synaptic loss; however, their presence probably makes this a protracted, rather than an acute, process.

Concluding remarks

NFTs, one of the major pathological markers in AD, are positively related to the progression of AD. However, we still do not know whether NFTs are action [84] or reaction [51] in the complex scenario of AD. Elsewhere in this issue, LaFerla and Oddo [18] eloquently discuss the current established notion that tau phosphorylation represents a pathologic process (i.e. action) that should be interrupted. Supporting this, mutations in tau can lead to neurodegeneration [85], albeit different from AD, and caspase cleavage of tau might be a significant event in disease progression [86,87]. However, as outlined in this article, the same evidence can also be re-interpreted to support a new function for NFTs in the neurodegenerative process and suggest that NFTs might be 'protective shields' for neurons facing adverse conditions (i.e. reaction). Supporting this, in addition to the evidence presented here, the secondary role of tau phosphorylation to oxidative stress is evident in sporadic AD [88], Down's syndrome [89] and even familial forms of AD [90]. According to some, the 'truth' might lie somewhere in the middle, such that phosphorylated tau is toxic (as considered in the classic concept) but its uptake into NFTs prevents it from causing harm. Although initially attractive, such a postulate fails to explain why most neurons that die in AD do so in the complete absence of any (aberrant) tau-phosphorylation events, thereby indicating that neither tau nor tau-phosphorylation is toxic. As with any novel and controversial hypothesis, we hope that this Opinion article opens the doors for further discussion and experimentation on the 'truth'. This is crucial in light of proposed therapeutic strategies envisaging the reduction of tau phosphorylation because, according to our hypothesis, this might actually be counterproductive and interfere with a normal stress response in neurons.

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