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PHOSPHORYLATED TAU: TOXIC, PROTECTIVE, OR NONE OF THE ABOVE

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Abstract

Identification of phosphorylated tau as the major protein component of neurofibrillary tangles (NFTs) led to the concept that phosphorylated tau was inherently toxic and, as such, intimately involved in Alzheimer's disease (AD) pathogenesis. While superficially logical, this construct ignores a number of key findings in AD, including i) that NFTs are encountered in viable neurons until late stage disease; ii) that NFTs persist within the neuronal cytoplasm for decades; iii) that NFTs are encountered, sometimes in significant numbers, in cognitively intact elderly; and iv) that neurons with NFTs contain normal content and structure of microtubules. Experimental data in transgenic animal models has further demonstrated that NFTs accumulate in neurons in spite of tau suppression and behavior normalization. These data call into question the inherent toxicity of phosphorylated tau, seemingly leaving the only viable hypothesis of the *ad hoc* "toxic intermediate" phosphorylated tau concept. However, since we also know that phosphorylated tau sequesters redox active heavy metals and protects against oxidative stress, here we suggest that phosphorylated tau serves a protective role against cellular toxicity.

Keywords

Alzheimer's disease; neurofibrillary tangles; phosphorylated tau; protective; toxic

INTRODUCTION

The identification of phosphorylated tau as the major protein component of neurofibrillary tangles led to a host of scientific investigations (reviewed in [40]) related to tau metabolism, mechanisms of phosphorylation and dephosphorylation, role of tau processing in disease, and in general the concept that phosphorylated tau was inherently toxic, such that the amelioration of phosphorylated tau from the aged brain may successfully treat Alzheimer's disease (AD). Lost in the process, however, were clinicopathological findings indicating that phosphorylated tau typically occupies viable cells and accumulates with age, often in significant amounts. More recent experimental and human data further call into question the notion of primary toxicity, while the presence of sequestered, redox-active heavy metals within neurofibrillary pathology suggests that tau lesions may be a manifestation of protection, or cellular adaptation with age.

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In this review, we discuss the role of phosphorylated tau in disease and argue against the standard notion of toxicity of AD lesions.

Tau and the Neurofibrillary Tangle

The structure of the neurofibrillary tangle (NFT) was elucidated by Terry in 1963 [52], while Kidd, in the same year, described paired helical filaments [17]. Some ultrastructural details of NFTs were subsequently added to the literature [51], but it was not until 1986 that NFTs were purified and the microtubule associated protein tau determined as the major protein component [12]. This finding initiated a substantial focus on tau pathophysiology; however, since the identification of tau in NFTs occurred about two years after the identification of amyloid-β (Aβ) in senile plaques, and since Down syndrome and familial AD had a genetic link to Aβ, it is not surprising that Aβ assumed primacy over tau as the important pathogenic, if not etiologic, factor in AD [21,25,39,54].

Tau is relatively abundant in neurons but is present in all nucleated cells and functions physiologically to bind microtubules and stabilize microtubule assembly for polymerization. The tau gene, comprised of over 100 kb and containing 16 exons [15], contains consensus binding sites for transcription factors such as AP2 and SP1. In the adult brain, alternative splicing of tau nuclear RNA transcribed on exons 2, 3, and 10, results in six tau isoforms, having either three or four peptide repeats of 31 or 32 residues in the C terminal region encoded on exon 10, comprising the microtubule binding domain or differing in the expression of zero, one, or two inserts encoded on exons two and three. These tau isoforms, as well as their phosphorylation status, change during development such that 3 repeat tau with no inserts is expressed in the fetus and early postnatal infant, while heterogeneous isoforms are expressed in the adult brain. This switch in RNA splicing also corresponds to a reduction in tau phosphorylation.

During neurodegeneration, tau is abnormally phosphorylated at proline directed serine/threonine phosphorylation sites [2,15,41], which can be detected using specific antisera, including Ser-202/Thr-205 (AT8 site), Ser-214 and/or Ser-212 (AT100 site), Thr-231 and/or Ser-235 (TG3 site), and Ser-396/Ser-404 (PHF-1 site). As noted above, the profile of alternative tau splicing differs among pathological phenotypes, such that tau accumulation in AD is a mixture of 3R and 4R tau, Pick disease tends to be 3R tau, corticobasal degeneration and progressive supranuclear palsy tends to be 4R tau, and so-called argyrophilic grain disease accumulates small inclusions comprised of 3R tau. The general term “tauopathy” encompasses the broad classification of neurodegenerative diseases that accumulate phosphorylated tau.

Tau as “Toxic Intermediate”

The correlation between regional distribution of phosphorylated tau and clinical signs suggests a close relationship between tau and AD pathogenesis. The increased tau phosphorylation that accompanies AD may result in separation of tau from the microtubule, possibly aided by other factors (Aβ, oxidative stress, inflammatory mediators), and sequestration in NFTs and neuropil threads. The loss of normal tau function (stabilization and maintenance of microtubules), combined with a toxic gain of function, could compromise axonal transport and contribute to the synaptic degeneration that is now a central theme [2,53]. Interestingly, the concept of NFT toxicity, like senile plaque toxicity, is increasingly being challenged [18,48]. In one transgenic model, mice expressing a repressible human tau developed NFTs, neuronal loss, and behavioral impairments [43]. After tau suppression, the behavioral deficits stabilized, yet NFTs continued to accumulate, suggesting that they are not sufficient to cause cognitive decline or neuronal death. In another AD-like model, axonal pathology with accumulation of tau preceded plaque deposition [49], while studies of a P301S tauopathy model demonstrated microglial activation and synapse loss prior to NFT formation [56]. Thus, the proponents of tau phosphorylation

appear to be headed toward an analogy of the updated Aβ cascade model, by suggesting that toxicity of hyperphosphorylated tau relates to the presence of toxic tau intermediates. This was predictable [5] but does not completely address the deficiencies in the original hypothesis. Indeed, it is important to note that NFTs (and presumable "intermediates") exist within the cytoplasm of *viable* neurons. Only in advanced disease are large numbers of extracellular NFTs identified, and when they are encountered, the largest numbers appear in the CA-1 region of the hippocampus - an area that is selectively vulnerable to ischemic injury. In another study, NFTs (and presumably tau oligomers) survive within the cytoplasm of neurons for decades [24], arguing strongly against any primary toxicity of phosphorylated tau. In another study, neurons with NFTs showed intact microtubules [6], calling into question the concept that accumulation of phosphorylated tau and destabilization of microtubule assembly go hand in hand. This is further confirmed in a recent study demonstrating that axonal transport rates are not affected by overexpression or diminishing of tau expression *in vivo* [57]. Consistently, axonopathy precedes tangle formation in a tau transgenic mouse model supporting the notion that NFT development is distinct from overt neuronal loss [19].

More likely, based on these data, phosphorylated tau is sequestered in the cytoplasm in a non-toxic state late in life, similar in many ways to lipofuscin, so not to hamper cellular metabolism.

Lessons from Diagnostic Criteria for Alzheimer's Disease

The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) convened a Neuropathology Task Force with the expressed long-term goal "to produce more accurate and reliable neuropathologic criteria for AD, to determine the neuropathologic spectrum of AD, and to establish the types and frequencies of other disorders coexisting with AD or occurring alone." The protocol was published in 1991 and was based on the a semi-quantitative assessment of "neuritic plaques" (i.e., plaques accompanied by phosphorylated tau) [22], since it was generally accepted that diffuse plaques, and cored plaques devoid of phosphorylated tau, were either less indicative of AD, or strictly age related. After sampling three neocortical areas and among other regions and impregnation with Bielschowsky silver, the numbers of neuritic plaques are categorized into sparse, moderate, or frequent, and this frequency is compared with age (younger than 50 years, 50-75 years, and older than 75 years).

Therefore, according to CERAD, the older the patient, the more neuritic pathology is acceptable, and the more neuritic pathology is required to establish a diagnosis of AD. It is then axiomatic that instances exist in which the same degree of neuritic pathology results in different diagnoses with application of standard criteria. A 49 year old patient with a sparse number of neuritic plaques and dementia would have histologic findings that "indicate" the diagnosis of AD by CERAD criteria (definite AD), while a 76 year old patient with the same number of neuritic plaques would have histological findings that are "uncertain" of the diagnosis of AD (possible AD). By extension, if pathology presumes toxicity, older patients would tolerate neuritic plaques better than younger patients. Few, of course, would accept this conclusion, yet many still believe in the notion of toxicity of phosphorylated tau.

The precursor Khachaturian criteria employed a similar concept but without specifying neuritic plaques. Moreover, with respect to NFTs, the Khachaturian protocol explicitly stated that NFTs may or may not be found in the neocortex, essentially indicating that, in terms of establishing a diagnosis of AD, NFTs were more of an age-related decoration of the brain than a meaningful indicator of toxicity [16]. The Braak method [4], on the other hand, presumes that everyone with neurofibrillary (phosphorylated tau) pathology has some degree of AD regardless of clinical signs, with the stage depending on the brain regions affected. Indeed, the Braak staging method was derived from pathological examination, irrespective of the degree of clinical dementia, or the presence or absence of clinical dementia. Because of the stereotyped progression from medial temporal lobe structures to neocortex, this method is more logical

than plaque-based methods. Nonetheless, it is important to consider that subjects with intermediate stage AD, and occasionally even advanced stage AD, by the Braak method, may be cognitively intact [3]. Overall, if the neuropathology, and its correlation with clinical dementia, is any indicator of tau toxicity, the only logical conclusion to be drawn is that the toxicity of phosphorylated tau is minimal to non-existent.

Is Tau Protective?

Since tau lesions persist in viable neurons for decades, and since clinico-pathological and experimental data now argue strongly against the toxicity of phosphorylated tau, the question then becomes whether phosphorylated tau is protective. The role of oxidative stress may answer this question definitively. Evidence for oxidative stress as an upstream pathophysiology process in AD and other neurodegenerative disorders is considerable [7-9,29-31,35]. Its role in AD is well documented, including the presence of oxidative stress in vulnerable but histologically normal neurons, younger individuals prior to the onset of pathology, and in familial AD and Down syndrome prior to the appearance of pathological lesions. We have also noted the role of heavy metals (e.g., iron, copper) as potent sources of free radicals, and that redox-active heavy metals co-localize with AD pathology, and in particular NFTs [7,10,44, 46]. These findings suggest that NFTs and neurofibrillary pathology are not only non-toxic, but function to ameliorate the effects of free radical-generating heavy metals through chelation. Indeed, it was shown that cells overexpressing tau exhibit marked reductions in oxidative stress *in vivo* [26] and *in vitro* resistance to apoptosis, while the high level of tau hyperphosphorylation was observed in those cells [20]. These data, combined with the clinicopathological data, increasingly support the concept that the phosphorylated tau in AD is a *response* to the underlying pathophysiology and functions as an adaptive phenomenon in an antioxidant capacity.

One interesting question is whether phosphorylated tau can sustain its putative protective function when hyperphosphorylated and aggregated. While further study is definitely required to make a clear conclusion for this interesting question, multiple lines of evidence indicate that hyperphosphorylated and aggregated tau is not harmful and could even be neuroprotective. Recent studies clearly demonstrate that hyperphosphorylation has an anti-apoptotic effect in neuronal cell culture [20] and that NFT formation in tau inducible transgenic mice is not sufficient to cause cognitive decline or neuronal death in this model of tauopathy [43]. Therefore, at its worse, aggregated tau is likely neutral; at its best, phosphorylated and aggregated tau may still retain protective attributes.

Linking Tau Phosphorylation to Disease Etiology

A close examination of the spatio-temporal relationship between NFT and the presence of oxidative modification would appear to present a paradox. Whereas stable glycation products and HNE-adducts are predominantly associated with NFT, reversible or rapidly degraded adduction products such as 8-hydroxyguanosine (8OHG) and nitrotyrosine are predominantly found in the cytoplasm of vulnerable neurons lacking cytopathology [26,28,47]. These data mean that oxidative stress, with its transient markers such as 8OHG and nitrotyrosine, precedes formation of long-lived lesions. In fact, when rapidly formed damage is examined, the damage is restricted to cytosolic compartments, with neurons containing NFTs having significantly lower levels of 8OHG, despite an obvious history of oxidative damage, i.e., advanced glycation endproducts (AGEs) or lipid peroxidation [27]. This suggests that NFT formation may represent a cellular response to increased oxidative stress and serve in an antioxidant function. Supporting this view is the observation that neurons containing NFTs can actually survive for decades [23]. Tau modification by HNE is phosphorylation dependent [50,55] and greatly increases its ability to form filaments similar to those found in NFT *in vitro* [34] and in cells [34] which suggest that lipid peroxidation and NFT formation may be regulated by signal

transduction pathways [38]; an obvious candidate is the stress-activated protein kinase (SAPK) pathway, which, being kinases, have the known ability to phosphorylate tau. In this regard, the entire JNK/SAPK pathway is altered in AD (reviewed in [59,62,63]). Further, JNK is not only activated but also redistributed in AD [45,61], from the nucleus to the cytoplasm, in a manner that correlates with the progression of the disease; phospho-JNK is exclusively localized in association with neurofibrillar alterations in severe AD cases [61]. Nuclear localization of active JNK/SAPK is almost uniformly detected in most susceptible neurons in early AD stages, a pattern that is similar to the oxidative marker 8OHG, which suggests that oxidative stress is a likely activator of the JNK/SAPK pathway in AD.

While in several models the activation of JNK/SAPK by oxidative stress is linked with consequent apoptosis, given the time course of apoptosis, the actual cell death by JNK/SAPK mediated apoptosis occurring in the AD brain at any point in time is likely rare [36,37,42,64, 65] given the large population of neuronal cells that demonstrate activated JNK/SAPK. Under conditions of chronic oxidative stress where induction of antioxidant enzymes may be inadequate, as is likely to be the case in a chronic neurodegenerative disease such as AD, neuronal cells may undergo structural adaptations such as phosphorylation of tau protein via JNK/SAPK activation and formation of NFTs which may serve an antioxidant function [18, 28,48]. An increase in activation of MKK6 and p38 and their association with neurofibrillary pathology is also well documented in AD and other tauopathies [1,11,13,14,32,33,58,60]. The nearly identical activation profiles for phospho-JNK and phospho-p38 in AD cases suggest that JNK and p38 are activated in neurons by the same signal [60].

Conclusion

It was not until about 20 years ago that AD lesions were regarded as inherently toxic. Driving this concept was the acquired knowledge that phosphorylated tau was the major protein component of AD. It is increasingly being recognized, however, that AD lesions, neurofibrillary pathology as well as senile plaques, are end-stage accumulations that have an inconsistent relationship with disease, both clinically and experimentally. For the moment, the toxic intermediate concept (i.e., TADDLs) is being pursued to explain the disconnect between pathology and pathogenesis, but this will most likely add more details to an already confusing area of investigation that is not verifiable by direct observation. Since oxidative stress is a major upstream pathophysiological process in AD, and since NFTs possess an inherent antioxidant function, NFTs are likely not only non-toxic, but are manifestation of cellular adaptation, likely necessary for normal aging in humans.

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