Commentary

Tau Pathology Generated by Overexpression of Tau

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Neurofibrillary changes of abnormally hyperphosphorylated tau are the key lesion in Alzheimer's disease (AD) and a number of other tauopathies. Recent developments in the field of autosomal dominantly inherited dementias, in particular the frontotemporal dementias and Parkinsonism linked to chromosome 17 (FTDP-17) group, have shown that abnormalities in the tau gene result in neurofibrillary degeneration and cell death. Clinically this disorder presents with behavioral abnormalities, which are followed by dementia and, depending on the affected areas, by motor dysfunction. The location of the lesions does not seem to depend so much on the type of mutation as on the individual's genetic background and may vary even in the same family with the identical mutation. For instance, in one family with a P 301 S mutation in exon 10 of tau, the father presented with frontotemporal dementia, whereas the son had corticobasal degeneration.¹

FTDP-17 is associated with both exonic and intronic mutations of the tau gene. The microtubule-associated protein (MAP) tau is a family of six proteins derived by alternative mRNA splicing^{2,3} from a single gene located on chromosome 17. These molecular isoforms of tau differ in whether they contain three or four tubulin binding domains/repeats of 31 or 32 amino acids each near the C-terminal end and no, one, or two inserts of 29 amino acids each at the N-terminal end of the molecule. There are nine missense mutations on tau exons 9 to 13; all but three are on exon 10.⁴⁻⁷ Exon 10 codes for the additional insert of the three 4-repeat tau isoforms. The resulting mutated taus possess an altered conformation⁸ and a somewhat reduced ability to bind to and assemble microtubules.^{9,10} In addition to the exonic mutations, mutations at several sites have been found in the predicted stem loop structure in the 5' splice site to exon 10. These intronic mutations and certain mutations in exon 10 that are close to the stem loop, and thus able to disrupt it, lead to two- to sixfold higher proportion of tau mRNA containing exon 10 than in control brains.⁵ The tau protein resulting from intronic mutations is normal, but the ratio of 4-repeat to 3-repeat isoforms is increased. Presently it is believed that due to the increased proportion of 4-repeat tau in the case of intronic mutations and the compromised biological activity of the tau with missense muta-

tions, excess tau is not bound to the microtubules, which can then be hyperphosphorylated and would lead to neurofibrillary degeneration.

In contrast to the FTDP-17 group of diseases, no mutations in the tau gene have been reported in AD at the time of writing this Commentary. In more than 90% of the AD patients the disease occurs sporadically above 60 years of age. In less than 5% of the cases the disease segregates with mutations in the amyloid precursor protein (APP), presenilin-1 (PS-1), or presenilin-2 (PS-2) genes.¹¹ Frameshift mutations of APP and ubiquitin at the level of transcription have been reported to be associated with sporadic and familial AD and Down's syndrome.¹² The occurrence of the apolipoprotein E4 allele¹³ and, most recently, mutations in the β 2 macroglobulin gene¹⁴ have been reported to be risk factors for the development of the late onset, sporadic AD. AD has two prominent neuropathological lesions, the extracellular deposits of the amyloid β peptide ($A\beta$) as plaques and the intraneuronal paired helical filaments (PHF) of abnormally hyperphosphorylated tau, which accumulate in the neuronal cell body as neurofibrillary tangles, in the neuropil (the so-called neuropil threads),¹⁵ and in the dystrophic neurites surrounding the neuritic plaques. The direct relationship, if any, between the tangles and β -amyloid is not yet understood. At the one extreme, in the normal aged brain there is the significant β -amyloid accumulation and minimal neurofibrillary degeneration, whereas in the early onset familial AD with mutations in the β -APP or presenilin gene, massive β -amyloid deposits, tau hyperphosphorylation, and tangle formation are always seen. The other extreme situation is represented by extensive neurofibrillary degeneration and minimal β -amyloidosis. These include tauopathies like the tangle-predominant form of senile dementia (tangle-only dementia), Guam Parkinsonism dementia complex, dementia with argyrophilic grains, Nieman Pick's disease type C, subacute sclerosing panencephalitis, Pick's disease, and the dementia group with mutations in the tau gene.¹⁶

Supported in part by the New York State Office of Mental Retardation and Developmental Disabilities and National Institutes of Health grants NS18105, AG05892, and AG08076.

Accepted for publication October 15, 1999.

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Although at present the exact role of PHF and β -amyloid in the pathogenesis of AD is not established, there is growing evidence from a number of laboratories that the intellectual deterioration in AD patients is associated with neurofibrillary degeneration.^{17–20} A third and well characterized phenomenon is synaptic loss and cell death exceeding 50% in certain areas of the brain.^{21,22}

Role of Abnormal Hyperphosphorylation of Tau in Neurofibrillary Degeneration

The MAP tau in abnormally hyperphosphorylated form is the major protein subunit of the paired helical filaments (PHF).^{23–25} These findings on the hyperphosphorylation of tau have been confirmed by a number of laboratories.^{26–29} In a normal neuron the biological function is dependent on an intact microtubule network through which much of the axoplasmic transport is supported. Tau is one of the major MAPs and its function is regulated by phosphorylation. In neurons with neurofibrillary tangles the normal cytoskeleton is disrupted and replaced by bundles of PHF.³⁰ The disruption of the microtubule network probably compromises the axonal transport and starts retrograde degeneration of the affected neurons. The degeneration takes place apparently over a long period of time and neurons devoid of most of their axonal and dendritic arborizations have been reported in brains of patients who suffered several years of this progressive disease.^{31,32} These neurons eventually die, leaving behind the extracellular tombstones, or ghost tangles.

Tau in PHF is posttranslationally modified. The earliest known modification seems to be its phosphorylation, which is followed at later stages of tangle formation by ubiquitination.³³ The very late stages of neurofibrillary tangles also stain immunocytochemically with antibodies to advanced glycation end products (AGE), suggesting that tau in PHF might be glycated.^{34–36} In addition, PHF-tau is glycosylated with both O- and N-linked glycans.³⁷ Apparently these molecules play a supportive role for the paired helical structure of the PHF, which, on digestion of the polysaccharides with endoglycosidase F/N-glycosidase F, untwist and collapse into tightly packed bundles of ~ 2.5 nm.

Besides being polymerized into PHF, a significant amount of abnormally hyperphosphorylated tau is also present as unpolymerized deposits (AD P-tau) in the neuronal cytoplasm of the AD brain.^{33,38} Tau in these so-called stage 0 tangles is not ubiquitinated, is soluble under nondenaturing conditions, and can be isolated from AD brain and separated from the accompanying normal tau.³⁹ Although not polymerized *in situ* the AD P-tau contains from 5 to 9 moles of phosphate per mole of tau, similar to the phosphorylation level of tau of the mature tangles, making it thus unlikely that the polymerization of tau into PHF might be catalyzed solely by the number of moles of phosphate.

A potential role of AD P-tau, and *in situ*, most probably, at least as important as its involvement in the polymerization of PHF, is its deleterious effect on the integrity of the microtubules. Hyperphosphorylated tau, when poly-

merized into PHF, is biologically inert whereas AD P-tau is toxic to the system. The AD P-tau competes with tubulin in binding to not only the normal tau but also the high-molecular-weight MAPs, MAP1 and MAP2, and this sequestration of normal MAPs results in inhibition of assembly and disruption of microtubules.^{40,41} The association between the abnormal and the normal taus leads to the formation of bundles of ~ 2.1 -nm tau filaments,⁴² whereas the association between the abnormal tau and MAP1 and MAP2 does not result in the formation of filaments. The binding of AD P-tau to the MAPs is even stronger than that between tubulin and MAPs because when AD P-tau is added to already formed microtubules, they are disrupted.^{41,42} The inhibition of the microtubule assembly by AD P-tau, its sequestration of normal MAPs and disruption of microtubules are solely due to its abnormal hyperphosphorylation, because AD P-tau or tau extracted from PHF, when dephosphorylated, lose these characteristics and become fully functional, indistinguishable from normal tau in promoting microtubule assembly.^{40–45} Furthermore, *in vitro* dephosphorylation of isolated PHF-tangles by protein phosphatases (PP) 2A and 2B disaggregates and disassembles them.⁴⁵

Protein phosphorylation is one of the major mechanisms for the regulation of cellular function.⁴⁶ The hyperphosphorylation of tau (see above), neurofilaments and MAP1b^{47–49} suggest a protein phosphorylation/dephosphorylation imbalance in the AD brain. Although increased kinase activities have not been shown as yet, it has been demonstrated that the activities but not the expression of both PP-1 and PP-2A are significantly (20–30%) reduced in AD neocortex.^{50–52} Furthermore, a reduction of PP-2B activity which correlated with neurofibrillary degeneration was also observed.⁵³ *In vitro* AD P-tau and PHF-tau are dephosphorylated mostly by PP-2A and PP-2B, to a lesser extent by PP-1 but not by PP-2C.^{54–56} Recombinant tau *in vitro* [³²P] phosphorylated can be dephosphorylated by PP-2A and PP-2B.^{57,58} Furthermore, treatment of neuroblastoma or primary neuronal cell cultures with the phosphatase inhibitor okadaic acid results in the hyperphosphorylation of tau and inhibition of its turnover.⁵⁹ In the human neuroblastoma cell line SY5Y the inhibition of PP-2A and PP-1 by okadaic acid is accompanied by a transient stimulation of a number of proline-directed protein kinases, hyperphosphorylation of tau at several sites, reduced binding of MAPs to microtubules, and microtubule destabilization.⁶⁰

Animal Models for Tauopathies

Multiple attempts to induce Alzheimer-type neurofibrillary degeneration in animals, mostly rodents, have only made relatively small inroads. Because, according to the amyloid cascade hypothesis⁶¹ tau pathology may be secondary and the result of amyloid disposition, this avenue has been primarily explored. Generation of transgenic mice expressing either the normal human amyloid precursor protein β -APP 751,⁶² the carboxy terminal 100 amino acids of the amyloid precursor protein, APP-C100,⁶³ or amyloid precursor proteins with mutations found in the

familial forms of AD^{64,65} in all cases resulted in the deposition of amyloid, in some cases neurotoxicity⁶³ but at the most only modest staining of the neuropil surrounding the plaques with antibodies to phosphorylated tau. The most convincing indication for a role of amyloid in neurodegeneration is the study of Geula et al⁶⁶ in which the injection into the brain of aged rhesus and marmoset monkeys of polymerized synthetic A β peptide fibrils resulted in neurotoxicity and the appearance of phosphorylated tau in neurons and neurites distal to the area with neuronal loss. The more direct approach to induce tauopathy in an animal model is the generation of transgenic mice expressing human tau. The first study in which the longest human tau isoform (two N-terminal inserts and four repeats) was expressed in mice under the control of human Thy-1 promoter was published in 1995.⁶⁷ In this study human tau was expressed in most brain regions, but the number of neurons immunolabeled with tau antibodies was relatively small. Moderate immunostaining of some neurons with mAb AT8 to phosphorylated tau was also visible. In contrast to the wild-type mice, tau was not only stained in the axon but also present in the somatodendritic compartment of the cells. Somatodendritic staining of tau with the AT8 antibody is one of the earliest changes in selected neurons of the entorhinal cortex where neurofibrillary degeneration can be first observed.¹⁵ Similar somatodendritic distribution of tau was also observed when the smallest isoform of tau (no inserts, three repeats) was expressed in mice under the control of the mouse 3-hydroxy-methyl-glutaryl CoA reductase promoter.⁶⁸ Although extensive immunolabeling of both neurons and astroglia with a battery of antibodies to different phosphorylation sites of tau was observed, antibody AT8 or the PHF-specific antibodies AP422 and AP10 did not react with the human tau-containing cells.

In this issue, Spittaels and coworkers⁶⁹ present a study in which they have again expressed in mice the longest human tau isoform under the control of Thy-1 promoter. However, in contrast to the previous studies in which the human tau represented only 10 to 20% of the endogenous mouse tau, in this case the human tau was threefold higher than the total mouse tau (ie, 300%). Human tau was expressed in all three cellular compartments, ie, not only in the axon, but also in cell body and dendrites, and stained with phosphorylation-dependent antibodies AT8, AT180, AT270, and PHF-1. Staining was also observed in a subgroup of neurons with antibodies Alz50 and MC-1, which recognize in tissue sections a conformational epitope that occurs in PHF. Most striking, however, was the widespread axonopathy with neurofilament and microtubule accumulations which occurred both in the brain in gray matter as well as the spinal cord. It was, therefore, somewhat puzzling that no sign of cell death was detectable, nor did the electron microscopy reveal any abnormal tau-positive filaments. Because the axonal pathology was gene dosage-dependent, it may be safely concluded that excess of tau interferes with the normal physiology of the cell. This is also seen in the AD brain, where tau is increased four- to eightfold over the normal

brain.⁷⁰ In contrast to the mouse brain the excess of tau in the human brain seems to elicit a different reaction, ie, hyperphosphorylation and polymerization of tau into PHF and cell death. Most probably the extent of abnormal hyperphosphorylation of tau that occurs in this transgenic mouse model is different from that in AD brain. PHF-tau is phosphorylated at more than 21 sites; however, not all these sites seem to be of equal biological importance.

As stated above, the unpolymerized abnormally hyperphosphorylated tau in AD has lost its ability to bind to tubulin and instead binds to normal tau and high-molecular-weight MAPs, thus causing not only the inhibition of microtubule assembly but also disruption of already formed microtubules. In the neuron this would in all likelihood result in the disruption of the axonal/dendritic transport, loss of synapses, dying back of cellular processes and cell death—all features suspected in the AD and FTDP-17 brain. The main reason why the reaction of the mouse brain to overexpression of tau is so different from that of the human brain is most probably its more stable protein phosphorylation/dephosphorylation balance. This is also indicated by the facts that the degenerating axons still contained microtubules and that tau was found associated with them. Tau can be phosphorylated by a large number of kinases and both stoichiometry of the phosphorylation and the specific sites on the tau molecule that are phosphorylated seem to be critical for its biological activity. Unphosphorylation at Ser 214, Thr 231, and Ser 262 seems to be important for the normal functioning of tau.^{71–73} In the case of the AD P-tau, it is not known yet whether it is the phosphorylation at these and other specific sites and/or the numbers of phosphates incorporated into a single tau molecule that transform tau into a potentially toxic molecule that sequesters normal MAPs. Thus a different activity profile of protein kinases/phosphates in the mouse brain as compared to the human brain might not lead to an optimally phosphorylated tau that can sequester normal MAPs to disrupt the microtubule network of the cell.

In vitro studies have shown that even unphosphorylated recombinant tau can be polymerized into 10-nm filaments by the addition of fatty acids⁷³ and into PHF by anionic polymers like RNA,⁷⁴ sulfated glycosaminoglycans,^{75,76} and polyglutamate.⁷⁷ Thus, even murine tau is capable of polymerizing into PHF and PHF-like structures.⁷⁴

Outlook

The regulation of protein phosphorylation in mouse brain appears to be considerably more stable than in aged human brain. Overexpression of tau alone in mouse brain does not appear to lead to AD-like abnormally hyperphosphorylated tau and thus the AD-like neurofibrillary pathology. Understanding of the relative differences in the regulation of intraneuronal protein phosphorylation between mouse and human brain might be required to generate mouse models of AD neurofibrillary pathology.

Acknowledgments

We thank Sonia Warren and Janet Biegelson for transcribing this manuscript.

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