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Mechanism of Neurofibrillary Degeneration in Alzheimer's Disease

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Abstract

Neurofibrillary degeneration associated with the formation of intraneuronal neurofibrillary tangles of paired helical filaments (PHF) and 2.1 nm τ filaments is one of the most characteristic brain lesions of Alzheimer's disease. The major polypeptides of PHF are the microtubule associated protein τ. τ in PHF is present in abnormally phosphorylated forms. In addition to the PHF, the abnormal τ is present in soluble non-PHF form in the Alzheimer's disease brain. The level of τ in Alzheimer's disease neocortex is severalfold higher than in aged control brain, and this increase is in the form of the abnormally phosphorylated protein. The abnormally phosphorylated τ does not promote the assembly of tubulin into microtubules in vitro, and it inhibits the normal τ-stimulated microtubule assembly. After in vitro dephosphorylation both PHF and non-PHF abnormal τ stimulate the assembly of tubulin into microtubules. The activities of phosphoseryl/phosphothreonyl protein phosphatase 2A and nonreceptor phosphotyrosyl phosphatase(s) are decreased in AD brain. It is suggested that

1. A defect(s) in the protein phosphorylation/dephosphorylation system is one of the early events in the neurofibrillary pathology in AD;
2. A decrease in protein phosphatase activities, at least in part, allows the hyperphosphorylation of τ; and
3. Abnormal phosphorylation and polymerization of τ into PHF most probably lead to a breakdown of the microtubule system and consequently to neuronal degeneration.

Index Entries: Alzheimer's disease; mechanisms of neuronal degeneration; neurofibrillary changes; paired helical filaments: biochemistry; microtubule-associated protein τ; abnormal phosphorylation; ubiquitination; microtubule assembly; axoplasmic flow; protein phosphorylation/dephosphorylation.

Introduction

Alzheimer's disease is the single major cause of dementia in adults in industrialized societies. At present, the etiology and the pathogenesis of this neurodegenerative disorder are not established. Alzheimer's disease probably has polyetiology, which includes genetic, environmental, and metabolic factors. The major form of Alzheimer's disease is sporadic and has a late onset, whereas a small percentage of cases are familial and have an early onset. An understanding of the basic mechanism(s) of degeneration of neurons in Alzheimer's disease will help in the development of rational therapeu-

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tic approaches to reverse or arrest the progression of the disease.

Histopathologically, Alzheimer's disease is characterized by the presence of two brain lesions, neurofibrillary tangles of paired helical filaments (PHF) admixed with 2.1 nm τ filaments *(1)* in the neurons and β-amyloid in the extracellular space. In addition to the neuritic plaques, β-amyloid also accumulates in the wall and the lumen of the brain vessels. Deposits of β-peptide, polymers of which form amyloid, are also seen as diffuse plaques throughout the affected areas of the brain. At present, the exact relationship between PHF and β-amyloid in the pathogenesis of Alzheimer's disease is not understood. However, there is growing evidence from a number of laboratories that dementia in Alzheimer's disease patients is associated with neurofibrillary degeneration *(2–4)*.

Many of the neurons with neurofibrillary changes may be only partially functional, and in some areas of the brain, such as neocortex, many of them may eventually die, leaving behind tangled masses of abnormal fibrils, the "ghost tangles." Unlike the intraneuronal tangles, the ghost tangles in the extracellular space are made up mostly of ~15 nm straight filaments and less defined amorphous filamentous material admixed with PHF and bundles of astroglial filaments *(5–7)*. All neuronal degenerations that occur because of Alzheimer's disease might not necessarily involve neurofibrillary changes. Furthermore, in certain areas of the brain in Alzheimer's disease, such as some of the hypothalamic nuclei, neurons affected by cytoskeletal protein alterations may undergo minimal degeneration *(8)*. In this review (an update of ref. *9*), we attempt to identify a sequence of some key molecular changes associated with neurofibrillary degeneration that might constitute a major mechanism of neuronal degeneration in Alzheimer's disease.

Abnormal Phosphorylation of τ and Disruption of Microtubules

In Alzheimer's disease brains there are two general populations of PHF, PHF I and PHF II *(10)*. PHF I are readily soluble in sodium dodecyl sulfate, whereas PHF II are solubilized by repeated heat extractions in sodium dodecyl sulfate and β-mercaptoethanol or by ultrasonication followed by extraction in the detergent *(10)*. PHF I and PHF II probably represent early and late maturation stages, respectively, of the neurofibrillary tangles. The major protein subunit of PHF is the microtubule associated protein τ *(11–15)*. Some of the τ in PHF II and not in PHF I is ubiquitinated *(13,16–17)*.

τ is a family of several closely related neuronal polypeptides that are generated from a single gene by alternative splicing *(18)*. In the adult human brain there are six isoforms of τ that differ from one another in containing three or four tubulin binding repeat domains and the presence or absence of two amino terminal inserts of 29 amino acids each *(19)*. τ in PHF is abnormally phosphorylated *(12,14)*. The abnormal phosphorylation of τ apparently precedes its polymerization into PHF/neurofibrillary tangles because (a) there is a pool of non-PHF and non-ubiquitinated soluble abnormally phosphorylated τ that can be isolated from Alzheimer's disease brains, and (b) some of the nontangle-bearing neurons in Alzheimer's disease brains and normal aged but not young adult cases are stained immunocytochemically for the abnormal τ *(17,20–21)*.

The abnormally phosphorylated τ from Alzheimer's disease brains contains 6–12 mol phosphate/mol of the protein, which is two- to threefold the level in normal τ; normal τ contains 2–3 mol phosphate/mol of the protein *(9,17,22)*. To date, nine abnormal phosphorylation sites on PHF τ have been recognized (Table 1).

Although in vitro several of these phosphorylation sites are accessible to alkaline phosphatase, the overall accessibility to the phosphatase in PHF is less than in normal microtubule τ *(14)*. The aberrant phosphorylation in Alzheimer's disease brains might be selective to a few neuronal proteins and not be a part of a generalized hyperphosphorylation. Levels of both total free phosphate and phosphoprotein phosphate are normal in Alzheimer's disease brains *(14,23)*.

One of the vital functions of the neuron is the transport of materials between the cell body and the nerve endings, and microtubules are required for this axonal transport. τ stimulates microtubule assembly by polymerizing with tubulin *(24)* and maintains the microtubule structure *(25)*. Phosphorylation of τ depresses τ's ability to promote microtubule assembly *(26)*. In Alzheimer's disease brains the levels of normal τ in the cytosol are decreased by about 40%, whereas the total tissue levels are increased severalfold, and this increase is in the form of the abnormally phosphorylated protein *(27,28)*. Binding of guanosine triphosphate (GTP) to the β-subunit of tubulin, which initiates microtubule assembly, is stimulated by τ. Lack of functional

Table 1
Phosphorylation Sites of PHF-τ

P-amino acid[a]	Phos. site[b]	Antibody used[c]	Reference
Ser 46	KESP	102c	Iqbal et al., 1989 *(14)*
Thr 123	HVTQ	TP30	Brion et al., 1991 *(39)*
Ser 199	YSSP	τ-1, AT8	Grundke-Iqbal et al., 1986 *(12)* Biernat et al., 1992 *(40)*
Ser 202	PGSP	τ-1, AT8	Grundke-Iqbal et al., 1986 *(12)* Biernat et al., 1992 *(40)*
Thr 231	VRTP	—	Hasegawa et al., 1992 *(41)*
Ser 235	PKSP	SMI33	Lichtenberg-Kraag et al., 1992 *(42)* Hasegawa et al., 1992 *(41)*
Ser 262	IGST	—	Hasegawa et al., 1992 *(41)*
Ser 396	YKSP	PHF-1, T3P	Greenberg et al., 1992 *(43)* Lee et al., 1991 *(15)*
Ser 404	DTSP	pτ 2	Kanemaru et al., 1992 *(44)*

[a]The phosphoamino acid is shown in bold print.
[b]The phosphorylation site is numbered according to the largest isoform of human τ, τ_{441}.
[c]The antibody usd to map the phosphorylation site.

τ in Alzheimer's disease brains might lead to decreased GTP binding and, consequently, decreased assembly of microtubules *(29)*. Microtubules are rarely seen in neurons with neurofibrillary tangles and microtubules are not assembled from brain cytosol of Alzheimer's disease cases *(30,31)*. Both PHF-τ and soluble abnormally phosphorylated τ require deposphorylation to stimulate in vitro assembly of tubulin into microtubules *(28,32)*. The abnormally phosphorylated τ inhibits the τ-stimulated assembly of microtubules by competing with tubulin for binding to normal τ *(28)*.

Protein Phosphorylation/ Dephosphorylation Systems Involved in Hyperphosphorylation of τ

The mechanism by which τ in Alzheimer's disease brains is abnormally hyperphosphorylated is not yet established. The state of phosphorylation of substrate proteins depends on the relative activities of protein kinases and phosphoprotein phosphatases. Studies *(12,14,33)* showing the dephosphorylation of the abnormally phosphorylated sites of τ after treatment with alkaline phosphatase in vitro suggest that the protein phosphorylation/ dephosphorylation defect might be the result, in part, of a deficiency in a protein phosphatase system or systems in the affected neurons in Alzheimer's disease. The activities of both protein phosphatase 2A (PP2A) and cytosolic phosphotyrosine phosphatase are decreased in Alzheimer's disease brains *(34)*, and PP2A dephosphorylates the abnormally phosphorylated τ in vitro *(35)*. The activity of MAP (mitogen activated protein) kinase, which might be involved in the phosphorylation of some of the abnormal sites *(36,37)* is inhibited by both PP2A and phosphotyrosine protein phosphatase *(38)*.

Conclusions

In conclusion, it appears that the protein phosphorylation-dephosphorylation system is defective in Alzheimer's disease brains, leading to abnormally phosphorylated τ and some other neuronal proteins, and that the abnormal phosphorylation of τ contributes to a microtubule assembly defect and consequent impairment of axoplasmic flow and neuronal degeneration (Fig. 1).

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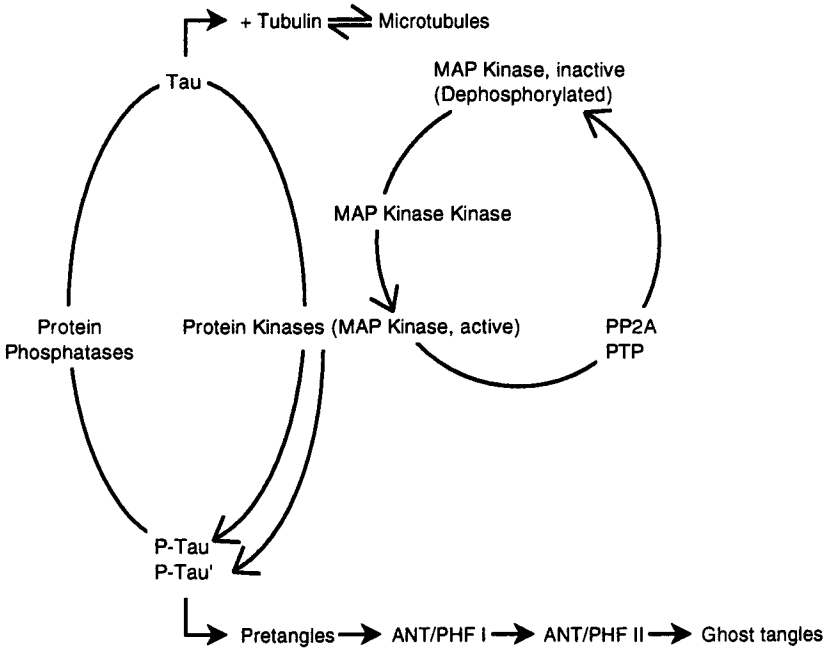

Fig. 1. A hypothetical scheme showing the mechanism of neurofibrillary degeneration in Alzheimer's disease. τ is phosphorylated by several protein kinases, including MAP kinase. Because of a decrease in the activities of protein phosphatase 2A (PP2A) and phosphotyrosine protein phosphatases (PTP) in affected neurons, some of the protein kinases, including the MAP kinase, may remain active for extended periods of time, thereby producing hyperphosphorylated τ. The latter does not bind to tubulin to form microtubules; competes with tubulin in binding to normal τ and inhibits the microtubule assembly; and becomes stabilized and polymerizes into PHF. The affected neurons degenerate both as a result of the breakdown of the microtubule system, and because of the accumulation of PHF as Alzheimer neurofibrillary tangles (ANT) filling the entire cell cytoplasm, leaving behind ghost tangles in the extracellular space.

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