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Review Article

Hyperphosphorylation determines both the spread and the morphology of tau pathology

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Abstract

Introduction: Neurofibrillary pathology of abnormally hyperphosphorylated tau (P-tau) is a hallmark of Alzheimer's disease (AD) and other tauopathies. Tau pathology can be experimentally induced and propagated. However, what induces the prion-like transmission character to tau and produces morphologically distinct tau lesions remains elusive.

Methods: We investigated the role of hyperphosphorylation in the spread of tau pathology in hTau transgenic mice.

Results: We found that intrahippocampal injection with AD P-tau, but not nonphosphorylated tau, produced numerous P-tau tangles and neuropil threads locally and in neocortex lateral to injection and upstream to the hippocampus. Dephosphorylation of AD P-tau with protein phosphatase-2A dramatically reduced and switched tau pathology from neurofibrillary tangles to argyrophilic grain-like morphology.

Conclusions: Our findings show that abnormal hyperphosphorylation of tau determines the spread and morphology of tau lesions and that the propagation of tau pathology takes place both locally and in axonally connected areas and highlight tau hyperphosphorylation as a potential drug target.

Keywords: Tau hyperphosphorylation; Tauopathies; Alzheimer's disease; Protein phosphatase-2A; Prion-like spread; Argyrophilic grains

1. Introduction

Alzheimer's disease (AD), the major neurodegenerative disease and cause of dementia, is a slow progressive disorder pathologically characterized by extracellular amyloid-β plaques and intracellular neurofibrillary tangles (NFTs). The neurofibrillary pathology in AD brain is initiated in the coeruleus/subcoeruleus complex and the transentorhinal area, from where it sequentially progresses to the limbic system and eventually the isocortex [1,2]. Hierarchical temporospatial progression of tau pathology has also been observed in argyrophilic grain disease (AGD), another age-related neurodegenerative tauopathy [3]. The stereotypical pattern of tau pathology progression is consistent with the hypothesis that tau pathology is transmitted from one area of the brain to another [4–9]. These studies suggest prion-like property of pathologic tau because host tau protein can be templated into filaments by intracerebral injection of brain extract containing pathologic tau prepared from either AD brain [6,10] or mutated tau-expressing mouse brain with end-stage tauopathy [4,11], or even by peripheral administration of pathologic tau [12]. In addition, synthetic aggregates produced from recombinant tau in vitro with heparin also show capability of inducing tau pathology in vivo [13,14]. Intriguingly, the morphology

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of tau pathology varies from tauopathy to tauopathy [15] and can be propagated in cultured cells and in brains of mice transgenic for human tau, indicating the involvement of different tau strains and further supporting the prion-like character of pathologic tau [6,8,10]. However, pathophysiologically what endows tau with prion-like character that produces neurofibrillary pathology remains elusive.

In AD and related tauopathies, the protein tau, instead of functioning to promote microtubule assembly, disassociates from microtubules and self-aggregates to produce morphologically distinct tau lesions [15,16]. Biochemical studies have revealed that the intracellular neurofibrillary pathology is made up of abnormally hyperphosphorylated tau (P-tau) in all tauopathies (for review, see [15]). For instance, in AD brain, P-tau is the major protein of NFTs [17] and is also seen as cytosolic oligomers, being approximately three- to four-fold more phosphorylated than normal tau from AD and control brains [18,19]. P-tau is also characterized as the main protein constituent of Pick bodies in Pick disease brain and of argyrophilic grains and coiled bodies in AGD brains [15].

Hyperphosphorylation may compensate for the positive charges [20] and thereby induce aggregation and self-assembly of tau [21,22], a highly soluble basic protein with little secondary structure [23,24]. Oligomeric hyperphosphorylated tau isolated from AD brain cytosol (AD P-tau), but not in vitro enzymatically dephosphorylated one, self-assembles into paired helical filaments (PHFs)/straight filaments [22], and all six isoforms of recombinant human tau can readily self-assembles into filaments after in vitro hyperphosphorylation [21]. Similar to being neutralized by hyperphosphorylation, tau can also be induced to aggregate and assemble when incubated in vitro with heparin [25], polyglutamate [26], RNA [27], or arachidonic acid [28]. In addition, the studies from our and other laboratories showed that tau self-assembles into filaments when pseudophosphorylated at some major sites by site-directed mutagenesis, that is, Ser/Thr to Glu [29,30]. Of particular note, our in vitro studies showed that unlike normal tau or PHF tau, oligomeric AD P-tau sequesters and templates normal tau into filaments, and enzymatic dephosphorylation inhibits this activity [31,32]. These findings suggest a potential role of abnormal hyperphosphorylation in conferring tau a particular conformation which favors its prion-like activity, yet not experimentally tested in vivo.

To study the role of hyperphosphorylation in the spread of tau pathology, we investigated the propagation of tau pathology after intracerebral injection of isolated AD P-tau, either untreated or in vitro dephosphorylated with protein phosphatase 2A (PP2A), into the hippocampi of hTau mice which express all six isoforms of nonmutated human tau encoded by the genomic human tau transgene [33]. We found that dephosphorylation both dramatically reduced the pathology and switched the tau lesion from NFTs to argyrophilic grain-like morphology.

2. Methods

2.1. Animals

The hemizygous human tau transgenic (B6.Cg-*Mapt*^{tm1} (EGFP)Klt Tg(MAPT)8cPdav/J, hTau) mice with murine tau knockout (tau$^{-/-}$) background [33] and tau$^{-/-}$ mice obtained from the Jackson Laboratory (Bar Harbor, ME, USA) were used in this study. The mice were housed under a 12-h light/dark cycle, with access to food and water ad libitum. All animal handling and use were as per the protocol approved by our Institutional Animal Care and Use Committee, in accordance with the PHS Policy on Human Care and Use of Laboratory Animals (revised February 15, 2015).

2.2. AD brain samples

Frozen brain tissue samples from autopsied and histopathologically confirmed AD cases were obtained from the Brain Tissue Resource Center, McLean Hospital, Belmont, Massachusetts.

2.3. Preparation of tau seeds

Oligomeric, AD abnormally hyperphosphorylated tau (AD P-tau) was isolated from the cerebral cortex of a frozen autopsied AD brain as described by us previously [19,31]. Briefly, 10% brain homogenate prepared in the buffer (20-mM Tris-HCl, pH 8.0, 0.32-M sucrose, 10-mM β-mercaptoethanol, 5-mM $MgSO_4$, 1-mM EDTA, 10-mM glycerophosphate, 1-mM Na_3VO_4, 50-mM NaF) in the presence of inhibitors to phosphatase and protease was centrifuged at 27,000*g* for 30 minutes. The supernatant was further centrifuged at 235,000*g* for 45 minutes, and the resulting pellet, that is, AD P-tau, was collected and washed three times and then resuspended in normal saline. The AD P-tau was bath sonicated using three bursts of 10 seconds each. A portion of the preparation was incubated with two doses of PP2A, each 400 mU/mL in phosphatase reaction buffer (50-mM Tris-HCl, pH 7.0, 2-mM $MnCl_2$, 0.01-mg/mL bovine serum albumin, 20-mM β-mercaptoethanol, 5-μg/mL leupeptin, 1.5-μg/mL pepstatin, 2-μg/mL aprotinin, 1-mM AEBSF, and 5-mM benzamidine) for 2 hours at 37°C. The dephosphorylated preparation was centrifuged as mentioned previously, and the pellet (PP2A-AD P-tau) was washed twice and resuspended in normal saline. The efficacy of dephosphorylation by PP2A in the PP2A-AD P-tau preparation was examined by using SDS-PAGE and Western blots developed with antibodies Tau-1, 12E8, and PHF-1, and tau concentration was assayed by immuno-dot blots.

2.4. Steorotaxic injection

Mice were deeply anesthetized with 1.25% Avertin (Sigma, St. Louis, MO, USA) and placed in a stereotaxic frame. After craniotomy, 1 mm in diameter, was made with a motorized minidrill, the tau seeds were injected using a