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p73 haploinsufficiency causes tau hyperphosphorylation and tau kinase dysregulation in mouse models of aging and Alzheimer's disease

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Abstract

Haploinsufficiency for the p53 family member p73 causes behavioral and neuroanatomical correlates of neurodegeneration in aging mice, including the appearance of aberrant phospho-tau-positive aggregates. Here, we show that these aggregates and tau hyperphosphorylation, as well as a generalized dysregulation of the tau kinases GSK3β, c-Abl, and Cdk5, occur in the brains of aged p73+/− mice. To investigate whether p73 haploinsufficiency therefore represents a general risk factor for tau hyperphosphorylation during neurodegeneration, we crossed the p73+/− mice with 2 mouse models of neurodegeneration, TgCRND8+/Ø mice that express human mutant amyloid precursor protein, and Pin1−/− mice. We show that haploinsufficiency for p73 leads to the early appearance of phospho-tau-positive aggregates, tau hyperphosphorylation, and activation of GSK3β, c-Abl, and Cdk5 in the brains of both of these mouse models. Moreover, p73+/−; TgCRND8+/Ø mice display a shortened lifespan relative to TgCRND8+/Ø mice that are wild type for p73. Thus, p73 is required to protect the murine brain from tau hyperphosphorylation during aging and degeneration.

Keywords: p73; p53 family; Tau phosphorylation; Pin1; Alzheimer's disease; Aging; Neurodegeneration; GSK-3β; c-Abl; Cdk5; Tauopathy; Paired helical filaments; TgCRND8

1. Introduction

The p53 family members are transcription factors with important cellular functions such as apoptosis, survival, cell cycle, cell arrest, differentiation, and senescence. The family consists of 3 members, p53, p63, and p73, which encode multiple isoforms including or lacking the major transcriptional transactivation domain (reviewed in McKeon and Melino, 2007). In the developing nervous system, p53 regulates naturally occurring cell death and stem cell maintenance, and protection from genotoxic stress (reviewed in Jacobs et al., 2006). We recently explored the function of this family in nervous system aging, degeneration, and postnatal stem cell function and found that p73 in particular plays salient roles. In stem cells, the transactivating (TA) form of p73 is required for neural stem cell renewal (Agostini et al., 2010; Fujitani et al., 2010; Talos et al., 2010; Tomasini et al., 2008) while the truncated (ΔN) form lacking the major transactivating domain is necessary for the survival and maintenance of many peripheral nervous system (PNS) and central nervous system (CNS) neurons (Lee et al., 2004; Pozniak et al., 2000, 2002; Tissir et al., 2009; Walsh et al., 2004; Wilhelm et al., 2010; Yang et al., 2000). Intriguingly, haploinsufficiency for p73 in aged but not young mice resulted in neuronal loss, increased microglial activation, aberrant cell cycle re-entry, lipofuscin accumulation, and motor and hippocampal-associated behavioral impairments (Wetzel et al., 2008). Surprisingly, in aged p73 mice, we also observed increases in phospho-tau-positive

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aggregates (Wetzel et al., 2008), a common feature in several neurodegenerative diseases and aging (Ballatore et al., 2007). Moreover, when p73+/− mice were crossed with TgCRND8+/Ø mice, a model of Alzheimer's disease that results in plaques but not neuronal loss (Chishti et al., 2001), the brains of these animals showed neuronal death and tau phosphorylation coincident with plaque formation (Wetzel et al., 2008). Together these findings implicated p73 as a major determinant of neuronal survival, aging, and degeneration.

Several questions were raised by these findings that we have addressed here. First, were the phospho-tau-positive aggregates observed in aged p73+/− mice and in p73+/−;TgCRND8+/Ø mice by immunohistochemistry an indication of a general increase in tau phosphorylation in the CNS? Second, how does haploinsufficiency for p73 cause these abnormal phospho-tau aggregates? Our previous work showed that in aged p73+/− and p73+/− mice crossed with the TgCRND8 mice, Jun N-terminal protein kinase (JNK) activity was enhanced (Wetzel et al., 2008). While JNK is one tau kinase, GSK3β and Cdk5 are thought to be the major kinases associated with the pathogenesis of Alzheimer's disease, playing roles in amyloid precursor protein (APP) processing, synaptic dysfunctions, behavioral impairments, neuronal death, and tau phosphorylation (Ballatore et al., 2007; Hooper et al., 2008; Lee et al., 2001; Su and Tsai, 2011). In this regard, c-Abl is another kinase that is known to regulate Cdk5 and that has been recently associated with tau phosphorylation (Cancino et al., 2008, 2011; Derkinderen et al., 2005; Lee et al., 2008; Lin et al., 2007; Zukerberg et al., 2000). We therefore asked whether these 3 kinases were dysregulated in p73+/− mice. Third, is p73 haploinsufficiency a general predisposition factor for neurodegenerative cues? To answer this question, we used a second mouse model of neurodegeneration, Pin1−/− mice (Liou et al., 2003). Pin1 is a peptidyl-prolyl cis/trans isomerase that shows reduced expression levels in human Alzheimer's disease (AD) brains (Sultana et al., 2006). Because Pin1−/− mice exhibit abnormal APP processing (Pastorino et al., 2006), motor and behavioral deficits, tau hyperphosphorylation, tau filament formation, and neuronal degeneration when they are over 9 months of age (Liou et al., 2003; Lu and Zhou, 2007; Pastorino et al., 2006), it has been proposed as an Alzheimer's disease model. We therefore asked whether p73 haploinsufficiency results in the early appearance of hyperphosphorylated tau in Pin1−/− mice, as it does in the TgCRND8 AD mouse model.

Here, we show that haploinsufficiency for p73 leads to aberrant tau hyperphosphorylation and tau kinase activation in the aged murine brain, and causes early phospho-tau accumulation and tau kinase dysregulation in the TgCRND8 and Pin1−/− neurodegenerative models. Thus, p73 is an important neuroprotective gene in the aged or degenerating mouse brain.

2. Materials and methods

2.1. Animals

This study was approved by the Hospital for Sick Children Animal Care Committee, in accordance with Canadian Council on Animal Care guidelines. p73+/− transgenic mice (Yang et al., 2000) were maintained on a C129SvJae background as described (Pozniak et al., 2000, 2002; Wetzel et al., 2008). Alternatively, the p73+/− mice were crossed into the C57/Bl6 background for more than 10 generations, and maintained in that background. TgCRND8 hemizygous mice (Chishti et al., 2001) containing the Swedish (K670N/M671L) and Indiana (V717F) APP mutations were maintained in the C129 background as crosses with the p73+/− mice. Pin1−/− mice (Liou et al., 2003) were originally in a C57/Bl6 background, were then crossed to the p73+/− mice in the C129SvJae background and were subsequently maintained and analyzed in this mixed background. For the lifespan studies, only littermates were included.

2.2. Neuroanatomy

For histology, mice were sacrificed by sodium pentobarbital and transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Brains were cryoprotected and sectioned at 18 μm. In some experiments, animals were perfused, and one brain hemisphere was cryoprotected and sectioned while the other half was prepared as paraffin sections by the Toronto Centre for Phenogenomics Pathology Facility. Prior to immunocytochemistry, these paraffin sections were deparaffinized with xylene, and hydrated with ethanol. Some sections were also treated for antigen retrieval using 10 mM citrate buffer; similar results were obtained with and without retrieval. For both cryosectioned and deparaffinized sections, immunocytochemistry was performed as described (Cancino et al., 2008). Briefly, sections were washed with Tris-buffered saline (TBS) buffer, permeabilized with TBS, 0.3% Triton X-100 solution, and then incubated in TBS, 5% bovine serum albumin (BSA), 0.3% Triton X-100 for 1 hour as a blocking solution. Slides were incubated with primary antibodies in blocking solution at 4°C overnight. After TBS washes, the sections were incubated with secondary antibodies in blocking solution for 1 hour at room temperature. Finally, after TBS washes, sections were mounted in Permount solution (Thermo, Waltham, MA, USA). Digital image acquisition was performed with AxioVision (version 4.8.2) software (Zeiss, Oberkochen, Germany) on a Zeiss Axioplan 2 microscope with a Hamamatsu (Bridgewater, NJ, USA) orca-R^2 CCD video camera.

2.3. Western blot analysis and densitometry

Extracts from adult hippocampi were prepared in ice cold radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid [EDTA], 1 mM ethylene glycol tet-