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Presenilins regulate the cellular level of the tumor suppressor PTEN

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Abstract

Alzheimer's Disease (AD) is characterized by amyloid plaques consisting of β-amyloid (Aβ) peptides and neurofibrillary tangles consisting of hyperphosphorylated tau protein. Aβ is proteolytically derived from its precursor protein through cleavages by β-secretase and γ-secretase complex comprising presenilins (PS, PS1/PS2), nicastrin, APH-1 and PEN-2. PS1 is also known to activate the PI3K/Akt cell survival pathway in a γ-secretase-independent manner. The tumor suppressor PTEN, which antagonizes the PI3K/Akt pathway, has increasingly been recognized to play a key role in neural functions and its level found reduced in AD brains. Here, we demonstrate that the protein level of PTEN is dramatically reduced in cultured cells and embryonic tissues deficient in PS, and in the cortical neurons of PS1/PS2 conditional double knockout mice. Restoration of PS in PS-deficient cells reverses the reduction of PTEN. Regulation of PTEN by PS is independent of the PS/γ-secretase activity since impaired γ-secretase by the γ-secretase inhibitor treatment or due to nicastrin deficiency has little effect on the protein level of PTEN. Our data suggest an important role for PS in signaling pathways involving PI3K/Akt and PTEN that are crucial for physiological functions and the pathogenesis of multiple diseases.

Keywords: Akt; Alzheimer's Disease; Phosphatase and tensin homologue deleted on chromosome 10; Phosphoinositide 3-kinase; Presenilin

1. Introduction

Alzheimer's Disease (AD) has two hallmark lesions: the extracellular β-amyloid (Aβ) plaques and the intracellular hyperphosphorylated tau fibrillary tangles (Golde, 2005; Hardy and Selkoe, 2002; Trojanowski et al., 1993). Mutations in *PSEN1* and *PSEN2* genes account for the majority of cases of early-onset familial AD (FAD) (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). *PSEN* genes encode polytopic membrane proteins termed presenilins (PS1 and PS2), which function as the catalytic subunit of γ-secretase, an intramembrane protease consisting of at least three other components: nicastrin (Nct), anterior pharynx-defective-1 (APH-1), and presenilin enhancer-2 (PEN-2). γ-Secretase has a wide spectrum of type I membrane protein substrates including Notch, ErbB4 receptor tyrosine kinase, CD 44, nectin-1α, E-cadherin, and low density lipoprotein receptor-related protein (LRP) (for review, see Refs. De Strooper, 2003; Vetrivel et al., 2006). Sequential cleavages of amyloid precursor protein (APP) by β-secretase (BACE) and γ-secretase release highly fibrillogenic Aβ peptides which accumulate in the brains of aged individuals and patients with AD (Dominguez et al., 2004; Greenfield et al., 2000; Hardy and Selkoe, 2002). FAD-associated presenilin variants are thought to exert their pathogenic function by selectively elevating the levels of highly amyloidogenic Aβ42 peptides (Borchelt et al., 1996; Golde, 2005; Hardy and Selkoe, 2002). PS null mice are embryonic lethal and show severe malformation resembling that of Notch deficiency (De Strooper et al., 1998; Donoviel et al., 1999; Shen et al., 1997; Wong et al., 1997).

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In addition to its roles in Aβ production and Notch cleavage, PS1 has been reported to play multiple physiological roles such as those in intracellular trafficking of membrane proteins, calcium homeostasis, neuronal development, neurite outgrowth, apoptosis, synaptic plasticity, and tumorigenesis (Sisodia et al., 1999; Thinakaran and Parent, 2004; Vetrivel et al., 2006; Xia et al., 2001). Recently, several studies have suggested that PS1 regulates the phosphoinositide 3-kinase (PI3K) signaling that governs a variety of crucial cellular functions including cell proliferation, migration and apoptosis (Baki et al., 2004; Kang et al., 2005; Weihl et al., 1999). PI3K phosphorylates phosphatidylinositol (4, 5) – diphosphate (PIP2) to generate phosphatidylinositol (3, 4, 5) – triphosphate (PIP3). Elevated PIP3 levels result in Akt activation by promoting its phosphorylation at residues serine 473 and threonine 308. Activated Akt in turn inactivates downstream substrate glycogen synthase kinase-3β (GSK-3β), which is strongly implicated in tau hyperphosphorylation (Alessi et al., 1996; Avila, 2006; Liang and Slingerland, 2003; Mandelkow et al., 1992; Mulholland et al., 2006). PS1 can positively regulate PI3K/Akt activation in a γ-secretase-independent manner, hence inactivating GSK-3β and reducing tau phosphorylation. FAD-linked mutations in PS1 conversely down-regulate the PI3k/Akt signaling (Baki et al., 2004; Kang et al., 2005; Weihl et al., 1999).

Pten (phosphatase and tensin homologue deleted on chromosome 10) is a tumor suppressor gene that mutates frequently in many sporadic and hereditary cancers (Stiles et al., 2004; Sulis and Parsons, 2003). *Pten*-null mice die at early embryonic stages, and heterozygous knockout mice develop a number of tumors (Di Cristofano et al., 1998; Podsypanina et al., 1999). PTEN contains a tyrosine phosphatase functional domain, exhibiting both protein and lipid phosphatase activity *in vitro* (Maehama and Dixon, 1998). PTEN dephosphorylates the 3′-position of PIP3 to generate PIP2, thus antagonizing the activity of PI3K/Akt (Maehama and Dixon, 1998; Myers et al., 1998; Stiles et al., 2004; Sulis and Parsons, 2003). In addition to its tumor suppressing function, PTEN has been found necessary for normal cerebellar architecture and for proper migration of neurons and glia (Marino et al., 2002). Mouse brains with conditionally inactivated *Pten* showed an increased soma size of neurons without altering proliferation (Fraser et al., 2004; Kwon et al., 2001). Mutations in PTEN-induced kinase 1 (PINK1) have been linked to hereditary early-onset Parkinson's disease (Valente et al., 2004), implying the importance of PTEN signaling in neurodegenerative diseases. Recent studies showed decreased levels and altered distribution of PTEN along with elevated PI3K signaling in AD patient brains (Griffin et al., 2005; Zhang et al., 2006a,b). In addition, our previous study demonstrated that PTEN affects the phosphorylation and aggregation of tau (Zhang et al., 2006a,b). These results suggest that a loss of PTEN function may contribute to neurodegeneration in AD. In the present study, we explored the effects of PS deficiency on PTEN and revealed a significant modulation of the cellular level of PTEN by PS.

2. Materials and methods

2.1. Cell lines

PS1 single knockout (PS1 KO), PS1/PS2 double knockout (PS DKO), and nicastrin knockout (Nct KO) mouse embryonic fibroblast cells, as well as the wild type cells derived from the respective control mice, were cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin (Hyclone, Logan, UT, USA). Nct KO cells stably expressing human nicastrin were kindly provided by Dr. G. Thinakaran and cultured in media supplemented with 0.4 mg/ml hygromycin (Roche, Indianapolis, IN, USA). Mouse embryonic stem cells isolated from PS1/PS2 double knockout as well as wild type mouse (PS wt) were maintained as previously described (Zhang et al., 2005). Mouse neuroblastoma N2a cells stably coexpressing the human APP Swedish mutant (N2a Swe) and one of the human PS1 variants (which includes a single amino acid substitution, D385A, in PS1 transmembrane domain 7 (Kim et al., 2001), a deletion of the first two PS1 transmembranes Delta 1-2 (Leem et al., 2002), and a deletion of PS1 exon 9 Delta 9 (Borchelt et al., 1996)) were maintained in medium containing 50% DMEM and 50% Opti-MEM (Invitrogen, Carlsbad, CA, USA), supplemented with 5% FBS, penicillin/streptomycin and 0.4 mg/ml G418 (Invitrogen).

2.2. Transfection and stable cell line establishment

Wild type fibroblast cells were transiently transfected with pcDNA (as control) or PS1 cDNA constructs using FuGENE 6 (Roche, Indianapolis, IN, USA), following the manufacturer's protocol. To establish stable cell lines, PS1 cDNA (or pcDNA) and pcDNA3/hygro vectors were co-transfected into PS1 KO cells; and transfected cells were selected with 0.4 mg/ml hygromycin. PS DKO cells were co-transfected with PS1 (or PS2) cDNA and pAG3zeo vectors, and selected with 0.2 mg/ml zeocin (InvivoGen, San Diego, CA, USA).

2.3. Immunoblotting and data analysis

Cells were lysed in lysis buffer (0.5% IGEPAL CA-630 and 0.5% deoxycholic acid in phosphate buffered saline, supplemented with protease inhibitors). Lysates of PS1 KO and PS1 heterozygous mouse embryos collected on embryonic day 15 (E15) (Wong et al., 1997) were kindly provided by Dr. G. Thinakaran. Total brain lysates of PS1/PS2 conditional double knockout (PS cDKO) mice (Saura et al., 2004) and littermate controls at 2 months of age were kindly provided by Dr. J. Shen. Equal amounts of proteins were separated by SDS-PAGE, transferred onto PVDF membranes, and immunoblotted with different antibodies. Monoclonal antibodies against PTEN and phospho-Akt and polyclonal antibody against PS2 carboxy-terminus were from Cell Signaling (Danvers, MA, USA). Monoclonal antibody against α-tubulin was from Sigma (St. Louis, MO, USA). Polyclonal antibodies against PS1 amino-terminus (Ab14) and