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Caspase-cleaved tau exhibits rapid memory impairment associated with tau oligomers in a transgenic mouse model

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ABSTRACT

In neurodegenerative diseases like AD, tau forms neurofibrillary tangles, composed of tau protein. In the AD brain, activated caspases cleave tau at the 421th Asp, generating a caspase-cleaved form of tau, TauC3. Although TauC3 is known to assemble rapidly into filaments *in vitro*, a role of TauC3 *in vivo* remains unclear. Here, we generated a transgenic mouse expressing human TauC3 using a neuron-specific promoter. In this mouse, we found that human TauC3 was expressed in the hippocampus and cortex. Interestingly, TauC3 mice showed drastic learning and spatial memory deficits and reduced synaptic density at a young age (2–3 months). Notably, tau oligomers as well as tau aggregates were found in TauC3 mice showing memory deficits. Further, *i.p.* or *i.c.v.* injection with methylene blue or Congo red, inhibitors of tau aggregation *in vitro*, and *i.p.* injection with rapamycin significantly reduced the amounts of tau oligomers in the hippocampus, rescued spine density, and attenuated memory impairment in TauC3 mice. Together, these results suggest that TauC3 facilitates early memory impairment in transgenic mice accompanied with tau oligomer formation, providing insight into the role of TauC3 in the AD pathogenesis associated with tau oligomers and a useful AD model to test drug candidates.

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1. Introduction

Tau is a neuronal microtubule-associated protein that binds to and stabilizes microtubules in axons. In AD and other tauopathies, tau is hyperphosphorylated and forms insoluble aggregates called neurofibrillary tangles (NFTs) in neurons (Lee et al., 2001). While NFTs are considered as a hallmark of AD, it is unlikely that NFTs are the major toxic tau species (Santacruz et al., 2005). Recently, tau oligomers were detected in the brain of AD model mice and proposed to play an important role in AD pathogenesis (Berger et al., 2007). It appears that the intermediate forms of tau, including tau oligomers, which precedes the NFT development, mediate neuronal dysfunction (Lasagna-Reeves et al., 2011).

Many tau mouse models have been established for studying tauopathy. Because normal tau protein is too soluble to form tau aggregates, however, transgenic (TG) mice expressing wild-type human tau or tau kinase do not show either NFTs or memory impairment in most cases (Duff et al., 2000; Gotz et al., 1995). A tau TG model expressing a missense mutation shown in a frontal temporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) patient reveals neuronal loss with hyperphosphorylated tau and an NFT-like tau aggregates, but no associated memory deficits (Lewis et al., 2000). rTG4510, a TRE-inducible mouse model overexpressing P301L tau mutant, shows memory loss with hyperphosphorylated tau at early age and NFT formation at middle age (Santacruz et al., 2005). However, overexpression of P301L tau mutant is too high in this case. Currently, existing mouse models are not sufficient to describe the spectrum of tauopathy, especially tau oligomer-associated memory impairment.

Because of amyloid beta (Aβ) and stress, caspases are activated in the AD brain and cleave multiple proteins, including tau (Rohn et al., 2002). The caspase-cleaved form of Tau, TauC3, is typically found in the brain of patients at the mild cognitive impairment stage of AD (Gamblin et al., 2003; Rissman et al., 2004) and those with other tauopathies, including Pick's disease (Mondragon-Rodriguez et al., 2008), corticobasal degeneration (Newman et al., 2005), progressive supranuclear palsy (Guillozet-Bongaarts et al., 2005), and traumatic brain injury (Gabbita et al., 2005). These data indicate that TauC3 is associated with the formation of tau aggregates. TauC3 has been shown to

Abbreviations: AD, Alzheimer's disease; DIV, days *in vitro*; *i.c.v.*, intracerebroventricular; *i.p.*, intraperitoneal; TauC3, caspase-cleaved form of tau.

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form tau oligomers and aggregates faster than wild-type tau *in vitro* (Gamblin et al., 2003), and localized within tau aggregates in P301S and rTG4510 mice (Delobel et al., 2008; Zhang et al., 2009). In the brain of rTG4510 mice, caspase activation leads to the formation of tangles in cells just a day after and TauC3 is found in tangles (de Calignon et al., 2010). These data indicate that TauC3 is associated with tau oligomers. However, the role of TauC3 in AD pathogenesis remains unknown.

In this study, we show that transgenic expression of TauC3 in the mouse brain induces memory impairment at a young age, which is accompanied by the appearance of tau oligomers and aggregates. Aggregation blockers, such as methylene blue and Congo red, reduce tau oligomers and prevent memory impairment in TauC3 mice. Thus, TauC3 oligomers are able to participate in AD pathogenesis.

2. Materials and methods

2.1. Generation of TauC3 mice

The human *tau* expression construct was injected into embryos and positive F_0 mice were identified by PCR analysis using a synthetic oligonucleotide (forward; 5′-GGA TGG CTG AGC CCC GCC CGG AGT TCG-3′) corresponding to the *BAI1-AP4* promoter and an oligonucleotide (reverse; 5′-GGG CTG CTC GCG GTG TGG CGA TCT TC-3′) of *tau* cDNA (Macrogen Inc., Korea). TauC3 mouse was identified and mated to BALB/c mice and raised under a 12:12 h of light:dark cycle with access to food and water *ad libitum*. All animal protocols were approved by the Seoul National University Standing Committees on Animals.

2.2. Western blot analysis

The cortex, hippocampus, striatum and cerebellum were prepared from TauC3 mice and age-matched littermates. Sub-sectioned brain tissues were ground with a mortar and pestle in liquid nitrogen and the powdered tissues were homogenized in 3 different extraction buffers for the detection of different state of tau species. First, TBS extraction buffer [20 mM Tris–Cl (pH 7.4), 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, and 20 mM NaF with 0.5% NP-40] to detect tau phosphorylation. Second, oligomer extraction buffer [TBS buffer containing 2 mM N-ethylmaleimide (NEM)] to detect 170 kDa Tau oligomer. Third, reassembly (RAB) buffer [0.1 M 2-(N-morpholino) ethanesulfonic acid (MES) pH 7.0, 1 mM EGTA, 0.5 mM $MgSO_4$, 0.75 M NaCl, 1 mM PMSF, 1 mM Na_3VO_4, and 20 mM NaF] and RIPA buffer, (50 mM Tris–Cl pH 8.0, 150 mM NaCl, 0.5% NP-40, 5 mM EDTA, 0.5% sodium deoxycholate, and 0.1% SDS), and 70% formic acid (FA) were prepared to solubilize RIPA-insoluble tau aggregates. Samples were then solubilized in SDS sample buffer with or without β-mercaptoethanol to examine different tau species with Western blotting. To avoid blood contamination, mice were sacrificed and perfused with heparinized PBS.

2.3. Antibodies

TG5 (total Tau: a.a.220–240, 1:1000), PHF-1 (p-Tau: Ser396/404, 1:3000), CP13 (p-Tau: Ser202, 1:1000), and MC1 (conformational abnormality of Tau, 1:500) were kindly gifted from Dr. P. Davies (Albert Einstein College of Medicine, NY, USA). 12E8 (p-Tau: Ser262/Ser356, 1:3000) was kindly gifted from Dr. P. Seubert (Elan Pharmaceuticals, MA, USA). AT8 (p-Tau: Ser262/Ser356, 1:3000), AT100 (p-Tau: Ser212/214, 1:5000), pT231 (p-Tau: Ser231, 1:3000), and HT7 (human Tau-specific: a.a.159–163, 1:10,000) antibodies were purchased from Thermo Scientific (IL, USA). TauC3 (cleaved tau: Asp421, 1:2500) antibody was from Invitrogen (CA, USA). ATG5 (1:2000), LC3 (1:3000), and p62 (1:1000) antibodies were from Novus Biologicals (CO, USA). P-S6 (1:1000), PSD95 (1:1500), NR1 (1:1000), NR2A (1:1000), and NR2B (1:1000) antibodies were from Cell signaling (MA, USA).

2.4. Cell culture, DNA construction, and DNA transfection

SH-SY5Y (human neuroblastoma) cells and primary hippocampal neurons were prepared as described previously (Park et al., 2012). Briefly, SH-SY5Y cells were cultured in DMEM (Hyclone) supplemented with 10% (v/v) fetal bovine serum (Hyclone). Primary hippocampal neurons were cultured from postnatal day 1 and incubated for 13 days in neurobasal media containing B27 (Invitrogen). SH-SY5Y cells were transfected using the Polyfect reagent (Qiagen), whereas primary neurons were transfected using the Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Tau-GFP and TauC3-GFP constructs were described previously (Chung et al., 2001).

2.5. Dendritic spine density analysis

Dendritic spine density analysis was described previously (Kam et al., 2013). In brief, cultured hippocampal primary neurons were transfected at 13 days *in vitro* (DIV) with EGFP-fusion construct for 24 h. Images of GFP-expressing cells were acquired by confocal microscopy (Carl Zeiss) and the numbers of spines on the axon (100 μm in length) of randomly chosen neurons were counted on the images ($n = 3$).

2.6. In vitro tau fibrillation and thioflavin T fluorescence assay

Tau construct was previously described (Chung et al., 2001). His-fusion human full-length tau protein was purified from bacteria (BL21-DE3) after transformation with His^{6x}-tau. Total 10 μM tau protein was incubated with 50 mM NH_4Ac buffer containing 20 μM heparin and 2 mM dithiothreitol at 37 °C. The reaction products were then incubated with 25 μM thioflavin T (Sigma) and fluorescence was measured with a LS-50 fluorimeter (Perkin-Elmer) using an excitation filter of 450 nm and an emission filter of 484 nm.

2.7. Filter trap assay

The filter trap assay of tau protein was performed as described previously (Park et al., 2012). Tissue extracts were prepared in TBS extraction buffer by sonication. After centrifugation at 15,000 × *g* for 30 min at 4 °C, the supernatants were incubated with 0.1% SDS for 30 min. After incubation, supernatants were filtered through nitrocellulose membrane (0.2 μm) (Pall Industries) by using a 96-well vacuum dot blot apparatus (Bio-Rad laboratories). The membrane was washed twice with Tris-buffered saline with 0.05% tween 20 (TBST), blocked with TBST containing 5% non-fat milk for 1 h, and analyzed by Western blotting.

2.8. Histology and immunohistochemistry

Mice were transcardially perfused with ice-cold PBS before dissection of the tissue post fixation. Histological and immunohistochemical analysis were performed on 25-μm thick coronal cut cryosectioned free-floating sections. Immunohistochemistry was done using standard immunoperoxidase procedures with Elite ABC kits (Vector Laboratories) and developed with 3,3-diaminobenzidine (DAB) or immunofluorescence with Alexa Fluor® 488-conjugated secondary antibody (Jackson Immunoresearch). In the immunohistochemistry, fluorescent intensity of synaptophysin was normalized by that of Hoechst dye signal. The average grayscale intensity of each section was calculated with Image J software. The following monoclonal antibodies were used to stain the free-floating brain sections: HT7 (1:100), TauC3 (1:50), PHF-1 (1:100), AT100 (1:1000), T22 (1:100), MC-1 (1:10), synaptophysin (1:100, Santa Cruz Biotechnology), and GFAP (1:1000, Santa Cruz Biotechnology).

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