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Early-onset cognitive deficits and axonal transport dysfunction in P301S mutant tau transgenic mice

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ABSTRACT

Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD) are neurodegenerative "tauopathies" characterized by hyperphosphorylated tau accumulation and neurofibrillary tangles. The P301S mutation of tau, a causal mutation of a familial type of FTLD, is believed to be involved in neurodegenerative progression. We developed a transgenic mouse, named TPR50, harboring human P301S tau. Tau phosphorylation in the hippocampus of TPR50 mice increased with age, particularly at S202/T205. Insolubilization and intracellular accumulation of tau were detected in the hippocampus by 9 months of age. Expression of calbindin was significantly reduced in 6- and 9-month-old TPR50 mice but not in 3-month-old mice. TPR50 mice demonstrated cognitive dysfunction at 5 months. At this age or earlier, although no intracellular tau accumulation was observed in the hippocampus, abnormally increased microtubule (MT)-related proteins and MT hyperdynamics in the hippocampus, and impaired axonal transport in the septo-hippocampal pathway were already observed. Therefore, cognitive dysfunction in TPR50 mice may result from early MT dysfunction and impaired axonal transport rather than accumulation of insoluble tau and neurodegeneration. TPR50 mice are a valuable new model to study progression of tauopathies at both the behavioral and neurocellular levels and may also prove useful for testing new therapies for neurodegenerative diseases.

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1. Introduction

Neurofibrillary tangles (NFTs), consisting of the microtubule (MT)-associated protein tau are a pathological hallmark of Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD) such as Pick disease, progressive supranuclear palsy, and corticobasal degeneration (Goedert et al., 1988; Lee et al., 2001). The strong temporal correlation between NFT formation, neurodegeneration, and symptom progression indicates that tau is a key molecule in disease pathogenesis. Indeed, these diseases are collectively termed tauopathies.

Under physiological conditions, tau functions as a modifier of microtubule stability (Drechsel et al., 1992; Lee et al., 1998; Dixit et al., 2008). Hyperphosphorylation of tau reduces the binding to MT. Consequently, the increased pool of soluble tau may trigger the disintegration of MTs (Grundke-Iqbal et al., 1986; Goedert et al., 1995). Mutations in the coding and intronic regions of *MAPT*, the gene encoding tau, are known to cause familial forms of FTLD (FTDP-17) (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). Several mutant tau transgenic (Tg) mouse lines have been generated to model tau pathology and neurodegenerative phenotypes (Götz and Ittner, 2008), some of which exhibit abnormal tau accumulation and impaired axonal function (Lewis et al., 2000; Zhang et al., 2004; Ittner et al., 2008).

In humans, the P301S mutation causes early onset of disease progression and strong functional influences (Bugiani et al., 1999; Goedert et al., 1999; Yasuda et al., 2000; Lossos et al., 2003; Werber et al., 2003). Biochemical studies have shown that the P301S mutation reduces the ability of microtubule assembly (Bugiani et al., 1999) and enhances heparin-induced tau filament formation (Goedert et al., 1999). Two lines of Tg mice expressing P301S mutant 4R0N and 4R1N tau isoforms have been reported (Allen et al., 2002; Yoshiyama et al., 2007), and both lines develop NFT-like pathology and neurodegeneration. Memory impairment was also reported in one of these lines (Takeuchi et al., 2011). However, definitive mechanism of toxicity by mutant tau is still unclear. We generated novel Tg mice (TPR50) expressing the longest form (4R2N) of tau

Abbreviations: NFTs, neurofibrillary tangles; FTLD, frontotemporal lobar degeneration; MT, microtubule; WT, wild type; Tg, transgenic; SEM, standard error of the mean.

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with a P301S mutation and assessed the biochemical and behavioral phenotypes to further investigate the mechanisms underlying age-dependent tau accumulation, neurotoxicity, and behavioral sequelae. We also examined tau pathophysiology, neural function, MT-related proteins, and axonal function of TPR50 mice by behavioral and biochemical approaches. These mice exhibited age-dependent abnormal tau accumulation and motor deficits as expected; in addition, they showed early-onset of cognitive impairment and disrupted axonal transport in the septo-hippocampal pathway.

2. Experimental procedures

2.1. Animals

TPR50 mice were generated by microinjection of a vector encoding a P301S mutant of the longest human tau isoform (4R2N) under the control of the mouse prion promoter (Supplementary Fig. S1) into single-cell embryos harvested from BDF1 mice and then backcrossed more than 10 times with C57BL/6J mice. Only male mice were used in this study. They were housed in groups and kept on a 12 h-light/12 h-dark schedule and provided *ad libitum* access to food and water. All animals were maintained and sacrificed according to the guidelines of the Takeda Experimental Animal Care and Use Committee.

2.2. Antibodies

Antibodies against the following proteins were used in this study: AT270, AT8, HT7 (Innogenetics, Ghent, Belgium), pS214-tau, pS262-tau, pS396-tau (Invitrogen, Carlsbad, CA), Ab-3 (Thermo Fisher Scientific, Fremont, CA), synaptophysin, acetylated tubulin, α-tubulin, β-actin (Sigma, St. Louis, MO), synaptotagmin (Enzo, New York, NY), kinesin heavy chain, PSD-95, and calbindin (Millipore, Bedford, MA).

2.3. SDS-PAGE and Western blotting

Hippocampi isolated from Tg and WT mice were homogenized in lysis buffer (50 mM Tris–HCl, 5 mM EDTA, 1 mM EGTA, 100 mM NaCl, 1% NP-40, and 2.5% sodium deoxycholate, pH 7.5) supplemented with protease inhibitors [1.37 mg/L pepstatin A, 25 KIU/mL aprotinin, 1 nM microcystin LR, 1 nM MG115, 40 nM leupeptin and 100 nM 4-(2-aminoethyl)benzenesulfonyl fluoride (ABSF) HCl] and phosphatase inhibitors (30 mM NaF, 5 mM sodium diphosphate, and 2 nM sodium orthovanadate). The homogenate was centrifuged at 10,000 × *g* for 10 min and the supernatant taken as the soluble protein fraction. Total protein concentration was determined using the BCA assay kit (Pierce, Rockford, IL). Equal amounts of protein (1–20 μg depending on the protein of interest) were separated by SDS-PAGE on 10% SDS gels, then electrophoretically transferred to 0.45 μm poly-vinylidene difluoride membranes (Millipore) and blocked for 1 h in BlockAce (DS Pharma Biomedical, Osaka, Japan). After blocking, the membranes were probed with primary antibodies followed by labeling with horseradish peroxidase (HRP)-coupled secondary antibodies (Amersham, Piscataway, NJ). Immunolabeling was visualized by a chemiluminescence reagent (Immunostar; Wako, Osaka, Japan) using a LAS1000 imaging system (Fujifilm, Tokyo, Japan) or ImageQuant LAS4000 (GE healthcare, Pittsburgh, PA). Quantitative densitometric analyses were performed with Image Gauge (Fujifilm) or ImageQuant TL (GE healthcare). Values presented are derived from densitometry arbitrary units (A.U.). With regard to immunoblot of tau, the bands including all tau species were selected and analyzed.

2.4. Preparation of insoluble tau

Insoluble aggregated tau was prepared by the sarkosyl extraction methods, described previously (Sahara et al., 2004; Taniguchi et al., 2005; Uno et al., 2009). In brief, lysis buffer-insoluble pellets were rehomogenized with 0.5 M NaCl containing 10% sucrose and incubated in 1% sarkosyl for 1 h at 37 °C. After centrifugation at 256,000 × *g* for 15 min, the pellets were resuspended by ultrasonication in phosphate buffered saline to yield the sarkosyl insoluble fraction.

2.5. RAB-RIPA-formic acid (FA) extraction

RAB-RIPA-FA extraction was performed by using a previously described method (Ishihara et al., 1999, 2001; Eckermann et al., 2007; Uno et al., 2009). Brain tissue was homogenized in ice-cold high-salt RAB buffer [0.1 M morpholineethanesulfonic acid (MES), 1 mM EGTA, 0.5 mM $MgSO_4$, 0.75 M NaCl, 0.02 M NaF, 100 μM ABSF, and protease inhibitors (Complete Mini, Roche Applied Science, Mannheim, Germany), pH 7.0], and the sample was centrifuged at 50,000 × *g* for 20 min at 4 °C. The supernatant was boiled for 5 min and then centrifuged at 10,000 × *g* for 20 min at 4 °C. The resulting supernatant contains the soluble tau fraction (RAB fraction). To remove myelin and associated lipids, RAB insoluble pellets were re-extracted with 1 M sucrose/RAB buffer and centrifuged at 100,000 × *g* for 30 min at 4 °C. The pellets were suspended in RIPA buffer (50 mM Tris–HCl, 5 mM EDTA, 1 mM EGTA, 30 mM NaF, 5 mM sodium diphosphate, 2 μM pepstatin A, 100 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 μM microcystin LR, 40 μM leupeptin, 100 μM ABSF, 2 mM sodium orthovanadate, 1 μM MG115, pH 7.6) and centrifuged as mentioned above to obtain the supernatants (RIPA fraction). Finally, the RIPA insoluble pellets were re-extracted with 70% FA.

2.6. Immunohistochemistry

Dissected hemibrains were fixed in 4% paraformaldehyde (Wako) for 24 h. Fixed hemibrains were embedded and frozen in freezing medium (OCT Tissue-Tek 4583; Sakura Finetechnical, Tokyo, Japan) and sliced into 20-μm thick sections using a cryostat (CM1850; Leica, Nussloch, Germany). These sections were mounted onto silane-coated slides (Matsunami, Osaka, Japan). After blocking with BlockAce containing 3% fetal bovine serum, the slides were probed with a primary antibody against total tau (HT7, 1:2000 dilution), followed by an HRP-coupled secondary antibody (Dako, Glostrup, Denmark). Immunolabeling was detected by 3,3′-diaminobenzidine tetrahydrochloride staining (Dako). Images were captured using Nanozoomer and NDP scan software (Hamamatsu Photonics, Hamamatsu, Japan).

2.7. Bielschowsky silver staining

Bielschowsky staining was performed as described previously (Bellucci et al., 2006), with minor modifications. The dissected brain was fixed in 4% paraformaldehyde (Wako) for 24 h. The right hemisphere was cut in the coronal plane and the left hemisphere was cut in the sagittal plane. Samples were embedded in paraffin and cut into 4-μm-thick sections. After deparaffinization, the sections were stained with 20% silver nitrate solution for 20 min, rinsed in distilled water, and stained with ammonium silver solution for 20 min. After washing with 0.1% ammonium hydroxide solution, the sections were put in ammonium silver solution containing formaldehyde, monohydrate citric acid, and concentrated nitric acid for optimal staining. The sections were then rinsed three times in distilled water, placed in 5% sodium thiosulfate solution for 5 min,

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