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Silencing of TREM2 exacerbates tau pathology, neurodegenerative changes, and spatial learning deficits in P301S tau transgenic mice

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ABSTRACT

Tau pathology is a pathological hallmark for several neurodegenerative diseases including Alzheimer's disease and frontotemporal dementia. As a novel susceptibility gene for these 2 diseases, triggering receptor expressed on myeloid cells 2 (*TREM2*) gene encodes an immune receptor that is uniquely expressed by microglia. Recently, a correlation between TREM2 expression and hyperphosphorylated tau has been revealed in the brain of Alzheimer's disease patients, suggesting a potential association between TREM2 and tau pathology. However, the role of TREM2 in tau pathology remains unclear thus far. Herein, using P301S mice, we showed that TREM2 was upregulated in microglia during disease progression. Silencing of brain TREM2 exacerbated tau pathology in P301S mice. This exacerbation might be attributed to neuroinflammation-induced hyperactivation of tau kinases. Additionally, more severe neurodegenerative changes and spatial learning deficits were observed following TREM2 silencing. Our results imply that TREM2 attenuates tau kinase activity through restriction of neuroinflammation, and thus protects against tau pathology. These findings further suggest that TREM2 may represent as a potential therapeutic target for tau-related neurodegenerative diseases.

1. Introduction

Tau pathology, usually refers to the intraneuronal hyperphosphorylation of tau protein, is considered as a pathological hallmark for a variety of neurodegenerative diseases, especially Alzheimer's disease (AD) and frontotemporal dementia (FTD) (Ballatore et al., 2007; Hanger et al., 2014). In the brain of AD and FTD animal models, tau hyperphosphorylation disrupts the stability of the cellular cytoskeleton, subsequently leading to dysfunction or even damage of neurons and synapses (Honson and Kuret, 2008). More importantly, compelling evidence indicates that tau pathology is closely associated with the severity of cognitive deficits in patients with AD or FTD (Giannakopoulos et al., 2003; Xu et al., 2014). In view of its critical role in the pathogenesis of these 2 diseases, more needs to be done to elucidate potential mechanisms affecting the formation and progression of tau pathology.

Recent genetic studies suggest that triggering receptor expressed on myeloid cells 2 (*TREM2*) gene may be associated with the susceptibility of AD because a low-frequency variant R47H increases the risk of this disease by nearly 3-fold in Caucasian populations (Guerreiro et al., 2013a; Jonsson et al., 2013). Interestingly, loss-of-function mutations in *TREM2* gene are also related with the clinical phenotypes and neuropathology of FTD, supporting the contribution of TREM2 to the pathogenesis and progression of this disorder (Guerreiro et al., 2013b; Rayaprolu et al., 2013). TREM2 is a 230 amino acid type I transmembrane receptor that belongs to the superfamily of immunoglobulin (Colonna, 2003). As its name implies, TREM2 is expressed by a subset of myeloid cells including monocyte, dendritic cells, osteoclasts, and tissue macrophages in blood and peripheral tissues (Hu et al., 2014; Jiang et al., 2013). In

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the brain, we and others showed that TREM2 was uniquely expressed by microglia, the main immune cell within central nervous system, and coupled with DAP12 for its signaling (Ma et al., 2015; Takahashi et al., 2005, 2007; Ulrich et al., 2014). More importantly, several lines of evidence suggest an essential role of TREM2 in modulation of microglial functions, especially the phagocytosis and the proinflammatory reactions (Kleinberger et al., 2014; Melchior et al., 2010; Takahashi et al., 2005, 2007).

Coincidentally, 3 recent studies have proposed a potential association between TREM2 protein and tau pathology in AD, the most common type of tau-related neurodegenerative disorder. Cruchaga et al. (2013) showed that *TREM2* R47H variant was related to higher levels of total tau and hyperphosphorylated tau protein in the cerebral spinal fluid of AD patients. Meanwhile, this finding has been further confirmed by Lill et al. (2015) in a larger cohort of AD patients. Furthermore, Lue et al. (2015) found that TREM2 expression was positively correlated with phosphorylated tau protein in postmortem brain samples from patients with AD. Despite these clinical findings, the precise role of TREM2 in the formation and progression of tau pathology remains largely unknown.

In this study, using P301S mice, a transgenic animal model of tau pathology, we showed that TREM2 was upregulated in microglia during disease progression. Silencing of brain TREM2 using a lentiviral-mediated strategy exacerbated tau pathology in P301S mice. This exacerbation of tau pathology might be attributed to neuroinflammation-induced hyperactivation of tau kinases. Additionally, more severe neurodegenerative changes and spatial learning deficits were observed after TREM2 silencing. Taken together, our results imply that TREM2 attenuates the activity of tau kinases through restriction of neuroinflammation, and thus plays a protective role against tau pathology. These findings further indicate that TREM2 may represent as a potential therapeutic target for tau-related neurodegenerative diseases, such as AD and FTD.

2. Materials and methods

2.1. Transgenic mice

Male P301S transgenic mice (Stock number: 008169, expressing P301S mutant human microtubule-binding protein tau on a C3H × C57BL/6 hybrid genetic background) and their age-matched wild-type (WT) mice (Stock number: 100010) were purchased from The Jackson Laboratory. They were maintained in individually ventilated cages at 20 °C–24 °C and relative humidity (30%–70%) with a 12-hour light-dark cycle and given free access to food and water. Animal Care and Management Committee of Qingdao Municipal Hospital approved the whole study protocol. All animal experiments were conducted in accordance with Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were reported in accordance with the Animal Research: Reporting of In Vivo Experiments guidelines (Kilkenny et al., 2010).

2.2. Isolation of microglia from adult mice

Adult microglia was isolated from 1-, 5-, and 9-month-old P301S mice and their age-matched WT mice according to a previously described protocol (Cardona et al., 2006). Briefly, mice were deeply anesthetized and perfused with ice-cold Hank's balanced salt solution (HBSS). The brains were placed into a 15-mL dounce homogenizer containing 3 mL of digestion cocktail to make a cell suspension. The cell suspension was then transferred to a 50-mL conical tube containing a total volume of 10 mL of digestion cocktail and was gently rocked at room temperature for 15 minutes. Afterward, the cell suspension was settled at room temperature and was centrifuged for 7 minutes at 300*g*. After discarding the supernatant, the pellet was washed with 10 mL of HBSS and was resuspended in 37% stock isotonic percoll. A total volume of 4 mL of the stock isotonic percoll was then transferred to a 15-mL conical tube containing 4 mL of 70% percoll. After pipetting 4 mL of 30% percoll and 2 mL of HBSS from the top of the 37% layer, the remaining gradients were further centrifuged for 40 minutes at 200*g*. The layer of debris was gently removed, and a total volume of 2.5 mL of the 70%–37% interphase was transferred into a new 50-mL conical tube. Afterward, the percoll containing the interphase was diluted for 3 times using additional HBSS and was centrifuged again for 7 minutes at 500*g*. Finally, the remaining pellet was resuspended in HBSS to get a suspension of adult microglia. The purity of the microglia was then determined by flow cytometry using an antibody against microglial marker ionized calcium-binding adapter molecule 1 (Iba1). In average, about 95% of isolated cells were immunostained with Iba1.

2.3. Lentiviral particles preparation

The following short hairpin sequences (TREM2 shRNA: 5′-TGATGCTGGAGATCTCTGGGTTCAAGAGACCCAGAGATCTCCAGCATCTTTTTTC-3′ and control shRNA: 5′-TGATGCTGAAGGTCGCTTGGTTCAAGAGACCAAGCGACCTCCAGCATCTTTTTTC-3′) were synthesized and cloned under the U6 promoter into the lentiviral vectors, respectively, as previously described (Jiang et al., 2014a). The lentiviral vectors were purified and then cotransfected with packaging vectors (Invitrogen) into 293FT cells (Invitrogen). The supernatant was collected after 48 hours, and the lentiviral particles in the supernatant were concentrated at 1:100 by ultracentrifugation and recovered by suspension in phosphate-buffered saline (PBS). Viral titers were determined by a commercial titration enzyme-linked immunosorbent assay (ELISA) kit (TaKaRa; titer for lentiviral particles containing TREM2 shRNA: 0.9×10^7 TU/μL; titer for control lentiviral particles: 1.1×10^7 TU/μL).

2.4. Intracerebral injection of lentiviral particles

Stereotactic intracerebral injection of lentiviral particles was conducted by technicians who were blinded to the experimental groups as previously described (Jiang et al., 2014a). Briefly, 5-month-old P301S mice and their age-matched WT mice were anesthetized by 80-mg/kg ketamine hydrochloride and 5-mg/kg xylasine hydrochloride and fixed on a stereotactic frame (David Kopf Instruments). Lentiviral preparation (2 μL) was injected into the cerebral cortex (2 deposits) and hippocampus (1 deposit) of each hemisphere using a 30-gauge blunt micropipette attached to a 10-μL Hamilton syringe (Hamilton) at a rate of 0.2 μL/min. Stereotactic coordinates of injection sites from bregma were (1) for the cerebral cortex: anteroposterior: −0.3 mm, mediolateral: 2 mm, dorsoventral: −1.5 mm; anteroposterior: −2 mm, mediolateral: 1.2 mm, dorsoventral: −1.2 mm and (2) for the hippocampus: anteroposterior: −2 mm; mediolateral: 1.2 mm; dorsoventral: −2 mm. The efficiency of lentiviral-mediated TREM2 silencing was determined by quantitative reverse transcription–polymerase chain reaction (qRT-PCR) and immunofluorescence staining at 2 months after injection. According to one of our preliminary experiments, the TREM2 expression in the brain of P301S mice was reduced to a relatively steady level at 1 month after lentiviral particle injection (data not shown). Meanwhile, as reported by Dodart et al. (2005), obvious neuronal loss was observed in the brain at 3 months after lentiviral vector-mediated gene silencing, possibly due to the long-term neurotoxicity of lentivirus. On consideration of these factors, "2-month" was selected as the time point for investigation.